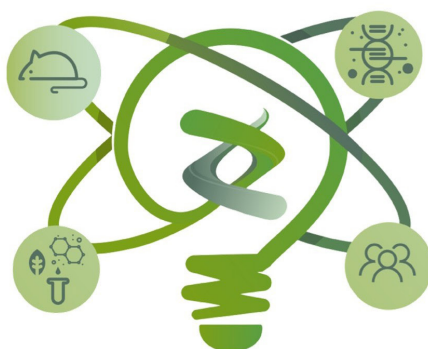


LIVRO DE RESUMOS



XXXIX REUNIÃO ANUAL DA FESBE
LX CONGRESSO ANUAL DA SBFIS
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

Inovações e Desafios nas Ciências Biomédicas: Novos Horizontes em Política Científica e Tecnológica

8 a 11 de Julho de 2025

Universidade Estadual de Campinas (UNICAMP), Campinas/SP

<https://2025.fesbe.org.br>



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Reunião Anual da FeSBE

A Federação de Sociedades de Biologia Experimental (FeSBE) realizou o XXXIX Encontro Anual de 8 a 11 de julho de 2025 no campus da UNICAMP. Reunindo 25 sociedades federadas o evento reafirmou seu papel como um dos mais tradicionais e relevantes da ciência brasileira.

Com uma programação ampla e diversificada, o encontro abordou temas gerais e específicos das áreas biomédicas e biológicas, fortalecendo o networking, o intercâmbio científico e a formação de estudantes e profissionais. O evento proporcionou um ambiente dinâmico para discussão de ideias, estabelecimento de colaborações e estímulo à inovação.

A edição de 2025 marcou mais um capítulo significativo na trajetória da FeSBE, contribuindo para o avanço da ciência no Brasil e reforçando o compromisso da Federação com a integração e o desenvolvimento da comunidade científica.

The Federation of Experimental Biology Societies (FeSBE) held its 39th Annual Meeting from July 8-11, 2025 on the UNICAMP campus. Bringing together 25 federated societies, the event reaffirmed its role as one of the most traditional and relevant in Brazilian science.

With a broad and diverse programme, the meeting addressed general and specific topics in the biomedical and biological fields, strengthening networking, scientific exchange, and the training of students and professionals. The event provided a dynamic environment for discussing ideas, establishing collaborations, and stimulating innovation.

The 2025 edition marked another significant chapter in the history of FeSBE, contributing to the advancement of science in Brazil and reinforcing the Federation's commitment to the integration and development of the scientific community.



**XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO**

**8 A 11 DE JULHO 2025
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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

EIXOS TEMÁTICOS

- 01. Biologia Celular e Molecular**
- 02. Sistema Cardiovascular**
- 03. Sistema Respiratório**
- 04. Sistema Urinário**
- 05. Sistemas Endócrino e Reprodutor**
- 06. Sistema Nervoso**
- 07. Exercício Físico**
- 08. Sistema Digestório, Nutrição e Metabolismo**
- 09. Fisiologia Comparada e Zoologia**
- 10. Farmacologia, Toxicologia, Produtos Naturais e Química Medicinal**
- 11. Imunologia, Microbiologia e Parasitologia**
- 12. Ecologia e Meio Ambiente**
- 13. Nanotecnologia, Biotecnologia, Biociências Nucleares e Terapias Avançadas**
- 14. Ciências de Animais de Laboratório e Modelos Alternativos**
- 15. Educação e Difusão do Conhecimento em Ciências Biomédicas**
- 16. Visão Oftalmologia**
- 17. Biociências Nucleares**
- 18. Anatomia**



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

ABSTRACT TITLES

01. Biologia Celular e Molecular

Molecular Insights of Myocilin Reveal Potential Role in Contraction and Growth of Fish Skeletal Muscle	1
Chrysin stimulates hypertrophy and glycolytic metabolism of the soleus muscle in old rats	2
Quantification Of HTLV-1 Proviral Load In Asymptomatic Carriers and Patients with Adult T-Cell Leukemia/Lymphoma: A Comparison Between Different Transmission Routes	3
Anti-inflammatory and neuroprotective effects of TNF-alpha inactivation in spinal motor neurons of Duchenne muscular dystrophy (DMD) mice	4
Molecular and Functional Characterization of the Role of the $\alpha 5 \beta 1$ Integrin Receptor in Hepatic Dysfunction During Amino Acid Restriction	6
Muscle Degeneration in dy^{2J} Mice, Model for Congenital Muscular Dystrophy type 1A: A Defect in Final Differentiation Rather Than Satellite Cell Exhaustion.....	7
Assessing the Cytotoxicity and Safety Profile of Trastuzumab: H9c2 Cardiomyoblast Exhibit no Evidence of Cytotoxic Damage	9
Inhibition of extracellular vesicles biogenesis as a novel action mechanism of Calix[6]arene	10
Melanopsin's participation in protection against cell death: Role in melanoma tumorigenesis	12
Individuals with long-COVID from mild and moderate cases presents negative impact on morphological and functional characteristics of the Vastus lateralis muscle.....	13
Investigation of Molecular Changes and the Role of the Tumor Microenvironment in the Progression and Metastasis of Childhood Neuroblastoma Using FISH Technique	14
Proteomic Landscape of Kidneys from Aged Rats Exposed to Maternal Protein Restriction: A DOHaD approach.....	15
The consequences of maternal malnutrition on the metabolism and thyroid morphophysiology in male young and old offspring rats	16
Complications of Maternal Protein Restriction on Hepatic Morphophysiology and Biochemical Activity in Old Male Rats: A DOHaD Approach.....	17
Maternal protein restriction affects molecular and metabolic parameters in the pituitary of post-weaning male and female rat offspring	18
Sex-specific Effects of Maternal Low-Protein Diet on Hypothalamus and Metabolism in the Offspring during Early Life	19
mTORC1 and mTORC2 mediate the increase in mass induced by constitutive activation of PI3K signaling in brown and white adipocytes	20
Maternal Protein Restriction promotes Cardiac Disorders by Disrupting Heart Developmental Morphophysiology in Young Male Offspring Rats	21
Sex-Specific Impacts of Maternal Protein Restriction on Cardiac Structure and Antioxidant Balance of Young Rats	22
The impacts of maternal protein restriction on maternal ovarian phenotypic and molecular aspects and those of young rat offspring: Possible transgenerational conditions.....	23
Maternal malnutrition and adverse maternal-fetal consequences: Placental impacts as a starting point for health or disease in offspring.....	25
Maternal Protein Restriction alters the lung morphology, molecular pathways, and proteomic landscape of male young offspring rats	26



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Mitoquinone Prevents Vascular Dysfunction by Modulating Mitochondrial-NADPH Oxidase Crosstalk Post-Myocardial Infarction	27
Role of KLK-14 as a Substrate in Matriptase-Dependent Cancer Progression	28
Proteomic Analysis of Skeletal Muscle Reveals Alterations in Ubiquitinated Sarcomeric Proteins in Parkin Knockout Mice	29
Evaluation of the Anti-Glioma Activity of Monepantel	30
Gene Editing of miR-146b as a Tool to Investigate Papillary Thyroid Carcinoma Oncogenesis	31
JAK/STAT pathway and its role in periodontal repair	32
Study of Molecular Variation Between SARS-CoV-2 Genomes Isolated and Sequenced in Residents of Macaé-RJ and Region	33
Gestational and Lactational Exposure to a Mixture of Phthalates and/or Nanoplastics and Sex-Specific Hepatic Repercussions in Offspring	34
Sperm Analysis of Adult Mice Exposed to Tebuconazole in the Prenatal Phase	35
Role of Annexin A1 in Gut Biology and NLRP3 Inflammasome Activation in a Murine Model of Type I Diabetes	36
mTORC1 and 2 Determine the Brown and White Adipose Tissue Mass in Mice Exposed to Cold	37
Annexin A1 Deficiency Attenuates Renal Injury in a Streptozotocin-Induced Model of Diabetic Nephropathy	38
Application of Bioinformatics: Analysis of Gene Expression and Identification of Functional Enrichment in Breast Cancer	39
Evaluation of oxidative metabolism and mitochondrial markers in the placenta of pregnant Walker 256 tumor-bearing rats, treated with the chemotherapeutics Doxorubicin and Cyclophosphamide ..	40
T-Cell Receptor (TCR) Rearrangement Study in HTLV-1 Carriers and ATLL Patients: Comparative Assessment Between Different Viral Transmission Routes	41
Mitochondrial bioenergetic function in bone cells exposed to polystyrene microplastics	42
Impact of Stress on Gene Expression: Differential Regulation of Apoptosis and Necrosis Pathways ..	43
The role of Hoxb7 in metastatic lung adenocarcinoma	44
Assessment of the role of kallikrein KLK7 and KLK5 in wound healing	45
Altered macromolecular orientation as assessed by birefringence in tendons of rats treated with sodium valproate	46
Effects of Polystyrene Microplastics on Bone-Related Protein Expression and Mineralization	47
Extracellular vesicles released by epithelial kidney cells induce cardiac cell stress	48
Identification of targets involved in skeletal muscle mass control through transcriptomic analysis in mice overexpressing microRNA-29c	49
Effects of Red LED Therapy in an Imiquimod-Induced Murine Model of Psoriasis	50
Collagen, Keratin, IGFR and EGFR expression in Epithelium-derived retinal progenitor cells	51
Platelet-Activating Factor Receptor Modulates Retinal Vasculature Development During Retinogenesis	52
EZH2 as a new regulator of lung cancer metastasis	53
Splenic Alterations Associated with Annexin A1 Deficiency in Streptozotocin- Induced Murine Type 1 Diabetes	55
CRISPR/Cas9 and Tol2-Based constructs cloning for functional assays of <i>WFDC1</i> in chicken limb development	56
Excess of Unsaturated Fatty Acids During Pregnancy Induces Gestational Diabetes and Compromises Muscle Development in Wistar Rats	57



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Metabolic and Epigenetic Reprogramming of Normal Human Keratinocytes by Melanoma-Derived Small Extracellular Vesicles.....	59
Standardization of a retinitis pigmentosa neurodegeneration <i>in vitro</i> model.....	60
Retina and Ciliary Epithelium Postnatal Development and Stem Cell Differentiation	61
Role of melatonin in suppressing tumor progression in MCF-7 and MDA-MB-468 breast carcinoma cell lines	62
Melatonin and Cisplatin Differentially Modulate the Expression of Genes Associated with Energy Metabolism in Hepatocellular Carcinoma Cells	63
Effects of microplastic exposure on the skeletal muscle tissue of zebrafish in relation to their reproductive success	64
An analysis of data on the Impacts of aging on Human Skin cells	65
Excess of Omega-3 and Omega-6 During Development Induces Metabolic Dysfunction and Oxidative Distress in Skeletal Striated Muscle.	66
CD2AP Expression After Albumin Overload in Podocyte Cell Culture with And Without Pan Injury	67
Adrenal disorders in response to maternal protein restriction: Immediate and long-term metabolic disturbances in male rat offspring.....	68
Characterization of a glutamatergic cell model derived from SHSY5Y to study glutamate excitotoxicity in Amyotrophic Lateral Sclerosis.....	69
Role of Osteoprotegerin from Apical Papilla Stem Cells in Monocyte Differentiation into Osteoclasts <i>In Vitro</i> : TRAP Activity and Staining Analysis	70
Decoding the Impact of Hepatic Fibrogenesis in Gap Junction: Insights from a Multicellular Spheroid Model.....	71
Parkin Controls K48 and K63 Ubiquitination levels During Skeletal Muscle Regeneration in Mice.....	73
Maternal Malnutrition Alters the Expression of LncRNAs That Regulate Interaction Networks with Targets that Contribute to the Developmental Origin of Prostate Cancer	74
Using therapeutic ultrasound in different cell lines exposed to Lipopolysaccharide	75
Assessment of hypothalamic inflammation and the blood-brain barrier in an experimental colitis model associated with high-fat diet consumption.....	77
Isolation and characterization of human limbus-derived stromal/mesenchymal stem cells for regenerative medicine and ophthalmological and treatment of ocular surface diseases	79
Antitumor action of ConBr lectin on glioma cells and relationship with p38MAPK activity.....	80
Exercise-improved muscle APLNR expression is impaired in kidney injury and restored by STAT3 inhibition.....	81
Walker 256 tumor induces histomorphological alterations in the rat plantaris muscle 14 days after inoculation.....	82
Melanoma spheroid age negatively affects response to single-dose treatment.....	83
The relevance of Nrf2 pathway in the progression of periodontitis and the effect of its activation/inhibition	84
Parkin overexpression alters myogenic factors during c2c12 differentiation in a dependent proteasomal activity manner	86
Mycoplasma Contamination Treatment Does Not Impact Murine Preadipocyte Differentiation	87
Hypoxia-conditioned Extracellular Vesicles Restore Mitochondrial Function in Ischemia/reperfusion-induced Acute Kidney Injury.....	88
Heterotypic Spheroid, 3D Model for Immunotherapy Studies for Triple Negative Breast Cancer.....	89
Evaluation of the effects of cyclic Semaglutide treatment in C57BL/6 mice with diet-induced obesity	90
Chondroitin Sulfate as a Mediator of Arthritis Severity in Chikungunya Virus Pathogenesis.....	91



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

The Wnt/Beta-Catenin Pathway and Cytoskeletal Filaments Are Involved in the Positioning, Size, and Function of Lysosomes during Chick Myogenesis	92
Generation of UHMK1 knockout in melanoma cells through CRISPR/Cas9	93
New Insights into the Role of the RAS Signal Transduction Pathway in Breast Cancer during Pregnancy	94
Generation of PX458 constructs for UHMK1 knockout via CRISPR/Cas9	95
Morphological Analysis of a Cyclopic Fetus: Study of Possible Etiologies	96
The Role of SET7 In Cardiorenal Syndrome Type 3 Induced by Acute Renal Ischemia	97
The effect of hypoxia on the activation of pathways related to the control of aging in hypothalamic proopiomelanocortin neurons	98
Potential inflammatory effect of mineral oil on intestinal parameters in an experimental model	99
Expression of HuC/D in the Early Embryogenesis of the Dorsal Root Ganglion	100
Impact of Lipid Composition on Embryonic Cryotolerance: Insights from Organellar Profiling and Cryopreservation	101
Molecular Study of the Spike Protein Receptor-Binding Domain in SARS-CoV-2 Variants in Porto Alegre/RS Post-Omicron	103
Applicability of Collagen Scaffolds Derived from Tilapia Skin Associated with Mangosteen Extract for the Treatment of Premaxillary Lesions	104
Repair of Pre-Maxillary Injury Using Collagen Matrices Derived from Tilapia Skin and Coated with Chitosan and Curcumin Extract	105
Analysis of mRNAs associated with neurodegeneration and neuroprotection in the hippocampus and hypothalamus from adult mice with type 2 diabetes and obesity	106
Dual effects of Metformin and Resveratrol on compromising / impairing viability of Endometrial Adenocarcinoma Cells	107
Highly invasive OSCC modifies fibroblast behavior and extracellular matrix organization	108
Mitoquinone Reestablishes Calcium Overload and Proton Leakage after Acute Myocardial Infarction	109
Analysis of the Effects of Interleukin 10 (IL-10) on Inflammatory Markers and on the Etiopathogenesis of Alzheimer's Disease in HT-22 Hippocampal Cells	110
Interference of Docosahexaenoic Acid with the response of PC3 prostate cancer cells to a PARP inhibitor	111
Glucose-6-phosphate dehydrogenase inhibitor alters cell interaction and migration dynamics in oral squamous cell carcinoma cell lines	112
Wnt signaling in the regulation of glucose transporters GLUT1 and GLUT3 in the hypothalamus of C57BL/6 mice with type 2 diabetes and obese	113
PD-L1 Blockade and MAPK Pathway Inhibition Modulate Mitochondrial Metabolism in Patients with BRAF-Mutant Melanoma	114
The influence of treatment with 5-FLUOROURACIL on viability and total ROS production at B16-F10, C2C12 myoblasts, and myotubes	115
Pineal melatonin differentially regulates the skin layers and <i>Per2</i>	116
DMSO induces major morphological and physiological alterations in zebrafish embryos.	117
Autologous Fat Enhances Muscle Recovery After End-to-Side Neuroorrhaphy Between the Sciatic and Femoral Nerves in Rats	118
Inhibition of the Oncogenic Enzyme PKM2 by Coumarin-Naphthoquinone Hybrids: Enzymatic Characterization and Autophagy-Induced Cancer Cell Death	119
NEGR1 Modulates Neuronal Survival Independently of mGluR5 in Excitotoxic Conditions	120
Exploring Heterogeneity in Magnocellular Neurons of the Supraoptic Nucleus: An Integrated Investigation of Electrical and Genetic Responses	122



**XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO**

**8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP**

Influence of Nanoplastics on the Response of Ovarian Tumor Cells to Chemotherapy Treatment ...	123
Expression of adhesion molecules in melanoma microenvironment.....	124
Avaliação do Perfil de Resistência aos Antimicrobianos e Diversidade Genética de Bactérias Gram Negativas Isoladas em Pinguins no Ambiente Antártico	126
Avaliação do Perfil de Resistência aos Antimicrobianos e Diversidade Genética de Bactérias Gram Negativas Isoladas em Pinguins no Ambiente Antártico	127
Impact of miR-181a-5p Upregulation on Cell Signaling and Apoptotic Pathways in Placental Tissue of Women with Breast Cancer During Pregnancy	128
Hepatic Metabolomic Profile of Male Rats Subjected to Maternal Protein Restriction	129

02. Sistema Cardiovascular

Functional and Molecular Study of Comorbidity between Neural Alterations, Heart Failure, and Metabolic Dysfunction: The Interaction between the Renin- Angiotensin System, Intestinal Microbiota, and Heart Disease.....	1
Physiological impact of multi-channel ion blockade on cardiac autonomic regulation	2
Cardiac autonomic regulation and mental health: Physiological markers for predicting post-infarction psychological outcomes.....	3
Perivascular adipose tissue protects the thoracic aorta from isoproterenol-induced hypercontractility in female mice	4
Testing Deactivation of Peripheral Chemoreceptors to Lower Arterial Pressure in Spontaneously Hypertensive Rats by Chronic Intermittent Hyperoxia.....	5
Meta-Analysis of Cardiac Transcriptome of Animals Treated with Doxorubicin Reveals Target Genes	6
Prevalence of Cardiovascular Complications in Children with Malaria at the General Hospital of Luanda/Angola	7
Submaximal Physical Capacity in Children and Adolescents with Congenital Heart Disease: A Cross-Sectional Study Using the 3-Minute Step Test.....	8
Short-term heat therapy promotes cardioprotection and reduces systolic blood pressure in spontaneously hypertensive rats	9
The impact of cardiovascular diseases in Brazil, 1996-2023: a retrospective study of epidemiology and impact on the unified health system	10
Proteomic profiling of preeclampsia: investigating correlations with soluble endoglin and soluble fms-like tyrosine kinase	11
Integrative Physiological Study of Radiation-Induced Lung Injury: Effects on Cardiac Function and Kidney Integrity	12
Reactive oxygen species mediate platelet hyperreactivity induced by oxidized collagen	13
Effect of Electrical Activation of the Carotid Sinus Nerve after Acute Myocardial Infarction in a Cardiorenal Syndrome Type 1 Model	14
Different doses of Bisphenol S alter the renin-angiotensin system and promote cardiac remodeling in adult male mice	15
Effects of CETP Expression on Endothelial Modulated Relaxation and Perivascular Adipose Tissue Function in male and female mice with Cardiac Hypertrophy.....	16
Cardiac and Renal Benefits of Empagliflozin in Heart Failure: Exploring Direct and Indirect Effects	17
Impact of uremic toxin indoxyl sulfate on cardiac cell viability and renal: type 3 cardiorenal syndrome model.....	18



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Does the disruption of the intestinal barrier promote the development of pre-eclampsia?	19
Short-term mitoquinone therapy prevents aorta elastin injury after myocardial infarction in rats..	20
Aerobic training improvements in cardiac dysfunction associated to type 2 diabetes are related to advanced glycation end products pathway	21
Structural and Molecular Aspects of Collagen upon Oxidation with Hydrogen Peroxide and its effect in platelet adhesiveness.....	22
The effects of Losartan on the memory of Hypertensive rats treated with the high-fat-diet.....	23
Increased platelet aggregation induced by oxidized collagen is potentially mediated by CD36.....	24
Collagen glycation reduces endothelial cell migration in vitro	25
Angiotensin-(1-7) and emotional regulation in allergic asthma: a new therapeutic approach	26
Fluid-electrolyte balance and anxiety-like behavior in spontaneously hypertensive rats	27
Role of Annexin A1 in Cardiac Remodeling and Dysfunction in a Murine Model of Type 1 Diabetes	28
Endothelial-derived catecholamines profile and its association with serum parathormone and blood pressure in a model of chronic kidney disease.....	29
Effects of probiotic administration on inflammation and cardiac and intestinal remodeling in spontaneously hypertensive rats	30
Effects of Different Concentrations of Magnesium on Vascular Reactivity in Wistar and SHR Rats .	31
Apocynin prevents the effect of Losartan on the contractile response to phenylephrine in aortas of treated SHR.....	32
Acute Exposure Effects of Trivalent Sodium Antimoniate and Pentavalent Sodium Antimoniate on Isolated Mitochondria from Heart of Mice	33
Functional Study of a <i>TNNC1</i> -Encoded Variant of Cardiac Troponin C, E135A, in a Multigeneration Family with Autosomal Dominant Dilated Cardiomyopathy.....	34
Intracranial and cerebral perfusion pressure changes induced by increases in intracerebroventricular volume in male and female Dahl Salt-Sensitive rats.....	36
Mitochondrial antioxidant Mitoquinone prevents cardiac dysfunction 7 days after myocardial infarction in rats.....	37
Effect of resistance training on cardiac function of spontaneously hypertensive rats (SHR).....	38
SGLT2 inhibitors attenuate iNOS activation and H ₂ O ₂ production in endothelial cells exposed to doxorubicin.....	39
Impacts of Hypoxia and the Uremic Toxin p-Cresyl Sulfate on Cardiorenal Syndrome Type 3.....	40
Chronic Apocynin Treatment Preserves Caveolar Integrity and Mitigates Endothelial Dysfunction in Spontaneously Hypertensive Rats	41
Sex-dependent changes in intracranial pressure and intracranial compliance in a rat model of renovascular hypertension.....	42
Mito-TEMPO treatment prevents progression of aortic PVAT dysfunction in spontaneously hypertensive rats.....	43
Protective Role of Crp3 Cysteine and glycine-rich protein 3 deletion against Age-Related Cardiac Dysfunction	44
Contribution of the <i>Timd4</i> gene in heart rate regulation in mice	45
Dengue Infection in Female Mice: Cardiovascular Disturbances Beyond the Usual Symptoms.....	46
Endothelial-dependent vascular relaxation is mediated by NO in the stimulation of α 1 adrenergic receptors, but not in the stimulation of serotonin receptors	47
Phenylalanine combined with mean blood pressure as a potential predictive biomarker for early-onset preeclampsia	48
Effect of ad libitum 0.3 M NaCl intake in renovascular hypertensive rats on the expression of microglia in brain areas involved in cardiovascular regulation	49



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Effect of the uremic toxin indoxyl sulfate on renal and cardiac tissue after ischemia-induced acute kidney injury.....	50
Functional Effects of Autoantibodies from Chagas Disease Patients on the Electrophysiology of Cardiomyocytes Derived from Human Induced Pluripotent Stem Cells.....	51
Comparison between different experimental protocols to induce hypothyroidism in mice: effects on the heart.....	52
Effects of high intensity interval training on neuro-cardiovascular dynamic changes and mitochondrial dysfunction induced by high-fat diet in rats	53
MrgD receptor deficiency induces cardiomyocyte atrophic response in adult male mice	54
The consumption of <i>Dipteryx alata</i> Vog. and goat whey during aging improves metabolism, reduces hepatic oxidative damage, and decreases atherogenic and coronary risk in aged rats.....	55
Chronic Magnesium Supplementation Attenuates Hypertensive Vascular Dysfunction via NO Enhancement and NOX Modulation	56
Maternal unsaturated fatty acid supplementation modulates cardiovascular and morphometric parameters in Wistar rat offspring	57
Secondary hypertension alters urinary biochemical mediators induced by DOCA-salt treatment..	58
Undernutrition as a Risk Factor for Cardiovascular-Kidney-Metabolic Syndrome: Role of Endogenous Cardiotonic Steroids*	59
Cardiovascular and neuroendocrine effects of LVV-H7 in hypertensive and normotensive rats	60
KDEL receptor blocker induces decrease in cardioprotection Induced by Human Plasma from Volunteers Undergoing Remote Ischemic Preconditioning	61
Effect of altered miRNA processing on the anticontractile function of perivascular adipose tissue in the aorta of males and females	62
Reactive oxygen species drive the preganglionic sympathetic activity in experimental renovascular hypertension	63
Role of the afferent renal nerves in the cardiovascular responses to acute renal ischemia-reperfusion in rats	64
Afferent renal nerves promote differential cardiovascular responses in response to furosemide in normotensive and hypertensive rats	65
Unraveling the role of muscarinic autoantibodies in the development of arrhythmias in Chagas Disease in a model of human cardiomyocytes derived from induced pluripotent stem cells (CM-hiPSCs).....	66
The stress-inducible protein (STI1) modulates physiological and pathological hypertrophy in neonatal cardiomyocytes treated with different biomolecules.....	67
Hypoxia-Induced Upregulation and Vesicular Localization of STI1 in Cardiac Fibroblasts.....	68
Outside the Brain: Cardiovascular Consequences of Zika Virus (ZIKV).....	69
Brain-spleen-kidney interaction in the evolution of hypertension in spontaneously hypertensive rats	70
Bk Channel Activation Induces Mitochondrial Protection Against Hypoxia/Reoxygenation Injury...	71
Cardiac remodeling in 6-OHDA Parkinsonism rat model: impact of estradiol treatment in ovariectomized rats.....	72
Does chronic exposure to air pollution influence cardiac lipofuscin deposition?	73
Investigation of the involvement of platelet-activating factor in the control of hypertension by aerobic training. a randomized controlled trial	74
Gq Protein Activation In Cardiomyocytes Modulates The Population Of Resident Macrophages Through In Situ Proliferation	75
Cardioprotection by transfer of human plasma after remote ischemic preconditioning: identification of cardioprotective humor factors through omics techniques	76



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Impact of chemotherapy on uterine artery reactivity during cancer in pregnancy	77
Collagen matricryptin improves cardiac function by mediating scar formation	78
Targeted Gi signaling activation exclusively in cardiomyocytes reverses cardiac damage induced by adrenergic overload	79
Vascular Effects of Perivascular Adipose Tissue are Preserved after Long-Term Androgen Depletion	80
Influence of obesity on cardioprotection induced by remote ischemic preconditioning	81
Mitochondrial imbalance drives persistent cardiac hypertrophy following adrenergic overload in estrogen-deficient mice	82
Evaluation of cardiovascular reactivity to acute stress following systemic GABA administration	83
Ovariectomized female mice are more susceptible to cardiac remodeling induced by Gq pathway activation	84
Effects of Dapagliflozin, a Sodium-Glucose Cotransporter 2 Inhibitor, on Mitochondrial Function ..	85
Alamandine-(1-5): A Novel Antihypertensive Peptide of the Renin-Angiotensin System	86
Efficacy of two treatment protocols for denture stomatitis: impact on inflammation, <i>Candida</i> load, and blood pressure.....	87
The Role of Cholinergic Signaling in Postnatal Cardiomyocyte Maturation.....	88
LEAP2, the endogenous inverse agonist of ghrelin receptor, modulates beta adrenergic-mediated cardiac effects in a GHSR-dependent manner	89
Parasympathetic Control and Beyond: Insights into Heart Rate Fragmentation from Experimental Animals.....	91
Myocardial Infarction-Induced Skeletal Muscle Remodeling Is Modulated by Orchiectomy.....	92
Mitochondrial Antioxidant Therapy with Mitoquinone does not Prevent Skeletal Muscle Atrophy Following Myocardial Infarction	93
Selective Gi Activation in Cardiomyocytes Confers Protection Against Isoproterenol-Induced Cardiomyopathy	94
Sex difference in progesterone action on the coronary bed in gonadectomized SHR rats	95
Vascular effects of exposure to accidental doses of cadmium in female rats - Protective effect of Red Quinoa Hydrolysate: A plant-based therapeutic alternative	96
The effects of Losartan on the memory of Hypertensive rats fed with the high-fat-diet	97
Ellagic Acid Reduces Oxidative Stress and Attenuates Endothelial Dysfunction in Aortas of Iron-Overloaded Rats	98
New Rho-kinase Inhibitor Reduces Diastolic Dysfunction Induced by Estrogen Depletion in Spontaneously Hypertensive Rats	99
Angiotensin-(1-7) attenuates cardiac hypertrophy and inflammation following adrenergic overload	100
Influence of glucose and oxygen deprivation on the function of isolated aortic arteries	101
Melatonin Therapy on Vascular Insulin Resistance in Males and Females with Pulmonary Arterial Hypertension	102
Similarities and Differences in Carotid Artery and Hemodynamic Reactivities Induced by Mental or Cold Stress: A Randomized Crossover Study	103
The Role of STI1 in the Regulation of Cardiac Hypertrophy.....	104
Cardioprotective Effect of SGLT2 Inhibition in Stress-Induced Cardiomyopathy.....	105
Evaluation of the interaction between Cerebral Dopamine Neurotrophic Factor (CDNF) and KDEL receptor at the cell membrane	106
The Cerebral Dopamine Neurotrophic Factor promotes cardioprotection via activation of the PI3K/AKT pathway and plasma membrane KDEL receptor.....	107



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Spontaneously hypertensive rats seek calories and a sweet taste after water deprivation	108
New acetophenones reduce oxidative stress in blood vessels	109
When Sex Matters: The Differentiated Role of the Nitric Oxide Pathway in Coronary Vasodilation of Diabetic Rats	110
Evaluation of the Functional Profile of Mitochondria in Rats with Post- Infarction Heart Failure ...	111
Modulation of endothelial glycocalyx by the pregnancy process in a model of essential hypertension.....	112
Study of the cardiovascular bulbar nuclei in the face of progressive heart disease in the MDX mouse, an experimental model of Duchenne muscular dystrophy	113
Action of Ethyl Ferulate on Muscarinic Receptors in Spontaneously Hypertensive Rats Females ..	114
Maternal Behavioral and Nutritional Factors Associated with Cardiometabolic Health in Children.	115
Impacts of maternal voluntary physical activity on cardiorespiratory and biochemical interventions of offspring subjected to perinatal protein malnutrition	116
Progesterone treatment attenuates the deleterious effects induced by hormonal deficiency in isolated aortic segments from ovariectomized rats.....	117
Nanoparticles of acetylated CGP containing essential oil from orange peel attenuate isoproterenol-induced cardiac hypertrophy in male mice.....	118
Effects of Walker-256 Tumour Ascitic Fluid on Mitochondrial Bioenergetics and Metabolic Profile of H9c2 Cardiomyocytes: The Modulatory Role of Leucine	119
Vascular Responses in Diabetic Rats: The Influence of Sex on Mesenteric Artery Reactivity	120
Cardiovascular effects of hypertonic potassium chloride infusion in normotensive rats.....	121
Cardiovascular Dysfunctions in a Rat Model of Preterm Birth	122
Effect of Dapagliflozin on cardiomyocytes of mice fed a high-fat diet and fructose-enriched water.....	123
Red Quinoa Hydrolysate: A Functional Food with Therapeutic Potential for Hypertension and Vascular Dysfunction in Rats Exposed to Mercury	124
Inverse association between circulating hexosylceramides and cardiometabolic risk factors in adult women with overweight	125
Comparative analysis of lipid profile, glycemic status, anxiety and stress levels in transcendental meditation practitioners at Universidade Estadual de Maringá.....	126
Indoxyl Sulfate in AKI and UT model: Dual Roles in Purinergic Vascular and Inflammatory Modulation	127
Neonatal history correlated with cardiac electrophysiological parameters and serum markers of metabolism in childhood	128
Effect of estradiol replacement and central injection of losartan on water and 0.3 M NaCl intake induced by water deprivation in ovariectomized renal hypertensive female rats	130
Gi Signaling Activation in Cardiomyocytes Preconditions the Heart and Enhances Recovery After Injury	131
Myocardial Infarction: Humoral Factors Arising from Remote Preconditioning and the Influence of Comorbidities on Cardioprotection	132
Cardiovascular impacts of an obesogenic diet supplemented with flaxseed oil in female offspring compared to males	133
Effects of leucine supplementation and Walker 256 tumour evolution on heart function and cardiac tissue metabolomic profile in pregnant rats	134
Exogenous 10 kDa-Heat Shock Protein Preserves Mitochondrial Function After Hypoxia/Reoxygenation	135
Impact of Ethyl Ferulate on Blood Pressure and Heart Rate: Investigation of Autonomic Mechanisms Potentially Involved in Wistar and SHR Rat Models	136



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Exacerbated vascular remodeling in TgPDIA1 overexpressing mice	137
Development of an experimental model of PRKAG2 cardiac syndrome: Cardiological evaluation of mice carrying the PRKAG2 P.His398Gln mutation	138
Parental fructose overload induces cardiorenal dysfunction associated with impaired blood pressure variability: a study of sex-dependent effects	139
Antithrombotic and antiplatelet effects of polysaccharide extract from <i>Libidibia ferrea</i> associated with rivaroxaban in arterial thrombosis model induced by iron chloride in rats.....	140
Spectral analysis of blood pressure and heart rate variability in spontaneously hypertensive rats (SHR): Study of sympathetic and hormonal modulation of blood pressure	141
Intracerebroventricular Administration of Alamandine Reduces the Compulsive-Like Behavior Observed in Spontaneous Hypertensive Rats	142
Effect of obesity associated with water deprivation on blood pressure maintenance in female rats	143
Impact of <i>Ananas comosus</i> L. Merrill residue flour on the cardiovascular system of the spontaneously hypertensive rats	144
Characterization of the hematopoietic system in deep vein thrombosis.....	145
Effect of Angiotensin-(2-7) on Vascular Reactivity in Diet-Induced Obese Mice.....	146

03. Sistema Respiratório

SGLT2 inhibitor reduces airway morphological changes in OVA-induced potentiated by methylglyoxal	1
Effects of lateral parafacial glutamatergic interneurons activation on the ventilatory parameters of mice during normocapnia and hypercapnia.....	2
Airway reflexes: review and proposal of teaching material	3
Ovariectomy Exacerbates Neurodegeneration and Respiratory Impairments in a Parkinson's Disease Mouse Model	4
Number of granular cells in the olfactory bulb of BALB/c mice may be dependent on oxidative stress in an experimental model of hyperoxia	5
The Role of GSK3- β and PKM2 in Respiratory Nuclei in Parkinson's Disease.....	6
Hyperoxia has the potential to modify the immune phenotype in an experimental asthma model through the influence of Treg cells.....	7
Effects of exposure to sodium hypochlorite on inflammatory and functional mechanisms in an experimental model of chronic allergic lung sensitization	8
Selective chemogenetic manipulation of Guanylyl Cyclase D+ neurons and their projections to the vNDB has no impact on the hypercapnic ventilatory response.....	9
Recruitment of inflammatory cells in the upper airways of BALB/c mice after an experimental model of acute asthma and hyperoxia opens a reflection on the concept of a single airway.....	10
Functional respiratory disorders in the MitoPark model of Parkinson's disease.....	11
Mitochondrial Dysfunction in Anxiety: The Impact of Early Life Stress and Hypercapnia	12
Impact of Hypoxemia, Normoxia, and Moderate to Severe Hyperoxemia on Brain-lung Crosstalk in Experimental Acute Focal Ischemic Stroke	13
Incidence of Long Covid syndrome: clinical, spirometric, metabolic and inflammatory profile after recovery from SARS-Cov-2 infection.	14



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

The effects of hypothalamic peptides vasopressin and oxytocin on breathing via interaction with the retrotrapezoid nucleus.....	15
Effects of chemoreceptors on arousal from obstructive sleep apnea in rats.....	16
Mechanisms underlying MuRF1 inhibition mediated protection against disuse-induced diaphragm dysfunction and atrophy	17
Repeated Seizures Attenuate CO ₂ Chemoreception in Sleep and Wakefulness in Wistar Rats due to partially reduced RTN sensitivity.....	18
Selective optogenetic stimulation of the vagus nerve prevents	19
respiratory dysfunction and astrogliosis in a Parkinson's disease model	19
Early and severe respiratory impairments in aged model of Parkinson's disease	20
Physical Exercise Prevents Cardiorespiratory Deficits and Neurodegeneration in the 6-OHDA Model of Parkinson's Disease	21
Adenosine A _{2A} receptors modulate respiratory responses to hemoreflex activation in mice exposed to 2 hours of sustained hypoxia.....	22
Progesterone and etonogestrel combined with estradiol increase ventilatory responses to hypercapnia in female rats	23
Optogenetic activation of somatostatinergic interneurons increases breathing in mice	24
Role of BK channels in the respiratory response to CO ₂ /pH during development in male and female mice.....	25
Post-natal development of hypoxic ventilatory response in normotensive and spontaneously hypertensive rats.....	26
Effects of chronic caffeine treatment on pulmonary ventilation and arterial blood pressure in spontaneously hypertensive rats	27
Cardioprotective effects of extracellular vesicles obtained from hypoxia-preconditioned mesenchymal stem cells in experimental pulmonary arterial hypertension	28
Impacts on function and vascular remodeling of combined sildenafil and mitochondrial transplantation (mitotherapy) in rats with pulmonary arterial hypertension.....	30
Potential effects of Lippia Grata plant extract complexed in β -cyclodextrin in a mice model of pulmonary emphysema.....	31
Influence of Maternal Inflammation and Vitamin D Supplementation on the Respiratory Responses of Mice.....	33
Elimination of noradrenaline synthesis in the Locus coeruleus neurons disrupts CO ₂ ventilatory and metabolic responses of mice in the early stages of development.....	34
New insights of aged alveolar cells under cyclic stretch that simulates mechanical ventilation....	35
Aging and Parkinson's Disease aggravation of anatomofunctional respiratory deficits: considerations upon the 6-OHDA rat model	36
NET measurement in the BALF of patients with interstitial lung disease: a possible biomarker in fibrosis?	37
Ventilatory, metabolic, and thermal responses to hypercapnia in female mice: effect of progesterone throughout the estrous cycle.....	38
Effects of Caffeine on Respiratory Disturbances in a Murine Model of Parkinson's Disease	39
Impairment in short-term memory is associated with increased Iba-1 and decreased nicotinic receptors in brain from mice with acute lung injury	40
Effect of peripheral BKCa channels blockade on respiratory and metabolic responses to CO ₂ /pH during mouse development	41
Involvement of ASIC1a channels in the locus coeruleus in the response to CO ₂ exposure in male mice	42
Electrophysiological properties of the Nucleus Tractus Solitarius neurons from mice activated by	



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

allergen-triggered pulmonary inflammation.....	43
CO2 activates astrocytes in the lateral parafacial region to restrain the tachypneic response in mice	44
Genetic Profile of Lung Adenocarcinoma: A University Oncologic Center-based Survey in Rio de Janeiro	45
Melanocortin receptor 4 agonist, setmelanotide, treats opioid-induced respiratory depression ...	46
Relationship Between Motor and Respiratory Function in Duchenne Muscular Dystrophy Patients	47

04. Sistema Urinário

Prevalence of chronic kidney disease in the indigenous population living in the reserve of Aracruz, Espírito Santo, Brazil	1
Role of p38MAPK and EGR1 in adriamycin-induced glomerulosclerosis.....	2
Contribution of the p38MAPK pathway to the modulation of MMP10 in an experimental cisplatin model	3
Non-steroidal mineralocorticoid receptor antagonist improves tubule-interstitial injury in subclinical acute kidney injury animal model	4
The Role of Probiotics in Modulating the Gut Microbiota as an Inhibitor of Diabetic Kidney Disease Progression	5
Tubule-interstitial injury in subAKI is attenuated by dapagliflozin treatment through modulation of O-linked Glc-NAcylation pathway	6
The role of SGLT2 inhibitor on the pathogenesis of acute kidney injury.....	7
Investigation of PKM2 expression in renal tissue of BTBR wild-type and ob/ob male and female animals.....	8
The lack of TRPV1 partially restores the effect of high sugar/high fat diet in kidney mice	10
Angiotensin-(1-7) ameliorates hyperglycemia-induced tubular dysfunction and injury in the early phase of Diabetic Kidney Disease	11
Development of tubular proteinuria associated with tubular injury induced by accumulation of fine particulate matter from air pollution in Wistar rats.....	12
RAGE inhibitor TTP488 (Azeliagon) reverses bladder dysfunction in leptin-deficient obese mice..	13
Acute Activation of the MGO-AGE-RAGE Axis Promotes Bladder Hypercontractility.....	14
Oral treatment with angiotensin-(1-7) ameliorates proteinuria in subclinical Acute Kidney Injury animal model.....	15
Tubular injury precedes modifications in glomerular function at early-stage of focal segmental glomerulosclerosis	16
Opioidergic transmission in the shell portion of the nucleus accumbens (sNAcc) is involved in cardiovascular and urinary bladder control	17
Hyperglycemia-Induced Tubular Proteinuria in Non-Obese Diabetic Mice: A Potential Role in Tubular Injury Development.....	18
Dapagliflozin and Oxidative Stress: Exploring the Role of SGLT2 Inhibition in the Proximal Tubule	19
Activation of the Angiotensin II Type 1 Receptor Mediates the Development of Kidney Injury and Proteinuria Observed in Obesity-Related Diabetic Kidney Disease.....	20
Activation of Ang II/AT1R axis mediates the tubular injury and fibrosis associated with tubular proteinuria observed in the early stage of Diabetic Kidney Disease	21



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Impact of Resistance Training on Renal Electrolyte Balance in Female Wistar Rats Under Cross-Sex Hormone Therapy	22
Impairment of postprandial glucagon-like peptide-1 bioavailability and renal glucagon-like peptide-1 receptor activation in experimental hypertension	23
Renal effects of a single intravenous irisin injection prior to bilateral kidney ischemia reperfusion injury	24
Angiotensin converting enzyme 2 enhances megalin-mediated albumin endocytosis in proximal tubule cells by restraining Angiotensin II/AT1R	25
Preventive exercise improves oxidative stress and inflammation in diabetic kidney disease through increased glutathione peroxidase activity and suppression of TNF- α regulated by the SDF-1 α /CXCR4 pathway	26
Intermittent fasting modulates renal function in association with estrous cycle variation and autophagy mechanisms	27
Valproic Acid Mitigates Hypertension Associated with Ischemia/reperfusion-induced Acute Kidney Injury	28
Hypovolemic states affect the urinary bladder responsiveness to the neurotransmitters of the autonomic nervous system and circulating hormones in female rats	29
Study of LEAP2 in fasting and renal ischemia and reperfusion in normotensive and hypertensive rats	30
Cellular dehydration-evoked models induce reduction in the responsiveness of the urinary bladder to neurotransmitters of the autonomic nervous system and vasopressin in female Wistar rats	31
Resistance training evokes inflammation and activates autophagy in the urinary bladder of female Wistar rats	32
Nephroprotective effect of a low molecular weight peptide fraction from common bean (<i>Phaseolus vulgaris</i>) against renal ischemia and reperfusion	33
Differential Effects of 48- and 72-Hour Fasting on Renal Redox Status and Function in Rats with Cisplatin-Induced Acute Kidney Injury	34
Evaluation of the effects of raas inhibitors on renal morphology in rats with renovascular disease induced by the two-kidney, one-clip model.....	35
SC1, a novel angiotensin II type 1 receptor antagonist, improves renal hemodynamic parameters in a rat model of chronic kidney disease	36
Modulatory Effects of Epithelium-Derived 6-Nitrodopamine on Human Ureter Contractility.....	37

05. Sistemas Endócrino e Reprodutor

Late impacts on sperm quality and quantity in rats exposed to a single dose of gadolinium during prepuberty	1
Influence of sleep deprivation on sperm function in mice	2
Basal release of 6-nitrodopamine and from in NOS knockout mice isolated vas deferens	3
Maternal obesity alters cannabinoid receptor content in pancreatic islets of rat offspring associated with changes in glycemic homeostasis.....	4
Reduction in Prostate Cancer Diagnoses During the COVID-19 Pandemic: Evidence from Brazil and the World	6
Chlorothalonil Disrupts Steroidogenic Pathway in Adult Male Mice.....	7
Three-dimensional model of ovarian granulosa cells for in vitro studies in female fertility	8
Effects of Testosterone Absence on Glucose Tolerance and Energy Metabolism	9



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Assessment of the functional impact of IL-17 exposure on human granulosa cells	10
Impact of perinatal exposure to plastic waste on prostate histology in adult rats	11
Effects of perinatal exposure to a phthalate mixture and nanoplastics on the reproductive health of male rat offspring	12
From pollution to placenta: How the silent invasion of plastics affects the maternal-fetal axis....	13
Maternal Exposure to Phthalates and Nanoplastics Impairs Blood-Testicular Barrier Integrity in Male Offspring	14
Association between hair cortisol levels and obesity in the ELSA-Brasil cohort	15
Effects of gestational and lactational exposure to plastic waste on biometric parameters of male rat offspring	16
Early overnutrition does not aggravate depressive-like behavior in a rat model of polycystic ovary syndrome.....	17
Perinatal exposure to plastic pollutants alters hypothalamic reproductive pathway in post-weaning male rats	18
Exploring the interaction between GLP-1 and androgens: an approach using published transcriptome analysis data	19
Gestational amplification of metabolic disruptions induced by excessive supplementation of polyunsaturated fatty acids	20
Fetal programming induced by unsaturated fatty acids: Delayed testicular development in offspring	21
Irisin chronic intracerebroventricular administration improves lipid metabolism in mice with diet-induced obesity	22
Assessment of reproductive parameters in hybrid male mice exposed to ethane dimethanesulfonate (EDS)	23
Lack of STAT3 signaling in ventromedial neurons accentuates the effect of estradiol deficiency on body weight.....	24
Inverse correlation of umbilical blood flow with umbilical cord diameter in gestational diabetes mellitus with insulin therapy	25
Evaluation of nuclear RAR receptors (Retinoic Acid Receptors- α and β) in the ventral prostate of vasectomized rats.....	26
Effects of pregnancy hormones in Achilles tendon biomechanics and biochemistry in female obese mice	27
Effect of estrogen hormone therapy on memory and hippocampal redox state in periostropause rats	28
Effect of maternal apical periodontitis on the activation of inflammatory pathways in the placenta of rats	29
Maternal true endo-periodontal lesion activates inflammatory pathways in the adult life of offspring	30
Evaluation of the oxidative stress in adult rats, offspring of rats with true endo-periodontal lesion32	
Different Estrogen Treatments in a BPA-Dysregulated Mammary Gland Microenvironment: Receptor Analysis	33
Vitamin D levels in the indigenous population living in the reserve of Aracruz, Espírito Santo	34
Effects of gestrinone treatment on body mass and renal morphofunction in female rats fed on a normo or a high-fat diet.....	35
Androgenic modulation affects neoplastic development in the gerbil male prostate following perinatal exposure to the endocrine disruptor bisphenol A	36
Evaluation of the Androgen Receptor and Estrogen Receptor Alpha in the ventral prostate of vasectomized rats.....	37



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Perinatal exposure to phthalates and susceptibility to prostatic inflammation in adulthood	38
Offspring birth weight classification in an experimental model of mild and severe hyperglycemia induced during pregnancy in rats.....	39
Gestrinone Administration Decreases Adiposity and Plasma Cholesterol but Increases Hepatic Cholesterol in Obese Female Mice.....	40
Prolactin attenuates stress-induced activation of the paraventricular nucleus but not corticosterone secretion in male rats	41
Does the past influence the future? Metabolic adaptations following repeated glucocorticoid treatments throughout life	42
Oral gestrinone treatment is refractory to obesity and its comorbidities in male mice	43
Chronic exposure to atrazine-based herbicides exacerbates liver steatosis in adult mice fed a high-fat diet.....	44
Chronic exposure to glyphosate-based herbicide aggravates hepatic steatosis in obese adult mice	45
Regulation of Foxo3 by Thyroid Hormones in Sepsis: Implications for Muscle Atrophy and Survival	46
The impact of growth hormone secretion on kisspeptin neurons activity	47
Effects of binge eating disorder on the metabolism of mice	48
Effects of GH Hypersecretion on mice Fertility.....	49
Sperm-Protective Action of Chrysin in Mitigating the Effects of Microplastics in <i>Zebrafish</i>	50
Calcitriol Suppresses Brown Adipose Tissue Thermogenesis in Lactating Rats	51
Influence of gestational and lactational exposure to phthalate and nanoplastics on the reproductive parameters and distribution of mast cells in the lactating mammary gland of Sprague-Dawley rats.....	52
Effects of estrogen therapies in a scenario of endocrine disruption on the female gerbil prostate	53
Ovarian steroids reverse depressive-like behavior induced by chronic unpredictable stress in female rats	54
T+E2 treatment induces proliferative lesions in the ventral prostate of adult male gerbils.....	55
Biometric data and sperm morphology in obese C57BL/6J mice treated with emulsifiers carboxymethylcellulose and polysorbate 80.....	56
Influence of women's lifestyle on the success of assisted reproduction protocols	57
Maternal low-dose caffeine intake during gestation or lactation disrupts the insulin signaling pathway in metabolic tissues of adult female offspring	58
Combined training improves physical performance and muscle irisin of senescent female rats ...	59
Effects of maternal apical periodontitis on fetal development and placental health	60
Evaluation of the nonenzymatic antioxidants in adult rats, offspring of rats with true endo-periodontal lesion	61
Impacts of Maternal Exposure to Bisphenol F (BPF) and AF (BPAF) on Thyroid Function in Wistar Rats: Effects During Lactation and Adulthood	62
Exposure to microplastics (MP) and nanoplastics (NP) during the perinatal life alters hormone levels in Wistar rat dams and offspring of both sexes and different ages.....	63
Melatonin and clock genes in interscapular brown adipose tissue: gene expression in adult rats.	64
Toxic Effects of Mercury and Cadmium Co-Exposure on Reproductive Function in Wistar Rats	65
Melatonin action in the initial and later steps of insulin signaling in rats with Apical Periodontitis, Periodontal Diseases and Endo-periodontal Lesion.....	66
Hypothyroidism differentially affects the bone microstructure of the maxilla in male and female mice.....	67



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Absence of the pineal gland alters the daily expression of clock genes and thermogenic molecular machinery genes in cervical brown adipose tissue	68
The impact of estradiol replacement on the browning of white adipose tissue and the maintenance of body temperature in high-fat-fed female mice.....	69
Effect of melatonin on the expression of TNF- α content in rats with apical periodontitis subjected to passive tobacco inhalation	70
Protective Effects of Female Sex Hormones Against CCl ₄ /HFD-Induced MASH	71
Analysis of bone microarchitecture and chemical structure of the femur neck in Wistar rats at different periods of reproductive aging	72
Effects of early exposure to polystyrene microplastics on metabolic parameters in zebrafish: impacts on the development of obesity and type II diabetes mellitus in adulthood	73
Sodium Saccharin Exposure During Gestation and Lactation Induces Prostate Histological Changes Across Generations in Rats.....	74
Fetal and placental morphometric alterations induced by prenatal stress	75
Effect of Melatonin on the Modulation of Skeletal Muscle Inflammation Induced by Apical Periodontitis and Smoking in Rats.....	76
Metabolic effects of chronic exposure to glyphosate at the acceptable daily intake dose in Wistar rats.....	78
Morphometric-stereological study of the dorsolateral prostate from perinatal exposure to a relevant environmental mixture of phthalates.....	80
Changes in hypothalamic norepinephrine activity associated with hot-flash symptoms in estrogen-deficient female rats.....	81
Raloxifene therapy attenuates the development of MNU-induced prostatic tissue lesions in rat ..	82
Toxic effects of tributyltin and cadmium co-exposure on the reproductive axis in Wistar rats	83
Impact of maternal periodontal disease on offspring: miRNA expression modulation and long-term health consequences	84
Effects of Peruvian Maca (<i>Lepidium meyenii</i>) on ovariectomized rats: Behavioral, Biometric and Brain Oxidative Stress Markers	85
Effect of perinatal exposure to BPA and glyphosate on prostate morphology in gerbil males	86
Follicle-stimulating hormone pulsatile secretion in female rats shows higher synchronicity with luteinizing hormone in the absence of estradiol	87
Selective celiac vagal transection avoids hyperinsulinemia without alter adiposity on hypothalamic obese rats	88
Selective activation of tuberoinfundibular dopaminergic neurons suppresses prolactin and stimulates luteinizing hormone secretion in male mice	89
Estrogen Replacement Therapy and Its Effects on BPA-Induced Ovarian Disruption in Mongolian gerbils	90
Vagus nerve modulates oral glucose patterns in hypothalamic-obese rats through a splenic-dependent mechanism.....	91
Role of GABAergic Transmission from Kisspeptin Neurons in Sexual Maturation and Fertility: Insights from Vgat Ablation Study	92
LH and FSH secretion in male and female rats determined by highly sensitive ELISA	93
Sexual dimorphic effect of the ablation B2R from kisspeptin cells in metabolic and reproductive profile thought development and adulthood in mice	94
Modulation of genes related to mitochondrial dynamics and mitophagy in the testis of hypothyroid rats	95
Estradiol modulates thermogenic activity and metabolic parameters in high-fat-fed female mice	96



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Potential of acCGP nanoparticles containing <i>Citrus sinensis</i> essential oil in the treatment of Type 1 Diabetes	97
Impacts of herbicide exposure on the adrenal cortex of Mongolian gerbils	98
Maternal apical periodontitis impact on insulin resistance and placental morphology in Wistar rats	99
Consequences of maternal high-fat/high-caloric diet associated with daily resistance training for placental oxidative stress and inflammatory gene expression	100
Effects of Peruvian Maca (<i>Lepidium meyenii</i>) on Hematological and Plasma Lipids Markers in Ovariectomized Rats	101
Hypothyroidism impairs glycogen metabolism machinery in the heart of male rats.....	102
Liraglutide reduces hypothalamic neuroinflammation in obese mice.....	103
Full-Spectrum CBD Oil as Adjunct to Insulin Improves Metabolic Outcomes in Type 1 Diabetes.....	104
Gestational Zika virus infection causes oxidative stress in murine and human placentae: a possible route for antioxidant preventive therapy	105
Reproductive biology of the simultaneous hermaphrodite.....	106
<i>Diplectrum formosum</i> (Teleostei: Perciformes)	106
Docosahexaenoic acid affects prostate cancer microenvironment by reduction of epithelium-to-mesenchymal transition markers and annexin A1 in cancer-associated fibroblasts	108
6-Cyanodopamine as a Novel Endogenous Modulator of Human Vas Deferens Contractility	109
Diabulimia em adolescentes com diabetes tipo 1: Uma revisão narrativa da literatura.....	110
Antidepressive-like effect of gestrinone in obese female rats	111

06. Sistema Nervoso

Defensive behavioral reactions in participants with interpersonal violence trauma.....	1
Systematic Review on the Effects of Valproic Acid (VPA) on Synaptic Plasticity in Animal Models of Autism.....	2
Neuropsychological and Biomarker Correlates in Mild Traumatic Brain Injury: Acute Cognitive Impairment and Subacute Psychiatric Complications.....	3
Effects of a Visuomotor Exergame on Executive Functions, Sensorimotor Integration, and Self-Regulation in Children with Autism Spectrum Disorder	4
GPR55 receptor as a modulator of cocaine-induced behavioral and molecular alterations	5
A treadmill exercise session performed during the late consolidation improves recognition memory by hippocampal protein synthesis regulation	6
Metabolomic Insights into the Effects of Brazilian Green Propolis in a Parkinson's Disease Model .	7
Evaluation of the role of B1 and B2 kinin receptors in the modulation of the inflammatory response induced by lipopolysaccharide (LPS) in astrocyte cell culture	8
Inactivation of the B2 kinin receptor in neurons GABAergic	9
Evidence that adenosine A2A receptor modulates Ca ²⁺ signaling in astrocytes of mice exposed to 24 hours of sustained hypoxia	10
Treatments with either Cannabidiol or Cannabigerol reduce Mechanical Hyperalgesia in Rats with Neuropathic Pain	11
Flavonoid rutin as a potential neuroprotective agent in autosomal recessive retinitis pigmentosa	12



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

High-fat Diet Consumption and Increasing Age Alter Hypothalamic MFSD2a.....	13
Co-Expression Networks Uncover Transcriptional Diversity in Excitatory and Inhibitory Neurons of the Lateral Hypothalamus	14
Effects of a single session of physical exercise on the consolidation and persistence of learning in basic education students.....	15
Sustained hypoxia increases the amplitude of spontaneous inhibitory currents and reduces the tonic GABAergic inputs in the NTS neurons of C57Bl/6J mice.....	16
Gestational Chronic Mild Stress Disrupts Sex-Specific Memory in Elderly Offspring	17
Impact of early auditory deprivation on memory and long-term potentiation in the hippocampus.....	18
Study of the enteric nervous system in the distal colon in an animal model of alzheimer's disease.....	19
Impacts of ICV administration of CS-6253 in the energy homeostasis of male C57BL/6J mice ...	20
Intracerebroventricular Irisin Treatment Alleviates Hippocampal Inflammation in Diet-Induced Obesity Mice	21
Impact of Sustained Hypoxia (SH) on the excitatory transmission in the nucleus Tractus solitarius (nTS) neurons of C57Bl/6J mice	22
Post-natal development of kv3.1 potassium currents mediated in the fusiform cells in the dorsal cochlear nucleus from mice	23
Modulation of Feeding Behavior by KATP Channels in Serotonergic Neurons of the Dorsal Raphe Nucleus.....	24
Adenosine A2B receptors regulate lactate release during brain hypoxia	25
Beyond the blindness: Glaucoma-induced circadian dysregulation in the endocrine system of the HPA axis	26
Interplay Between Sleep Deprivation and the Gut-Brain Axis mediated by the vagus nerve in Feeding Behaviour	27
The consumption of cactus flour (<i>Opuntia ficus-indica</i>) during aging preserves memory, increases fatty acid levels, and reduces carbonyl levels in brain tissue.....	28
Expression of biochemical markers after hippocampal histamine infusion in the reconsolidation of object recognition memory in Wistar rats.....	29
Neurochemical Profile of Hypothalamic PVN Neurons Targeting the DMV in Response to Central Insulin Action.....	30
Neuropeptidergic neurons of the hypothalamus are differentially activated by acute salt loading	31
Effects of adenosine triphosphate administration in OVLT neurons on blood pressure and heart rate of rats	32
Physiological responses reveal that COVID-19 impacts psychological health	33
Risk and Protective Factors for Suicidality in the Homeless Population of Belo Horizonte	34
Therapeutic hypothermia for the treatment of neonatal hypoxic-ischemic brain injury in male and female rats	35
Role of lactate in the inflammasome NLRP3 signaling pathway after neonatal hypoxia-ischemia.	36
Exercício agudo reduz neurotoxicidade induzida por 6-OHDA e possíveis implicações para estágios iniciais da Doença de Parkinson experimental	37
Study of Inhibitors of DNA Binding Protein ID1 and ID3 in Pitt-Hopkins Syndrome, a neuropsychiatric disorder in the autistic spectrum	38
The NMDA receptor antagonist MK-801 inhibits the sensitization of salt intake independent of alterations in salt taste	39
Cafeteria Diet Induces Obesity and Increases Anxious Behavior in Male Rats.....	40
Investigating the role of ephrin receptors in the neuronal migration process in a monogenic autism spectrum disorder.....	41



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Evaluation of microglial cell response after rutin treatment in an retinitis pigmentosa animal model	42
Biodegradable hydrogel membranes as treatment after spinal cord injury.....	43
KLK6 Expression in a Neonatal Asphyxia Model: Implications for Neuroinflammation and Hypoxic-Ischemic Encephalopathy	44
Evaluation of the effect of pharmacological blockade of TLR4 in the medial prefrontal cortex and small intestine of mice subjected to maternal immune activation combined with perinatal asphyxia	45
Effect of maternal neglect on emotional behavior in male and female rats.....	46
The role of sleep in the acquisition of cocaine conditioned place preference.....	47
The effect of Maternal Neglect on the body and reflex development of male rats	49
Carotid Bodies Mediate Sympathetic Control of Inflammation During Systemic LPS Challenge....	50
OVLT-PVN purinergic pathway contributes to blood pressure control during acute hyperosmotic stimulation	51
Maternal Consumption of a High-sucrose or High-fat Diet Alters Cognition and Memory Behavior and Hippocampal Transcriptome in Offspring	52
Early acoustic deprivation does not substantially affect the electrophysiological properties of fusiform neurons of the dorsal cochlear nucleus (DCN) of mice.....	53
Sympathetic denervation aggravates hyperalgesia but does not affect synovial TNF- α levels in zymosan-induced temporomandibular joint inflammatory pain in rats	54
Screen use in children aged 12 to 42 months attending daycare with an enriched environment .	55
Effects of Dance on Daily Activities of Children with Autism Spectrum Disorder: Randomized Controlled Triple-Blind Clinical Trial.....	56
Effects of chronic exposure to glyphosate-based herbicides on behavior and short-and long-term memory of female and male Wistar rats	57
Melatonin promotes significant reduction in TNF- α levels in the hippocampus of rats with apical periodontitis	59
Virtual Reality and aspects of sleep and cognition in overweight/obese children in Northeastern Brazil	60
Evaluation of the involvement of TLR4 receptors in nociception induced by SARS-COV-2 peptide 3 in mice.....	62
Oxidative balance in the brainstem in the context of cancer	63
Impacts of enriched environment on the modulation of oxidative balance in the hypothalamus in the context of cancer.....	64
Enhancing Quality of Life: Lithium as a Neuroprotective Agent in Chemotherapy-Induced Neuropathy	65
Epigenetic mechanisms regulating bdnf expression that underlie the relationship between early-life stress and cognitive function across sexes	67
Assessment of working memory in rats subjected to chronic sleep deprivation treated with statins	68
Retinal Dystrophin Deficiency Drives Flash Electroretinogram Alterations in Duchenne Muscular Dystrophy Mouse Models.....	69
Multicomponent training prevents memory deficits induced by early neurodegeneration related to Alzheimer's Disease	70
Effects of maternal ultra-processed foods on neuronal expression of serotonin transporter in offspring of rats.....	71
The modulatory effect of novelty on the persistence of reconsolidated recognition memory depends on the activation of protein kinase a in the dorsal hippocampus of rats	72



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Early life stress, L-DOPA and their interplay have distinct effects in the behavior of prepubertal female and male mice	73
Impact of Cannabidiol on Novel Object Recognition Test After Traumatic Brain Injury in Rats	74
Single-Cycle Binge Drinking During Pregnancy Alters Offspring's Motor Neurodevelopment in Rats in a Sex-dependent Manner.....	75
The efficacy of GLP-1 receptor agonists in the treatment of cognitive impairments in preclinical models of diabetes mellitus: a systematic review and meta-analysis	76
Brain reactivity in victims of interpersonal violence and its relationship with peritraumatic tonic immobility	77
Environmental Enrichment Effects on Anxiety-/Depression-Like Behaviors in Rats Submitted to Osmotic Stress.....	78
Ketamine exhibits a long-lasting antidepressant effect in the early weaning model in male mice	79
Influence of environmental enrichment on the thirst during water deprivation.....	80
Anxiolytic and antidepressant effects of ketamine treatment on behavioral changes during adolescence and adulthood induced by an early-life immune activation model	81
Paternal anabolic steroid exposure promotes autism-like behavior in adult mice offspring.....	82
Evaluation of the effect of nanoparticles containing bioactive peptides from hardened beans in the prevention of neuroinflammation and oxidative stress.....	83
Levels of vitamin B12, cognitive aspects, and gross motor coordination in children	84
The Extract of Pequi (<i>Caryocar coriaceum</i>) Shows Anticonvulsant Effects in Adult Zebrafish (<i>Danio rerio</i>).....	85
Effects of supplementation with <i>Spirulina in natura</i> on metabolic parameters and brain morphology in rats treated during lactation: sex-specific analysis	87
Cognitive performance across different dystrophins in Duchenne muscular dystrophy: evidence from WASI assessments	88
The potential action of Oleoylethanolamide on fear memory reconsolidation.....	89
Clinical and Molecular Profile of Duchenne Muscular Dystrophy in a Single Center in Brazil	90
Catecholaminergic Modulation of LEAP2-Mediated Behavioral and Thermogenic Responses to Aversion in Rats	91
Non-Toxic and Non-Sedative Anxiolytic Effects of <i>Syzygium cumini</i> (Jamelão) Extract in Adult Zebrafish (<i>Danio rerio</i>).....	92
Maternal acetaminophen treatment during pregnancy induces autism-like behavior in adult mice offspring	94
Statin-induced recovery of hippocampal neuronal activity and inflammatory regulation in chronically sleep-deprived rats	95
Relationship Between Birth Weight and Oxidative-Antioxidant Balance in Early Childhood	96
Role of irisin in the brains of irradiated mice: effects on behavior, neuronal plasticity and neuroinflammation.....	97
Histomorphometry of AgNORS in the CA1 hippocampal area of <i>rattus norvegicus</i> with Alzheimer's disease and subjected to progressive resistance training	98
Investigation of migration defects in neurons derived from patients with a monogenic autism spectrum disorder.....	99
Behavioral effects of maternal separation stress are sex-specific and distinct in prepuberty and early adulthood	100
Evaluation of cerebral neovascularization and neuroinflammation in spontaneously hypertensive rats fed a high-fat diet and treated with sitagliptin	101
Maternal separation disrupts olfactory memory via ERK/CREB/ $\alpha 7$ -nAChR signaling in the olfactory bulb and hippocampus.....	102



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Maternal separation disrupts fear memory consolidation via AChE/CREB/Na ⁺ /K ⁺ -ATPase pathways: Rescue by galantamine treatment	103
Anxiolytic Effects of Carvacrol in Adult Zebrafish (<i>Danio rerio</i>): Behavioral Assessment Using Light-Dark and Open Field Tests	105
Rosuvastatin attenuates the negative effects on aversive memory of sleep-deprived rats subjected to the elevated plus maze	106
Dose-Dependent Effects of Simvastatin on Barnes Maze Performance in Sleep-Restricted Rats ..	107
Ramped Plus Maze as a Behavioral Test for Anxiety in Zebrafish Larvae (<i>Danio rerio</i>)	108
Dissociating the contribution of C1 and DMV neurons to glycemic challenge responses in rats ..	109
Comparative Module-Based Analysis Identifies Conserved Pathways Involved in Amyloidogenic Regulation and Neurodegeneration in Magnocellular Neurons	110
Acute effects of Transcutaneous Vagus Nerve Stimulation in women with Fibromyalgia: a randomized, crossover, simulation-controlled clinical trial	111
Nanoencapsulated Oleoylethanolamide Restores Short-Term Spatial Memory in Alzheimer's Disease Mice	112
Utilization of Oleoylethanolamide Associated with Phosphatidylcholine Nanoparticles: Effect on Glycemic Metabolism in an Experimental Model of Alzheimer's Disease	113
Effects of progressive resistance training on astrocytic and neuronal density in <i>Rattus norvegicus</i> Wistar induced to Alzheimer's disease	114

07. Exercício Físico

Physiological adaptations to long-term circuit resistance training: cardiovascular risk modulation in aging	1
Physical Activity Improves Mood States in Military Personnel During a Ship Expedition to Antarctica	2
Impact of vegetarian diets on the muscle recovery process after exercise-induced muscle damage in trained men	3
Time-restricted feeding combined with exercise training promotes metabolic improvements, reduces adiposity, and fat accumulation in the liver of obese mice	4
Exercício físico atenua alterações metabólicas e renais em camundongos com doença renal diabética experimental	6
Effects of Short-Term Wet Static Apnea Training on Well-Being, Internal Load, Recovery, and Physical Performance of Para Swimmers	7
Effects of physical training and/or a carbohydrate-restricted and high-protein diet on muscle morphometric parameters and irisin/FNDC5 expression of obese rats	9
Effects of high-intensity interval training versus continuous aerobic training on nitro-oxidative stress markers in female rats with cisplatin-induced hepatotoxicity	10
Effects of Aerobic Exercise on Immune Cell Profile of Perivascular Adipose Tissue and Vascular Function in Obese Female Mice	11
Interactive effect of a plant-based omega-3 fatty acid intake and exercise training on hippocampal GPR120 of obese mice	12
Association of decorin levels and cardiometabolic benefits in the context of post-cardiopulmonary exercise test in endurance athletes	13
Skeletal Muscle Adaptations to Fluoroquinolone Treatment and Voluntary Running in Obesity Prevention	14



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Effects of aerobic physical training over Achilles tendon on obese female mice	15
Effect of inorganic nitrate on microvascular function, angiogenesis and muscle endurance in healthy adults	16
Muscle tissue adaptations after eccentric exercise injury: Morphological, biochemical and functional aspects	17
Effects of curcumin supplementation associated with strength training on muscle thickness, maximal strength and training biomarkers: a randomized, double-blind clinical trial	18
Effects of Physical Training and Cocoa Flavanols on Memory and Neurotrophic Factors in a D-Galactose-Induced Aging Model	20
Longer Sleep Duration Explains Less Perceived Exertion in Para-Athletes During High Thermal Strain Training Sessions	23
Effects of systemic vibratory therapy on sarcopenia parameters of frail older adults: preliminary findings	25
Treinamento físico e suplementação com probióticos reduzem rigidez arterial em animais hipertensos por diferentes mecanismos	26
Effects of systemic vibratory therapy on body fat mass and waist-to-hip ratio in adults with obesity: preliminary results	27
Role of extracellular calcium in fatigue resistance of type-II skeletal muscle fibers during repeated submaximal stimulation	29
Acute effects of combined systemic vibratory therapy and osteopathic manipulative treatment on parasympathetic activity in individuals with obesity: preliminary results	30
Effects of Aerobic Exercise and Semaglutide on Lipid Profile in a Classical Menopause Model	31
Effects of a high-fat diet on glucose responses and on physical performance in female mice evaluated at different times of the day	32
Home-based inspiratory muscle training improves cardiac autonomic response to postural stress in Parkinson's disease: a preliminary study	34
Effects of creatine supplementation in zebrafish	35
A Zero-Cost System to Evaluate Swimming Performance in Zebrafish Adults and Embryos	36
Strength training improves body composition, muscle metabolism and hypertrophy in high-fat-fed ovariectomized mice	37
Aerobic Exercise Training and BACH1/HO-1 Regulation in Colon and Melanoma Cancer Cells: <i>in vitro</i> model	38
In obese rats, physical training showed more effectiveness than carbohydrate-restricted and high-protein diet in antioxidant defenses in subcutaneous adipose tissue	39
Resistance exercise for mice: a new approach to training rodents	40
Climb training modifies the effects of arterial insufficiency in the pelvic limb of elderly rats	41
Physical exercise changes expression of mRNA- mitochondrial dynamics and biogenesis related in heart of overnourished juvenile male rats	42
Effects of moderate physical exercise on the redox homeostasis of white adipose tissue of overnourished juvenile male rats	43
Assessment of the Effect of Acute Caffeine Supplementation on Affective Parameters and Cognition During a Resistance Training Session	44
Analysis of the Quality of Life of Elderly People Included in a University Extension Program of the Undergraduate Course in Physical Education	45
Gestational resistance training affects fetal development differently depending on fetal sex with minimal impact on maternal health in rats submitted to an obesogenic diet	46
Relationship Between Mood State and Sleep in Rugby 7's Athletes During a Regional Championship	47



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Anti-adiposity effects of swimming training are accentuated by vagus nerve and spleen ablation on male Wistar rats without alter iron or hepcidin circulating levels	48
Impact of whey protein and mild aerobic training on renal function in rats.....	49
Influence of Maternal Lifestyle Habits on the Determinants of Cardiovascular and Metabolic Health in Children.....	50
Effect of Endurance Running Training in a Hot Environment on Phenotype and Heat Shock Protein in the Skeletal Muscles of Mice.....	51
Resistance training and secondhand smoke negatively modulate FIS1 protein expression in mitochondrial dynamics in glycolytic muscle	52
Impact of resistance training on mitochondrial fission of glycolytic fibers in obese rats	53
Effects of Immobilization and Exercise Training on Muscle Carnosine and Anserine content in Rats	54
Effect of pharmacological and non-pharmacological therapy on spatial learning and memory in an experimental model of ADHD in ovariectomized females.....	55
The influence of physical exercise in mice with severe generalized lipoatrophy	56
Effect of resistance training on behavioral responses in the experimental model of female ADHD .	57
Effects of maca (<i>Lepidium meyenii</i>) supplementation on sports performance in amateur runners: a randomized, double-blind, cross-over, clinical trial.....	58
Semaglutide combined with physical training: impact on diabetic kidney disease morbidity.....	59
De IGF-1 em fibras musculares do tipo II e a adaptação óssea e muscular à natação em camundongos fêmeas.....	60

08. Sistema Digestório, Nutrição e Metabolismo

Gestational Amplification of Metabolic Disruptions Induced by Excessive Supplementation of Polyunsaturated Fatty Acids.....	1
Time course of hepatic bioconversion of alpha-linolenic acid into long- chain polyunsaturated fatty acids	2
The short- or long-term exposition to a high-fat diet does not change the behavioral profile of adolescents C57BL/6 mice.....	3
Cardiometabolic responses to Vitamin D supplementation in elderly ribeirinhos	4
Preventive effect of <i>Citrus bergamia</i> on pathophysiological processes involved in metabolic hepatic steatosis in animals.....	5
The effect of bergamot on adipose tissue modulates changes in the redox and inflammatory state in animals fed a high sugar-fat diet.....	6
Effect of Ora-Pro-Nóbis supplementation in tilapia diets: Amino Acid Metabolism and Mitochondrial Energy Metabolism.....	7
Evaluation of safety and effects of <i>Pereskia aculeata</i> on cellular carbohydrate metabolism in Nile tilapia liver	8
The modulatory effect of Ora-pro-nóbis on the lipid metabolism of the Nile tilapia	9
Effect of Ora-pro-nobis on growth and body composition of Nile tilapia	10
Gut microbiota composition of non-obese type 2 diabetic rats (Goto-Kakizaki)	11
Gestational protein restriction accelerates male bone loss during aging	12
Effects of lycopene supplementation on oxidative stress and collagen deposition in the liver of diabetic and normal rats.	13



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Metformin controls oxidative stress, inflammation, and collagen deposition in the liver of normal and diabetic rats.....	14
Impact of Different Dietary Patterns on Inflammatory and Immune Response Following Exercise-Induced Muscle Damage in Trained Men	15
Beta-hydroxy-beta-methyl-butyrate (HMB) supplementation enhances mitochondrial capacity in hepatic stress induced by partial hepatectomy (PHx) in mice.....	16
Reversal of metabolic dysfunction-associated steatohepatitis by galectin-3 inhibition	17
Gamma-oryzanol Modulates Hepatic Metabolism and Prevents MAFLD: An Integrative Omics and Molecular Approach.....	18
Cholesterol exacerbates the pathophysiology of metabolic dysfunction associated steatotic liver disease through upregulation of hypoxia-inducible factor 1 and modulation of microcirculatory dysfunction.....	19
Mutation in the GPNMB protein induces negative regulation of energy metabolism and of rhythmic profile in mice fed a high-fat diet supplemented with fructose	20
Effects of alpha-linolenic fatty acid and physical exercise on long-chain polyunsaturated fatty acids metabolism	21
The Impact of Air Pollution in Combination with Ultra-Processed Foods on Glucose Metabolism in C57BL/6J Mice	22
Morphometric changes in colon fragments exposed to dexamethasone in utero and subjected to excess fructose intake in adulthood	23
Effects of periodontal disease during gestation and lactation on salivary flow, biochemical composition, and redox status in rats.....	24
Vagus nerve neural circuit regulates bile acid secretion in the liver	25
Mice with experimental Ulcerative Colitis treated with Sodium Butyrate had reduced distal colon collagen fiber damage	26
Reduction of advanced glycation end products formation by Pyridoxamine improves adipose tissue inflammation and microvascular dysfunction in C57BL/6 obese mice.....	27
The role of cholesterol on renal microcirculatory dysfunction in diet-induced obesity (DIO) model	28
Feeding pattern of rats submitted to the maternal neglect model.....	29
Effects of gestrinone administration on the gastrointestinal tract of rats fed on a hyperlipidic diet ...	30
Cannabinoid Receptor 1 (CNR1) expression in the gastric antrum and fundus of patients with obesity or overweight	31
Effects of the systemic inflammation on the intestinal tone of endotoxemic mice: functional evidence of disordered contractile regulation involving calcium- sensitization	32
Sex-related differences in the gut function: evidence of increased contractile activity in the colon of females.....	33
Effects of chronic central ghrelin infusion on the glycemic profile of offspring of mild hyperglycemic rats.	34
Differential effects of maternal obesity and metformin on male and female Wistar rats	35
Butyrate attenuates enteric neuron loss and reduces caspase-3 cleavage in experimental ulcerative colitis	36
Probiotic in periostropause: impact on cognition and behavior Wistar rats.....	37
Influence of nicotinic cholinergic receptor alpha-7 deletion in.....	38
Aging and inflammatory bowel disease: The role of P2X7 receptor in enteric neuronal loss after experimental ulcerative colitis	39
Antagonism of RAGE ameliorates cholesterol-induced exacerbation of metabolic dysfunction-associated steatotic liver disease.	40
Targeting AGE/RAGE Pathway with Pyridoxamine Attenuates Inflammatory and Microcirculatory	



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Abnormalities in Metabolic dysfunction- associated Steatohepatitis.....	41
PNPLA3 polymorphism as a possible determinant of bile acid dysregulation in MASLD progression	42
Chronic intracerebroventricular treatment with irisin increases lipid metabolism in adipose tissues in diet-induced obesity mice.	43
Lipidomic Signature of Somatic Muscles in Diet-Induced Metabolic Syndrome Animal Model Supplemented with Gamma-Oryzanol.....	44
Glycolytic Muscle Metabolomic Signature of Obese Animals Submitted to Western Diet Supplemented with Gamma-Oryzanol	45
Effects of chronic central ghrelin infusion on food preference of male offspring of mild hyperglycemic rats.....	46
Role of cholesterol in triggering the AGE-RAGE pathway in an in vitro steatosis model	47
Effects of estradiol and fish oil on white and brown adipose tissue of ovariectomized mice	48
Effects of Gestrinone Administration on Body Composition and food intake in Female Rats Fed a High-Fat Diet	49
Supplementation preserves muscle functionality and morphology in aging wistar rats.....	50
Effects of maternal curcumin supplementation on offspring in an experimental model of protein restriction during the gestational period.....	51
Supplementing with n-3 fatty acids to balance the n-6:n-3 ratio leads to metabolic dysfunction, oxidative stress, and liver structural changes in rat offspring, caused by a pro-inflammatory response	52
Chronic supplementation with polyunsaturated fatty acids induces histopathological liver alterations in wistar rats.....	53
Parkin Deficiency Alters Ubiquitination Levels in Liver Regeneration in Mice	54
Association between inflammatory foods, excess weight and quality of life of patients with autoimmune disease	55
Abbreviation of preoperative fasting in patients with heart disease	56
Impact of Chemotherapy Treatment on the Nutritional and Behavioral Status of Oncology Patients	57
Maternal administration of curcumin protects the liver of offspring at 31 days postnatal age of females subjected to protein restriction.	58
Impact of fish oil supplementation on obesity and metabolic syndrome in a rat model of litter size reduction: effects on oxidative stress and adiposity	59
Effects of Amazon açai (Euterpe precatoria Martius) supplementation on biochemical parameters and oxidative stress in the serum and liver of healthy Wistar rats	60
Diet-Induced Insulin Resistance Reduces Brain BDNF Levels in Female but Not Male Mice Without Affecting Memory	61
Influence of Yerba Mate Extract (<i>Ilex paraguariensis</i>) on Weight Gain and Metabolism in Wistar Rats	62
Impact of physical training on diabetes renal dysfunction: role of advanced glycation end products	63
Fetoplacental microcirculatory and morphometric abnormalities in mice with preeclampsia.....	64
Intermittent Cafeteria Diet in Adolescent Rats: Metabolic Effects in Adolescence and Adulthood...	65
Effect of physical training on renal microcirculatory changes and metabolic parameters in preclinical models of diabetes.....	66
Lack of Sexual Dimorphism in Hepatic Enzyme Alterations Induce by Diet-Induced Insulin Resistance in Mice	67
Characterization of a Model of Steatohepatitis Associated with Metabolic Dysfunction for Use in	



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Post-Transplant Evaluation Studies of Acellular Hepatic Scaffolds	68
Influence of Different Macronutrients on Skin Temperature in Para Athletes.....	69
High-Protein Diet Modulates Hepatic Gluconeogenesis: From CREB to FoxO1	70
Influence of high-Fat diet (HFD) consumption combined with colon inflammation on hepatic miR-122 expression in mice	71
Effects of Amazonaçaí (Euterpe precatoria Martius) pulp supplementation on body composition and bone parameters in Wistar rats	72
Effects Baru oil (Dipteryx alata Vog.) supplementation on body composition and biochemical parameters in Wistar rats.....	73
Bisphenol-A alters the expression of genes involved in lipogenesis, inflammation, and oxidative stress in the liver of adult zebrafish.	74
Acute Effect of Nutrition on Physical Performance in Para Athletes.....	75
The effect of early weaning on eating and emotional behavior in adult Wistar rats.....	76
Brazil nut oil (Bertholletia excelsa) supplementation on biochemical parameters and bone composition of healthy adult male rats and those programmed by early weaning.....	77
The role of the MrgD receptor and Angiotensin-(1-7) in adipose tissue remodeling in obese mice.	78
Effects of tauroursodeoxycholic acid (TUDCA) on adiposity and brown adipose tissue activation in malnourished mice fed with high-fat diet	79
Maternal Vit.D status plays a crucial role in determining bone and fat mass in male offspring but not in females	80
Liraglutide, a GLP-1 analog, enhances brown adipose tissue sympathetic activity of obese mice independently of food intake.....	81
Maternal obesity increases endocannabinoid signaling and triglyceride content in the liver of rat offspring at weaning.....	82
Effect of Galactoglucomannan on the Protection of Intestinal Barrier Integrity in Sodium Dextran Sulfate-Induced Colitis	83
Acute physical exercise decreases the P2X7 purinergic tone, facilitating cholinergic activity to delay solids gastric emptying	84
Consumption of high-fat diet impairs the cholinergic anti-inflammatory system and tight junctions proteins in dextran sulfate sodium (DSS)-induced colitis in mice.....	85
An interplay between the Sympathetic Nervous System and the Hypothalamic- Pituitary-Adrenal axis in the regulation of glycemia during acute cold stress.....	86
Therapeutic Potential of Angiotensin-(1-7) in Gut Dysbiosis-Induced Metabolic Disorders: Experimental Insights in Mice	87
Cold and restraint stress aggravates 5-fluorouracil-induced intestinal mucositis in mice	89
A new murine model of chemically-induced ileitis: Insights into inflammation, antioxidant imbalance and gastrointestinal dysfunction.....	90
Sleep deprivation prevents ethanol-induced gastric injury in mice: A dynamic and reversible effect modulated by rebound sleep.....	91
Splenectomy attenuates 5-fluorouracil-induced intestinal mucositis in mice: Evidence for a key role of gut-spleen axis	92
Subdiaphragmatic vagotomy improves obesity, vagal activity and leads to browning in the iBAT..	93
Bile acids and the P2Y1 purinoceptor play complementary roles to decrease esophageal motility in a gastroesophageal reflux disease (GERD) rat model.....	94
Proteolytic pathways in protein metabolism of skeletal muscles in diabetic mice.....	95
Effects of post-weaning protein restriction in the intestinal morphometry and motility of C57BL/6	



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

male mice	96
Effect of maternal obesity and perinatal fish oil supplementation on endocannabinoid system in the small intestine of adolescent rat offspring	97
Serum Metabolomic Analyses in Colorectal Cancer Patients: Impact of Cachexia.....	98
Resveratrol exerts molecular effects on insulin resistance induced by maternal obesogenic diet and fatty acids in c2c12 myotubes.....	99
Assessment of the Glycemic Response to Various Culinary Preparations of White Potato in Healthy Adults.....	100
Pentyl- <i>p</i> -Coumarate Prevents Ethanol-Induced Gastric Injury by Modulating Oxidative Stress ...	101
Effects of guava (<i>Psidium guajava</i>) supplementation on biochemical, bone and oxidative stress parameters of healthy Wistar rats.....	102
Effect of non-alcoholic fatty liver disease and fibrosis on the hepatic endocannabinoid system of obese patients undergoing bariatric surgery	103
Time without endogenous melatonin impacts food intake and sleep aspects in patients treated for pineal tumor.	104
Effectsofwatermelon(<i>Citrulluslanatus</i>)supplementationon metabolic parameters, oxidative stress and bone health in Wistar rats.	105
VLDL-associated miR-122 mediates liver–adipose tissue crosstalk in humans with obesity	106
The effects of maternal obesogenic diet on the hypertrophic capacity of skeletal muscle in adult rat offspring subjected to compensatory hypertrophy	107
Nutritional transition from a low-protein to a high-fat diet reduces the expression of antioxidant enzymes and activates inflammatory pathways.....	108
Vagus nerve and alteration on iron-hepcidin axis may be involved on the splenic iron deposition in the hypothalamic obese rats.....	109
7 Month old Alzheimer’s disease animal model double transgenic mice (APPswe/PS1DE9) show differences in metabolic and locomotor activity but age comparably to healthy controls	110
Impact of dietary adequacy on redox status in the cardiac ventricle in aged and obese rats	111
High-fat maternal diet enriched with omega 3: increases activity and expression of antioxidant enzymes and reduces inflammatory markers.	112
The PKA/CREB pathway stimulates interleukin-6 production by skeletal muscle during atrophy..	113
Role of subdiaphragmatic vagotomy on gastrointestinal motility, feed behavior, autonomic balance and oxidative stress in rats with Crohn's disease	114
Pineapple (<i>Ananas comosus</i>) supplementation changes biochemical parameters, oxidative stress and bone health in Wistar rats	115
Combination of benznidazole and benzoylthioureas attenuates tissue lesions caused by chronic <i>T. cruzi</i> infection in the esophagus of BALB/c mice.....	116
Angiotensins and a 3D Liver model: A Novel Approach to Investigate Fibrosis.....	117
Relationship between metabolic syndrome components and sleep quality in adults attended in primary health care.....	118
Metabolic Syndrome And Its Association With Mental Health And Executive Functions: A Study In Adults From Vitória De Santo Antão, Pe	119
Effect of dapagliflozin administration on masld induced by a high-fat diet and fructose-rich water in C57BL/6 mice	120
Antibesogenic and Antioxidant Effects of Botryosphaeran (a Fungal Exocellular (1-3) (1-6)-B-D-glucan) in Treating Obese and Non-Obese Female Wistar Rats.....	121
Antibesogenic and metabolic beneficial effects of botryosphaeran, an (1-3)(1- 6)-β-D-glucan, in offsprings from obese rats during pregnancy	122
Effect of Hand Immersion in Cold Water on the Thermographic Response of Supraclavicular	



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Skin in Para Athletes	123
The impacts of IUGR in hepatic and mesenteric adipose tissue cytokines and its response to acute glucose injection.....	124
Maternal malnutrition during pregnancy increases O-GlcNAc in arcuate nucleus astrocytes in adult Offspring.....	125
Effects of sitagliptin on mitochondrial remodeling and glycemic parameters in spontaneously hypertensive rats fed a high-fat diet	126
Maternal vitamin B9 status may modulate serum biochemical and oxidative stress markers in children	127
Heat Exposure Combined with Whey Protein Supplementation Enhances Strength and Fatigue Resistance in the Diaphragm Muscle of Mice	129
TRPV1 Channel does not participate in β 3-adrenergic induced UCP1 increase in brown adipose tissue but contributes to the thermoregulatory responses	131
Introdução Alimentar, Estado Nutricional e Hábitos Alimentares De Crianças De 2 A 5 Anos do Município da Vitória de Santo Antão	132
Fetal programming by high-fat diet induces alterations in somatic growth and delays in the parameters of the neurodevelopment in young rats	133
Impacts of protein malnutrition and overnutrition during the lactation period on offspring growth	134
6-nitrodopamine is an endogenous mediator of the rat gastric fundus relaxation	135
Maternal Low-Protein Diet Impairs Skeletal Muscle Hypertrophic Capacity In Adult Rats.....	136
Maternal obesity and placental development: the role of insulin and insulin-like growth factors .	137
Therapeutic Peptides Target Calcium Signaling Dysregulation in Hepatic Steatosis.....	138

09. Fisiologia Comparada e Zoologia

Early adaptation to an unusual host in the bean weevil <i>Zabrotes subfasciatus</i> is associated with changes in body size and reproductive physiology	1
Ovarian activation dynamics in <i>Zabrotes subfasciatus</i> (Boh.) (Coleoptera: Chrysomelidae, Bruchinae): The need for seeds and males.....	2
Effects of fipronil contamination on cardiovascular responses and autonomic control of the heart in rainbow trout during aerobic exercise.....	3
Impact of Settleable Atmospheric Particulate Matter (SePM) on metal bioaccumulation and histopathological changes in the main absorption pathways, gills and intestine, in Nile Tilapia.....	4
Living Airless: Metabolic and oxidative responses of the Tree Frog <i>Boana pulchella</i> under anoxic conditions	5
Ventilatory responses in heat dissipation in <i>Tropidurus catalanensis</i> (Squamata: Tropiduridae)	6
Functional and morphological characterization of the reproductive cycle of the rodent <i>Cerradomys goytaca</i>	7
Electroretinography for Retinal Function Evaluation in Pediatric Patients with Nystagmus	8
Comparative transcriptomics of anurans' liver: Insights on hepatic metabolism	9
Metabolic progression during embryonic development of the red- winged tinamous <i>Rhynchotus rufescens</i> (Palaeognathae)	10
Thermoregulatory Mechanisms in the Red Junglefowl, <i>Gallus gallus</i> : Metabolic and Ventilatory Adjustments to Cold.....	11
Investigating seasonal variation in mitochondrial function of erythrocytes in the tegu lizard <i>Salvator merianae</i>	12



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Development and structure of the respiratory system in the lizard <i>Tropidurus catalanensis</i> Gudynas & Skuk, 1983 (Squamata, Tropiduridae)	13
The relationship between structure and function of the avian respiratory system	14
Comparative analysis of air-breathing frequency (ABF) and critical thermal maximum (CT _{max}) in relation to physiological and behavioral responses between cave-dwelling and surface-dwelling armoured catfish species of the genus <i>Ancistrus</i>	15
Morphological diffusion capacity of the eggshell in the red-winged tinamous <i>Rhynchotus rufescens</i> (Palaeognathae)	16
Mitochondrial responses to nutritional and immunological challenges in Japanese quail	17
Evaluation of Evoked Visual Potentials in children with tumors affecting the visual pathway	18
Descrição de espécie inédita do gênero <i>Loksaphorus</i> Hirschmann, 1979 (Acari: Uropodina: Eutrachytidae)	19

10. Farmacologia, Toxicologia, Produtos Naturais e Química

Effect of varespladib on the reversal of crotoxin-induced neuromuscular blockade.....	1
Gestational Exposure to Low Doses of Glyphosate-Based Herbicide Induces Transient Neurodevelopmental Delays in Rats Offspring	2
Ruthenium (II) complex with venilpyridine induces oxidative stress, cell cycle arrest and apoptosis in melanoma cells	3
Antihypertensive effects of the aqueous extract (AE) of the leaves of <i>Saccharum officinarum</i> L. (sugarcane) in a model of primary (SHR) and secondary (2K1C) hypertension	4
GlyT1 inhibition promotes neuroprotection in the middle cerebral artery occlusion model through the activation of GluN2A-containing NMDAR	5
Neurorepair Induced by treatment with Sarcosine in an Animal Model of Cerebral Ischemia.....	6
Neurorestorative effect of a new l-proline transporter inhibitor (MCX13) in an animal model of cerebral ischemia (MCAO)	7
GlyT1 inhibition by NFPS post-treatment promotes neuroprotection in the middle cerebral artery occlusion model.....	8
3D cell culture establishment to better understand crotoxin cytotoxic effect on breast tumor cell lines	9
Effects of <i>Delonix regia</i> galactomannan on neuroinflammation and microglial activation in animal model of osteoarthritis.....	10
Impacto óleos essenciais encapsulados em levedura no desenvolvimento do mosquito <i>Aedes aegypti</i>	11
Isolation and characterization of mangrove fungi and prospecting for bioactive polypeptides	12
The impact of a mixture of contaminants (TBT, Hg, and Cd) exposure in the reproductive axis of female rats.	13
Chronic exposure to a mixture of phthalates changes white adipose tissue morphology in female mice.....	14
Probenecid Potentiates cGMP-Mediated Relaxation in Isolated Rat Corpus Cavernosum	15
Pharmacologic Characterization of Probenecid in the Prostate of Healthy Rats: An In Vitro Study. 16	
Effects of the Biopesticide Spinosad on Epididymal Morphology and Sperm Activity	17
Synthesis and study of single crystal 1,10-phenanthroline complexed with Cu(II) and Zn(II) for antibacterial actions	18



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Synthesis and study of single crystal 1,10-phenanthroline complexed with Cu(II) and Zn(II) for antitumoral actions	19
Pharmacological characterization of Pannexin-1 channels in rat corpus cavernosum: an in vitro study.....	20
Correlation Between Cardiac, Hepatic and Renal Oxidative Stress Markers in Wistar Rats Subjected to an Acute Dose of Doxorubicin.....	21
Exposure to atomoxetine hydrochloride decreases testosterone biosynthesis, colony formation, induces genotoxicity and cell death in TM3 Leydig cells	22
The insecticide malathion causes oxidative stress in the placenta and uterus of Wistar rats and increases the number of micronuclei at different gestational ages	23
Nicotine reward sensitivity in high sugar-induced binge-eating mice and the role of cannabidiol in food intake	24
Investigation of the effects of mesotherapy with yohimbine in mice.....	25
Behavioral effects of neonatal aluminum exposure in <i>Caenorhabditis elegans</i>	26
<i>Ilex paraguariensis</i> post-exposure to Al reestablishes sociability patterns in zebrafish.....	27
<i>Ilex paraguariensis</i> post-exposure to aluminum reestablishes biomarkers in <i>Caenorhabditis elegans</i>	28
Exposure to Low Dose NDMA Causes Structural and Inflammatory Changes in the Prostate of Adult Rats in a Transgenerational Effect, in the Context of the Paternal Origins of Health and Disease (POHaD)	29
Alteration of stimulated flow rate, biochemical composition, and salivary redox state in male Wistar rats caused by the antiepileptic drug Carbamazepine	30
<i>Trans</i> -Aconitic Acid, bioactive compound isolated to sugarcane, enhances dopaminergic activity in VTA subregions related to emotional and motor circuits in Wistar male rats submitted to forced acute physical activity	31
Effects of unsaturated fatty acid supplementation during gestation: delays in motor development and alterations in muscle characteristics in offspring rats.....	32
Sperm mitochondrial activity was impaired by <i>in vitro</i> exposure to polystyrene nanoplastics.....	32
Intra-Articular Oxygen Reduces Temporomandibular Joint Arthritis-Related Pain and Inflammation	34
Bisphenol S and High Fat Diet-Induced Brown Adipose Tissue Plasticity.....	35
Analysis of the proliferative effect of <i>Lavandula dentata</i> essential oil on HaCaT keratinocytes.....	36
Impact of Exposure to N-Nitrosodimethylamine (NDMA) on Fertility and Embryo Fetal Development in Wistar Rats	37
Norepinephrine and angiotensin II stimulation alter the cell proliferation in human oral squamous cell carcinoma and keratinocytes by different mechanisms.....	38
Spike protein induces cytokines production mediated by RAS components expression on human three-dimensional bronchiole co-culture	39
Spinosad exposure during puberty impairs testis morphophysiology in Wistar rats	40
Virtual screening of natural products for identifying novel antiviral candidates targeting the VP39 protein of the Mpox virus.	41
Integrating bioisosterism and virtual screening in the search for Adenosine A2A Receptor therapeutics.	42
Vasorelaxant effects of <i>trans</i> -3-methoxy- β -nitrostyrene in rat thoracic aorta: involvement of soluble guanylate cyclase.....	43
Prophylactic effect of Ascorbic Acid or Ketamine on Anxiety-Related Behavior induced by DSS	44
Omega-3 and omega-6 in metabolic regulation: dose-dependent effects on body, plasma, and inflammatory parameters.....	45



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

β -Caryophyllene derived from clove oil, carried in a self-emulsifying drug delivery system, potentiates antirheumatic effects in rats	46
p-Syneprine inhibits adipogenic differentiation and induces UCP-1 expression in 3T3-L1 preadipocytes	47
Evaluation of the isoflurane effects on the inflammatory response and redox state of the mice liver fed a high-fat diet.....	48
Application of Dandelion in Ovarian Cancer: A Promising Natural Approach	49
Investigation of molecular interactions between agmatine and its potential targets in depression, anxiety and suicide	50
Effect of Chlorothalonil on the gene expression of mouse (<i>Mus musculus</i>) brain cortex	51
Structural Characterization and Biological Activity of a Potential toxin-peptide Extracted from the Sea Anemone <i>Bunodosoma caissarum</i>	52
IL-6/JAK/STAT3 pathway and its possible role in ketamine antidepressant and antisuicidal effects	53
Multigenerational Study of Paternal F2 Generation Male Rats: Evaluations of Reproductive Development after Exposure of the F0 Generation to Nitrosodimethylamine.....	54
Consequences of Nitrosodimethylamine (NDMA) Exposure on Reproductive and Toxicological Parameters in Adult Male Wistar Rats.....	55
Role of full-spectrum <i>Cannabis sativa</i> oil in lymphoid organs of rats exposed to crack cocaine during pregnancy.....	56
Effects of Incretin-Based Therapies on NeuroCardiovascular Dynamic Changes Induced by High Fat Diet in Rats	57
Neuroprotective Effect of Citicoline in the Alzheimer's Disease Model of.....	58
Modulatory Effects of Omega-3-Derived Lipid Mediators and Acetylsalicylic Acid on Skeletal Muscle Regeneration and Fatigue Response in a Snake Venom-Induced Injury Model	59
Antimelanoma Potential and Selectivity of Cerumen and Propolis from <i>Nannotrigona testaceicornis</i> (Hymenoptera, Apidae, Meliponini)	60
Antioxidant Properties of <i>Xanthosoma sagittifolium</i> (Taioba) Constituents Related to the Endogenous System of the Yeast <i>Saccharomyces cerevisiae</i>	61
Protective Activity of Chalcone Derivatives in a Parkinsonian Model of <i>Saccharomyces cerevisiae</i>	62
Influence of Cu(II) toxicity in eukaryotic study models expressing neurodegenerative disease peptides.....	63
Influence of antioxidant compounds derived from chalcones and coumarins in <i>Saccharomyces cerevisiae</i> cells with autophagic dysfunction	64
Development of active compounds against A β 42 peptide aggregation:.....	65
Neuroprotective effect of sarcosine in an animal model of Alzheimer's disease.....	66
Adolescent Binge Drinking Withdrawal Leads to Long-lasting Spinal Cord Neuron Damage and Oxidative Stress in Rats.....	67
Adolescent Binge Ethanol Consumption Impairs Motor Function and Induces Long-Lasting Oxidative Stress in Female Rats.....	68
Virtual screening of natural products targeting TNF- α for psoriatic.....	69
The potential of decanoic acid as a neurorepair agent in an animal model of cerebral ischemia....	70
In vitro study of leucine supplementation and acerola (<i>Malpighia emarginata</i> DC.) hydroethanolic extract effects on the modulation of cancer cachexia.	71
Aqueous extract of <i>Vernonia condensata</i> flowers prevents DNA oxidation and protects <i>Caenorhabditis elegans</i> from oxidative stress	72
Formulation Strategies to Enhance Cannabidiol Solubility and Bioavailability: A Preliminary Scoping Review.....	73



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Investigation of alternative therapeutic strategies for treatment of oral squamous cell carcinoma employing ruthenium(II) complex.....	75
<i>Tillandsia loliacea</i> : Antimelanoma activity, selectivity, and in vivo toxicity.....	76
Impact of Bergamot Leaf and Fruit Extracts on Metabolic Syndrome Parameters in Wistar Rats ...	77
Green Propolis prophylaxis improves liver function in diabetic rats	78
Alcohol metabolism in a repeated binge drinking pattern induces biochemical alterations in adolescent female rats: focus on liver enzymes and lipid profiles	79
Long-term hepatic oxidative imbalance and tissue damage induced by binge drinking from adolescence to adulthood in female rats	80
Neuroprotection Induced by Pre-treatment with Sarcosine in an Animal Model of Cerebral Ischemia	81
Evasins EVABm14B and EVABm1A from <i>Rhipicephalus microplus</i> as anti-inflammatory potential and interaction with HIV gp120 protein	82
Innovating Antibiotic Research and Development Through Brazil's Biodiversity	83
Clinical and obstetric profile of normotensive and pre-eclamptic pregnant women attended at the Botucatu Maternity - SP, Brazil.....	84
Metabolic Effects of <i>Baccharis dracunculifolia</i> DC on an Innovative Animal Model of Cardiometabolic Syndrome	85
Exploring the antiproliferative activity of essential oil from lemon myrtle using HEPG2 cells in 2d and 3d cultures	87
Selecting molecular targets to investigate the role of glyphosate exposure in the pathogenesis of Alzheimer's disease using the Comparative Toxicogenomics Database	88
The XDH gene of the population of São Paulo	89
Effects of oral administration of Brazilian Red and Faveleira propolis on acute nephrotoxicity induced by cisplatin in juvenile Wistar rats.....	90
Evaluation of the effects of <i>in vitro</i> exposure to different concentrations of polystyrene nanoplastics on human sperm motility	91
Binge Drinking on Adolescence: Neurobehavioral and Gut-Brain Axis Alterations in Female Rats ..	92
Novel apocynin analogues improve endothelial function in aorta rings.....	93
Binge Drinking and Motor Function: A Global Bibliometric Analysis and Mapping of Experimental Studies	94
Orchiectomy Induces Selective Memory Deficits and Dendritic Spine Loss in Mice	95
Effects of oral administration of Brazilian Red and Faveleira Propolis on acute hepatotoxicity induced by dimethylnitrosamine in juvenile Wistar rats	97
Participation of the NO/GMC/K+ATP pathway in the antinociceptive effect of the hydroethanolic extract of <i>Megaskepasma erythrochlamys</i> flowers	98
Study of the immunomodulatory activity of components of crude <i>Pouteria caimito</i> seed extract ..	99
Single-dose oral treatment with field rosemary (<i>Baccharis dracunculifolia</i>) extract fails to produce anxiolytic effects in Wistar rats	100
Multigenerational Impacts of Nitrosodimethylamine on the Adult Life of Female F1 Generation Rats	101
Effect of the hydroalcoholic extract from <i>Calycophyllum spruceanum</i> barks on mice anxiety and depression behaviors.....	102
Single-dose administration of <i>Baccharis dracunculifolia</i> extract does not elicit antidepressant effects in Wistar rats	103
Effect of metformin on behavioral and biochemical changes in female mice induced to autism spectrum disorder by valproic acid.....	104



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Effect of homeopathic formulation of <i>Arnica montana</i> L in experimental model of rat tendinopathy	105
Physicochemical characterization and evaluation of cytotoxic and hepatoprotective effects of sulfated polysaccharides from <i>Fucus vesiculosus</i> and <i>Spirulina</i> sp.	106
Doxorubicin causes histomorphometric changes and oxidative stress in the parotid glands of rats	107
Evaluation of the insecticidal activity of Brazilian pepper tree (<i>Schinus terebinthifolius</i> Reddi) fruit extract on the development of <i>Aedes aegypti</i> mosquito larvae.....	108
Dynamics of epididymal protease inhibitor (EPPIN) compartmentalization and colocalization with its binding partner semenogelin-1 in human spermatozoa.....	109
Cytotoxic effect of plant polysaccharide extracts on pancreatic tumor cells (PANC-1).....	110
Trans-Anethole Preserves Pulmonary Function and Architecture in Cecal Ligation/Puncture-Induced Sepsis	111
Trans-Anethole as a Multi-Target Therapeutic Strategy for Sepsis: Attenuation of Systemic Inflammation and Oxidative Stress	112
Evaluation of the toxicity of Aroeira oil (<i>Schinus terebinthifolius</i>) and the virulence of the entomopathogenic fungus <i>Metarhizium anisopliae</i> in the development of mosquito larvae of the genus <i>Aedes</i>	113
Metabolites of the endophytic fungus <i>Ochrocladosporium elatum</i> protect biomolecules and <i>Caenorhabditis elegans</i> from oxidative stress	114
Effect of methotrexate conjugated to chitosan nanocomplexes on zymosan-induced arthritis	115
Propolis from <i>Melipona quadrifasciata anthidioides</i> and <i>Scaptotrigona depilis</i> reduces lipid accumulation in 3T3-F442A cells	116
Erythroxyllum anguifugum and Erythroxyllum pelleterianum protect against oxidative stress in a <i>Caenorhabditis elegans</i> preclinical model	117
Tocolytic effect of the aqueous extract of <i>Lippia origanoides</i> H.B.K. aerial parts and the action mechanisms involved in different contraction response.....	118
Semenogelin-1 targets the Ca ²⁺ channel CatSper to inhibit sperm hyperactivation in mice	119
Gastroprotective activity in vivo and in silico of myrtenyl laurate obtained by biotransformation	120
Technological prospecting of the medicinal use of <i>Pectis brevipedunculata</i>	121
Biomonitoring of Extracts from the Marine Sponge <i>Dysidea janiae</i> with Anti-Inflammatory Potential for the Treatment of Osteoarthritis	122
Study of the psychopharmacological effects of <i>Illicium verum</i> essential oils by inhalation in mice	123
From <i>in silico</i> to <i>in vivo</i> : novel PKM2 inhibitor with antineoplastic effect against oral squamous cell carcinoma mitigates tumor growth.....	124
Target Fishing based on Phytochemicals with Neuroprotective Potential for Neurodegenerative Diseases	125
Protective Effects of Citronellol on Ethanol-Induced Gastric Lesions: Morphological and Histological Analyses	126
Developmental Effects of NSAIDs on Male Rat Offspring Following Intrauterine and Lactational Exposure.....	127
Evaluation of the antiulcerogenic potential of Cannabis sativa-derived Cannabidiol and Cannabinol through <i>in silico</i> protein interaction profiling	128
Maternal toxicity assessment in Wistar rats exposed to COX-1 and COX-2 inhibitors during gestational and lactational periods and their possible repercussions on female offspring.....	129
Cardiovascular effects of (±)-Propranolol, (±)-4-NO ₂ -propranolol, and (±)-7-NO ₂ -propranolol in anesthetized rats.....	130



**XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO**

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

11. Imunologia, Microbiologia e Parasitologia

Modulating Bronchial Vascular Remodeling In An Experimental ACO Overlap Model Through IL-17 Inhibition	1
Malaria-Induced Brain and Kidney Injury: A Novel Approach Using Young BALB/c Mice	2
Expression of COX-2 and IL-6 and inflammatory infiltrate as immunological markers in neuroblastoma	3
Monotherapy with 4-bromo-N-(3-nitrophenyl) carbamathiol benzamide during the acute phase of experimental Chagas disease prevents cardiovascular alterations and reduces cardiac lesions in the chronic phase.....	4
Antibiofilm and Antifungal Activities of Tritrpticin (Trp3) Analogs Revealed by Confocal Laser Scanning Microscopy (Clsm) Assays and In Vivo Assays Using a Galleria Mellonella Model	5
Activity of the Bioactive Peptide IbktP-Nh2 on Multispecies Biofilms of Candida albicans and Staphylococcus aureus and in vivo Studies Using Galleria mellonella Larvae for the Treatment of Multispecies Infections and Identification of Immunological Alterations	6
Hyperglycemia and Endothelin-1 Affect Endothelial Cell Homeostasis by Increasing Glycocalyx Permeability and Degradation In Vitro	7
Immunoregulation and effects of pre-treatment with aspirin in mice with metabolic syndrome during severe sepsis.....	8
Effect of VACHT reduction on pulmonary alterations induced by exposure to iron particles in an asthma model	9
Silymarin exerts protective effects on arthritis and myositis induced by Chikungunya virus.....	10
Albumin Transport Triggers Microglial Activation Before BBB Breakdown in Early Stages of Cerebral Malaria	11
Effects of Anti-IL-33 on Pulmonary Mechanics and Inflammation in an Experimental Model of Asthma Exacerbated by LPS	12
Gestational Exposure to Phthalates and Nanoplastics in Rats: Impacts on Gut Microbiota Composition and Intestinal Histology.....	13
OM-85 bacterial lysate reduces P. aeruginosa susceptibility in cystic fibrosis while exacerbating tissue damage in a preclinical model	14
Rational design of immunogenic peptides for a multiepitope vaccine targeting structural proteins of Oropouche virus	15
Conserved immunogenic epitopes in host entry and exit proteins of the reemergent Monkeypox virus and the emerging Alaskapox virus.....	16
Implications of the Heme Group in the bioenergetics of Trypanosoma cruzi isolates from patients with different clinical forms of Chagas disease	17
Detection and identification of microorganisms in ticks of the families Argasidae and Ixodidae from different Brazilian States (Amazonas, Mato Grosso do Sul, and So Paulo) by MALDI-TOF/MS	18
Purification and Sequencing of a Paralyzing Protein Obtained from the Salivary Glands of the Tick Ornithodoros brasiliensis (Acari: Argasidae)	19
Ivermectin impairs the microbicidal capacity of macrophages in vitro by binding to the TLR-4/MD2 complex, but is beneficial in acute pneumonia in TLR-4 knockout mice.....	20
Histopathological analysis of liver and lung tissues in mice with metabolic syndrome induced to polymicrobial sepsis pretreated with aspirin.....	21
Selective Cytotoxicity of Novel Ruthenium Metallodrugs Against Leishmania Species Causing Cutaneous Leishmaniasis	22
Chronic suppression of beta-adrenergic signaling exaggerates the inflammatory response to	



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

systemic LPS in rats.....	23
Biotechnological Evaluation of BthTX-II for the Control of Toxoplasma gondii: A Novel Therapeutic Approach for Congenital Toxoplasmosis	24
Cannabidiol reduces nitric oxide and pro-inflammatory cytokine levels in 2-kidney, 1-clip (2K1C) hypertensive rats.....	25
Antibacterial activity of melittin against Acinetobacter baumannii, Enterococcus faecium, and Klebsiella pneumoniae	26
Temporal dynamics of murine brain B lymphocytes distribution during life.....	27

12. Ecologia e Meio Ambiente

Sub-chronic histopathological damage induced by the fungicide carbendazim in Danio rerio (Zebrafish)	1

13. Nanotecnologia, Biotecnologia, Biociências Nucleares e

Organ Masses for Mice Dosimetry Models: A Review	1
Lipid Nanoparticles Conjugated to Docetaxel Combined with Aerobic Exercise Reduce Tumor Burden and Enhance Apoptotic Pathways in Bladder Cancer.....	2
Hydrogel Derived from Bovine Bone Extracellular Matrix Enriched with PRP and Its Influence on Fibroblast Culture	3
Evaluation of myelin content by PET with [11C]PIB in an animal model of Down syndrome.....	4
Macrophage Differentiation Induced by Phosphatidylserine / 7 - Ketcholesterol Nanoparticles	5
Food-derived bioactive compounds and their effect on currently applied chemo and chemo plus immunotherapy regimens for colorectal cancer: an <i>in vitro</i> approach	6
Implementation of Perinatal Stem Cells Biobank Employing Good Laboratory Practices for Pre-clinical Research in Ophthalmology.....	7
Immunogenicity and Standardization of a Method for Quantification of Anti-Cocaine Antibodies Induced by the UFMG-VAC-V4N2 Vaccine in a Non-Human Primate Model.....	8
Production and characterization of biocuratives derived from human amniotic membrane for clinical application in ophthalmology	9
Application of Good Manufacturing Practices in Blood Component and Tissue Amniotic Membrane Based Products for Ophthalmology Applications.....	10
Mesenchymal Stem Cells from Dental Tissues: A promising Alternative for Regenerative Medicine	11
Evaluation of platelet-rich plasma adhesion to therapeutic contact lenses	12
Gold nanorods functionalized with Angiotensin-(1-7) promote neuroprotection in an <i>in vitro</i> stroke model	13
Development of an algorithm to design and validate interference RNAs of altered genes in Rett syndrome.....	14
Evaluation of oligomycin-preconditioned umbilical cord mesenchymal stromal cells and extracellular vesicles in experimental sepsis	15
Tracking 50 Years of Knee Osteoarthritis Treatments: A Keyword Analysis Using AI and Constellate.org.....	16



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Effects of IFN- γ preconditioning of bone marrow-derived mesenchymal stromal cells in a murine model of pulmonary sepsis caused by <i>Klebsiella pneumoniae</i>	17
Assessment of elastin degradation in a 3D study model of COPD/emphysema	18
Modulating effect of systemic laser therapy on inflammation and oxidative stress in a stroke model in mouse	19
Biological Activity of Porcine Placental Extracellular Matrix-Derived Hydrogels: In Vitro Validation and Wound Healing Activity Evaluation.....	20
Establishment of a Bioprocess for the Production of a Hydrogel Resulting from the Combination of Alginate and Pequi Oil (<i>Caryocar brasiliense</i> Camb.) as a Potential Therapeutic Alternative for Cutaneous Ulcers	21
Effects of platelet-rich fibrin and platelet-rich plasma on protein expression in experimental arthritis in Wistar rats.....	22
Ultrastructural and Physicochemical Characterization of Extracellular Matrix-Derived Hydrogels from Different Porcine Reproductive Tissues	23
Development of the Full-Length IgG Non-Glycosylated Version of the Monoclonal Anti-PD-L1 as an Immunobiological Tool for Prospecting New Antitumor Immunotherapy	24
In Vitro Biocompatibility and Adhesion of MRC-5 Cells to Chitosan-Based Biomaterials	25

14. Ciências de Animais de Laboratório e Modelos

Influence of Omega-3 Fatty Acid Supplementation on Oxidative Stress, Glycemia, and Lipid Profile in Rats Chronically Treated with Doxorubicin.....	1
Effect of whole-body vibration exercise on the hematological profile of diabetic <i>Wistar</i> rats: a preliminary study.....	2
Virulence of Atypical Enteropathogenic <i>Escherichia coli</i> (aEPEC) Strains Isolated From Dogs in an Experimental Model With <i>Galleria mellonella</i> Through Intrahemocelic and Oral Infection	3
Avaliação de resistência à deltametrina em Linhagens Endogâmicas Recombinantes de <i>Aedes aegypti</i>	4
The Effects of Moderate Physical Training on Renal Function in Male Rats Treated with Tenofovir Disoproxil Fumarate	5
Anti-TSLP Treatment Modulates Pulmonary Response in an Experimental Model of Asthma COPD Overlap.....	6
Sex Differences in Memory Consolidation and Persistence at Different Intensity Stimulus' Levels in Contextual Fear Conditioning	7
Osteocyte number density can predict bone maturation.....	8
Low-intensity laser therapy has the potential to promote bone neof ormation by decreasing the number of osteocytes	9
Guide to the husbandry of mice strains at the Instituto de Ciência e Tecnologia em Biomodelos/ Fiocruz.....	10
Determination of the Ammonia Concentration and Change Frequency of the B6.Cg-Tg(K18-Ace2)2prlmn/J Strain at the Institute of Biomodels Science and Technology.....	11
Effects of swimming and resistance training on the diaphragm of male and female dystrophic mice	12
Effects of the ketogenic diet on gut microbiota of rats with cerebral palsy.....	13
Weight and metabolic outcomes of light-phase time-restricted feeding combined with western-style diet in Wistar rats	14
Physicochemical characterization of two types of collagen and their use in the construction of 3D	



**XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO**

**8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP**

skin-equivalent models	15
Establishment and characterization of a 3D hepatic steatosis spheroid model for MASLD studies and preliminary phytotherapeutic screening with <i>Pereskia grandifolia</i> extract	16
Therapeutic Potential of Ora-Pro-Nóbis (<i>Pereskia aculeata</i>) Extract Supplementation in Ischemia/Reperfusion-Induced Muscle Injury	18
Technological development of a purified, high-fat, pelletized experimental diet for laboratory rodents	19
Bacteriological profile of mice and rats in Brazilian animal facilities	20
Evaluation of chemotherapy response in precursor B-cell acute lymphoblastic leukemias (B-ALL) with different transcriptional profiles in the IL7rCPT in vivo model	22

15. Educação e Difusão do Conhecimento em Ciências

It's not rocket science: talking to society about aging factors on social media	1
Neuroscience in Teacher Training: Enhancing Pedagogical Innovation for Adolescent Education	2
The Impact of Neuroscience Outreach through Neuroblitzes in Primary Schools in Uruguaiana/RS	3
DoHAD Bulletin: a bulletin for the promotion of science and culture	4
Engaging Cell Biology Students through Interactive and Contextualized Online Resources	5
VHISTO: A mobile and web application for histology learning in undergraduate education	6
Empowering Undergraduates to Confront Cancer Misinformation: An Interdisciplinary Project-Based Educommunication Approach	7
Biomedical Action Project: new playful methodologies in the awakening of future scientists	8
From the Brain to the Classroom: Neuroeducation and Pedagogical Innovation in Teacher Training	9
Medical students' perception of the time spent on social media and its consequences on study time	10
Obesity and endocrine disruptors: an e-book approach for teaching science in high school	11
Autonomy and satisfaction in human physiology: a study on student experiences in Brazil	12
Recording and blocking of electric signal in annelids – a valuable teaching tool in physiology and pharmacology	13
Effect of Innovative Educational Strategies on Learning About Cells	14
Medical students' perception of Team-Based Learning (TBL) as a collaborative learning tool in Human Physiology	15
Science Laboratories in High School: Advances and Setbacks Throughout the 2000	17
Take a look: Scientific dissemination about eye health and research in vision science	18
SintomaBot: an AI-powered virtual tutor for enhancing physiology-based clinical reasoning in health science education	19
Use of Active methodologies to encourage the participation of caregivers of People with disabilities in the collaborative construction of knowledge	20
Gamification as a strategy for teaching Viral Hepatitis to families of People with Disabilities	21
Teaching of Anatomy through the extension project Laboratório Didático de Anatomia (PEAnato): implications of inequality between public and private institutions	22
Can active methodologies like scavenger hunt be interesting in learning physiology?	23
Scientific dissemination and education about blood donation for deaf people	24
Assessment of emotional activation levels of university students in different periods of the	



**XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO**

**8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP**

semester.....	25
The impact of ALDH2 polymorphism on endothelial function in young healthy East Asians	26
Affiliations:	26
Spatial abilities modulate students' performance in interpreting cladistic graphical representations: a 3D-printed cladogram as a haptic educational tool	27
Tiktok and epilepsy disorder: a cross-sectional study of social media content quality	28
Understanding Obesity Through Podcasts: Neuroendocrine Regulation of Appetite and New Therapeutic Strategies.....	29
Scientific Journalism Research Project: Energy balance in the context of body mass regulation and its impact on the development of obesity	30
Contribution of formative assessments to study routines in university: student perception	31
Active Methodology in the Teaching of Human Physiology: An Experience Report.....	32
The Myth of High-Load Training for Strength Gains: A Research-Based Approach to Teaching Muscle Physiology	33
Reels as a Didactic Tool for Young Learners: An Experience Report from Faciologia	34
Can The Methodology Motivate Tutoring? A Practical Strategy For Teaching Human Physiology: An Experience Report.....	35
The Impact of Active Methodologies in Health Education: An Experience Report of the Faciologia Extension Project.....	36
"Oncologia Descomplicada: Scientific Communication as a Tool for Healthy Education and Social Transformation"	37
Use of the Kahoot Platform in Reducing Anxiety and Autonomic Regulation in Academic Assessments: A Pilot Study	39
Multisensory Approach to DNA Structure and Extraction: An Inclusive Educational Strategy.....	40
Nexus: A Gamified Approach to Teaching Cell Biology	41
The surface-area-to-volume-ratio approach with jelly cubes: innovations on a protocol to adress interdisciplinary studies for basic and higher education	43
Brazilian Brain Bee for Undergraduates as a Platform for Disciplinary Integration: A Historical and Educational Analysis (2018–2024)	44
Brazilian Brain Bee Participant Profile in 2024: Female Predominance and Sociodemographic Diversity	45
Analysis of life habits of basic school students and development of a teaching resource for teaching physiology and health.....	46
Perception of undergraduate students about the use of biosensors associated with an online teaching platform in human physiology teaching	47
XVI Brain Awareness Week in Rio de Janeiro: Science, Evolution, and Diversity in Interactive and Intergenerational Practices.....	48
Impact of Sleep Quality on Academic Performance in Children With and Without Learning Difficulties	49
Boosting Executive Functions Through Rubik's Cube Training: A Neuropsychopedagogical Approach in Early Childhood Education.....	50
Proposal for a digital game for learning respiratory physiology.....	51
Obesity, genetics and the sweetener saccharin: use of e-books as a didactic resource in context of DOHaD theory.....	52
Knowledge of Human Physiology among Physical Education Students: Perception and Applicability in the School Context	53
From health basic sciences experiments to data analysis: an interdisciplinary proposal for scientific	



**XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO**

**8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP**

initiation in high school conducted by M.Sc. and Ph.D. students as a part of "Anísio Project"	54
Influence of Instagram on the level of physical exercise of followers	55
"What Hormone Is This?": A Didactic Game to Support Learning of Endocrine Physiology in Health Education	56
Tutoring In Basic Disciplines In Undergraduate Courses In The Health Field: An Experience Report	58
Perception of sus users about epidemiology and risk factors of human papillomavirus in São Félix do Xingu, PA, Brazil.....	59
Epidemiological Situation of Visceral Leishmaniasis in São Félix do Xingu-PA, Brazil in the last five years (2020-2024).....	60
Health Under the Microbial Lens: Unveiling Invisible Threats in the School Routine.....	61
Bringing rare diseases to light: the importance of awareness and disclosure	62
Visuomotor Impairments and Reading Difficulties in Schoolchildren: Unveiling a Hidden Educational Crisis.....	63
The Impact of Neuroscience Outreach through Neuroblitzes in Primary Schools in Uruguaiana/RS64	
Impact of Using a 3D Model of Cell Membrane Proteins Combined with an Online Platform in Human Physiology Education	65
Effects of a Cardiac Cycle Puzzle on Learning and Perceptions of Veterinary Medicine Students ...	66
Exploring gustatory and olfactory perception and their contributions to methodological advances in teaching in public schools in Macaé	67
Accessibility and usability in the development of technological solutions for the SUS	68
Carrapatos e febre maculosa: jogo educativo como uma proposta de instrumento pedagógico de ensino	69
Tratamento documental em projetos de pesquisa: gestão de documentos e gestão de acervos para preservação, acesso e difusão.....	70
O movimento e gênero musical BRock e sua importância durante a ditadura militar: Possibilidades por meio da CienciArte e Pesquisa Baseada em Artes.....	71

16. Visão Oftalmologia

Microperimetry as a valuable tool for assessing changes in visual function following voretigene neparvovec-rzyl gene therapy.....	1
Insulin nanoemulsion eye drops for treatment of dry eyes in Sjögren's Disease: A randomized clinical trial Phase I/II	2
Salivary and urinary concentrations of metals in Sjögren's Disease.....	3
Clinical study of nanoemulsion eye drops containing insulin for the treatment of ocular damage due to dry eye diseases	4
Inhibition of FAAH, the anandamide-degrading enzyme, provides neuroprotection in photoreceptor degeneration in rd10 mice.....	5
Cannabidiol administration mitigates photoreceptor degeneration in rd10 mice, a model of retinitis pigmentosa	6
Inhibitor of anandamide-degrading enzyme (FAAH) preserved photoreceptors via CB1 and CB2 receptors in rd10 mice.....	7
Enhanced P2Y1 receptor gene expression and its blockade increased photoreceptor degeneration in a murine model of retinitis pigmentosa.....	8



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

FAAH inhibition as a potential therapy to alleviate oxidative stress in a murine model of retinitis pigmentosa	9
Sex Influence on Retinal Degeneration in the RD10 Model of Retinitis Pigmentosa	10

17. Biociências Nucleares

Effect of varespladib on the reversal of crotoxin-induced neuromuscular blockade	1

18. Anatomia

Omega-3 and prednisolone therapy enhances the efficiency of limb movements during gait	1



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Molecular Insights of Myocilin Reveal Potential Role in Contraction and Growth of Fish Skeletal Muscle

Authors:

Lucas Camilo Moraes ALVES¹,
Eduardo Rosa da SILVA¹,
Erika Stefani PEREZ²,
Daniel GARCIA DE LA SERRANA³,
Bruna Tereza Thomazini ZANELLA⁴,
Maeli DAL-PAI-SILVA²,
Bruno Oliveira da Silva DURAN¹

Affiliations:

¹Federal University of Goiás ²São Paulo State University ³University of Barcelona ⁴Federal University of Jataí

Keywords: Bioinformatics. Fishes. Muscle growth. Myocilin. Skeletal Muscle.

Abstract:

The skeletal muscle of fish corresponds to 60% of their body mass, being the main edible part and providing nutrients for the human diet. Numerous factors (amino acids, IGF1, hormones) stimulate fish muscle growth, impacting aquaculture. However, several genes and proteins activated in this process are still poorly characterized, such as myocilin (*myoc*). This study aimed to elucidate the role and relevance of *myoc* in the fish skeletal muscle using omics and bioinformatics data. We analyzed public single-cell RNA data and the transcriptome from muscle cells of pacu (*Piaractus mesopotamicus*) and seabream (*Sparus aurata*) treated with amino acids or IGF1. We also evaluated molecular networks and enrichment of ontological terms from differentially expressed genes related to *myoc* in fish muscle cells. Our results show high expression of *myoc* in skeletal muscle, especially in fibroblasts (1126 nTPM) and myocytes (42 nTPM) within the tissue environment. Additionally, we observed high expression of *myoc* in pacu muscle cells treated with amino acids (80.3 counts), while seabream cells showed no statistical differences, despite a large number of counts. Gene networks showed interactions of *myoc* with members of the same family (*olfactomedin-like*) and with genes mainly related to muscle development and maturation, metabolic and biosynthetic activity, extracellular matrix and cell signaling, and transcriptional regulation. Protein networks revealed interactions with Pdlim7, Gas7a, Plk1, Plk3, Mybphb, Col18a1a and Gpc1a, required for proper muscle differentiation and structure. We identified the enrichment of several biological processes, cellular components, and molecular functions relevant to skeletal muscle, especially 'muscle contraction', 'sarcoplasm', and 'calcium binding', respectively. Our findings help clarify the molecular pathways modulated by *myoc* and its possible regulation of fish muscle growth, offering new perspectives for applications in aquaculture.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Chrysin stimulates hypertrophy and glycolytic metabolism of the soleus muscle in old rats

Authors:

Eduardo Rosa da SILVA¹
Lucas Camilo Moraes ALVES¹
Jane Sousa NAVES¹,
Erika Stefani PEREZ²
Fernanda Cristina Alcantara dos SANTOS¹
Bruna Tereza Thomazini ZANELLA³
Maeli DAL-PAI-SILVA²
Rafaela Alves RIBEIRO¹
Bruno Oliveira da Silva DURAN¹

Affiliations:

¹Federal University of Goiás ²São Paulo State University ³Federal University of Jataí

Keywords: Skeletal muscle; Sarcopenia; Muscle hypertrophy; Chrysin.

Abstract:

The maintenance of skeletal muscle is directly related to healthier life condition. Aging promotes muscle mass loss and atrophy of muscle fibers, a condition known as sarcopenia, resulting in decreased muscle strength, mobility problems, and falls. Chrysin is a natural compound with anti-inflammatory and antioxidant properties, in addition to inhibitory effects on the aromatase enzyme, responsible for converting testosterone into estradiol. Thus, chrysin has potential beneficial effects for attenuating sarcopenia, since testosterone is known for its anabolic effects, inducing muscle fiber hypertrophy and increasing protein synthesis. Our objective was to evaluate the soleus muscle of old rats treated or not with chrysin. 10-month-old rats were divided into two groups: CTR (treated with saline solution, via gavage) and CHR (treated with 100 mg/kg of chrysin, via gavage) (n=5 per group; CEUA 064/20). After soleus muscle sampling, we evaluated relative muscle weight, morphology and morphometry of muscle fibers, collagen distribution, and carbohydrate content. The rats treated with chrysin showed a greater number of large fibers (increased area) and fewer small fibers (reduced area), indicating muscle hypertrophy compared to untreated animals. Additionally, chrysin did not affect collagen content, but it altered the metabolic profile of the fibers to a more glycolytic state. Our results show that chrysin induces greater hypertrophy of soleus muscle fibers, possibly attenuating, at the cellular level, the muscle mass loss characteristic of sarcopenia. Treatment with chrysin may serve as a potential treatment aiming to improve muscle function and a healthier life condition during aging.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Quantification Of HTLV-1 Proviral Load In Asymptomatic Carriers and Patients with Adult T-Cell Leukemia/Lymphoma: A Comparison Between Different Transmission Routes

Authors:

Giulia Sales GUIMARÃES¹
Davi Ferreira JOFRE¹
Juliana PEREIRA²
Luís Alberto de Pádua Covas LAGE²
Cadiele Oliana REICHERT²
Jessica Bilar CAVALCANTE²
Priscila de Lima BARROS²
Hebert Fabrício CULLER²

Affiliations:

¹ Faculdade de Medicina da Universidade de São Paulo (FMUSP)
² Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HC/FMUSP)

Keywords: Adult T-cell leukemia/lymphoma (ATLL). HTLV-1. Proviral load. Real-time PCR (qPCR)

Abstract:

Study approved by the Research Ethics Committee (CEP) of HCFMUSP under process number 60994322.4.0000.0068 and funded by FAPESP (process 2023/18474-0). The study of HTLV-1 infection and molecular mechanisms linked to lymphomagenesis in Adult T-cell Leukemia/Lymphoma (ATLL) is scarce. Breastfeeding-acquired HTLV-1 increases neoplastic transformation risk; however, sexual and parenteral transmission, which may also become malignant, are poorly studied. HTLV-1 diagnosis involves serological screening detecting specific antibodies, mainly via ELISA. A positive ELISA requires confirmation, often through real-time PCR (qPCR), which also quantifies and monitors proviral load. Lymphocytes from peripheral blood were separated using Ficoll® Paque Plus. The DNA extraction followed the QIAamp DNA Mini Kit (Qiagen, Germany) protocol, and proviral load quantification was performed via qPCR with TaqMan probes. Proviral load was quantified in 47 samples, including asymptomatic HTLV-1 carriers and ATLL patients. To compare proviral load between transmission routes and between asymptomatic carriers and ATLL patients, the Mann-Whitney test was applied. No significant difference was observed between transmission routes ($p = 0.4274$), but proviral load was significantly higher in ATLL patients than in asymptomatic carriers ($p = 0.0054$).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Anti-inflammatory and neuroprotective effects of TNF-alpha inactivation in spinal motor neurons of Duchenne muscular dystrophy (DMD) mice

Authors:

Anna Oller BONANI¹

Valquiria Aparecida MATHEUS²

Ana Laura Midori Rossi TOMIYAMA¹

Alexandre Leite Rodrigues de OLIVEIRA¹

Affiliations:

¹ Laboratory of Nerve Regeneration, Department of Structural and Functional Biology, Institute of Biology, UNICAMP / Brazil.

² Advanced Therapies Laboratory, Faculty of Pharmaceutical Sciences, UNICAMP / Brazil.

Keywords: Central Nervous System (CNS). Duchenne muscular dystrophy. MDX. Spinal cord. TNF- α .

Abstract:

Duchenne muscular dystrophy (DMD) is a severe neuromuscular disorder caused by the absence of functional dystrophin, leading to muscle degeneration, inflammation, and retrograde changes in the Central Nervous System. Inflammation in DMD increases tumor necrosis factor- α (TNF- α), which contributes to the degradation of dystrophic myofibers. We investigated the anti-inflammatory and neuroprotective effects of Etanercept (a chimeric protein blocking the TNF- α receptor) in the spinal cord microenvironment of MDX mice. Adult male MDX mice (n=5/group) and C57BL/10Unib control mice (n=5/group) were divided into 4 groups: G1 received vehicle, while the other groups received Etanercept at 3 (G2), 6 (G3), and 12 mg/kg (G4) every 72 hours (i.p.). Daily walking track tests (CatWalk system) were conducted. After two weeks of treatment, lumbar intumescence, tibialis anterior, and soleus muscles were collected for flow cytometry, immunofluorescence (Synaptophysin, GAD65, VGLUT1, IBA-1, GFAP), and H&E staining. Procedures were approved by the Institutional Animal Ethics Committee (CEUA/IB/ UNICAMP, protocol number 6340-1/2023). Results showed Etanercept at 12mg/kg reduced astrogliosis by 30% (Vehicle vs Etanercept-12mg; $p<0.0001$) and microglial activation by 50% (Vehicle vs Etanercept-12mg; $p<0.0001$) in MDX mice. Additionally, Etanercept increased synaptophysin expression (Vehicle MDX vs. 12mg MDX - 80% increase; $p<0.001$), while VGLUT-1 expression was downregulated in MDX (Vehicle BL10 vs. Vehicle MDX - 35% reduction; $p<0.01$) and increased with treatment (Vehicle BL10 vs. 12mg MDX - 25% reduction; $p<0.05$). TNF- α levels were reduced by 30% compared to MDX vehicle to Etanercept-treated MDX (12mg/kg; $p<0.05$). Similar results were seen for Th1 polarization (Vehicle MDX vs. 12mg MDX - 30% decrease; $p<0.05$). These effects were reflected in functional outcomes (Sciatic Functional Index improvement $p<0.05$), suggesting that Etanercept could be an effective clinical intervention for DMD.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Mimicking the circadian rhythm in astrocytes *in vitro* using melatonin

Authors:

Leslie Liandra Duarte da ROSA¹

Márcio Henrique Mello da LUZ¹

Fernanda Gaspar do AMARAL¹

Marimélia Aparecida PORCIONATTO¹

Kil Sun LEE¹

Affiliations:

¹Escola Paulista de Medicina - Universidade Federal de São Paulo

Keywords: Alzheimer's disease. Astrocytes. Clock genes. Melatonin. Rhythmicity.

Abstract:

Sleep deprivation is highly prevalent in the Brazilian population and can increase susceptibility to many health problems, including neurodegenerative disorders such as Alzheimer's disease. The sleep-wake cycle and circadian rhythm are synchronized across all cells in the body, with melatonin (MT) playing a key role in this process. However, different cell types may exhibit distinct responses to MT. Studying the circadian behavior of individual cell types in the brain is particularly challenging due to its structural and cellular complexity. Given the critical role of astrocytes in maintaining brain homeostasis and A β clearance, understanding how rhythmicity affects their function is crucial, and *in vitro* models can be helpful for this purpose. Therefore, we attempted to entrain astrocytes *in vitro* through intermittent MT treatment and clock gene expression analysis. iPSC-derived astrocytes were cultured and treated intermittently with 1 nM MT for three days, with each treatment lasting 12 hours. Then, one plate was lysed every four hours for RNA extraction (n=5 per timepoint). Control cultures had their media replaced at the same intervals without melatonin and were lysed at the same timepoints (n=5 per timepoint). The expression of *CLOCK*, *BMAL1*, *PER1*, *PER2*, *CRY1*, *CRY2* and β -*Actin* was evaluated by RT- qPCR. Statistical analyses were carried out using Jamovi v 2.3.28 software (p<0.05). MT treatment significantly induced oscillation in *BMAL1* expression (p=0.035) but not in the expression of other genes. MT appeared to reduce *CLOCK* and *CRY1* expression compared to untreated cells, particularly during the period corresponding to diurnal phase (p<0.001; p=0.015). Among the analyzed genes, *CLOCK*, *BMAL1* and *CRY1* showed the highest expression levels compared to *PER1*, *PER2* and *CRY2*. Curiously, genes with highest expression were most affected by MT treatment. Further analyses are ongoing to better understand the role of melatonin in simulating circadian rhythmicity in culture.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Molecular and Functional Characterization of the Role of the $\alpha 5\beta 1$ Integrin Receptor in Hepatic Dysfunction During Amino Acid Restriction

Authors:

Jakeline de Oliveira CARVALHO¹

Dr. Everardo Magalhães CARNEIRO²

Mariana Roberta Rodriguez MUNIZ²

Dr. Thiago dos Reis ARAÚJO²

Affiliations:

¹Ribeirão Preto Medical School - University of São Paulo (USP) ²Departament of Structural and Functional Biology, Endocrine Pancreas and Metabolism Laboratory – University of Campinas (UNICAMP)

Keywords: Integrin. MAFLD. Metabolism. Lipids. Undernutrition.

Abstract:

Undernutrition, characterized by energy and nutrient deficiency, increases the susceptibility to metabolic disorders like Metabolic Dysfunction-Associated Fatty Liver Disease (MAFLD), which is responsible for fat accumulation, inflammation, and hepatic impairment. Given its severity, this study investigates the $\alpha 5\beta 1$ integrin receptor pathway as a possible mediator of MAFLD, focusing on its role in glucose and lipid metabolism in HepG2 cells under amino acid restriction. Focal adhesion kinase (FAK), a downstream effector of this receptor, was included as a key molecular target due to its regulatory role in lipid metabolism. HepG2 cells were cultured in DMEM with 10% fetal bovine serum and incubated under control conditions (100% amino acids) or restricted (10% amino acids) for 48 hours. In the last 24h, cells received 150 μ M tauroursodeoxycholic acid (TUDCA) as an $\alpha 5\beta 1$ receptor agonist and 10 μ M ATN161 peptide as an antagonist or were left untreated. Lipid accumulation and cell viability were analyzed, and Western blot was used to analyze FAK phosphorylation and will be used to assess markers of insulin signaling. Data are presented as mean $\pm$ SEM and were analyzed using the Mann-Whitney U test (* $P < 0.05$ versus control). Preliminary results show effective standardization of $\alpha 5\beta 1$ receptor modulators. Treatment with 10 μ M ATN161 significantly reduced the phosphorylation of focal adhesion kinase (FAK) (50%), a downstream protein in the pathway, without compromising cell viability but increasing lipid accumulation, suggesting a possible effect on hepatic lipid metabolism (9%). Conversely, TUDCA progressively increased FAK phosphorylation (100%) and reduced hepatic lipid deposition even under restriction (70.94%). Our data indicate that reduced $\alpha 5\beta 1$ -FAK pathway activation leads to higher hepatic lipid deposition and reduced cell viability, findings similar to those observed in liver disease associated with protein undernutrition.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Muscle Degeneration in dy^{2J} Mice, Model for Congenital Muscular Dystrophy type 1A: A Defect in Final Differentiation Rather Than Satellite Cell Exhaustion

Authors:

Luiza Albuquerque COELHO¹
Antônio Fernando RIBEIRO JR¹
Letícia Nogueira FEITOSA¹
Felipe Tadeu Galante Rocha de VASCONCELOS¹
Mariz VAINZOF¹

Affiliations:

¹Universidade de São Paulo

Keywords: Dystrophies. Dy^{2J} . Regeneration. Skeletal muscle.

Abstract:

Humans and mice with a deficiency in the $\alpha 2$ subunit of laminin-2 develop merosin-deficient congenital muscular dystrophy (MDC1A), characterized by severe and progressive muscle weakness. Muscle deterioration include cycles of degeneration and regeneration, and this last process involves the activation of satellite cells (SCs), the muscle stem cells. When quiescent SCs express only *Pax7*, while myoblast precursor determination depends on *Myf5* and *MyoD*, and terminal muscle differentiation requires expression of *Myog*, *MyoD* and *Mrf4*. This study investigated muscle regeneration in gastrocnemius muscle of dy^{2J} mice, which carry a mutation in LAMA2 gene. Approved by the Animal Ethics Committee of IBUSP (CEUA no. 413/2023), the research included four age groups with at least six animals per group: 1) < two months; 2) 2-8 months; 3) 8-15 months; 4) > 15 months. We analyzed SCs proportion, including total and activated cells per muscle fiber (PAX7 and KI67 Immunofluorescence [IF]), gene expression of *Pax7*, *Myf5*, *Myod*, and *Myog* (qPCR, using adult wild-type [WT] mice as reference), and the proportion and diameter of newly regenerated fibers, expressing developmental myosin (dMyHC+ IF). No significant differences were found in the proportion of total SCs among age groups, however group 1 showed significantly more activated SCs than group 2 ($p=0.034$). Considering the expression of the myogenic factors, *Myog* was increased 5-10 fold in the affected groups ($p<0,001$) while *Pax7*, *Myf5* and *Myod* expression showed a discrete increase (up to 2x), that was not statistically significant compared to WT. The percentage of dMyHC+ fibers declined with age ($p<0.001$), and group 1 had significantly larger dMyHC+ fiber diameters than the groups 2 ($p<0.001$) and 3 ($p=0.005$). These findings suggest that the progression of the dystrophic process in dy^{2J} mice could be more related to a failure in the later stages of regeneration, worsening with age, rather than to SCs depletion. FAPESP- CEPID, CNPQ-INCT.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Impact of microbiota depletion in the T cells CD4⁺ profiles in lean type 2 diabetic rats (Goto-Kakizaki)

Authors:

Henrique de Souza FALCÃO¹,
Gabriela Mandú GIMENES¹,
Thaís Martins RODRIGUES³,
Tiago Bertola LOBATO¹,
Ana Carolina Gomes PEREIRA¹,
Maria Janaína Leite de ARAUJO¹,
Giovanna Batista LISBOA¹,
Annelize Leal PACHECO¹,
Alyssa Helena Caratanasov MORENO¹,
Tania Cristina PITHON-CURI¹,
Sandro Massao HIRABARA¹,
Marco Aurelio Ramirez VINOLO²,
Rui CURI^{1,4},
Renata GORJÃO¹

Affiliations:

1Cruzeiro do Sul University (São Paulo, SP)
2Laboratory of Immunoinflammation, Institute of Biology, University of Campinas (São Paulo, SP)
3Laboratory of Endocrine Physiology, Institute of Biomedical Sciences, University of São Paulo (São Paulo, SP) 4Butantan Institute (São Paulo, SP)

Keywords: Gut microbiota. T lymphocytes. Gastrointestinal tract. Inflammation.

Abstract:

Alterations in gut microbiota composition can trigger an inflammatory state characterized by a predominance of pro-inflammatory cells, contributing to the development of insulin resistance and type 2 diabetes mellitus (T2DM). The Goto-Kakizaki (GK) rat, a non-obese T2DM model, exhibits both gut microbiota dysbiosis and a pro-inflammatory CD4⁺ T helper (Th) cell profile. To investigate if the role of gut microbiota in T2DM is independent of obesity and Th cell profile, we depleted microbiota using an antibiotic (ABX) cocktail administered via drinking water for 30 days. Wistar (WT) and GK rats were divided into control (CT) and ABX-treated groups (ethics committee: Cruzeiro do Sul University, 001/2024). Th cells from mesenteric lymph nodes (MLN) and Peyer patches (PP) were collected. The Th cell profile was evaluated by flow cytometry and gene expression of lymphocytes profile was analyzed by real time-PCR. Cecum enlargement in both GK^{ABX} and WT^{ABX} groups confirmed the successful microbiota depletion. Glucose tolerance test results showed a reduced area under the curve in GK^{ABX} compared to GK^{CT} (P=0,0188), indicating improved glucose tolerance. However, serum endotoxin levels remained unchanged. Immunophenotyping of PP cells revealed an increased Th1 population in GK^{ABX} vs WT^{ABX} (P=0,0123), while Th17 and Treg (P=0,0319 and P=0,0064, respectively) cells were reduced in WT^{ABX} compared to WT^{CT}. No significant changes were detected in MLN Th cell populations. Gene expression analysis of IL35 showed upregulation in GK^{ABX} vs WT^{ABX} (P=0,0052), with no significant alterations in other genes. Our findings suggest that gut microbiota depletion influences T cell profiles in PP and IL-35 expression in MLN, potentially affecting systemic inflammation in T2DM. The improved glucose tolerance in GK^{ABX} rats indicates a microbiota-dependent metabolic effect. Further studies are needed to clarify the underlying mechanisms in this process and long-term impacts of microbiota depletion.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Assessing the Cytotoxicity and Safety Profile of Trastuzumab: H9c2 Cardiomyoblast Exhibit no Evidence of Cytotoxic Damage

Authors:

Guilherme Curi Aiub CASAGRANDE¹

Karoline dos Santos RODRIGUES²

Michael ANDRADES^{1 2}

Affiliations:

¹ Hospital de Clínicas de Porto Alegre - Porto Alegre, Brazil

² Programa de Pós-Graduação em Ciências da Saúde: Cardiologia e Ciências Cardiovasculares - Universidade Federal do Rio Grande do Sul - Porto Alegre, Brazil.

Keywords: Breast cancer. Deferoxamine. H9c2. N-acetylcysteine. Trastuzumab.

Abstract:

Breast cancer is the most common cancer in women worldwide, with HER2+ subtypes representing a particularly aggressive form, accounting for approximately 20% of new cases. Trastuzumab (TRZ), a humanized monoclonal antibody, has significantly improved survival outcomes in HER2+ patients; however, its use is associated with an increased risk of cardiotoxicity, typically resulting in reduced left ventricular ejection fraction and heart failure. This TRZ-induced cardiotoxicity limits treatment and may require early discontinuation, compromising patient outcomes. Here, we investigated the effects of TRZ on H9c2 cardiomyocytes and assessed the potential of N-acetylcysteine (NAC) and deferoxamine (DFX) to mitigate TRZ-induced damage. In this pilot study, H9c2 cells were treated with TRZ (0.5, 1.0, 1.5, 2, 4, 8, and 16 μ M), NAC (100, 1000, and 10,000 μ M), and DFX (10, 100, and 1000 μ M), individually and in combination. Cytotoxicity was assessed using MTT assays and flow cytometry. The results indicate that DFX and NAC alone did not induce significant cytotoxicity up to a concentration of 1 μ M, although high-dose NAC (10 μ M) resulted in a non-significant 56% reduction in cell viability, accompanied by reduced cell size without propidium iodide incorporation. Interestingly, TRZ did not induce cytotoxic damage, even at doses exceeding therapeutic concentrations, and at some doses, it increased cell viability. This observation contrasts with existing studies reporting TRZ-induced cardiac toxicity in H9c2 cells, suggesting that the effects of TRZ may depend on variables such as exposure time and experimental context. In conclusion, given the unexpected lack of cell viability observed with certain concentrations of TRZ, further studies are needed to elucidate these effects in H9c2 cells and to evaluate whether H9c2 cells are suitable models to observe TRZ-induced cardiotoxicity.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Inhibition of extracellular vesicles biogenesis as a novel action mechanism of Calix[6]arene

Authors:

Helon Guimarães CORDEIRO^{1*}
Jordana Maria Azevedo MARTINS¹
Karin Juliane Pelizzaro ROCHA-BRITO¹ Ângelo de FÁTIMA²
Carmen Veríssima FERREIRA-HALDER¹.

Affiliations:

¹ University of Campinas (UNICAMP), São Paulo, Brazil.

² Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.

* Main speaker who participated in the conference. email: heloncordeiro@ufs.j.edu.br

Keywords: Calix[6]arene. Pancreatic Cancer. Extracellular Vesicles.

Abstract:

Generally, patients with pancreatic cancer are diagnosed in the metastatic stage, which contributes to treatment failure. Extracellular vesicles (EVs) are double-membrane-bound structures released by cells that cooperate with metastasis. The main goal of this study was to validate a platform of small molecules screening that target EV biogenesis in pancreatic cancer cells, in which we combined microRNA profiling and EV biogenesis in response of a broad number of small molecules. PANC-

1 and MiaPaca cells were treated with different small molecule families for 24h, followed by the analysis of the global expression of miRNAs. For that, GeneChip® miRNA 3.0 Arrays were used followed by analysis in miRpath 3.0 from DIANA database. For EVs isolation, PANC-1 and MiaPaca cells were cultured in serum-free DMEM medium for 24h. PANC-1 cells showed higher secretion of EV (8.27×10^{11} particles/mL; size 147.7 ± 2.3 nm) compared to Mia Paca cells (4.83×10^{11} particles/mL; size 163.7 ± 3.5 nm). In addition, the presence of TSG101, CD81, CD63 proteins confirmed that the vesicles were exosomes. Surprisingly, PANC-1 showed higher expressing of proteins involved in exosomes biogenesis and secretion compared to Mia Paca cells. Importantly, when treated with Calix[6]arene (CLX6), the EVs biogenesis mediators expression dropped and the microRNA expression was affected. CLX6 increased the expression of miRNAs that usually are down-regulated in pancreatic cancer, such as, miR-198, let-7b/d/f. Further functional analysis of differentially expressed miRNAs indicated that they are involved in vesicles trafficking. Our findings showed that (CLX6) decreases the EVs production by PANC-1 cells, through diminishing the level of biogenesis mediators and increasing the expression of microRNAs involved in vesicles trafficking. These findings explain the strong antimetastatic action of CLX6, once the EVs are key players in the communication between the tumor cells and microenvironment.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: T3 increases bone marrow neutrophils *in vivo*, splenic IL-6 *in vitro* and *in silico* analysis reveals TRE in genes of neutrophil cytokines and plasma cell differentiation transcription factors

Authors:

Beatriz C.J. Miranda-da-Silva^{1*}
Flavia F. Bloise^{1,2*}
Huila Fonseca¹
Maria Belio³
Samuel Valença¹
Felipe Leite de Oliveira¹
Carmen Pazzos-Moura²
Valeria de Mello-Coelho¹

Affiliations:

Institutes of ¹Biomedical Sciences; ²Biophysics Carlos Chagas Filho; ³Microbiology Paulo Goes; Federal University of Rio de Janeiro/ UFRJ, Health Sciences Building, Av. Carlos Chagas Filho 373, Rio de Janeiro, RJ, 21941-902

Keywords: Hyperthyroidism, B Cell, Plasma Cell, Neutrophils, Cellular Differentiation

Abstract:

High circulating levels of T3 stimulate differentiation of plasma cells in mice. It is known that B lymphocyte development and differentiation can be regulated directly via T3 receptor and activation of key transcriptional regulatory genes; or via secretion of cytokines by neutrophils, including IL-6 and BAFF. However, the cellular and molecular mechanisms of T3 excess in this process are still unknown. Herein, we treated young male C57/BL6 mice with T3 (5µg/10g of body weight/day) or vehicle solution for 14 days (CEUA/CCS protocol# DAHEICB046 - A09/19-048-18). By flow cytometry, T3 excess increased total bone marrow cellularity and, in particular, mature neutrophils number (Ly6G+CD11b+cKit-). Treatment also trend to increase splenic neutrophil number (Ly6G+CD11b+) and significantly enhance plasmablasts (B220+CD138+cKit-) and plasma cell (CD138+B220-cKit-) numbers in the bone marrow and spleen. Nitroblue tetrazolium (NTB) assay revealed increased reactive oxygen production by splenic cells under T3 indicating activity of granulocytes. While splenic long-lived plasma cells (CD138+CXCR4+B220-) were increased, it was reduced in the bone marrow of T3-treated mice. Next, using *cell sorting* and *western blotting*, we verified that splenic TCR-B220+ B cells express TRb1. Then, using the NRH-SCAN platform, we identified thyroid hormone-responsive elements (TRE) in genes of transcription factors neutrophil cytokines inducers of plasma cell differentiation, including DR4 and ER6 in BLIMP-1, IL-4 and BAFF and IL-6 as well as TRE IR0 in IRF4. Using the WebLogo program for probability analysis, we confirmed that identified TRE nucleotide sequences were conserved. Finally, we verified a significant increase of IL-6 in splenic cell culture treated with T3(10-9M) by ELISA. In conclusion, our data support the hypothesis that, in hyperthyroidism, plasma cell differentiation might occur directly and indirectly, possibly through regulation of cytokines produced by neutrophils.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Melanopsin's participation in protection against cell death: Role in melanoma tumorigenesis

Authors:

Maria Eduarda Palma SAVINI¹

Eliz Maria de Oliveira FURTADO²

Renato BARBOZA¹

Maria Nathalia MORAES¹

Affiliations:

¹ Institute of Environmental, Chemical and Pharmaceutical Sciences, Department of Biological Sciences, Federal University of São Paulo – UNIFESP ² Institute of Biosciences, University of São Paulo - USP

Keywords: Melanoma, Apoptosis, Melanopsin.

Abstract:

Melanopsin (OPN4), a light-sensitive protein in melanocytes from skin cells, influences both photodetection and non-photoc processes such as proliferation and apoptosis in melanocytes. Given its oncogenic role in melanoma, this study investigates OPN4's function in protecting melanoma cells from oxidative stress-induced cell death. To do this, we used a culture of tumor melanocytes that had undergone CRISPR genome editing to express non-functional melanopsin. We performed a cell death assay by H₂O₂ treatment, and evaluated the apoptosis pathways by analyzing gene expression by RT-PCR and protein expression by Western Blot. Upon H₂O₂-induced oxidative stress, *Sod1* and *Sod2* expression significantly increased only in OPN4 KO cells, suggesting enhanced antioxidant response in the absence of melanopsin. In KO cells the pro-apoptotic gene *Bid* was upregulated compared to WT. In response to H₂O₂ the expression of *Bid* in both WT and KO was reduced. *Bax* increased only in treated WT cells, indicating differential apoptotic activation. The anti-apoptotic gene *Bcl2* was downregulated by H₂O₂ in both WT and KO cells, with no differences between lineages. *Pro-caspase-3* was significantly reduced in treated KO cells, suggesting greater caspase-3 activation and apoptosis in cells without melanopsin. *Mitf*, key for melanocyte function, showed lower expression in KO cells. In WT cells *Mitf* was repressed by H₂O₂, but not in KO, supporting melanopsin-dependent regulation. *p53* decreased in treated WT cells and increased in KO cells, suggesting that melanopsin negatively regulates *p53* under oxidative stress. In this way, OPN4 is related to protective pathways against cell death. Thus, understanding the mechanisms in which melanopsin contributes to increased resistance to cell death could pave the way for the development of drugs targeting OPN4-related death pathways, potentially enhancing treatments for melanoma.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Individuals with long-COVID from mild and moderate cases presents negative impact on morphological and functional characteristics of the Vastus lateralis muscle

Authors:

Rosângela Aline ANTÃO¹;
Emilly SIGOLI¹;
Giovana BERNARDES ¹;
Alessandra Jungers OKUYAMA ¹;
Dilson E. RASSIER ²;
Anabelle S. CORNACHIONE ¹

Affiliations:

¹ Department of Physiological Sciences, Federal University of Sao Carlos, Sao Paulo, Brazil.

² Simon Fraser University, Canada.

Keywords: Covid-19. Immune system. SARS-CoV-2. Single Cell. Skeletal muscle.

Abstract:

Skeletal muscle symptoms are common in long COVID, marked by fatigue and muscle weakness consequently impairing day to day activities and studies have expansively targeted these symptoms in grave cases, however, mild and moderate cases also present alterations regarding muscle fiber characteristics and function, with unknowns cause. We aimed to understand the impact of the COVID-19 disease in the morphological and functional alterations of the skeletal muscle from individuals with Long-COVID from mild and moderate cases. Participants (18–50 years) were divided into control and long COVID groups, both sedentary and without comorbidities (CAAE: 39913520.3.0000.5504; opinion number: 4.509.688). Six days post-biopsy, isokinetic tests assessed functional parameters of the right leg, including peak torque, time to peak torque, number of repetitions, total session work, and fatigue index. No significant differences were found ($p > 0.05$). Vastus lateralis samples were obtained via needle biopsy and divided for: histological and immunofluorescence techniques to assess morphological alterations, inflammatory agents, fiber types, and trophism; permeabilized single fibers that were dissected, and maximum active force (pCa^{2+} 4.5; sarcomere length 2.5 μm) was measured. Connective tissue was unchanged comparing both groups, but fibrotic regions were qualitatively more pronounced. Macrophages were reduced ($p < 0.01$), while T lymphocytes showed no differences. Morphological and trophism alterations were noted. FTI and FTIIA fibers exhibited reduced minimal ferret diameter ($p < 0.0001$), whereas FTIIAD fibers showed increased trophism ($p < 0.0001$), but fiber type distribution remained unchanged. Single fibers showed a reduced isometric force ($p < 0.05$). Our findings provide insights into skeletal muscle morphological and functional impairment in Long-COVID, which may contribute to its symptoms. These findings could help the understanding of the condition and developing effective treatments.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Investigation of Molecular Changes and the Role of the Tumor Microenvironment in the Progression and Metastasis of Childhood Neuroblastoma Using FISH Technique

Authors:

Gabriel Pereira RIBEIRO^{1,2};

Vitor Botafogo^{3, 4};

Cristiane Ferreira e Facio³;

Ines Motta⁴;

Cristiane Millito⁵;

Leonardo Bustamante⁶;

Fernando Werneck⁷;

Jose Maria Sayagués⁴;

Alberto Orfao⁴;

Elaine Sobral da Costa³;

Daiana Vieira Lopes^{1,2,3}

Affiliations:

1Medical Science Institute, Federal University of Rio de Janeiro (UFRJ), Macaé, RJ; 2Integrated Morphology Laboratory, Biodiversity and Sustainability Institute, Federal University of Rio de Janeiro (NUPEM/UFRJ), Macaé, RJ

3Institute of Pediatrics IPPMG, Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil

4Service of Cytometry, Department of Medicine and Cancer Research Center (IBMCC, University of Salamanca-CSIC and IBSAL), University of Salamanca, Salamanca, Spain 5Department of Pathology, Faculty of Medicine, UFRJ, Rio de Janeiro, Brazil 6Children's Hospital, Rio de Janeiro, Brazil

7Pediatric Oncology Clinic (COPE), Rio de Janeiro, Brazil.

Keywords: Clonal evolution. IFISH. Neuroblastoma. Tumor microenvironment.

Abstract:

Neuroblastoma (NB) represents 7-10% of pediatric cancer. Its mortality rate exceeds 90% in advanced stages. NB is highly heterogeneous, from aggressive forms to spontaneous regression. Genetic alterations such as MYCN amplification and ALK gain, drive tumor progression, but the mechanisms within the tumor microenvironment are poorly understood. This study analyzes genetic alterations in NB and the role of tumor microenvironment in progression and metastasis. Solid tumor samples of patients with NB were characterized by immunophenotyping, by flow cytometry, and fluorescence in situ hybridization (iFISH) to detect genetic alterations and profile tumor heterogeneity. The study was approved by the Institutional Review Board (CAAE 35130014.6.3003.5249/35130014.5264). Among the 35 tumor samples of NB analyzed by iFISH, only 20% amplification of MYCN and 2,8% of ALK were found. The analysis revealed MYCN and ALK gain (49% and 46%, respectively) and hyperdiploidy (11%) as the main genetic alterations. Also, tumors without MYCN amplification, but which presented joint gain of MYCN/ALK (37% 2p23_24), frequently associated with a high mortality rate, were found. Tumor metastasis samples (n=2) had an immunophenotyping and clonal profile similar to the primary tumor, but with different percentages of clones presenting specific genetic alterations, especially in the gain of 17q22 (about 20% more in the tumor metastasis samples). The study reveals that the sequence of genetic alterations in NB varies according to the degree of tumor cell differentiation, providing new insights into its clinical heterogeneity. Studying the tumor microenvironment through FISH and co-culture models will enhance the understanding of metastasis mechanisms, offering potential for better risk stratification and development of more effective therapies. To study the tumor microenvironment, co-culture models of NB cell lineage with bone marrow stroma are being used, together with three-dimensional in vitro models to investigate tumor interactions and metastasis mechanisms. **Funding support:** FAPERJ and Cancer Foundation



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Proteomic Landscape of Kidneys from Aged Rats Exposed to Maternal Protein Restriction: A DOHaD approach

Authors:

Marina Pereira PIRES¹,
Matheus Naia FIORETTO¹,
Luisa Annibal BARATA¹,
Isabelle Tenori RIBEIRO¹,
Renato MATTOS¹,
Flávia Alessandra MACIEL¹,
Luis Antonio JUSTULIN¹.

Affiliations:

¹ São Paulo State University "Júlio de Mesquita Filho" – UNESP, Department of Structural and Functional Biology, Morphology Sector

Keywords: Aging. DOHaD. Kidney. Maternal Malnutrition.

Abstract:

The Developmental Origins of Health and Disease (DOHaD) concept postulates that adversities on susceptibility windows of development can affect multiple systems with long-term consequences in the descendants. One model articulated in this context is Maternal Protein Restriction (MPR), which reduces the nephrons and glomerular filtration, increasing the risk of metabolic diseases in the offspring. Therefore, the aim was to evaluate the consequences of MPR on the renal morphophysiology of male rats at postnatal day (PND)540. Sprague Dawley rats were divided into two groups: Born from dams fed a normoprotein diet (CTR, 17% protein) or a hypoprotein diet (GLLP, 6% protein) during gestation and lactation. After weaning, the rats were fed a normoprotein diet until PND540, when the kidneys were collected for morphological (n=6), molecular (n=5), and proteomic analyses (n=5) (CEUA 5119280121). We used the Shapiro-Wilk normality test, followed by the Student's t-test or Mann-Whitney, with significance when $p < 0.05$. We observed fibrotic regions ($p = 0.0002$), inflammatory infiltrates, Vimentin ($p = 0.0465$), AMACR ($p = 0.0230$), and PECAM-1 (0.0239) immunostaining in the kidney of the GLLP group. In the proteomic landscape, we identified 26 differentially expressed proteins (6 with $p \leq 0.05$ and 20 with $p \geq 0.95$) in the GLLP group. The pathways enriched showed downregulation in muscle contraction and metabolism, and upregulation in stress response and immune system. There were no differences in the protein expression of TNF α , TGF β 1, and IL10, however, we showed a reduction in SOD2 ($p = 0.0036$) and matrix metalloproteinase 9 ($p = 0.0004$) in the GLLP group. We also observed a decrease in *Vegf-r* ($p = 0.0044$) gene expression, despite no difference in *Ang*, *Tubb*, and *Vegf* in the GLLP group. Therefore, MPR modulates the renal proteomic profile during aging, impacting structural, tissue remodeling, and antioxidant parameters, impairing renal function and systemic aspects, leading to premature aging.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: The consequences of maternal malnutrition on the metabolism and thyroid morphophysiology in male young and old offspring rats

Authors:

Ana Livia Silvério VIEIRA¹, Matheus
Naia FIORETTO¹,
Luisa Annibal BARATA¹,
Isabelle Tenori RIBEIRO¹,
Luis Antonio JUSTULIN¹.

Affiliations:

¹Structural and Functional Biology Department (Morphology), Institute of Biosciences -
UNESP Botucatu/SP, Brazil.

Keywords: Dohad. Maternal protein restriction. Thyroid.

Abstract:

Maternal malnutrition is a political and social problem that violates human rights and impacts not only the health of the mother but also the descendants. This correlation is supported by the concept of the Developmental Origins of Health and Disease (DOHaD). In this context, experimental studies demonstrate that Maternal Protein Restriction (MPR) can disrupt several organs and systems in the offspring during their lifespan, including thyroid and metabolic disorders. Here, we aim to investigate the consequences of MPR on thyroid morphophysiology in male offspring rats at postnatal day (PND)21 and during aging. The Sprague Dawley rats were divided into two groups: Born from dams that received a normoprotein diet (CTR, 17% protein) or hypoprotein diet (GLLP, 6% protein) during gestation and lactation. Part of the animals were euthanized at PND21, and another part at PND540. The thyroids and blood were collected at PND21 and PND540 for metabolic (n=8), histopathological (n=5), and molecular analyses (n=5). For statistics, we used Shapiro-Wilk for normality, and a posteriori Student T test or Mann-Whitney, considering $p < 0.05$ for significance (CEUA 5119280121). At PND21, we observed a decrease in the thyroid follicles ($p = 0.0120$), serum T3 ($p = 0.0002$), and T4 levels ($p = 0.0036$), alongside an increase in the genic expression of *Tsh-r* ($p = 0.0294$) and *Ar* ($p = 0.0173$) in the GLLP group, indicating a potential to early-life hypothyroidism. At PND540, we showed an increase in the serum T4 ($p = 0.0166$) and mast cells ($p = 0.0202$), and a decrease in the elastic fibers ($p < 0.001$) in the GLLP group. At PND540 we observed proliferative lesions (parafollicular cell adenoma, diffuse follicular cell hyperplasia, and focal parafollicular cell hyperplasia) in the GLLP rats. Therefore, our findings suggest that MPR impacts thyroid morphophysiology early in life, leading to long-term hormonal imbalances and proliferative lesions that may impair thyroid and systemic homeostasis during aging.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Complications of Maternal Protein Restriction on Hepatic Morphophysiology and Biochemical Activity in Old Male Rats: A DOHaD Approach

Authors:

Pedro Menchini VITALI¹,
Isabelle Tenori RIBEIRO¹,
Matheus Naia FIORETTO¹,
Luis Antonio JUSTULIN¹.

Affiliations:

¹ São Paulo State University "Júlio de Mesquita Filho" – UNESP, Department of Structural and Functional Biology, Morphology Sector.

Keywords: DOHaD. Maternal Malnutrition. Liver.

Abstract:

Epidemiological and experimental data have elucidated that exposure to stressors during pregnancy and lactation can permanently deregulate the physiological organ-specific profile of the offspring, increasing susceptibility to non-communicable diseases. This panorama is associated with the Developmental Origins of Health and Disease (DOHaD) concept. One adverse condition linked to this context is Maternal Protein Restriction (MPR), which leads to negative impacts on the liver metabolism, the central organ of metabolism and detoxification. We aim to evaluate the consequences of MPR on the liver of old male offspring rats. For this, Sprague Dawley rats were divided into two groups: CTR: rats born from mothers fed a normoprotein diet (17% protein), and GLLP: rats born from mothers fed a hypoprotein diet (6% protein) during gestation and lactation. After weaning, the rats were fed a normoprotein diet until postnatal day 540, when they were euthanized and the livers collected for morphological and molecular analysis (n=6/group - CEUA 5119280121). The Shapiro-Wilk test was used for normality, with subsequent statistical analysis using the Student's t-test (parametric) or Mann-Whitney test (non-parametric), considering significant differences when $p < 0.05$. We observed an increased incidence of inflammatory focus ($p < 0.0001$), tissue expression of GST-pi ($p = 0.0252$), and hepatic glycogen ($p = 0.248$) in the liver of the GLLP group, despite no differences in collagen deposition, mast cells, macrophages, and reticular fibers. In the protein expression, we observed a significant decrease in TNF α ($p = 0.0152$) in the GLLP group, with no differences in IL10 and TGF β . In the liver antioxidant defense, we found a reduction in superoxide dismutase ($p = 0.0159$), reduced glutathione ($p = 0.0161$), and catalase ($p = 0.0273$). Therefore, MPR affects the liver morphophysiology and the antioxidant response, predisposing to metabolic and liver disorders, which can negatively impact aging.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Maternal protein restriction affects molecular and metabolic parameters in the pituitary of post-weaning male and female rat offspring

Authors:

Gustavo Monezzi CORDEIRO¹,
Matheus Naia FIORETTTO¹,
Flávia Alessandra MACIEL¹,
Luisa Annibal BARATA¹,
Isabelle Tenori RIBEIRO¹,
Luis Antonio JUSTULIN¹.

Affiliations:

¹Structural and Functional Biology Department (Morphology), Institute of Biosciences - UNESP Botucatu/SP, Brazil.

Keywords: DOHaD. Maternal protein restriction. Pituitary.

Abstract:

Epidemiological and experimental studies highlight the determining role of critical developmental windows such as pregnancy and lactation for the health or disease of offspring. This panorama is based on the concept of Developmental Origins of Health and Disease (DOHaD), with one of the models used being Maternal Protein Restriction (MPR), which is extremely important because it mimics conditions of hunger and food insecurity. The MPR could impact several organs and systems in descendants throughout developmental biology, increasing the risk for metabolic and chronic diseases. Therefore, we aim to evaluate the sex-specific consequences of MPR on the metabolic and molecular pituitary parameters in young rats. Sprague Dawley rats (CEUA 5119280121) were divided into two groups: CTR: Rats born from mothers that received a normoprotein diet (17% protein) and GLLP: Rats born from mothers that received a hypoprotein diet (6% protein) during gestation and lactation. At postnatal day 21, the male and female rats were euthanized and the blood and pituitary glands were collected for metabolic (n=8), histological (n=5), molecular (n=5), and statistical analyses (normality, and a posteriori Student's t test or Mann-Whitney – considering $p < 0.05$ for significance). We observed that MPR did not affect the pituitary structure in both sexes. However, we showed a decrease in the insulin receptor ($p = 0.0490$) gene expression, despite no difference in the *Neurod1*, *Neurod4*, *Igf1*, *Ppara*, *Ppar γ* , *Prl*, and *Tshr* gene expression in the female GLLP rats. In male rats, we showed an increase in *Neurod1* ($p = 0.0173$) and a decrease in *Prl* ($p = 0.0020$) gene expression in the GLLP group, despite no differences for *Neurod4*, *Igf1*, *Ppara*, *Ppar γ* , *Ir* and *Tshr* gene expression. In the metabolic results, there was a decrease in serum T3 ($p = 0.0002$) and T4 ($p = 0.0036$) in the male GLLP rats, however no difference in corticosterone. In the female rats, we did not find differences in serum T3, T4, and corticosterone. In conclusion, MPR affects the molecular and metabolic functions of the pituitary glands, with more notable systemic effects in males, mainly on the axis regulated by the hypothalamus and pituitary gland on the thyroid and basal metabolism. This may create a window of susceptibility to long-term pituitary and metabolic disorders.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Sex-specific Effects of Maternal Low-Protein Diet on Hypothalamus and Metabolism in the Offspring during Early Life

Authors:

Carolina Beatriz Pinheiro BASSO¹,

Matheus Naia FIORETTO¹,

Flávia Alessandra MACIEL¹,

Isabelle Tenori RIBEIRO¹,

Luis Antonio JUSTULIN¹.

Affiliations:

¹Structural and Functional Biology Department (Morphology), Institute of Biosciences - UNESP Botucatu/SP, Brazil.

Keywords: DOHaD. Hypothalamus. Metabolism

Abstract:

The Developmental Origins of Health and Disease (DOHaD) establish that adversities during critical periods can cause permanent effects on organs and systems in the descendants. One of these circumstances is maternal malnutrition, and the literature demonstrated that Maternal Protein Restriction (MPR) negatively influences the organ-specific homeostasis in the offspring, increasing the risk of chronic and metabolic diseases. The aim was to evaluate the sex-specific consequences of MPR on the hypothalamus and metabolism of rats in early life. Sprague Dawley offspring rats (CEUA 5119280121) were divided into two groups: CTR (17% protein diet) or GLLP (6% protein diet), receiving the diets throughout gestation and lactation. At postnatal day 21, the male and female offspring were euthanized and the blood and hypothalamus were collected for metabolic and molecular analyses. For statistical analyses, the normality test was used, followed by the Student's t- test or Mann-Whitney test, with $p < 0.05$ for significance. We observed hypothalamus morphological alterations for collagen and reticulin in the GLLP group for both sexes. We showed that aquaporin 1, AR, and BDNF protein expression did not differ in the males and females, although there was an increased Era ($p = 0.0317$) in the females of the GLLP group. There were no differences in the matrix metalloproteinases 2 and 9 in either sex. We observed a decrease in the circulating levels of IGF1 ($p = 0.0007$) and T3 ($p = 0.0003$) in the males, in addition to an increase in estrogen ($p = 0.0061$) and testosterone ($p = 0.0148$) in the GLLP group. However, we did not observe significant differences in corticosterone. In the females, there were no differences for T3, T4, and corticosterone. Our results demonstrate that MPR partially affects the hypothalamic structure of male and female rats early in life, leading to negative metabolic consequences in males, which may increase the risk of metabolic diseases throughout life.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: mTORC1 and mTORC2 mediate the increase in mass induced by constitutive activation of PI3K signaling in brown and white adipocytes

Authors:

Ana Carolina Pereira Baptista¹
Thayna dos Santos Vieira¹
Natália Corte¹
Marina Honda¹
Nathália Monteiro¹
Loreana Silveira¹
Victor Xavier¹
Vicência Monteiro¹
Erick Silveira¹
Julia Franca¹
William Festuccia¹

Affiliations:

¹Institute of Biomedical Sciences (ICB), University of São Paulo (USP), São Paulo, São Paulo (SP)

Keywords:

Adipose tissue. Mtorc1. Mtorc2

Abstract:

The PI3K-mTORC2-Akt-mTORC1 pathway regulates lipid metabolism in white and brown adipocytes (WAT and BAT, respectively). To advance our understanding of the individual role of mTORC2-Akt and mTORC1 in the regulation of this metabolism, genetically modified mice were studied under the following conditions: constitutive activation of PI3K signaling due to Pten deletion in adipocytes [(PtenKO); (n=11)] associated with mTORC1 or mTORC2 deficiency due to adipocyte Raptor or Rictor deletion, respectively [(PtRapKO (n=6) and PtRicKO (n=7), respectively)] and littermate floxed controls [(WT); (n=15)] (CEUA, ICB/USP 6160250820). The data were analyzed using GraphPad Prism version 9 for normality (Shapiro -Wilk test) and homogeneity of variances (Bartlett's test), followed by ANOVA. A p-value <0.05 was used as the significance level. Despite similar weight gain (p=0.9254) and food intake (p=0.5338), the group with Pten deletion in adipocytes showed increased BAT mass, an effect abolished by Rictor or Raptor deletion [(WT: 40.57±1.429; PtenKO: 60.96± 2.528; PtRicKO:39.49± 7.193; PtRapKO:17.52± 1.172); (p<0.0001)]. A similar response pattern was observed for inguinal [(WT:192.4± 8.821; PtenKO:451.5± 13.64; PtRicKO: 274.2± 12.02; PtRapKO:152.3±11.18);(p<0.0001)], and retroperitoneal WATs [(WT: 40.77± 2.805; PtenKO: 76.41± 4.721; PtRicKO:51.13± 2.371; PtRapKO:24.18± 3.078); (p<0.0001)]. The exception was epididymal WAT, where increased mass was observed in mice with Pten deletion, an effect blocked by Raptor deletion but not by Rictor deletion [(WT: 279.4± 11.23; PtenKO:343.3± 13.28; PtRicKO: 356.3± 17.71; PtRapKO: 132.9± 19.28); (p<0.0001)]. mTORC1 and 2 appear to be important mediators in the regulation of lipid metabolism in white and brown adipocytes.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Maternal Protein Restriction promotes Cardiac Disorders by Disrupting Heart Developmental Morphophysiology in Young Male Offspring Rats

Authors:

Matheus Naia FIORETTO¹,
Lucas Sobrinho LEMOS¹,
Isabelle Tenori RIBEIRO¹,
Luísa Annibal BARATA¹,
Flávia Alessandra MACIEL¹,
Renato MATTOS¹,
Luiz Marcos Frediani PORTELA¹,
Sérgio Alexandre Alcantara DOS SANTOS^{1,3},
Clélia Akiko Hiruma LIMA¹,
José Ricardo de Arruda MIRANDA²,
Elena ZAMBRANO^{4,5},
Luis Antonio JUSTULIN¹

Affiliations:

¹Department of Structural and Functional Biology, Institute of Biosciences, Sao Paulo State University, Botucatu, SP, Brazil.

²Department of Biophysics and Pharmacology, Institute of Biosciences, Sao Paulo State University, Botucatu, SP, Brazil.

³Cancer Signaling and Epigenetics Program, Fox Chase Cancer Center, Philadelphia, PA, USA

⁴Reproductive Biology Department, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán

⁵Faculty of Chemistry, National Autonomous University of Mexico.

Keywords: DOHaD. Heart. Maternal Malnutrition.

Abstract:

Maternal malnutrition is a serious problem that affects thousands of people, violating human rights. Several epidemiological and experimental studies associate adverse intrauterine conditions with the susceptibility of cardiovascular diseases in the descendants, highlighting the theory of Developmental Origins of Health and Disease (DOHaD). One of its associated models is Maternal Protein Restriction (MPR), a condition that mimics food insecurity and malnutrition, bringing adverse organ-specific consequences such as hypertension and risk for cardiorespiratory diseases. We evaluated the consequences of MPR on the cardiac morphophysiology of male offspring in early life. Sprague Dawley rats were divided into 2 groups: Born from dams fed a normoprotein diet (CTR, 17% protein) or a hypoprotein diet (GLLP, 6% protein) during gestation and lactation (CEUA 5119280121). At postnatal day 21, the offspring were euthanized. The normality test was used, followed by the Student's t-test or Mann-Whitney, with $p < 0.05$ for significance. There was a decrease in serum levels of IGF1 ($p = 0.0007$), an increase in testosterone ($p = 0.015$), and a decrease in the size of cardiomyocytes ($p < 0.0001$) and their nuclei ($p = 0.004$), collagen ($p = 0.004$), reticular ($p = 0.045$) and elastic fibers ($p = 0.002$). MPR led to electrical disorders with decreased cardiac frequency ($p < 0.0001$) and increased R-R ($p < 0.0001$) QRS, and P-R ($p < 0.0001$) waves ($p < 0.0001$). There was an increase in vascular (Aquaporin1 and PECAM-1 $p = 0.0147$) and structural proteins (Fibronectin $p = 0.011$), in addition to unbalance in cellular control proteins (increased BCL2 $p = 0.019$ and decreased BAX $p = 0.034$). We showed disorders in the antioxidant system, with decreased Peroxiredoxin4 ($p = 0.028$), and increased GSTp ($p = 0.002$) and SOD activity ($p = 0.021$). These adverse effects early in life increase the risk of pathophysiological remodeling of the heart, with the potential for hypertension, hypertrophy, and cardiovascular disorders later in life.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Sex-Specific Impacts of Maternal Protein Restriction on Cardiac Structure and Antioxidant Balance of Young Rats

Authors:

Luísa Correia GOMES¹,
Matheus Naia FIORETTTO¹,
Lucas Sobrinho LEMOS¹,
Flávia Alessandra MACIEL¹,
Isabelle Tenori RIBEIRO¹,
Luisa Annibal BARATA¹,
Luis Antonio JUSTULIN¹.

Affiliations:

¹Structural and Functional Biology Department (Morphology), Institute of Biosciences
- UNESP Botucatu/SP, Brazil.

Keywords: Cardiovascular system. DOHaD. Maternal Malnutrition.

Abstract:

Epidemiological and experimental studies have demonstrated a strong association between maternal malnutrition and an increased risk of disease development in offspring, including cardiovascular disorders. These conditions are associated with the Developmental Origins of Health and Disease (DOHaD), a theory that establishes the intrauterine and postnatal environment as a determinant of offspring developmental biology. One of the studied models is Maternal Protein Restriction (MPR), a condition that leads to hypertension and the risk of cardiorespiratory disorders in the offspring. Therefore, we aim to evaluate the effects of MPR on the cardiovascular morphophysiology of male and female rats at postnatal day (PND) 21. For this purpose, Sprague Dawley rats (CEUA 5119280121) were divided into two groups: Rats born from dams fed a normoproteic diet (CTR, 17% protein) or a hypoproteic diet (GLLP, 6% protein) during gestation and lactation. At PND 21, the rats were euthanized and the hearts were collected for morphological (n=6) and molecular analyses (n= 6). For statistical analyses, the normality test was used, followed by the Student's t-test or Mann-Whitney test, with $p < 0.05$ for significance. The results showed a decrease in collagen deposition ($p = 0.0041$), as well as in the length and width of cardiomyocytes ($p < 0.0001$) and nuclei ($p = 0.0041$) in GLLP males, but no differences in GLLP females. We observed a decrease in Peroxiredoxin 4 ($p = 0.0280$) and an increase in GSTpi ($p = 0.002$) and PECAM-1 ($p = 0.0055$) protein expression in the hearts of males. In females, we found a decrease in Peroxiredoxin 3 (0.0411), although no differences for GSTpi, and PECAM-1 protein expression. Our results indicate that MPR impacts the cardiac structure of male rats early in life and leads to an imbalance in vascular and antioxidant parameters in the hearts mainly in males, establishing sex-specific consequences of MPR on early-life cardiac biology.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: The impacts of maternal protein restriction on maternal ovarian phenotypic and molecular aspects and those of young rat offspring: Possible transgenerational conditions

Authors:

Ana Luiza Silva de ABREU¹,
Matheus Naia FIORETTO¹,
Lucas Sobrinho LEMOS¹,
Luísa Annibal BARATA¹,
Flávia Alessandra MACIEL¹,
Isabelle Tenori RIBEIRO¹,
Luis Antonio JUSTULIN¹.

Affiliations:

¹Structural and Functional Biology Department (Morphology), Institute of Biosciences
- UNESP Botucatu/SP, Brazil.

Keywords: Dohad. Developmental biology. Maternal malnutrition. Ovaries.

Abstract:

The Developmental Origins of Health and Disease (DOHaD) stipulates that the gestational and/or lactational environment can impact several organs and systems in the offspring throughout developmental biology. One model used to investigate such conditions is Maternal Protein Restriction (MPR), which leads to increased maternal glucocorticoids, delayed development in early life, impacts on folliculogenesis, fertility problems in both male and female offspring, and risk of metabolic diseases. Considering the ovaries as essential for metabolism and reproductive aspects, we investigated the consequences of MPR on the ovary of dams and offspring in early life. Sprague Dawley rats (CEUA 55119280121) were divided into 2 groups: Dams fed a normoprotein diet (CTR, 17% protein) or a low-protein diet (GLLP, 6% protein) during gestation and lactation. The dams and the offspring were euthanized at the end of lactation and the ovaries were collected. For the statistics, we used the normality test, with subsequent analysis by the Student's t-test or Mann-Whitney test, considering significance when $p < 0.05$. In the dams, we observed a decrease in chow (17.74 ± 4.06) and water (48.46 ± 4.68) intake, in addition to an increase in *Aromatase* genic expression ($p = 0.0134$), although no differences for *Star* and *Hsd3bl*. In the offspring, there was a decrease in *Pi3K* ($p = 0.0408$) and *AKT* ($p = 0.0159$) genic expression, despite no differences in *MAPK*. In conclusion, MPR appears to impact maternal water intake and satiety behavior during lactation, in addition to potentially impacting ovarian function and aromatization. In contrast, MPR leads to immediate consequences on the offspring, with a decrease in molecules important for growth, differentiation, and insulin response, which may lead to greater susceptibility to damage to the ovarian follicular reserve, with long-term and even transgenerational consequences.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Preservation of Heart Mitochondrial Bioenergetics During Aging in NAD(P)⁺ Transhydrogenase (NNT) Deficient Mice

Authors:

Ana P. DALLA COSTA¹ -
Edilene S. SIQUEIRA-SANTOS¹
Luiz A. C. PASSOS²
Andréia R. SALGADO²
Daniele M. R. DEMOLIN²
Annelise FRANCISCO³
Roger F. CASTILHO¹

Affiliations:

- 1- Laboratory of Bioenergetics and Cellular Metabolism, FCM, State University of Campinas (UNICAMP), Campinas, SP, Brazil
- 2- Multidisciplinary Center for Biological Research in the Area of Laboratory Animal Science (CEMIB), State University of Campinas (UNICAMP), SP, Brazil
- 3- Department of Experimental Medical Science, Lund University, Lund, Sweden

Keywords: Aging, Heart, Mitochondria, NADPH, Transhydrogenase

Abstract:

Introduction: NAD(P)⁺ transhydrogenase (NNT) is highly expressed in heart mitochondria and serves as a primary source of NADPH, which is essential for antioxidant defense systems and biosynthetic pathways. However, the role of NNT in maintaining heart mitochondrial function during aging remains unclear.

Objectives: The aim of this study was to evaluate mitochondrial bioenergetics and Ca²⁺ retention capacity in isolated organelles from wild-type (*Nnt*^{+/+}) and NNT-deficient (*Nnt*^{-/-}) mice.

Methods: Mitochondria were isolated from the hearts of 5-, 12-, and 22-month-old *Nnt*^{+/+} and *Nnt*^{-/-} mice (CEUA/UNICAMP 6068-1/2022). These mice have a C57BL/6JUnib genetic background. Bioenergetic function was assessed using high-resolution respirometry (Oroboros Oxygraph-2k), with substrates targeting individual respiratory complexes. Ca²⁺ retention capacity was measured by monitoring extramitochondrial Ca²⁺ concentrations using the fluorescent probe Calcium Green 5N.

Results: At 12 months of age, there was an increase in yield of isolated mitochondria from *Nnt*^{-/-} hearts. No significant differences in respiratory states were observed between *Nnt*^{+/+} and *Nnt*^{-/-} mitochondria at any age. Ca²⁺ retention capacity was similar between *Nnt*^{+/+} and *Nnt*^{-/-} mitochondria, with a slightly non-significant trend for greater capacity in *Nnt*^{-/-} mitochondria.

Conclusion: NNT deficiency does not impair the bioenergetic function and Ca²⁺ retention capacity of mouse heart mitochondria. This may be due to mitochondrial adaptation to NNT loss, which could include the use of alternative NADPH sources and increased mitochondrial biogenesis and turnover.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Maternal malnutrition and adverse maternal-fetal consequences: Placental impacts as a starting point for health or disease in offspring

Authors:

Luisa Annibal BARATA¹,
Matheus Naia FIORETTO¹,
Flávia Alessandra MACIEL¹,
Natalia MAGOSSO¹,
Luis Antonio JUSTULIN¹.

Affiliations:

¹Structural and Functional Biology Department (Morphology), Institute of Biosciences
- UNESP Botucatu/SP, Brazil.

Keywords: DOHaD. Maternal Protein Restriction. Placenta.

Abstract:

Maternal nutrition during pregnancy plays a crucial role in fetal development and the long-term health of the offspring, a scenario associated with the Developmental Origins of Health and Disease (DOHaD) theory. One of its models is Maternal Protein Restriction (MPR), a condition that can impact placental homeostasis, compromising nutrient transport and affecting fetal growth, which has the potential to metabolically program the fetus, increasing the risk of chronic diseases. Therefore, the aim was to evaluate the consequences of MPR on the morphological profile of the placenta. Sprague Dawley rats (CEUA No. 7376171224) were divided into two groups based on diet: CTR (17% protein) and GLP (6% protein), receiving the diets throughout gestation. At gestational day 20, the rats were euthanized and the placentas were collected for histological (n=5) and molecular (n=5) analyses. For statistical analysis, the normality test was used, followed by the Student's t-test or Mann-Whitney test, with significance when $p < 0.05$. The results showed that MPR did not alter maternal reproductive aspects. In the offspring parameters, there was a significant decrease in anogenital distance in the GLP group (m: $p = 0.004$; f: $p = 0.0014$). We also showed a significant decrease in placental weight ($p = 0.0211$) and an increase in placental efficiency ($p = 0.0036$), in addition to a reduction in the labyrinthine zone ($p = 0.0279$), in the total placental area ($p < 0.0001$) and the percentage of glycoprotein area ($p = 0.0002$) in the GLP group. Molecular analyses revealed no significant differences in the gene expression of *Vegf a*, *Pgf*, *Hif-1*, *Pecam-1*, *Igf-1*, and *Ppar γ* , nor the protein expression of Aquaporin-1, PI3K, HIF-1 and Pecam-1. Therefore, MPR affects placental structure. However, it can be suggested that adaptive mechanisms occur in response to these changes to support and maintain fetal growth even under suboptimal intrauterine conditions.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Maternal Protein Restriction alters the lung morphology, molecular pathways, and proteomic landscape of male young offspring rats

Authors:

Flávia Alessandra MACIEL¹, Matheus
Naia FIORETTO¹,
Luísa Annibal BARATA¹,
Isabelle Tenori RIBEIRO¹,
Wellerson Rodrigo SCARANO¹,
Luis Antonio JUSTULIN¹

Affiliations:

¹Structural and Functional Biology Department (Morphology), Institute of Biosciences - UNESP Botucatu/SP, Brazil.

Keywords: DOHaD. Maternal Malnutrition. Lung Development. Omics Sciences.

Abstract:

The Developmental Origins of Health and Disease (DOHaD) concept elucidates that adverse conditions during gestation and lactation can impact offspring development. One model associated with this condition is Maternal Protein Restriction (MPR), which increases the risk of metabolic and cardiorespiratory diseases throughout the offspring's life. The aim was to investigate the effects of MPR on the morphological and molecular aspects of the lungs of post-weaning male rats. The animals were divided into two groups: CTR (17% protein) and GLLP (6% protein) which received the diets during gestation and lactation (CEUA N^o. 5119280121). At postnatal day 21, the rats were euthanized and the lungs were collected for morphological, molecular, and proteomic (LC- MS/MS) analyses. For statistics (n=5/group), the Shapiro-Wilk test for normality was used, followed by the Student's t-test or Mann-Whitney test, considering $p < 0.05$ statistically significant. We observed a decrease in alveolar diameter ($p = 0.0001$) and an increase in elastic fibers ($p = 0.0001$) and CD68 macrophages ($p = 0.0052$) in the GLLP group, with no differences in collagen, reticular fibers, and mast cells. A total of 244 differentially expressed proteins (116 up/128 down) were identified, with positive regulation of the stress response, muscle contraction, and carbohydrate metabolism ($p \geq 0.95$) and negative regulation of the cell cycle, cell junctions and protein metabolism ($p \leq 0.05$). The GLLP group showed an increase in the expression of AKT123 ($p = 0.0298$), mTOR ($p = 0.0492$), CD8 ($p = 0.0015$), and catalase activity ($p = 0.0259$). In matrix metalloproteins (MMP), we showed that MMP-2 isoforms (pro, inter, and active) were increased in the GLLP group ($p < 0.0001$). Therefore, MPR alters lung growth pathways, modulating protein expression, antioxidant action, and MMP activity, as a possible compensatory effect in development, which creates a window of susceptibility to respiratory diseases during lifespan.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Mitoquinone Prevents Vascular Dysfunction by Modulating Mitochondrial-NADPH Oxidase Crosstalk Post-Myocardial Infarction

Authors:

Marlon Ramos R. MACHADO¹, Carmen CASTARDELI¹,
Carolina Falcão XIMENES¹,
Pietra Zava LORENCINI¹,
Katyana Kaline Silva FERREIRA¹,
Ingridy Grafit R. SCHEREIDER¹,
Eduardo Hertel RIBEIRO¹,
Sara Bianca TAUFNER¹,
Aurelia Araujo FERNANDES²,
Silva GUATIMOSIM³,
Ivanita STEFANON¹

Affiliations:

1 Federal University of Espirito Santo, Department of Physiological Sciences, Health Sciences Center, Vitória, ES, Brazil.

2 Federal University of Espirito Santo, Department of Morphology Sciences, Health Sciences Center, Vitória, ES, Brazil.

3 Federal University of Minas Gerais, Department of Physiology and Biophysics, Belo Horizonte, MG, Brazil.

Keywords: Mitochondrial Antioxidant. Myocardial Infarction. Oxidative Stress. Vascular Reactivity.

Abstract:

Myocardial infarction (MI) is a critical condition triggering inflammatory responses and oxidative stress, leading to vascular complications and endothelial dysfunction. Our research explores the role of mitochondrial-NADPH oxidase (NOX) crosstalk in aortic vascular reactivity 7 days post-MI. Male Wistar rats underwent surgical induction of MI and were categorized into Myocardial Infarction (MI), Myocardial Infarction Mitoquinone (MIM), Sham (S), and Sham Mitoquinone (SM) groups. Animals received Mitoquinone (100 μ M), a specific mitochondrial antioxidant, in drinking water for seven days (CEUA-UFES 17/2021). Vascular reactivity was evaluated in isolated aortic rings under phenylephrine stimulation. Results showed no change in infarct area with Mitoquinone treatment (MI=46.6 $\pm$ 2.8; MI MitoQ=43.6 $\pm$ 2.6%), but a prevention of weight loss in the MI group (MI=-6 $\pm$ 2.7*; MI MitoQ=16 $\pm$ 4 g*, p<0.01). Reactivity to phenylephrine increased in the MI (Rmax S:100.1 $\pm$ 5.5 vs MI:127.3 $\pm$ 8.7*% KCl 75 mM, p<0.05). Mitoquinone treatment prevented the increase in vascular reactivity in the MI (Rmax MI:127.3 $\pm$ 8.7 vs MIM:100 $\pm$ 8.5*% KCl 75 mM p<0.05). L-NAME 100 μ M had no effect on Rmax in the MI, suggesting a decrease in NO bioavailability. However, when the MI group was treated with MitoQ, superfusion with L-NAME resulted in an increased response to phenylephrine, suggesting higher NO bioavailability. Mitoquinone treatment reduced mitochondrial (Mitosox) and cytosolic ROS (DHE) and increased vascular NO production (DAF). The specific NOX inhibitor, ML-171 5 μ M, reduced vascular reactivity and decreased NOX1 expression in the MI group. In conclusion, the mitochondrial antioxidant mitoquinone prevented vascular dysfunction, oxidative stress dependent on mitochondrial -NADPH Oxidase crosstalk 7 days after MI.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Role of KLK-14 as a Substrate in Matriptase-Dependent Cancer Progression

Authors:

Camila Aparecida Coelho BRAZÃO¹
Katiuchia Uzzun SALES¹

Affiliations:

¹ University of Sao Paulo

Keywords: Head and Neck Carcinoma; Kallikrein 14; Matriptase; PAR2.

Abstract:

Head and neck squamous cell carcinoma (HNSCC) is an aggressive malignancy with high mortality rates, primarily due to the lack of specific biomarkers and targeted therapies. Serine proteases, a class of enzymes predominantly localized at the plasma membrane or in the extracellular milieu, orchestrate proteolytic cascades that regulate key oncogenic signaling pathways. Among these, matriptase, a type II transmembrane serine protease, activates substrates such as pro-hepatocyte growth factor (pro-HGF) and protease-activated receptor-2 (PAR-2), thereby promoting tumor cell invasion and proliferation. Notably, kallikrein 14 (KLK14), another serine protease, functions as a downstream substrate of matriptase *in vitro* and plays a critical role in epidermal differentiation and inflammatory responses by modulating the Hippo and NF-κB signaling pathways. This study investigates the hypothesis that KLK14 functions as an *in vivo* substrate for matriptase and examines its role in the PAR2-NF-κB and YAP1/TAZ-TEAD signaling pathways using transgenic mouse models subjected to a DMBA-induced carcinogenesis assay. Specifically, mice overexpressing matriptase in the basal epithelial layer, either with or without KLK14 expression, will be evaluated for phenotypic alterations and tumor progression. Furthermore, KLK14-knockout (KO) HNSCC cell lines will be treated with recombinant matriptase to determine whether matriptase-dependent activation of the PAR2-NF-κB and YAP1/TAZ-TEAD pathways is mediated through KLK14. These effects will be assessed using western blotting, indirect ELISA, and immunofluorescence. Our findings demonstrate that the phenotypic abnormalities observed in K5-matriptase transgenic mice are significantly rescued following the genetic ablation of KLK14, suggesting a critical role for KLK14 in mediating matriptase-driven effects. Moreover, to investigate the molecular mechanisms underlying this rescue, we employed CRISPR/Cas9 gene editing to generate KLK14-knockout (KO) human HNSCC cell lines. Specifically, RNA guides were cloned into LentiCrisprV2-GFP vectors and used for the transduction of viral particles into Cal27 and SCC25 cell lines. Analysis of these KLK14-deficient cells revealed a marked reduction in the production of key cytokines, including IL-8, indicating a potential link between KLK14 activity and NF-κB-dependent inflammatory signaling pathways in HNSCC. In conclusion, our study identifies KLK14 as a critical substrate in the matriptase-driven proteolytic cascade in HNSCC, highlighting its role in modulating the tumor microenvironment and inflammatory responses. Given their extracellular localization, kallikreins, including KLK14, represent promising therapeutic targets for modulating tumor progression. Our findings provide valuable insights into the molecular mechanisms underlying HNSCC and may contribute to the development of novel biomarkers and targeted therapeutic strategies to improve patient prognosis.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Proteomic Analysis of Skeletal Muscle Reveals Alterations in Ubiquitinated Sarcomeric Proteins in Parkin Knockout Mice

Authors:

Michelle Barbosa ROSÁRIO¹

Ana Laura Vieira DA SILVA¹

Isabela Aparecida DIVINO¹

Igor Luchini BAPTISTA¹

Affiliations:

¹ Laboratory of Cell and Tissue Biology (LaBCeT), School of Applied Sciences, University of Campinas, Limeira, Brazil.

Keywords: E3 ligase, Parkin, Proteomic. Mass Spectrometry

Abstract:

Parkin is an important E3 ubiquitin ligase involved in mitophagy and energy regulation, contributing to several tissues physiology. Proteomic analyses aimed to identify possible new targets of ubiquitination by Parkin, based on ubiquitinated protein expression. Thus, this study aims to identify ubiquitinated protein by Parkin in skeletal muscle, based on proteomic data derived from mass spectrometry. For this, tibial muscle samples (n=2) from wild-type (WT) C57BL/6J mice and Parkin^{m1Shn} (Parkin ^{-/-}) mice (CEUA #6444-1/2024), were extracted using the TUBEs technique (ubiquitinated protein enrichment). The extracts were stored at -80°C and sent for mass spectrometry analysis using an LC-MS/MS system. Mass spectrometry analyses revealed significant differences in the ubiquitinated proteome between WT and Parkin^{-/-}. Out of a total of 43 proteins identified, 18 proteins (41.86%) were present in both groups. 32 (71.41%) showed a reduction in Parkin^{-/-}, with notable decreases in ubiquitin, which was reduced approximately 45 times compared to WT, and ATPases ATP2A1 and MT-ATP8, which showed reductions of 60% and 70%, respectively. Among the 32 proteins that were reduced in Parkin^{-/-}, 14 were not identified (100% reduction), including the following: heavy chain myosins (MYH2, MYH8, MYH4), light chain myosin (MYL1), and tropomyosin (TPM2), all of which are essential for sarcomere organization, as well as muscle contraction proteins such as actin (ACTA2), and the transcriptional repressor MXD3, which plays a role in cell cycle modulation. Thus, to date, our results suggest that the absence of Parkin alters the ubiquitination pattern in skeletal muscle tissue, significantly affecting the ubiquitination of sarcomeric proteins, intracellular pathways crucial for energy metabolism, and transcriptional repressors. Therefore, we propose that Parkin functions as a relevant E3 ligase for the maintenance of skeletal muscle physiology.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Evaluation of the Anti-Glioma Activity of Monepantel

Authors:

Nicole SARTORI¹
Valéria Bresolin da ROSA²
Ana Carolina dos SANTOS³
Rudimar MARTINS JUNIOR⁴
Rodrigo BAINY LEAL⁵

Affiliations:

¹ Universidade Federal de Santa Catarina **Keywords:**

Autophagy. Glioblastoma. Monepantel. **Abstract:**

Glioblastoma is an aggressive malignant primary brain tumor treated with surgical resection, radiotherapy, and chemotherapy with temozolomide. However, the efficacy of these treatments remains limited, highlighting the need for novel therapeutic strategies. Autophagy is essential for cellular homeostasis and may contribute to tumor survival. However, when excessively induced, it can trigger cell death, making it a promising therapeutic target. Monepantel, an anthelmintic developed by Novartis®, has demonstrated antitumor activity in ovarian cancer through autophagy induction. This study aims to characterize its antiglioma activity in vitro by assessing cell death, autophagy, migration, and the associated signaling pathways (MAPKs, Akt/mTORC1). Rodent (C6) glioma cell lines were treated with Monepantel (1-50 μ M) for 6-48h. The following parameters were analyzed: cell viability (MTT assay), autophagy (LC3-II levels by western blot and acridine orange staining microscopy), cell migration (Scratch assay), and cell signaling proteins (western blot). The results showed that treatment with Monepantel reduced cell viability by 20-25% after 24 h (N=8; 25 μ M $p < 0.01$; 50 μ M $p < 0.0001$) and 20% after 48 h (N=6; 50 μ M $p < 0.01$) as compared to control cells. Moreover, cell migration, evaluated after 24 h (N=3; 5 μ M $p < 0,1$; 12,5 μ M $p < 0,001$; 25 μ M $p < 0,0001$; 50 μ M $p < 0.0001$) and 48 h (N=3; 25 μ M $p < 0.0001$; 50 μ M $p < 0.0001$) was also impaired by Monepantel. Noteworthy, Monepantel (50 μ M; 24 h) induced autophagy, as evidenced by increased LC3-II levels (N=4; $p < 0.05$) and the formation of acidic vesicular organelles. Analysis of the AKT/mTORC1/p70, ERK1/2, and p38^{MAPK} pathways revealed a significant reduction in ERK2 phosphorylation (N=3; 50 μ M $p < 0.05$). These findings indicate that Monepantel exhibits antiglioma activity, supporting further investigation to identify the molecular targets involved in the modulation of autophagy and cell death in rodent and human glioma cell models.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Gene Editing of miR-146b as a Tool to Investigate Papillary Thyroid Carcinoma Oncogenesis

Authors:

Geovanna DE PAULA¹
Vitor de Sousa AMORIM¹
Kelly Cristina SAITO¹
Cesar Seigi FUZIWARA¹
Edna Teruko KIMURA¹

Affiliations:

¹Department of Cell and Developmental Biology, Institute of Biomedical Sciences, University of São Paulo, São Paulo-SP, Brazil.

Keywords: CRISPR/Cas9. Exosome. Gene editing. MicroRNAs. Thyroid Cancer.

Abstract:

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression post-transcriptionally and are often dysregulated in cancer due to genomic alterations. miR-146b is the most upregulated miRNA in thyroid cancer (TC), strongly associated with BRAF mutations and aggressive tumor phenotypes. However, the lack of a solid *MIR146B* knockout model limits a deeper understanding of its role in TC. Therefore, we aim to develop a CRISPR/Cas9-induced *MIR146B* edition model to disrupt miR-146b expression in papillary thyroid carcinoma (PTC) cells, and investigate its intracellular and extracellular effects, particularly in exosome-associated miRNA secretion. To inhibit miR-146b expression in the PTC cell line, we edited the *MIR146B* gene using the CRISPR/Cas9 system. B-CPAP cells, a PTC-derived cell line, were transfected with the PX459 plasmid (Addgene), carrying four distinct sgRNAs targeting *MIR146B*, designed with the ChopChop tool. After transfection, cells underwent puromycin selection (1 µg/mL) to generate stable populations, which were screened for intracellular miR-146b expression by qPCR. Single-cell-derived clones were established for further analysis. Moreover, to assess miR-146b secretion in the culture medium, total RNA was extracted using a modified Trizol-based protocol with glycogen as a carrier and cel-miR-39 as a spike-in control. Four cell populations were generated from sgRNAs A, B, 7, and 8, showing reduced miR-146b expression. Clones from guides A and B exhibited over 90% miR-146b suppression, with changes in cell morphology suggesting a more epithelial-like phenotype. miR-146b was detected in the culture supernatant, indicating a correlation with intracellular levels and its potential as a biomarker. Conclusion: CRISPR/ Cas9 effectively silences miR-146b expression in PTC cells, providing a valuable tool to investigate its intracellular and extracellular dysregulation in TC.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: JAK/STAT pathway and its role in periodontal repair

Authors:

Andréa Cardinali Romanelli ROSA¹
Angelo Constantini CAMILLI¹
Mariely Araujo de GODOI¹
Vitória Bonan COSTA¹
Morgana Rodrigues Guimarães STABILI¹

Affiliations:

¹ São Paulo State University "Julio de Mesquita Filho" School of Dentistry at Araraquara. São Paulo, Brazil.

Keywords: Host modulatory therapy. JAK inhibitors. Periodontitis

Abstract:

The Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway plays a central role in modulating multiple immune axes involved in the immunopathogenesis of several inflammatory disease. The aim of this study is to investigate the role of JAK in tissue repair in an in vivo periodontitis model through the systemic administration of the JAK1-3 and JAK-3 inhibitors. Periodontitis was induced by placing ligatures around the first mandibular molars of 60 rats. The ligatures were removed after 10 days, and oral administration of the two inhibitors at a concentration of 6.2 mg/kg was initiated via gavage. The inhibitors were administered daily for 5 and 15 days after ligature removal. Following euthanasia, the mandibles were assessed using micro-computed tomography (μ CT) to evaluate bone loss and stereometric analysis to assess inflammatory infiltrate. Gingival tissue was analyzed for evaluating the expression of biological mediators through RT-PCR (RUNX2, collagen, and MMP-13) and ELISA (lipoxin A4). Data were subjected to the ANOVA homoscedasticity test with Tukey's post-hoc test, establishing a significance level of $p < 0.05$. JAK1-3 inhibition stimulated bone formation during the early repair phase. Both inhibitors reduced the amount of cellular infiltrate in gingival tissue, decreased MMP-13 gene expression, increased RUNX2 expression, and enhanced Lipoxin A4 levels. These findings suggest that the inhibition of the JAK-STAT pathway may have therapeutic potential in periodontal tissue repair. Ethics Committee number: 27/2020.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Study of Molecular Variation Between SARS-CoV-2 Genomes Isolated and Sequenced in Residents of Macaé-RJ and Region

Authors:

Amanda Cristina Veiga Fernandes da SILVA¹,

Artur Nunes PAES¹,

Carina Azevedo Oliveira SILVA¹,

Graziele Fonseca de SOUSA¹,

Viktória Aparecida Gomes Silva COELHO¹,

Raquel de Souza GESTINARI¹,

Cintia Monteiro de BARROS¹,

Flávia Borges MURY¹

Affiliations:

¹Instituto de Biodiversidade e Sustentabilidade (NUPEM/UFRJ)

Keywords: COVID-19. Genetic variation. Genomic surveillance. SARS-CoV-2.

Abstract:

The coronavirus is a single -stranded, positively coated, enveloped RNA genome virus of the family Coronaviridae. The emergence of new variants of SARS-CoV-2 that carry mutations in the spike protein, a protein responsible for infection and interaction with receptors (ACE2) in human cells, may affect its transmissibility. Therefore, the project aims to study the circulating variants in samples from volunteers infected with the SARS-CoV-2 virus, correlating them with clinical conditions and the production of antibodies and cytokines, in the city of Macaé -RJ and region (approved by CEP/UFRJ- Macaé; CAAE: 57373422.8.0000.5699). The experimental groups were: control (vaccinated without infection) and infected. Nasopharyngeal swab samples were stored in Dulbecco's modified Eagle's medium and used for viral RNA extraction and nucleic acid detection by reverse transcription followed by real-time polymerase chain reaction for viral RNA detection. This is followed by RNase H dependent PCR (rhAmp PCR). If the rhAmp is unsuccessful, the viral genome is analysed by third -generation sequencing using the shotgun metagenomics approach. Blood samples are taken at the time of confirmation of infection. After centrifugation of the blood, plasma is collected for detection of anti-S IgM, IgG and IgA antibodies, neutralising antibody testing and cytokine dosage. In preliminary studies involving 1001 patients, SARS-CoV-2 RNA was extracted from the samples. Using the rhAmp technique and/or sequencing, the variants were identified in 289 samples. The gamma, delta and omicron variants were observed in the identified samples. The infected group showed higher IgM production (0.2833 ± 0.09768) compared to the control group (0.1035 ± 0.03625). IgG levels were lower in the infected group (1.391 ± 0.1563) compared to the control group (1.770 ± 0.1393). These analyses make it possible to monitor the predominant variants in the community and to associate them with the most relevant clinical cases.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Gestational and Lactational Exposure to a Mixture of Phthalates and/or Nanoplastics and Sex-Specific Hepatic Repercussions in Offspring

Authors:

Gabriel Aquino de Souza MAIA¹,
Patrick Vieira de SOUZA¹,
Natália MAGOSSO¹,
Mirella Franco MOREIRA¹,
Vanessa Aguiar ROCHA¹,
Matheus Naia FIORETTO¹,
Viktória Cristina PINHA¹,
Wellerson Rodrigo SCARANO¹

Affiliations:

¹ São Paulo State University (UNESP), Botucatu-SP – Department of structural and functional biology – Institute of Biosciences

Keywords: DOHaD. Liver. Nanoplastics. Phthalates.

Abstract:

Plastic production and improper disposal result in the release of micro/nanoplastics and plasticizers (phthalates) into the environment. These contaminants are widespread and have been identified in several biological tissues, suggesting their ability to cross biological barriers. The liver, due to its central role in metabolism of xenobiotics, is susceptible to the action of these substances, which can trigger functional, structural and epigenetic changes. Based on the DOHaD theory, which postulates that early exposures can modulate structural and functional aspects throughout life, we aimed to evaluate the impacts of maternal exposure to an environmentally relevant phthalate mixture (PM) and/or nanoplastics (NPs) on the liver of both male and female offspring. Pregnant SD rats (CEUA-1174020523) were divided into 4 groups (n=5-8/group): Ctrl (corn oil); T1: 20 µg/kg/day of the PM (21% DEHP, 35% DEP, 15% DBP, 8% DiBP, 5% BBzP, 15% DiNP); T2: 1 mg/kg/day of polystyrene NPs (100 nm); T3: combination of the PM (20 µg/kg/day) with NPs (1 mg/kg/day). Exposure took place from the 10th gestational day (GD) to the postnatal day (PND) 21, orally. The male and female pups were euthanized at PND22 for histological (liver) and biochemistry (plasma ALT and AST) analysis. The data was statistically analyzed by ANOVA/ Kruskal-Wallis tests (mean ± SD, with p<0.05). In the exposed males, there was no differences in the liver structure, despite a decrease in the TGO plasma levels) in T2 vs. Ctrl (p=0.0195). In the exposed females, it was observed a significant increase in glycogen deposition (P.A.S. positive) in T1 when compared to the Ctrl (p=0.0297), suggesting a possible imbalance in hepatic glucose metabolism and stress or liver damage. These results highlighted to the sex-specific alterations that occur in the liver of females and the metabolism of males early in life, and it is important to extend these findings to older ages to understand the effect of long-term exposure.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Sperm Analysis of Adult Mice Exposed to Tebuconazole in the Prenatal Phase

Authors:

Raysa Sá Teles FERNANDES¹
Catarina Conrado de BRITTO¹
Amanda Seraphim OLIVEIRA¹
Leisiane Pereira MARQUES¹
Danilo Roman CAMPOS¹
Samara Urban de OLIVA¹

Affiliations:

¹ Federal University of São Paulo (EPM/ UNIFESP)

Keywords: Mouse. reproductive toxicity. sperm. tebuconazole

Abstract:

Introduction: Tebuconazole (TEB) is a fungicide widely used in agriculture and can accumulate in the environment and food. Triazoles interfere with cytochrome P450 activity, which can lead to health risks, including for humans. TEB can cause pre- and postnatal development disorders, reproductive and endocrine dysfunctions. Considering that environmental insults during critical periods of development can cause long-term effects, this study aimed to investigate the late effects of TEB on spermatogenesis and sperm quality of adult mice, derived from the offspring of females treated during pregnancy and lactation. Materials and methods: Adult Swiss Webster mice were divided into 2 groups: treated group (TEB), consisting of male offspring of females treated with TEB (10 mg/kg/day) throughout gestation; and control group (C), which received the vehicle (corn oil), following the same experimental protocol. At 90pnd, quantitative and qualitative sperm analyses were performed. The project was approved by the Ethics Committee for Animal Use (CEUA) n.: 3642070622. Results: In the TEB group, there was a reduction in progressive motility (TEB: 47.6%±3.2; C: 83.3%±5) associated with an increase in non- progressive, immobile spermatozoa with abnormal morphology (TEB: 18.1%±5.9; C: 4.2% ±2,5). There was also a decrease in the number of spermatids in stage 19 of spermiogenesis (TEB: 15.3x10⁶±2.1; C: 16.7x10⁶±1.8) and in daily sperm production (TEB: 3.2x10⁶±0.4; C: 3.4x10⁶±0.3). Conclusion: The results suggest that exposure to TEB can alter sperm qualitatively and quantitatively, possibly affecting fertility.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Role of Annexin A1 in Gut Biology and NLRP3 Inflammasome Activation in a Murine Model of Type I Diabetes

Authors:

Lara Dariolli ROSSI¹
Luiz Philipe de Souza FERREIRA¹
Kátia de VASCONCELOS¹
Rafael André da SILVA² Cristiane
Damas GIL^{1,2}

Affiliations:

¹Departamento de Morfologia e Genética, Escola Paulista de Medicina, Universidade Federal de São Paulo (UNIFESP), SP.

²Programa de Pós-Graduação em Biociências, IBILCE-UNESP, São José do Rio Preto, SP.

Keywords: ANXA1. Inflammation. Intestinal epithelium. Streptozotocin.

Abstract:

Type 1 Diabetes Mellitus (DM1) involves a complex interaction between the intestinal microbiota, immune system, and intestinal permeability. Annexin A1 (AnxA1), identified in inflamed intestinal tissues, regulates inflammatory responses, zonula occludens 1 (ZO1), claudin-1, and NLRP3 (nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3) inflammasome activation, which is crucial for immune modulation and epithelial barrier integrity. This study examines the impact of AnxA1 deficiency on NLRP3 activation and intestinal morphology in a DM1 model. Male C57BL/6 AnxA1^{+/+} and AnxA1^{-/-} mice underwent streptozotocin-induced diabetes (65 mg/kg for five days) and were monitored for 12 weeks. Body weight and blood glucose levels were monitored (CEUA 2125200323). After 90 days, intestinal samples were collected for histological and immunohistochemistry. Western blotting was employed to analyze the levels of AnxA1 and components of the NLRP3 inflammasome in intestinal homogenates. Additionally, cytokines and chemokines levels in intestinal homogenates were evaluated by multiplex assay. Diabetic WT and AnxA1^{-/-} mice exhibited weight loss, hyperglycemia, and reduced intestinal ZO1 and EGF (epidermal growth factor) levels. AnxA1 deficiency led to fewer goblet cells, increased epithelial height, and lower cadherin expression compared to controls and/or WT diabetics. In DM1, the absence of AnxA1 attenuated the intestinal inflammatory response, as seen by decreased mMCP6, CD68, CD4 expression, and reduced inflammatory cytokine production. However, it exacerbated NLRP3 inflammasome activation, increasing its components (ASC, caspase-1 and NLRP3) and IL-1 β levels. Our findings suggest that AnxA1 is crucial for regulating inflammation and preserving intestinal epithelial integrity in DM1 by modulating cytokine production. Beyond classical inflammation, AnxA1 may also influence inflammasome regulation, epithelial integrity, and cellular stress in diabetes.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: mTORC1 and 2 Determine the Brown and White Adipose Tissue Mass in Mice Exposed to Cold

Authors:

Marina Akemi Abe HONDA¹
Érique de CASTRO²
Thayna dos Santos VIEIRA³ Natália
Monteiro PESSOA⁴
Érika Vicência Monteiro PESSOA⁵
Natália Pontara CORTE⁶
Camila Nogueira Alves BEZERRA⁷ Julia
Vitor FRANÇA⁸
William T. FESTUCCIA⁹

Affiliations:

¹ Universidade de São Paulo (USP)
² Instituto de Ciências Biomédicas da USP

Keywords: Cold, Brown Adipose Tissue, mTORC1, mTORC2, adrenergic signaling

Abstract:

Futile metabolic cycles composed of antagonistic processes such as fatty acids de novo synthesis and β -oxidation are activated simultaneously with brown adipose tissue UCP-1 and thermogenesis. Thus, it is our goal in this project to investigate the signaling pathways regulating fatty acids de novo synthesis and β -oxidation in brown adipose tissue (BAT) with a special focus on the role of mTORC2 and 1 as mediators of adrenergic signaling. For this, we investigated the impact of unilateral BAT sympathetic denervation, as well as adipocyte Rictor (mTORC2) and Raptor (mTORC1) deficiencies on body weight, food intake, energy expenditure, mass, DNA and triacylglycerol contents, intracellular signaling, de novo lipogenesis, β -oxidation, mitochondrial mass and function in the BAT of mice kept at thermoneutrality (30°C) or exposed to either acute (10°C, 6 and 24 hours) or chronic (10°C, 14 days) cold (CEUA Protocols: 6160250820 and 6071181120). Male C57BL/6J mice with unilateral BAT sympathetic denervation exposed to cold for 14 days show increased weight of the innervated side of BAT when compared to innervated BAT of mice from other groups ($n = 4-5$) (Cold 14 days $33,96 \pm 2,99$ vs Cold 24h $21,58 \pm 2,29$ vs Cold 6h $20,62 \pm 2,28$). Mice with adipocyte Rictor deletion exposed to cold show reduced weight of BAT when compared to mice with the same genotype maintained at thermoneutrality ($n = 11$) (Thermo A-Rictor KO $75,95 \pm 9,28$ vs Cold A-Rictor KO $43,51 \pm 2,50$). Male C57BL/6J mice with Raptor deletion in adipocytes exposed to cold showed reduced weight of brown (Cold A-Raptor WT $59,67 \pm 2,55$ vs Cold A-Raptor KO $21,10 \pm 1,17$), inguinal (Cold A-Raptor WT $159,5 \pm 11,38$ vs Cold A-Raptor KO $107,2 \pm 5,83$) and epididymal (Cold A-Raptor WT $182,6 \pm 18,36$ vs Cold A-Raptor KO $104,7 \pm 9,78$) adipose tissue when compared to controls exposed to cold ($n = 6-10$). In conclusion, mTORC2, mTORC1 and the time exposed to cold are important determinants of brown and white adipose tissue mass.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Annexin A1 Deficiency Attenuates Renal Injury in a Streptozotocin-Induced Model of Diabetic Nephropathy

Authors:

Larissa SUZUKI¹

Diego Dias dos SANTOS¹

Laura Santana de CHIARA¹

Rafael André da SILVA²

Luiz Philipe de Souza FERREIRA¹

Caroline Silvério FÁRIA³

Kátia de VASCONCELOS¹

Cristiane Damas GIL¹

Affiliations:

1: Division of Histology and Structural Biology, Department of Morphology and Genetics, Escola Paulista de Medicina – EPM, Universidade Federal de São Paulo - UNIFESP, SP, Brazil.

2: Biosciences Graduate Program, Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista - UNESP, São José do Rio Preto, Brazil.

3: Medical Research Laboratory (LIM03), Hospital das Clínicas (HCFMUSP), Faculdade de Medicina, Universidade de São Paulo - USP, SP, Brazil.

Keywords: Fpr. Immunohistochemistry. Kidney. Oxidative stress. Urea.

Abstract:

Diabetic nephropathy (DN) is a major complication of diabetes mellitus (DM), in which oxidative stress plays a pivotal role. Annexin A1 (AnxA1) is a 37 kDa anti-inflammatory protein involved in inflammation resolution and apoptosis, but its role in renal oxidative stress remains unclear. This study evaluated the impact of AnxA1 deficiency on renal morphology, function, and oxidative stress in a streptozotocin (STZ)-induced model of type 1 diabetes. Wild-type (WT) and AnxA1 knockout (AnxA1^{-/-}) male C57BL/6 mice were treated with STZ for five consecutive days and analyzed 90 days post-induction. Both diabetic groups developed sustained hyperglycemia; however, AnxA1^{-/-} diabetic mice exhibited lower blood glucose levels and milder weight loss compared to WT diabetics. Histologically, the kidneys of both diabetic groups showed morphological alterations, including nuclear vacuolization in tubular epithelial cells and tubular degeneration. Notably, WT diabetic mice displayed a significant increase in glomerular diameter and elevated plasma urea and creatinine levels when compared to control WT and diabetic AnxA1^{-/-} mice, suggesting worse renal impairment in the presence of AnxA1. Immunohistochemistry revealed enhanced expression of Fpr1 and Fpr2 receptors in diabetic AnxA1^{-/-} kidneys. Additionally, antioxidant analysis showed that renal superoxide dismutase levels were significantly higher in WT diabetic mice than in their AnxA1^{-/-} counterparts. In summary, the absence of AnxA1 attenuated key structural and functional features of DN, indicating a context-dependent role for this protein in diabetic kidney injury. These findings open new perspectives on AnxA1 as a potential modulator in DN pathogenesis. (CEUA: 8518230821).

Funding: FAPESP (2024/07185-0).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Application of Bioinformatics: Analysis of Gene Expression and Identification of Functional Enrichment in Breast Cancer

Authors:

Lailton Oliveira da Silva¹
Carolina Panzarin¹
Marciane Milanski Ferreira²
Leticia Ignacio De Souza Zimmermann²
Adriana Sousa Torsoni²
Marcio Alberto Torsoni²

Affiliations:

¹Graduate Students, Nutrition, Sport, and Metabolism Graduate Program (CNEM), FCA/ UNICAMP.
²Faculty Members, School of Applied Sciences – FCA/UNICAMP.

Keywords: $\alpha 7$ nAChR. Breast neoplasms. Genes. Bioinformatics.

Abstract:

The incidence of breast neoplasms is rising at an alarming rate, accompanied by high mortality rates. Understanding the biological aspects and cellular mechanisms involved is crucial for developing new therapeutic alternatives. This study aimed to analyze gene expression and oncogenic biological processes (via Functional Enrichment - FE) involving the target genes: SIRT1, CHRNA7, SRC, PPARG, BAX, BCL2, VEGFA, mTOR, HIF-1 α , LIN28A, LIN28B, RAF1, CD36, and SP1. Gene expression (RNA-seq) data from the TCGA TARGET GTEx and GDC TCGA Breast Cancer (BRCA) databases, available through Xena Browser, were analyzed by comparing Normal Breast Tissue (NBT) vs. Primary Tumor Tissue (PTT). The Enrichr platform was used to correlate the genes of interest with Biological Processes (BPs), with selection based on a p-value < 0.05, filtering through Revigo, and final manual selection. In the TCGA TARGET GTEx dataset, using Welch's t-test, the genes SRC, BAX, BCL2, mTOR, LIN28A, LIN28B, and SP1 showed upregulation in PTT vs. NBT, while CHRNA7, PPARG, VEGF, RAF1, and CD36 were downregulated. In the BRCA dataset, the upregulated genes were BAX, SRC, and VEGFA, while the downregulated ones were mTOR, SIRT1, CD36, SP1, CHRNA7, BCL2, and PPARG. The HIF-1 α gene showed no significant changes in expression. Following FE analysis, the genes with the strongest associations with BPs in 2023 and 2021 included SRC, mTOR, PPARG, CHRNA7, BAX, CD36, LIN28A, LIN28B, and SP1, linked to processes such as regulation of cell death, apoptotic process, positive regulation of angiogenesis, among others (56 BPs in total). In conclusion, various genes are involved in BPs that regulate pathways associated with the tumor microenvironment.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Evaluation of oxidative metabolism and mitochondrial markers in the placenta of pregnant Walker 256 tumor-bearing rats, treated with the chemotherapeutics Doxorubicin and Cyclophosphamide

Authors:

Beatriz Cassaniga TALASSI¹
Beatrice Candido ALVES¹
Bianca de Fátima Pereira de SOUSA¹
Carla de Moraes SALGADO¹
Leisa LOPES-AGUIAR¹
Gabriela Fontana de MESQUITA¹
Rogério Willians dos SANTOS¹
Laís Rosa VIANA¹
Maria Cristina Cintra GOMES-MARCONDES¹

Affiliations:

¹Laboratory of Nutrition and Cancer, Department of Structural and Functional Biology, Biology Institute, University of Campinas, Campinas, SP, Brazil

Keywords: Cancer. Chemotherapy. Fetal growth. Placenta. Pregnancy.

Abstract:

Cancer is one of the leading causes of morbidity and mortality in Brazil and worldwide, and the incidence of cancer during pregnancy has been increasing. This condition can result in worse outcomes for the mother and baby due to the tumor and/or chemotherapy treatment. These outcomes are strongly influenced by the placenta, which responds to the adverse intrauterine environment and regulates fetal metabolic programming. This study evaluated molecular changes in pathways related to oxidative metabolism, mitochondrial content, and function in the placenta of Walker 256 tumor-bearing rats under chemotherapy. The study, approved by the Ethics Committee on the Use of Animals (6268-1/2023), involved four groups of pregnant rats: control (treated or not with chemotherapy) and tumor-bearing dams (treated or not with chemotherapy). The females received Doxorubicin (0.1 mg/Kg) and Cyclophosphamide (1 mg/Kg) on the 14th and 17th days of pregnancy. Morphometric, RT-qPCR, and immunohistochemical analyses were performed. The statistical analysis was conducted using two-way ANOVA, followed by Tukey's post-hoc test, with a *P*-value <0.05. The results showed that the tumor- chemotherapy combination reduced weight gain by 42.8% compared to control. Placental and fetal weights were reduced in all groups compared to control, indicating that both the tumor and chemotherapy negatively influence placental-fetal growth. Although there were no differences in gene expression of *citrate synthase*, *HIF-1α*, and *COX5A*, immunohistochemistry analyses revealed higher expression of citrate synthase in the placental labyrinth of the tumor and tumor with chemotherapy groups (*P*=0.007), as well as higher expression of HIF-1α in the tumor-treated group (*P*=0.026). These findings suggest that Walker 256 tumor and chemotherapy negatively affect placental-fetal development, with metabolic adaptations to hypoxia and/or cellular stress in the labyrinth zone to minimize harmful effects to maintain its function.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: T-Cell Receptor (TCR) Rearrangement Study in HTLV-1 Carriers and ATLL Patients: Comparative Assessment Between Different Viral Transmission Routes

Authors:

Davi Ferreira JOFRE¹ Giulia
Sales GUIMARÃES¹ Hebert
Fabrício CULLER² Jessica Bilar
CAVALCANTI¹ Juliana
PEREIRA^{1,2}
Karine Sobral MARQUES¹
Luis Alberto de Pádua Covas LAGE^{1,2} Priscila
de Lima BARROS¹
Youko NUKUI²

Affiliations:

Faculdade de Medicina da Universidade de São Paulo¹
Hospital das Clínicas da Faculdade de Medicina da USP²

Keywords: ATLL. HTLV-1. T-cell receptor. T-cell subpopulations.

Abstract:

The study of the gamma T-cell receptor rearrangement (TCR γ) is a molecular assay of major importance in the diagnosis of many lymphomas and leukemias. It is known that the infection of the retrovirus Human T Lymphotropic Virus type 1 (HTLV-1) may cause diseases such as Adult T Cell Leukemia/Lymphoma (ATLL), HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP), immune sensitivity, uveitis and arthritis. HTLV-1 has tropism mainly for CD4⁺ T cells with potential viral reservoir in a rare population of long-lived lymphocytes known as T Memory Stem Cell CD4⁺ (Tscm-CD4⁺). Therefore, we have been investigating the profile of T cell clonality in the gamma rearrangement of HTLV-1 and ATLL patients, and correlating a possible clonal evolution outcome in different subpopulations of lymphocytes. In accordance with the Research Ethics Committee of the Hospital das Clínicas da Faculdade de Medicina da USP (approval number 60994322.4.0000.0068), peripheral blood was collected from 20 healthy individuals (HI), 41 HTLV-1 carriers (HC), and 4 ATLL patients (AP). DNAs were extracted. Multiplex PCR assays for TCR γ were performed.

Subsequently, the samples were subjected to capillary electrophoresis. Subpopulations were then sorted based on their membrane antigens using flow cytometry. A Mann-Whitney test was performed to compare monoclonality results between HI and HC, HI and AP, as well as between sexual and vertical transmission routes in HC. The degree of clonality was significantly higher in HC compared to HI ($p = 0.01$), with an odds ratio of 3.85 for monoclonality in PBMCs. Clonality was also significantly higher in AP than in HI ($p = 0.005$). Regarding transmission routes, no statistically significant differences were observed between the groups ($p = 0.8$). Monoclonality results in HTLV carriers were predominantly exclusive to the terminally differentiated effector T-cell (TTE) population (33.3%), suggesting a possible marker of early malignant T-cell proliferation.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Mitochondrial bioenergetic function in bone cells exposed to polystyrene microplastics

Authors:

Daniela Annunziata MASARO^{1,2}

Mariana Cassani OLIVEIRA¹ Pedro
CHITECULO¹

Edilene de Souza SIQUEIRA-SANTOS³

Jéssica CAMARGO¹

Lauter PELEPENKO¹

Roger Frigério CASTILHO³

Rodrigo Bueno de OLIVEIRA¹

Affiliations:

¹ Laboratory for Evaluation of Mineral and Bone Disorders in Nephrology (LEMON), School of Medical Sciences, University of Campinas (UNICAMP), Campinas, Brazil.

² Medical School, Pontifícia Universidade Católica, Campinas, Brazil.

³ Department of Pathology, School of Medical Sciences, University of Campinas (UNICAMP), 13083 887 Campinas, SP, Brazil.

Keywords: Cellular respiration. Lactate. Microplastics. Osteoblastic cells. Polystyrene.

Abstract:

Introduction: Microplastics, such as polystyrene (PS-MPs), raise concerns about human health and mitochondrial dysfunction. PS-MPs may impair mitochondrial function by inducing oxidative stress and reducing ATP production. Their effects on the bioenergetic processes of osteoblast-like cells remain unclear. **Methods:** MG-63 cells (CRL-1427) were exposed to PS-MPs at concentrations ranging from 5 to 50 µg/mL for 24 h. Mitochondrial function was assessed by monitoring oxygen consumption using high-resolution respirometry (Oroboros™), with the addition of an oxidative phosphorylation inhibitor (oligomycin) and an uncoupler (carbonyl cyanide m-chlorophenylhydrazone). Glucose consumption and lactate production were measured using colorimetric enzymatic kits. A normality test was performed and either one-way ANOVA or the Kruskal-Wallis test was used. **Results:** No significant differences were observed in oxygen consumption between control and PS-MPs-treated cells ($p > 0.05$), regardless of the respiratory state. The median O₂ flux at the H⁺ leak state (i.e. oligomycin present) (pmol O₂/s*10⁶ cells) for control, 5 µg/mL, and 50 µg/mL were 38.7 (33.1-45.3), 38.6 (32.8-60.4), and 30.4 (25.4-59.7). The median maximum O₂ flux was 93.9 (39.2-140.0) for control, 105.0 (56.3-153.0) for the 5 µg/mL exposure, and 101.0 (78.4-145.0) for the 50 µg/mL concentration. Glucose concentrations (mg/dL) in the cell culture medium at 48 hours were 40.4±21.4 for the control, 49.9±20.25 for 5 µg/mL group, and 45.26±19 for 50 µg/mL group, with no significant differences between the groups. Lactate concentrations were significantly higher at 48 hours ($p < 0.001$) for all conditions when compared with their 24-hour baseline, but there were no significant differences between groups. **Conclusion:** MG-63 cells appear to be resistant to the tested PS-MPs concentrations, as no significant differences in mitochondrial respiration, glucose consumption, or lactate release were observed across the conditions.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Impact of Stress on Gene Expression: Differential Regulation of Apoptosis and Necrosis Pathways

Authors:

Marco Antonio Cordeiro Carvalho DOS SANTOS¹

Regina Célia SPADARI²

Ana Elisa Teófilo Saturi DE CARVALHO³

Affiliations:

1-Federal University of São Paulo

2-Federal University of São Paulo

3-Federal University of São Paulo

Keywords: Stress, Genes, Apoptosis and necrosis

Abstract:

Stress has been defined as the organism's reaction to any real or potential threat to its integrity (Selye, 1936). The stress response is triggered when genetically programmed expectations, established by previous learning or inferred from circumstances, do not match the actual or potential perceptions of the external and internal environments (McEwen, 2000). When this response is very intense, prolonged, or repeated, or when it leads to frustration, stress can trigger diseases (McEwen, 2000). Rats were subjected to one daily session of foot shock stress for three consecutive days, as previously described (Moura et al., 2021). Hearts were collected, and the left ventricle was used for gene expression analysis based on two-color microarrays, performed using the Agilent-074036 SurePrint G3 Rat GE v2 8x60K Microarray G4858A platform (GEO GPL22145) from Agilent Technologies (Santa Clara, CA, USA). A total of four samples per group were analyzed, with two experimental groups: control and stress. Data analysis was conducted using the Genevestigator software. According to the results, we observed that stress causes alterations in the expression of several genes. We identified 29 genes related to apoptosis, a process of programmed cell death, and 28 genes related to necrosis, a non-programmed (uncontrolled) form of cell death. After analysis, we found that 8 genes were exclusive to apoptosis, 3 genes were exclusive to necrosis, and 21 genes were involved in both cell death processes. Of the 57 genes related to apoptosis and necrosis, 46 were upregulated and 11 were downregulated. We conclude that stress induces significant changes in gene expression, directly affecting cell death processes. The identification of 57 genes involved in these pathways, with the majority (46) showing upregulation, suggests that stress may activate cellular mechanisms that promote both programmed and unprogrammed cell death. These findings reinforce the relevance of stress as a potentially harmful factor to cellular integrity and may contribute to a better understanding of the molecular pathways involved in stress responses.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: The role of Hoxb7 in metastatic lung adenocarcinoma

Authors:

Pedro Lucas da Silva OLIVEIRA¹
Guilherme Alves de GOIS¹
Edna Teruko KIMURA¹
Cesar Seigi FUZIWARA¹

Affiliations:

¹ Instituto de Ciências Biomédicas, Universidade de São Paulo

Keywords: CRISPR, Epithelial-Mesenchymal Transition, Hoxb7, Lung Adenocarcinoma.

Abstract:

Lung cancer, while second in prevalence among human cancers, ranks first in lethality, largely due to late-stage diagnosis. Asymptomatic early progression often delays clinical investigation, resulting in over 50% of patients presenting metastasis at diagnosis, which drives rapid disease advancement and poor survival rates. Elucidating the molecular mechanisms of metastasis is therefore critical for improving prognosis and therapeutic strategies. To address this, we performed RNA sequencing on a paired murine cell line model: an aggressive liver metastasis-derived line (KPT4-LM) and its primary lung adenocarcinoma counterpart (KPT4). Bioinformatic analysis identified differentially expressed genes ($|\log \text{fold-change}| \geq 1$), followed by Gene Ontology enrichment (EnrichR) and survival correlation studies (GEPIA2). Notably, the 200 most upregulated genes in KPT4-LM revealed significant enrichment for *HOX* family members (32 genes, mainly *Hoxb7*, *Hoxb9* and *Hoxa10*), validated via qPCR. To investigate Hox functional role, we designed three single-guide RNAs (CHOPCHOP) to edit *Hoxb7* with CRISPR/Cas9 that were cloned into PX459-Cas9-puro plasmids, and transfected into KPT4-LM cells. Successful editing was confirmed by Sanger sequencing and Tricine-SDS-PAGE Western blotting. The clones lacking *Hoxb7* showed reduced mitochondrial activity (MTT assay), impaired colony formation. Luciferase reporter assays showed increased activity of miR-200c, miR-200b/a/429 clusters, and the E-cadherin promoter—key markers of epithelial-mesenchymal transition (EMT) reversal in KPT4-LM edited cells. These findings implicate *HOX* genes reactivation as an additional key factor for metastasis in lung adenocarcinoma, potentially through EMT suppression, while differentiation pathways are downregulated. Our work highlights *HOXB7* as a potential therapeutic target and underscores the prognostic value of *HOX* dysregulation as a biomarker in metastasis.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Assessment of the role of kallikrein KLK7 and KLK5 in wound healing

Authors:

Laura Miguel RODRÍGUEZ¹
Katiuchia Uzzun SALES²

Affiliations:

¹ University of Sao Paulo ²
University of Sao Paulo

Keywords: healing, inflammation, kallikreins, skin, wound

Abstract:

The skin constitutes the body's first line of defense, and its integrity is essential for maintaining health and overall well-being. However, damage is inevitable due to constant exposure to external aggressors. Cellular and molecular events ensure the healing of damaged areas, but significant disturbances can lead to serious complications, such as chronic infections, persistent pain, and even impaired healing. Serine proteases play a crucial role in the homeostatic mechanisms of the skin. Previous studies have identified kallikreins 5 (KLK5) and 7 (KLK7) as being associated with certain dermatological diseases, primarily those involving inflammatory profiles. During the healing process, prolonged inflammation is a key factor in wound chronicity, as frequently observed in elderly and diabetic patients. In this context, we hypothesize that KLK5 and KLK7 are involved in skin regeneration by modulating the inflammatory profile of the healing microenvironment. To test this hypothesis, we employed CRISPR/Cas9 technology to design unique RNA guides targeting the genes encoding KLK5 and KLK7, which were cloned into the LentiCRISPRv2GFP vector. Lentiviral particles were then used to transduce immortalized human keratinocyte cell lines (HaCaT and NOK), generating KLK5 and KLK7 knockout (KO) cell lines, that will be used to study proliferation and migration by bioimpedance. In parallel, the "Ethics Committee for Animal Use" (CEUA) approved the use of double KLK5/KLK7 KO mice to perform wound healing assays. These mice were wounded using a 6 mm dermatological punch and compared to wild-type littermate controls. Our results show reduced migration and proliferation in cells lacking KLK5 and/or KLK7. Similarly, wounds in double KO mice took longer to close compared to WT mice. Moreover, the wound edges in double KO mice were more inflamed than those in WT controls. In conclusion, our findings improve the understanding of these enzymes' roles, highlighting their potential as therapeutic targets for wound healing.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Altered macromolecular orientation as assessed by birefringence in tendons of rats treated with sodium valproate

Authors:

Eli Heber Martins DOS ANJOS¹,
Andrea Aparecida DE ARO²
Benedicto de Campos VIDAL¹
Maria Luiza Silveira MELLO¹

Affiliations:

¹Department of Structural and Functional Biology, Institute of Biology, University of Campinas, Campinas, SP, Brazil

²Department of General and Applied Biology, Institute of Biosciences, Sao Paulo State University, Rio Claro, SP, Brazil

Keywords: Birefringence. Sodium valproate. Tendon collagen bundles

Abstract:

Sodium valproate (VPA) is an anti-convulsant drug and a classic histone deacetylase inhibitor favoring epigenetic changes. Recent reports highlight VPA effects on skin fibrosis reducing the density of collagen types I and III that increases during this pathogenesis. Considering that tendons are highly organized structures primarily composed of collagen type I and proteoglycans, we investigated whether VPA affected the macromolecular orientation of these structures, by assessing their birefringence, an optical anisotropic property. Wistar rats aged 21 days were used for receiving daily doses of VPA by gavage for 9, 14, and 39 days after which effects on calcaneal tendon collagen bundles were followed with their advancing development and macromolecular organization using polarization microscopy. Each studied group, including saline control group, consisted of three animals. Birefringence brilliance intensity was measured as average gray values linearly correlated with optical retardations in tendon sections immersed in deionized water using an Olympus BX51 polarizing microscope equipped with a Q-color 5 camera and Image-Pro Plus 6.3 software. Statistics was conducted using GraphPad Prism 8.0.1. A highly significant birefringence reduction was demonstrated in collagen bundles from rats which received VPA for 39 days, indicating a structural remodeling of the tendons characterized by alterations in the macromolecular orientation of their collagen bundles. It is suggested that the VPA exposure time was prevalent to affect the collagen bundle organization. Studies on linear dichroism of stained collagen and proteoglycan substrates are in progress to further elucidate which components of the collagen fibers are responsible for the birefringence reduction detected in the whole structure of the tendon collagen bundles. All protocols involving animal care and use were approved by CEUA/UNICAMP (6269-1/2023). (FAPESP, grant no. 2007/58251-8; CNPq, grant no. 304797/2019-7).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Effects of Polystyrene Microplastics on Bone-Related Protein Expression and Mineralization

Authors:

Mariana Cassani OLIVEIRA¹
Daniela Annunziata MASARO^{1,2}
Pedro CHITECULO¹
Jéssica CAMARGO¹
Lauter PELEPENKO¹
Luciene Machado dos REIS³
Loïc LOUVET⁴
Rodrigo Bueno de OLIVEIRA¹

Affiliations:

¹ Laboratory for Evaluation of Mineral and Bone Disorders in Nephrology (LEMON), School of Medical Sciences, Universidade Estadual de Campinas – UNICAMP, Campinas, Brazil.

² Medical School, Pontifícia Universidade Católica, Campinas, Brazil.

³ LIM 16–Laboratório de Fisiopatologia Renal, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HCFMUSP), Universidade de São Paulo, São Paulo, Brazil.

⁴ Pathophysiological Mechanisms and Consequences of Cardiovascular Calcifications (MP3CV), Picardie Jules Verne University, Unité de recherche (UR) 7517 Université de Picardie Jules Verne (UPJV), Amiens, France.

Keywords: Microplastics. Polystyrene. Osteoblasts. Osteocalcin. DKK1.

Abstract:

Introduction: Recent research evidenced microplastics (MPs) in the human skeleton. Meanwhile, several pre-clinical studies suggested a negative role of MPs in biological processes. Therefore, the effects of MPs on bone metabolism now require further understanding. We aimed to evaluate the impact of polystyrene (PS) on bone-related proteins expression and in the mineralization process. **Methods:** Osteoblast-like cells (MG-63; CRL 1427) were exposed to fluorescent PS-MPs at concentrations from 5 to 50 µg/mL. MPs cell internalization was analyzed by confocal microscopy. Osteoprotegerin, osteocalcin, osteopontin, and DKK1 levels in cell lysates and/or supernatants were evaluated by a Milliplex™ human bone panel. Cell mineralization capacity was estimated by hydrolysis of *p*-nitrophenyl-phosphate (pNP, alkaline phosphatase activity) and calcium incorporation by the o-cresolphthalein complexone method. **Results:** MG-63 internalized PS-MPs into the cytoplasm. Compared to the control, cells exposed to PS-MPs at 10 and 25 µg/mL significantly decreased osteocalcin (137 vs. 109 pg/mL, $p=0.04$; and 137 vs. 100 pg/mL, $p=0.03$) and osteoprotegerin (8719 vs. 7402 pg/mL, $p=0.04$; 8719 vs. 6950 pg/mL, $p=0.01$) expression. DKK1 concentration was reduced following PS-MPs exposure with 25 µg/mL (10431 vs. 7789 pg/mL, $p=0.04$). No significant changes were observed for osteopontin. Yet, cellular mineralization remained preserved, as indicated by similar pNP production and calcium uptake. **Conclusion:** PS-MPs affected key bone protein expression without affecting cellular mineralization capacity.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Extracellular vesicles released by epithelial kidney cells induce cardiac cell stress

Authors:

Raquel Silva Neres dos SANTOS¹

Dylan BURGER²

Marcela Sorelli Carneiro RAMOS¹

Affiliations:

¹ Cardio Immunology Laboratory, CCNH, Federal University of ABC, Santo André, São Paulo, Brazil.

² Department of Cellular and Molecular Medicine and School of Pharmaceutical Sciences, University of Ottawa, Ottawa, Ontario, Canada.

Keywords: Extracellular vesicles, Hypoxia, Kidney disease, Viability.

Abstract:

Extracellular vesicles (EVs) are blebs released by all cell types, exerting intercellular communication function due to their cargo. EVs have been reported to play a role in the pathophysiology of several diseases, such as cancer, cardiovascular, and renal diseases. However, if EVs contribute to the pathological crosstalk between the kidney and the heart is unknown. We aimed to characterize EVs from in vivo and in vitro acute kidney disease models and to assess whether they would impact cardiac cellular viability. C57Bl/6 male mice (UFABC animal facility commenting number: 9019180122) left kidney were submitted to 60 minutes of warm ischemia and 1 and 8 days of reperfusion (IR1 and IR8) (n=3). The sham animals (control) only had the left flank opened. HK-2 cells were exposed to hypoxia for 24 hours, followed by 3 hours of reperfusion (HR) (n=7). The normoxia cells were kept under 21% O₂ throughout the HR period. EVs were isolated by ultracentrifugation from the plasma and conditioned medium, and characterized by NTA. H9C2 cells were treated with 6000 EVs/cell from both biofluids for 48 hours, followed by MTT assays. The results were expressed as mean±standard deviation and analyzed by t-test and ANOVA, p-values < 0.05 were considered statistically significant. The EVs characterization showed that the IR protocol did not induce changes in the total particles/mL (IR1:2.54 ±0.52 and IR8:2.03±0.51 vs Sham:2.43±0.54) but promoted the particle size (nm) decrease (IR1:126.33±2.37 and IR8:128.90±0.86 vs Sham:154.90±4.80). HR-induced HK-2 cells release 2-fold more EVs with bigger sizes (nm) than the control group (Hypoxia:14.52±7.47 vs Normoxia:5.30±1.34 particles/mL) (Hypoxia:183.92±3.90 nm vs Normoxia:159.55±10.20 nm). Plasma EVs did not affect the cellular viability of H9C2 cells (Sham:0.94±0.07, IR1:1.04±0.10 and IR8: 0.98±0.98 vs Untreated:1.00±0.09), nevertheless, both normoxic and hypoxic EVs induced the reduction of the cellular viability (Normoxia:0.81±0.02 and Hypoxia:0.88±0.04 vs Untreated:1.00±0.04). The characterization results suggested that hypoxia stimulates EVs' biogenesis, release in renal epithelial cells, and modification in size. The MTT results indicated that EVs caused stress in cardiac cells, affecting their viability.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Identification of targets involved in skeletal muscle mass control through transcriptomic analysis in mice overexpressing microRNA-29c

Authors:

Luis Dutra CHINAIT¹
Robson Francisco CARVALHO
Anselmo SigariMORISCOT¹

Affiliations:

¹ University of Sao Paulo, USP
² Paulista State University, UNESP

Keywords: Bioinformatic. Transcriptome. MicroRNA. Mir-29c. Skeletal muscle.

Abstract:

Muscle atrophy is characterized by a reduction in the size and function of its fibers, and is associated with several clinical conditions such as denervation, limb immobilization, renal failure, aging, cancer, among others. Cachexia, muscle atrophy associated with chronic diseases, is responsible for 20% of cancer deaths and is present in more than half of cancer patients. These and other atrophy conditions are related to increased mortality, loss of quality of life, and increased hospital expenses. Therefore, protection against muscle mass loss is of utmost importance. The present study hypothesizes a protective role of miR-29c in the skeletal context, a microRNA that our group described as hypertrophic (30% increase in muscle mass and strength) without the need for any other classic intervention such as the use of mechanical overload. For this purpose, c57bl/6 mice were divided into two groups (n=3): one group had the tibialis anterior muscle electroporated with an empty vector and the other group with a vector containing the miR-29c mimetic sequence. The tibialis anterior of these animals was used to generate a transcriptome and the differentially expressed genes (DEGs) were compared with the DEGs of patients with atrophy conditions: Cachexia, immobilization and exposure to dexamethasone, using the GEO (Gene Expression Omnibus) database; only one gene remains in the intersection between the groups. This gene (MYO10) is negatively regulated by miR-29c and positively regulated by atrophy conditions, and can be hypothesized in a first analysis as a gene capable of reducing muscle mass. Protein-protein interaction (PPI) analyses with MYO10 also showed that it has a direct link with calcium signaling pathways, through connections with CALM1, CALM2 and CALM3, and it is potentially through this pathway that the atrophic effect is produced. DEGs: logFoldChange > 0.4, p < 0.05 obtained by DESeq2 analysis.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Effects of Red LED Therapy in an Imiquimod-Induced Murine Model of Psoriasis

Authors:

Nathália Rodrigues da SILVA¹
Rebeca D. CORREIA-SILVA¹
Diego Dias dos SANTOS¹
Mab Pereira CORRÊA¹
Cristiane Damas GIL¹

Affiliation:

Division of Histology and Structural Biology, Department of Morphology and Genetics, Universidade Federal de São Paulo - UNIFESP, SP, Brazil.

Keywords: Spleen. Inflammation. Mast cells. Skin. **Abstract:**

Psoriasis (Pso) is an immune-mediated skin disease characterized by chronic inflammation and scaling. Due to the complexity of its pathogenesis, identifying biomarkers is essential for the development of personalized treatments. Among alternative therapies, LED phototherapy has gained attention. This study evaluated the effects of red LED (LEDV) light on Pso progression in an imiquimod (IMQ)-induced murine model. C57BL/6 mice were divided into four groups: control (CTR), CTR+LEDV, Pso, and Pso+LEDV (n=5-7 animals/group). On day 0, animals were shaved; from days 1 to 8, Pso groups received topical IMQ (50 mg/kg), while CTR groups received Vaseline. LEDV treatment was applied on days 4, 6, and 8. Skin thickness (double skinfold), body weight, and PASI score were assessed on days 0, 2, 4, 6, and 8. Twenty-four hours after the last IMQ and LEDV application, animals were euthanized for skin and spleen collection (CEUA nº 5387071123). IMQ-induced Pso led to a significant increase in PASI score in the Pso+LEDV group on days 4 and 6 compared to CTR, whereas on day 8, this increase was observed only in the untreated Pso group. Skinfold thickness analysis showed a marked increase between days 4 and 6 in both Pso and Pso+LEDV groups. Histological analysis revealed significant epidermal thickening in LEDV and Pso+LEDV groups compared to CTR and Pso, respectively. In contrast, mast cell quantification in the dermis showed a significant increase only in the Pso group compared to the others. Systemic response was indicated by an increase in relative spleen weight and white pulp area in both Pso and Pso+LEDV groups (One-way ANOVA). Although LEDV did not prevent Pso progression, it suggests a potential modulatory effect on the cutaneous inflammatory response, highlighting the need for further studies to elucidate its mechanisms.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Collagen, Keratin, IGFR and EGFR expression in Epithelium-derived retinal progenitor cells

Authors:

Giovanna Serra da SILVA¹
Bárbara DALMASO¹
Ivanir Santana de Oliveira PIRES¹
Matheus Andrade BETTIOL¹
Carolina Beltrame DEL DEBBIO¹

Affiliations:

¹Department of Cell and Developmental Biology, Biomedical Sciences Institute - University of São Paulo (ICB-USP), São Paulo (SP).

Keywords: Pigmented epithelium. Retina. Stem cells

Abstract:

The retina is a nervous tissue susceptible to degenerative stimuli that can lead to the development of diseases such as glaucoma. Although retinal degeneration is irreversible and incurable, some mammalian retinal epithelial cells located at the Ciliary Epithelium (CE) exhibit stem cell potential, with the ability to reprogram themselves into retinal progenitor cells after stimulation with growth factors, and subsequently differentiate into functional retinal neurons. Despite the ability of these cells to dedifferentiate, they preserve some characteristics of their original adult cells. In this study, we investigated the expression of the cellular structural components keratin and collagen, as well as EGFR and IGFR in stem cells derived from ciliary Pigmented Epithelium (PE) cells to assess whether these markers are preserved during the process of stem cell activation. The experiments in this project were approved by the Ethics Committee on Animal Experimentation, CEUA protocol no. 6771020823. The transcriptional analysis (PCR) evaluated by ANOVA test in Graphpad Prism software with a threshold of $P < 0.05$, and immunohistochemistry analysis ($N=3$), indicated that the adult WT Balb/c mice neurospheres (stem cells PE-derived) expressed higher transcriptional levels of collagen 15 (Col15a1), keratin 14 (Krt14), IGFR and EGFR when compared to the differentiated PE. These data indicate that, despite being reprogrammed into stem cells, some epithelial characteristics are maintained in these progenitor cells, suggesting that they play a role in retinal stem cells proliferation and differentiation processes. In addition, they raise the need to characterize the functioning of the IGF and EGF pathways in the CE and possible interferences in those pathways after PE reprogramming, considering that IGF-1 and EGF are important neurotrophic factors involved in both embryonic development and retinal regulation, in addition to EGF acting as a dedifferentiation factor.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Platelet-Activating Factor Receptor Modulates Retinal Vasculature Development During Retinogenesis

Authors:

Enzo Costa ZONTA¹
Barbara DALMASO¹
Matheus Andrade BETTIOL¹
Ivanir Santana de Oliveira PIRES¹
Carolina Beltrame DEL DEBBIO¹

Affiliations:

¹ Departamento de Biologia Celular e do Desenvolvimento, Instituto de Ciências Biomédicas, Universidade de São Paulo

Keywords: PAFR. Retina stem cells. Vascular development.

Abstract:

Vision is a critical sense for vertebrates with the retina playing a key role in visual processing. In certain animals, retinal regeneration is mediated by resident progenitor cells that are maintained by the retinal vascular network driven by growth factors like PDGF, VEGF and unknown factors. The signaling mediated by platelet-activating factor receptor (PAFR) influences retinal stem cell maintenance, and its absence may delay cell differentiation and alter electrophysiological responses. To understand the PAFR's role in retinal vascular development and stem cell regulation, we investigated the formation of the retinal vascular network in PAFR knockout animals (PAFR-KO) during the postnatal period, and the gene and protein factors related to vasculogenesis in the visual system. The experiments of this project were approved by the Ethics Committee on Animal Experimentation, CEUA protocol no. 6771020823. Eyes from Wild-Type and PAFR-KO Balb/c mice were collected at postnatal days 1 (P1), P10, and P21. RNA was isolated, converted to cDNA, and qPCR was performed for VGFA, VGFR, and angiopoietin genes, normalized to beta-actin. Western Blotting was done for VEGF and SRC proteins. Eyes were processed for immunohistochemistry using DAPI and isolectin B4, with 12 µm sections for vascular network analysis via confocal microscopy. Our results indicated that PAFR-KO mice presented reduced inner retinal vessels in immunohistochemistry analysis. VGFR transcriptional expression was significantly lower in PAFR-KO at P1 and P10 in comparison to control animals. At P21, the VGFR transcriptional levels were equally expressed in controls and PAFR-KO animals. No significant differences were found in VGFA ligand transcriptional expression. In conclusion, we observed that the receptor expression of the main growth factor involved in retinal vascularization development is regulated by PAFR signaling, which may impact the formation of the retinal vascular network.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: EZH2 as a new regulator of lung cancer metastasis

Authors:

Guilherme A. GOIS¹
Pedro Lucas da Silva OLIVEIRA¹
Joice M. MENEZES¹
Diego C. DE MELLO¹
Edna T. KIMURA¹
Cesar S. FUZIWARA¹

Affiliations:

¹Department of Cell and Developmental Biology, Institute of Biomedical Sciences, University of São Paulo

Keywords: Lung cancer. Metastasis. MicroRNA. EZH2

Abstract:

Lung cancer is the second most common cancer and the leading cause of death. However, the molecular mechanism underlying the early activation of metastasis in lung cancer is still to be elucidated. Increased activity of the EZH2/PRC2 complex has been reported in different types of cancer, mainly in more aggressive histotypes, including lung cancer. EZH2 is responsible for the trimethylation of lysine 27 in histone H3 (H3K27Me3), which induces chromatin compaction and leads to gene silencing. One potential target of EZH2 is the miR-200 family of microRNAs, whose loss of expression is associated with the activation of the epithelial-mesenchymal transition (EMT) process and, consequently, associated with the metastatic process in lung cancer. Thus, this project aimed to investigate the role of EZH2 in metastatic lung cancer and its relationship with miR-200 regulation. We edited the gene encoding murine *Ezh2* with CRISPR/Cas9 and validated the editing by Sanger sequencing. We obtained clones that presented lower EZH2 levels compared to unedited control by Western Blotting. We selected two clones with epithelial phenotype for functional assays. We performed a luciferase reporter promoter assay and observed greater activation of the miR-200 promoters in the clones with *Ezh2* editing. In addition, we performed a cell counting assay, in which we observed a lower number of cells in the edited clones at 72h, and a mitochondrial activity assay, that showed contrasting results between the clones. We then cultured the cell lines in suspension and observed that the clones formed smaller cell clusters than the unedited control. Finally, we performed transwell migration assay, which revealed lower cell migration in the edited clones compared to control. Thus, we conclude that *Ezh2* editing was successful, and that the reversal of the epithelial phenotype is correlated with greater activation of miR-200 promoters. In addition, we revealed that *Ezh2* silencing has an antitumor effect.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Molecular insights into *AMH* and *NR5A1* genes in a rare double diagnosis of 46,XY Partial Gonadal Dysgenesis and Persistent Müllerian Duct Syndrome

Authors:

Felipe Rodrigues DE OLIVEIRA^{1,2}
João Victor VIRGILIO-DA-SILVA¹ Geovana
CARDOSO-DE-ASSIS³
Mara Sanches GUARAGNA^{1,4,5}
Andrea Trevas MACIEL-GUERRA^{4,5}
Gil GUERRA-JUNIOR^{5,6}
Helena FABBRI-SCALLET^{1,5,6}

Affiliations:

¹Center for Molecular Biology and Genetic Engineering (CBMEG), University of Campinas, Campinas, Brazil

²Postgraduate Program in Child and Adolescent Health, School of Medical Sciences, University of Campinas, Campinas, Brazil

³Pontifical Catholic University of Campinas, Campinas, Brazil

⁴Department of Medical Genetics and Genomics, School of Medical Sciences, University of Campinas, Campinas, Brazil

⁵Interdisciplinary Group for the Study of Sex Determination and Differentiation (GIEDDS), University of Campinas, Campinas, Brazil

⁶Department of Pediatrics, School of Medical Sciences, University of Campinas, Campinas, Brazil

Keywords: *AMH*. Differences of sex development. *NR5A1*. 46,XY Gonadal Dysgenesis.

Abstract:

Disorders/Differences of sex development (DSD) are congenital conditions involving atypical chromosomal, gonadal, or anatomical sex characteristics. DSD with a 46,XY karyotype represent a broad and heterogeneous group of disorders. Among them, only Complete or Partial Gonadal Dysgenesis (CGD or PGD), Ovotesticular DSD and Persistent Müllerian Duct Syndrome (PMDS) may have uterus. An 8-month-old boy was referred to our service due to the presence of uterus and gonads resembling testicles during left herniorrhaphy. The parents were first cousins. At first evaluation, he also presented a subcoronal hypospadias. Investigations revealed a 46,XY karyotype, normal testosterone secretion, low levels of Anti-Müllerian hormone (AMH) and dysgenetic gonads bilaterally. By performing whole exome sequencing, variants in two DSD-related genes were identified: the homozygous c.556-2A>G in *AMH* and the heterozygous c.877G>A, in *NR5A1*, inherited from the father. These variants are likely responsible for the double diagnosis of PMDS and PGD, respectively. *AMH*, which follows an autosomal recessive inheritance pattern, is essential for the regression of Müllerian ducts, while *NR5A1* is crucial for testicular differentiation. The c.877G>A variant, previously described in PGD and CGD cases, was classified as pathogenic (ACMG: PP3_S + PM2 + PS3_P), and functional studies confirmed reduced *NR5A1* activity as a transcription factor. The c.556-2A>G variant in *AMH*, located at the splice acceptor site, is predicted to cause a loss of function and was also classified as pathogenic (ACMG: PVS1 + PM2 + PP4). To validate the impact of this variant, we proposed a minigene strategy, and functional studies are currently ongoing. Using the Exontrap System vector, our aim is to investigate the formation of the *AMH* transcript and its subsequent effect on protein translation. These findings will help us to clarify the role of each variant in the patient's phenotype (CAAE 69225123.3.0000.5404).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Splenic Alterations Associated with Annexin A1 Deficiency in Streptozotocin- Induced Murine Type 1 Diabetes

Authors:

Laura Santana de CHIARA^{1;2};
Larissa SUZUKI²;
Diego Dias dos SANTOS²;
Rafael André da SILVA;
Luiz Philipe de Souza FERREIRA;
Katia de VASCONCELOS²;
Cristiane Damas GIL²;
Adriana DEGROSSOLI²

Affiliations:

¹ Paulista University (UNIP);

² Department of Morphology and Genetics, Paulista School of Medicine (EPM), Federal University of São Paulo (UNIFESP), São Paulo, SP.

³ Biosciences Graduate Program, Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista - UNESP, São José do Rio Preto, Brazil.

Keywords: immune cells, diabetes mellitus, streptozotocin, oxidative stress, inflammation.

Abstract:

Diabetes mellitus (DM), a serious public health problem, affects various tissues, including the spleen. Patients with type 2 diabetes (T2D) have high levels of annexin A1 (AnxA1), a protein that mediates inflammation. In murine models of T2D, AnxA1 improves insulin resistance; however, its role in the spleen in type 1 diabetes (T1D) is poorly studied. The objective of this study was to assess the role of AnxA1 in spleen biology in a murine T1D model. T1D was induced by intraperitoneal injection of streptozotocin (STZ) in wild-type (WT) and AnxA1 knockout (KO) male C57Bl/6 mice (CEUA:3916170624). After 90 days of T1D induction, the spleens of the control (CT) and diabetic (DB) animals were harvested for histological analysis. This included hematoxylin and eosin (HE) staining for general morphology, Perls staining for iron deposits, and Masson's trichrome for collagen. Immunohistochemistry was used to identify CD3⁺ and CD20⁺ cells. The number of lymphoid nodules in the spleens among the groups was similar. However, the perimeter of the white pulp in the spleens of the WT DB mice (1516±1137 mm²) was found to be smaller compared to the WT CT mice (1831±1137 mm²). Hemosiderin deposition, revealed by Perls staining, was greater in KO DB (121±20,3 a.u.) compared to KO CTR (110±3,14 arbitrary units - a.u.) mice and WT CTR (118±6,57 a.u.) compared to KO CTR (110±3,14 a.u.) mice. Masson's Trichrome staining revealed higher collagen deposition in KO DB (161±13 mice) compared to other groups. WT CTR (195,5±28,3 a.u.) mice showed higher CD3 expression, while CD20 expression was similar across all groups. The results indicate that animals with DM1 and deficient in AnxA1 present alterations in the spleen, including atrophy of the white pulp, greater hemosiderin deposition and fibrosis, as well as a reduction in the number of T cells (CD3⁺). These findings suggest that AnxA1 plays a role in maintaining the structural integrity of the spleen, even in DM1.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: CRISPR/Cas9 and Tol2-Based constructs cloning for functional assays of *WFDC1* in chicken limb development

Authors:

Thaís METZKER-PINTO¹ Igor
BUZZATTO-LEITE¹
Thaís Holtz THEIZEN¹ Lúcia
Elvira ALVARES¹

Affiliations:

¹University of Campinas, Institute of Biology, Department of Biochemistry and Tissue Biology

Keywords: Chicken embryo. Cloning. CRISPR/Cas9. Gain- and loss-of-function. *WFDC1*.

Abstract:

The *WFDC1* gene encodes the ps20 protein, a component of the extracellular matrix involved in regulating cell proliferation, migration, and epithelial-mesenchymal transition in cancer. These processes also occur during limb development. However, the specific roles of *WFDC1* in this context remain undescribed, despite its expression being previously identified in chicken limb buds by our research group. To investigate these roles, we will perform gain- and loss- of-function assays in chicken (*Gallus gallus*) embryos. For this purpose, we will use the CRISPR/Cas9 system to inactivate the gene and an integrative expression construct based on the Tol2 transposon system to overexpress it for several days post-transfection. These constructs will be introduced into the lateral plate mesoderm of chicken embryos via *ex ovo* electroporation. The first step toward these functional assays was the cloning of constructs for CRISPR/Cas9-mediated inactivation and Tol2-based overexpression. For gene inactivation, we cloned target sequences for four *WFDC1* exons into a vector containing the U6.3 promoter, essential for sgRNA expression. In parallel, we are optimizing the electroporation of these sgRNA mini-vectors along with the pCAG_Cas9-2A-Citrine vector, which carries the Cas9 nuclease under the CAG promoter and the 2A-Citrine reporter gene. For overexpression, we used the pCAGGS-Myc-IRES-eGFP integrative vector, which contains Tol2 sequences, the CAG promoter, and the eGFP reporter gene. The full-length *WFDC1* cDNA was amplified from a clone generated using the TOPO cloning system and subsequently subcloned into the overexpression construct. The electroporation of this vector is also being optimized. With the cloning completed, the next steps will involve testing the efficiency of the constructs *in vivo* and evaluating potential morphological alterations resulting from *WFDC1* manipulation during limb development.

CEUA nº 321/2024 - Dispensa de Análise de Projeto



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Excess of Unsaturated Fatty Acids During Pregnancy Induces Gestational Diabetes and Compromises Muscle Development in Wistar Rats

Authors:

Matheus Felipe ZAZULA¹
Ana Carla COSTA E SILVA¹
Mônica MACIEL¹
Heloisa Deola CONFORTIM²
Luiz Claudio FERNANDES³
Katya NALIWAIKO¹

Affiliations:

¹ Laboratório de Plasticidade Morfofuncional, Departamento de Biologia Celular, Universidade Federal do Paraná – Curitiba, Paraná, Brasil.

² Laboratório de Plasticidade Morfofuncional, Universidade Federal do Paraná – Toledo, Paraná, Brasil.

³ Laboratório de Metabolismo Celular, Departamento de Fisiologia, Universidade Federal do Paraná – Curitiba, Paraná, Brasil.

Keywords: Maternal health; Unsaturated fatty acids; Muscle development; Metabolic programming; DOHaD.

Abstract:

Maternal health is essential for the proper development of offspring, particularly during pregnancy and lactation. Increased fat consumption, driven by industrialized diets, has been linked to higher prevalence of metabolic diseases such as diabetes and obesity. These metabolic changes in the mother can negatively impact fetal and postnatal development. This study aimed to evaluate the effects of supplementation with unsaturated fatty acids on maternal health and muscle development in the offspring of Wistar rats. Forty-eight rats were divided into four groups: Control (CT), supplemented with fish oil (FO), soybean oil (SO), and olive oil (OO). The supplementation was administered for 21 days before mating, throughout pregnancy, and lactation. After weaning, the offspring were assessed for muscle development, strength, muscle morphology, and metabolic characteristics (CEUA 1452/2022). The results showed that rats supplemented with FO, SO, and OO had a significant reduction in peripheral glucose tolerance on gestational day 18. In muscle development, the offspring from supplemented rats exhibited delayed development, with reduced muscle strength and mass. These animals showed fewer muscle fibers, with less differentiation by type and significantly smaller fiber sizes. These findings suggest that maternal supplementation with unsaturated fatty acids in excess negatively impacts the muscle development of offspring, impairing fiber formation, differentiation, and functionality. Exposure to these fatty acids during embryonic and neonatal stages leads to a compromised muscle development pattern, indicating a metabolic programming effect that affects muscle performance in the long term. In conclusion, the results suggest that maternal supplementation with unsaturated fatty acids, particularly during pregnancy and lactation, may have detrimental effects on offspring muscle development, affecting muscle morphology, functionality, and overall metabolic and motor health.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Optimisation of *ex ovo* electroporation targeting the lateral plate mesoderm for *in vivo* functional analysis of *DACT2* during chicken limb development

Authors:

Igor BUZZATTO-LEITE¹ Thaís
METZKER-PINTO¹
Thaís Holtz THEIZEN¹ Lúcia
Elvira ALVARES¹

Affiliations:

¹ University of Campinas, Institute of Biology, Department of Biochemistry and Tissue Biology

Keywords: Chicken embryo. *DACT2*. *Ex-ovo* electroporation. *In vivo* functional assays. Limb development

Abstract:

DACT2 belongs to a family of adaptor proteins, which orchestrates a myriad of developmental processes. Evidence suggests that this molecule modulates TGF- β and Wnt signalling pathways in diverse cellular contexts. However, its precise molecular role in limb development remains uncharacterised. Our *in situ* hybridisation data indicate that *DACT2* may play a key role in limb patterning. To address this matter, we will perform *in vivo* functional assays using the chicken embryo as a model. Accordingly, *ex ovo* electroporation will be employed to introduce both an overexpression system (CAG-GFP-T2A-Tol2-ITR + Tol2-Transposase) containing the full-length *DACT2* cDNA and a CRISPR/Cas9-mediated knockdown system into the lateral plate mesoderm of HH14 embryos. Thus far, the *ex ovo* system and the electroporation technique were optimised. After trying out a few materials, we found a square weighing bowl and square Petri dish as the most effective setup for embryo survival. Regarding electroporation, after testing multiple parameters, we achieved high fluorescence levels of all systems with minimal limb malformation rate by applying three pulses at 10V, 50ms duration, and 100ms intervals using homemade platinum electrodes (0.4mm). Embryos stained with Alcian blue at stages HH30-32 revealed no qualitative differences in appendicular skeletal morphology between electroporated and contralateral control limbs. However, further quantitative analysis is required to fully validate the feasibility of these electroporation parameters. Our next step is to perform in batch limb bud electroporation using the experimental constructs to evaluate their expression efficiency and conduct validations. CEUA nº 323/2024.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Metabolic and Epigenetic Reprogramming of Normal Human Keratinocytes by Melanoma-Derived Small Extracellular Vesicles

Authors:

Sophia Nora BAPTISTA¹ Rodrigo
Augusto DA SILVA²
Carmen Veríssima FERREIRA-HALDER¹

Affiliations:

¹Department of biochemistry and tissue biology, Biology Institute (IB), Universidade Estadual de Campinas (UNICAMP)
²Environmental and Experimental Pathology Program, Universidade Paulista (UNIP)

Keywords: Epigenetics. Extracellular Vesicles. Keratinocytes. Melanoma. Metabolism.

Abstract:

Melanoma is the most aggressive and treatment-resistant form of skin cancer, with its incidence rising significantly over recent years. The tumor microenvironment plays a crucial role in melanoma progression, comprising both cellular and non-cellular components. Among these, keratinocytes and small extracellular vesicles (sEVs)—particles smaller than 200 nm involved in intercellular communication—are of particular importance. Accumulating evidence supports the role of sEVs in the reprogramming and metabolic modulation of target cells through a process known as tumor education. Previous studies by our group demonstrated that melanoma-derived sEVs influence keratinocyte morphology, migration, and MTT reduction capacity. Based on these findings, we hypothesized that sEVs may affect other keratinocyte features, such as the epigenetic and metabolic profile of these cells, in a process that ultimately leads to enhanced melanoma cell survival. To validate our hypothesis, sEVs derived from melanoma cell lineage Skmel-103 were isolated and characterized. Experiments to guarantee the quality control of the extracted particles were also performed. The impact of sEVs co-culture on human keratinocytes metabolism and epigenetics was analyzed through western blotting and qPCR assays, respectively. Results displayed a reduction in the expression of proteins linked to cellular glycolytic metabolism. In particular, pyruvate dehydrogenase and glucose transporter 1 showed a reduction of over 40%, pointing to a decrease in the keratinocyte's glucose uptake. Epigenetic assays revealed a general overexpression of Line 1, DNA demethylases (TETs) and methyltransferases (DNMTs) on keratinocytes exposed to the melanoma's sEVs, suggesting the involvement of these particles in the development of major genomic instability. These findings provide compelling evidence that melanoma sEVs actively modulate the behavior of keratinocytes.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Standardization of a retinitis pigmentosa neurodegeneration *in vitro* model

Authors:

Julia de Almeida PEREIRA¹; Gabrieli Bovi dos SANTOS¹; Marília Inês MÓVIO¹; Mariana Sacrin Ayres FERRAZ¹; Alexandre Hiroaki KIHARA¹

Affiliations:

¹Center of Mathematics, Computing and Cognition, UFABC, São Paulo.

Keywords: Cell death. Neurodegeneration. Phosphodiesterase 6 beta. Retina. Retinitis pigmentosa.

Abstract:

Retina is essential for vision and is affected by retinitis pigmentosa (RP), an inherited retinopathy caused by mutations as in PDE6b, which leads to intracellular Ca^{2+} and cGMP accumulation, triggering cell death pathways and glial activation. This study aimed to standardize an *in vitro* model of light-induced neurodegeneration to investigate these pathways in RP. C3H/HeJ (RD1) and C57BL/6J (WT) mice were used in P0-P3. Retinas were extracted, and primary cultures were exposed to 12k lux light at DIV11, for dark, 2 and 5 hours. Viability and cell death were assessed by MTT, PB, TUNEL, and immunofluorescence (IF). Morphology was analyzed using the Sholl Analysis in Fiji. Procedures were approved by CEUA/UFABC (9965240217). Statistical analysis used 1-way ANOVA, T-test, and K.W. test. When exposed to light, WT mice showed no differences in viability, but it was reduced in RD1 cultures after 5h ($F(1,003,2.005)=17.77$; $p=0.05$, $n=3$, PB). TUNEL confirmed increased cell death in 5h group ($F(2,15)=11.66$, $p=0.0009$; Darkvs5h: 0.013 ± 0.004 vs 0.096 ± 0.021 , $p=0.001$; 2hvs5h: 0.026 ± 0.007 vs 0.096 ± 0.021 , $p=0.004$; 1-wayANOVA; $n=6$). IF rhodopsin showed similar rod concentration between groups ($F(2,8)=1.485$; $p=0.28$; $n=3-4$), but colocalization with TUNEL increased with light exposure, indicating more rod death (K.W:15,92, $p=0.0003$; Darkvs5h: 0.77 ± 0.23 vs 5.33 ± 1.37 , $p=0.001$; 2hvs5h: 1.13 ± 0.41 vs 5.33 ± 1.37 , $p=0.002$; $n=13-15$). Caspase-3 and RIPK-3 IF revealed apoptosis and necroptosis in RD1 cultures, with necroptosis activating in 3h ($p=0.003$; Darkvs3h: 0.302 ± 0.077 vs 0.865 ± 0.181 ; $n=8-45$), while apoptosis occurred after 5h ($p=0.0438$; Darkvs5h: 0.233 ± 0.036 vs 0.360 ± 0.043 ; $n=22-37$). RD1 glial cells showed reduced morphological diversity with increased exposure, with 61% of similarity of the 5h group morphology, compared to the more diverse control group. These results indicate that PDE6b mutation makes RD1 photoreceptors more light-sensitive than WT, triggering apoptosis, necroptosis, and glial activation.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Retina and Ciliary Epithelium Postnatal Development and Stem Cell Differentiation

Authors:

Matheus Andrade BETTIOL¹
Ivanir Santana de Oliveira PIRES¹
Carolina Beltrame DEL DEBBIO¹

Affiliations:

¹Departamento de Biologia Celular e do Desenvolvimento, Instituto de Ciências Biomédicas, Universidade de São Paulo

Keywords: Eye development. Postnatal development. Stem cell. Retina.

Abstract:

The Ciliary Epithelium (CE) of adult mammalian eyes contains a quiescent population of Retinal Progenitor Cells (RPC), which are epithelial-derived and capable of differentiating into functional retinal neurons upon induction. Despite this regenerative potential, the efficiency of regeneration remains low due to unknown mechanisms and/or intrinsic epithelial characteristics that persist in RPC derived from the CE. Therefore, we evaluated the expression profile of retinal progenitor cells differentiating into CE cells, focusing on understanding the differentiation mechanisms involved in the transition of stem cells into epithelial cells. The experiments in this project were approved by the Ethics Committee on Animal Experimentation, CEUA protocol no. 6771020823. Eyes from wild-type Balb/c mice at postnatal days 1, 5, 10, and 21 were collected, fixed in 4% paraformaldehyde, and incubated in a 30% sucrose solution for 24 hours. Subsequently, the eyes were processed for immunohistochemical analyses on histological cross-sections and flat-mounted eyes. Primary antibodies against Pax6 were used to detect retinal progenitor cells, and antibodies against pancytokeratin were used as an epithelial cell marker. Our results showed that Pax6 was detected in the retina and CE cells during early postnatal differentiation, with decreased reactivity at day 21. Pancytokeratin was diffusely expressed in the neuroblast layers during development, becoming restricted to retinal pigmented cells and pigmented CE cells in young adults. In conclusion, RPC and epithelial marker expression shifted during postnatal differentiation. RPC expression decreased and became confined to a select progenitor population in the CE, while pancytokeratin, initially weak and diffusely expressed in neuroblasts, became more prominent in adult retinas. These findings suggest that keratin plays a role in retinal differentiation, becoming more specific in differentiated epithelial cells at later stages.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Role of melatonin in suppressing tumor progression in MCF-7 and MDA-MB-468 breast carcinoma cell lines

Authors:

Roberta Carvalho CESÁRIO¹, Karolina da Silva TONON¹,
Maria Luiza Gonçalves AGNEIS¹,
Fábio Rodriguez Ferreira SEIVA¹,
Débora Aparecida Pires de Campos ZUCCARI², Luiz Gustavo de Almeida CHUFFA¹

Affiliations:

¹ Universidade Estadual Paulista "Júlio de Mesquita Filho" ²
Faculdade de Medicina de São José do Rio Preto

Keywords: Melatonin. Breast cancer. Tumor progression.

Abstract:

Melatonin, a hormone produced by the pineal gland, regulates circadian rhythms and shows antitumor properties in several cancers, including breast cancer. This study aimed to evaluate its effects on cellular metabolism and tumor progression in the MCF-7 and MDA-MB-468 breast carcinoma cell lines. Cells were divided into control and melatonin-treated groups (7 μ M) for 24 hours, with the dose and exposure time determined by cell viability assays (MTT). Melatonin inhibited cell migration and invasion in MCF-7 and MDA-MB-468 cell lines. In MCF-7, the control group achieved 100% wound closure, whereas melatonin-treated cells exhibited only 10% closure. In MDA-MB-468, the control group showed 12% closure, while no closure (0%) was observed in the treated group. In MCF-7 cells, the number of invading cells decreased from 179.33 ± 90.98 in the control group to 123 ± 13.07 in the treated group. In MDA-MB-468 cells, invasion dropped markedly from 2073.33 ± 253.85 in controls to 256.33 ± 25.89 after melatonin treatment ($p < 0.05$). Melatonin treatment also reduced the expression of GAPDH, HIF-1 α , and citrate synthase, with reductions of 74%, 93%, and 59% in MCF-7 and 55%, 90%, and 45% in MDA-MB-468, respectively ($p < 0.05$). The treatment induced oxidative stress, as indicated by increased levels of MDA, protein carbonyls, and GSH. In MCF-7 cells, these markers rose by 14%, 38%, and 48%, respectively, while in MDA-MB-468 cells, the increases were 18%, 71%, and 57% ($p < 0.05$). Notably, biogenic amines — melatonin, serotonin, and agmatine — were detected exclusively in treated groups. In MCF-7, their levels were 28.897 ± 0.935 μ g/ μ L, 2.973 ± 2.353 , and 58.158 ± 25.523 , respectively; and in MDA-MB-468, 34.342 ± 0.867 μ g/ μ L, 2.342 ± 1.886 , and 1.154 ± 0.750 . These findings suggest that melatonin may be a promising therapeutic agent for combating breast cancer, with the ability to reduce cell migration and invasion while modulating oxidative stress and tumor-associated metabolic proteins.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Melatonin and Cisplatin Differentially Modulate the Expression of Genes Associated with Energy Metabolism in Hepatocellular Carcinoma Cells

Authors:

Wesley Ladeira CAPUTO¹,
Maria Luisa Gonçalves AGNEIS¹,
Matheus Ribas de ALMEIDA¹, Milena
Cremer DE SOUZA¹,
Celio Junior da Costa FERNANDES¹,
Gerson Santos de ALMEIDA¹, Roberta
Carvalho CESARIO¹, Karoliny Alves das
NEVES¹,
Érika Novais OLIVEIRA¹,
Fábio Rodrigues Ferreira SEIVA¹.

Affiliations:

¹ Department of Chemistry and Biochemistry, Institute of Biosciences (IB), São Paulo State University (UNESP), Botucatu, Brazil.

Keywords: Complementary therapy. Gene expression. Liver cancer

Abstract:

Hepatocellular carcinoma (HCC) is associated with hepatitis, excessive alcohol consumption, and obesity, with limited treatment options due to chemoresistance. Metabolic reprogramming, a hallmark of tumor cells, enables the adaptation of energy metabolism for survival. Melatonin exhibits antitumor effects and influences cellular metabolism. Cisplatin acts by modifying DNA and inducing apoptosis. This study evaluated the cytotoxic effects of melatonin (2 mM) and cisplatin (0.5, 1.0, and 2.0 µg/mL) on Huh7.5 and HepG2 cells after 24 and 48 hours of treatment. Cytotoxicity and modulation of energy metabolism-related genes were assessed in three independent experiments performed in triplicate (n = 3), with gene expression analyzed via RT-qPCR (relative expression normalized to GAPDH). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test (p < 0.05). At 24 hours, melatonin significantly reduced PI3K (0.77 ± 0.10) and LDH (0.57 ± 0.01) expression in Huh7.5 cells, while cisplatin reduced PI3K (0.71 ± 0.15) and PFK1 (0.54 ± 0.03). In HepG2 cells, cisplatin increased PFK1 (3.46 ± 0.16), PDH (2.75 ± 0.22), whereas melatonin reduced PI3K (0.18 ± 0.01). At 48 hours, melatonin increased Akt (9.58 ± 0.91) and PFK1 (3.06 ± 0.31) in Huh7.5 cells, while cisplatin elevated HIF1α (7.64 ± 0.30) and PI3K (5.35 ± 0.23). In HepG2 cells, cisplatin increased GSK3β (2.87 ± 0.20) and PDH (3.22 ± 0.33), while melatonin reduced LDH (0.66 ± 0.04) and PI3K (0.65 ± 0.06). These findings suggest that melatonin and cisplatin modulate glycolytic and mitochondrial pathways, influencing tumor energy metabolism. Cisplatin affected genes associated with cellular respiration, while melatonin reduced PI3K, AKT, and LDH expression, indicating inhibition of anaerobic glycolysis. Both treatments showed cell line-specific effects. Co-treatment with melatonin and cisplatin could reduce cisplatin doses, minimizing side effects while maintaining chemotherapy efficacy.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Effects of microplastic exposure on the skeletal muscle tissue of zebrafish in relation to their reproductive success

Authors:

Anna Alice Souza Vilela COSTA¹,
Ana Karla Lopes Domingos de ANDRADE¹, Davi
Araújo FERNANDES¹,
Talita Sarah MAZZONI¹

Affiliations:

¹ Universidade Federal de Alfenas

Keywords: Microplastics. Reproduction. Skeletal muscle tissue. Zebrafish.

Abstract:

The widespread and indiscriminate use of plastic over decades has led to environmental exposure to high concentrations of fragments resulting from its degradation, known as microplastics (MPs). These particles enter the body of any living organism through the ingestion of water, food, or indirect exposure, circulate throughout the organism, and accumulate in organs and tissues, causing harmful effects. It is known that MPs can affect various organs, but their effects on muscle tissue remain poorly understood. This study aimed to analyze the correlation between morphological and functional changes in skeletal striated muscle tissue and the reproductive success of *Danio rerio* (zebrafish) after exposing adult fish to a concentration of 0.5g/L of MPs for 30 days (CEUA - nº 4503250320 - IBB - UNESP, BOTUCATU). The treated group exhibited anatomical alterations, such as muscle atrophy after 15 days of exposure (de), with muscle damage worsening after 30de. Histological analysis of the skeletal muscle tissue of these animals revealed severe morphological alterations, including structural disorganization of myofibrils and muscle fibers, morphological changes in cell nuclei, an increased collagen fiber content, and fragmentation of muscle bundles and fibers. These changes led to a decrease in muscle fiber diameter, a reduction in the number of muscle cells, and muscle tissue rupture, which led to muscle atrophy and fibrosis formation. MPs, detected in muscle tissue through acid digestion, caused significant damage to the zebrafish's body structure. Despite the muscle tissue was severely affected, the animals were still able to reproduce, producing a large number of eggs, similar to the control group. However, the offspring mortality rate exceeded 70% in the treated group. Given that muscle tissue is vital for maintaining physiological homeostasis, it was concluded that MPs can significantly compromise the health and overall well-being of living organisms, including humans. Financial Support: Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) – Process No APQ-02933-21. Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) – Process No 163231/2024-9.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: An analysis of data on the Impacts of aging on Human Skin cells

Authors:

Esther Midori YABUUTI¹,
Vinícius de Rezende RODOVALHO²,
Hosana Gomes RODRIGUES¹

Affiliations:

¹Faculdade de Ciências Aplicadas, Universidade Estadual de Campinas, Limeira, São Paulo, Brasil
²Laboratório Nacional de Computação Científica, Petrópolis, Rio de Janeiro, Brasil

Keywords: Epidermal cells. Python. Senescence. Single-cell. Transcriptomic profile.

Abstract:

The aging process induces morphological and physiological changes in human skin, with a decrease in tissue elasticity, thickness, and resistance. Regions such as the face and the area around the eyes are the most affected because, in addition to senescence related to biological age, there are effects associated with photoaging caused by excessive exposure to ultraviolet radiation. Considering the complexity of the skin and the need for more in-depth characterization of these changes during aging, the technique of single-cell RNA sequencing and the analysis of these data allows the capture of unique transcriptomic profiles. Raw data was obtained from a public database (National Genomics Data Center - GSA) as part of a dataset identified as HRA000395, available at <https://ngdc.cnbc.ac.cn/gsa-human/browse/HRA000395>. FASTQ files were downloaded using FTP transfer software and processed with CellRanger and Scanpy. During the quality control step, outliers were filtered based on a threshold of 5 median Absolute deviations (MADs) for low quality cells and 3 MADs for mitochondrial and hemoglobin genes. Cells with mitochondrial counts greater than 25% and hemoglobin counts greater than 1% were also removed. Summarized pipeline consists of doublet removal (scDbtFinder), ambient RNA removal (SoupX), normalization (Scran, Shifted logarithm and Analytic Pearson residuals), feature selection, dimensionality reduction and data integration (scVI). Cell type annotation combined manual and automatic resources with marker gene input and use of CellTypist. In this analysis, the cells were grouped into two groups (young and middle-aged) revealing 10 cell types. Continuing the analyses will further contribute to characterizing the complexity of human skin using both Python- and R-based tools. Financial support: FAPESP, CNPQ, FAEPEX-PRP/UNICAMP, CAPES (finance code: 001)



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Excess of Omega-3 and Omega-6 During Development Induces Metabolic Dysfunction and Oxidative Distress in Skeletal Striated Muscle.

Authors:

Matheus Felipe ZAZULA¹
Mônica MACIEL¹
Heloisa Deola CONFORTIM²
Luiz Claudio FERNANDES³
Katya NALIWAICO¹

Affiliations:

¹ Laboratório de Plasticidade Morfofuncional, Departamento de Biologia Celular, Universidade Federal do Paraná – Curitiba, Paraná, Brasil.

² Laboratório de Plasticidade Morfofuncional, Universidade Federal do Paraná – Toledo, Paraná, Brasil.

³ Laboratório de Metabolismo Celular, Departamento de Fisiologia, Universidade Federal do Paraná – Curitiba, Paraná, Brasil.

Keywords: Polyunsaturated fatty acids; Maternal supplementation; Oxidative damage; DOHaD

Abstract:

The increased availability of nutrients during embryonic development can lead to metabolic changes in the offspring. With the Westernization of diets, lipid consumption has significantly increased, representing 30% to 40% of daily caloric intake in industrialized populations. Maternal supplementation with omega-3 polyunsaturated fatty acids is widely recommended due to its benefits for brain development. However, little research has been conducted on the potential adverse effects of excessive consumption of these fatty acids during the offspring's development. This study aimed to evaluate the impact of maternal supplementation with high doses of n-3 and n-6 on the energy metabolism and antioxidant system of the soleus and extensor digitorum longus muscles. Thirty Wistar rats were divided into three experimental groups: control (CT), supplemented with 4 g/kg of fish oil (FO) or soybean oil (SO), administered before mating, during pregnancy, and lactation. At 60 days of age (n = 12 per group), the animals were euthanized, and the muscle soleus (SOL) and extensor digitorum longus (EDL) were collected, and the enzymes related to energy metabolism and antioxidants were analysed (CEUA 1303/2019). Data were analyzed using ANOVA followed by Tukey's post hoc test; for non-parametric data, the Kruskal-Wallis test followed by Dunn's post hoc test was applied. The data were also evaluated through Principal Coordinates Analysis (PCoA) and subsequently by cluster analysis. The results showed that the offspring of mothers supplemented with FO and SO exhibited changes in energy metabolism ($p < 0.0001$ in PCoA), activation of the antioxidant system ($p < 0.0001$, in PCoA), and accumulation of oxidative damage ($p < 0.0001$, in PCoA). The alterations were more pronounced in EDL, compared to the SOL, which showed greater adaptive capacity ($p < 0.005$, in cluster analysis). Thus, although excessive FO and SO supplementation caused harmful effects in both muscles, the predominance of glycolytic fibers in the EDL was decisive for its lower adaptation capacity. These findings suggest that maternal supplementation with omega-3 and omega-6 fatty acids in high doses during pregnancy and lactation can induce significant changes in energy metabolism and the antioxidant system of offspring, with the EDL muscle being more susceptible due to its predominance of glycolytic fibers.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: CD2AP Expression After Albumin Overload in Podocyte Cell Culture with And Without Pan Injury

Authors:

Heber da Silva ANTONIO¹ Daiani
Pontes de LIMA¹ Igor da Silva
SANTANELLO¹ Raphaella Farah
GIGLI¹ Taís Nitsch MAZZOLA¹
João Victor VIRGILIO-DA-SILVA¹
Mariana Conceição da SILVA¹ José
Ronaldo Alves TROLEZE¹ Luana dos
SANTOS¹
Maricilda Palandi de MELLO¹ Vera
Santoro BELANGERO¹ André
Alexandre de THOMAZ¹ Aline Mara
dos SANTOS¹
Luiz Fernando ONUCHIC² Mara
Sanches GUARAGNA¹

Affiliations:

¹Universidade Estadual de Campinas

²Faculdade de Medicina da Universidade de São Paulo

Keywords: Proteinuria. Albumin overload. Puromycin aminoglycoside. CD2AP expression.

Abstract:

Proteinuria, a key marker of glomerular disorders and chronic kidney disease (CKD), typically results from damage to the glomerular filtration barrier, which consists of fenestrated endothelium, glomerular basement membrane, and podocytes. Podocyte injuries that cause proteinuria are known as podocytopathies. Podocytes are exposed to multiple physiological stressors that can lead to injury and proteinuria. However, the molecular effects of prolonged albumin exposure in podocytopathies remain unclear. This study examined the effects of albumin overload on CD2AP expression in podocytes under normal and pre-damaged conditions. The project received a waiver from CEP-CONEP (CEP No. 058/2023) since it involves immortalized cells and was approved by the local CIBIO (01/2016). Human immortalized podocytes were exposed to albumin concentrations (0, 3, 20 and 40mg/mL) for 24h with and without prior damage with puromycin aminoglycoside (PAN). CD2AP expression was analyzed by RT-qPCR, western blotting (WB) and immunofluorescence (IF). WB band densitometry and IF intensities were quantified using ImageJ. Statistical analyses were performed with GraphPad Prism, employing Wilcoxon and Mann-Whitney tests. No significant differences were identified in either mRNA or protein levels after albumin overload, in the conditions with and without PAN ($p > 0.05$, $n = 5$, Wilcoxon). Conversely, CD2AP immunostaining revealed a significant reduction in the fluorescence intensity at 40 mg/mL of albumin without PAN ($p < 0.0001$), more than 2.5 fold to control levels (0mg/mL); and at all albumin concentrations with PAN ($p < 0.05$), more than 2.0 fold at the 40 mg/mL compared to control levels (Mann-Whitney, $n = 14$ to 33 cells). As revealed by our IF analysis, the injury caused by PAN amplified the effects of albumin on CD2AP expression level and localization. This finding is consistent with some previous clinical reports involving glomerular injury.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Adrenal disorders in response to maternal protein restriction: Immediate and long-term metabolic disturbances in male rat offspring

Authors:

Vinícius Alexandre de Andrade FELIPE¹,

Matheus Naia FIORETTO¹, Luisa

Annibal BARATA¹, Isabelle Tenori

RIBEIRO¹,

Luiz Marcos Frediane PORTELA¹, Renato

MATTOS¹,

Flávia Alessandra MACIEL¹, Wellerson

Rodrigo SCARANO¹, Luis Antonio

JUSTULIN¹

Affiliations:

¹São Paulo State University "Júlio de Mesquita Filho" – UNESP, Department of Structural and Functional Biology, Morphology Sector

Keywords: Adrenal Gland, DOHaD, Maternal Malnutrition.

Abstract:

Malnutrition and food insecurity still affect many people worldwide, violating human rights. From this perspective, the concept of the Developmental Origins of Health and Disease (DOHaD) establishes that adversities during pregnancy and lactation can increase the risk of diseases in offspring. As a model that mimics these conditions, Maternal Protein Restriction (MPR) has already been associated with increased metabolic diseases in descendants. Considering the fundamental role of the adrenal glands in systemic characteristics and this panorama, the aim was to evaluate the consequences of MPR on the adrenal glands in male offspring rats during early life and senescence. Sprague Dawley rats were divided into 2 groups: Rats born from dams that received a normoprotein diet (CTR, 17% protein) or a low-protein diet (GLLP, 6%) during gestation and lactation. The rats were euthanized at postnatal day (PND) 21 or 540 (CEUA 5119280121). Adrenal glands and blood were collected for metabolic (n=8), histological (n=6), and molecular (n=5) analyses. Data were analyzed using Shapiro-Wilk, followed by the Student's t-test or Mann-Whitney tests, considered statistically significant when $p < 0.05$. At PND21, we observed a decrease in the fasciculate zone ($p < 0.0001$) and medulla ($p = 0.017$) in the GLLP group. At PND540, there was an increase in the fasciculate zone ($p = 0.012$), microvessels ($p = 0.014$), apoptotic immunostaining, and collagen ($p < 0.0001$) in the GLLP group. At PND21, we showed increased reduced glutathione ($p = 0.032$) and catalase activity ($p = 0.016$). There was a reduction in the *Cyp11b1* ($p = 0.043$) and an increase in the *Cyp11b2* ($p = 0.045$) gene expression in the GLLP group at PND540, in addition to high levels of corticosterone ($p = 0.006$) and lower levels of aldosterone ($p = 0.0005$). These findings suggest that MPR influences adrenal morphophysiology in early life, with long-term consequences for cellular stress and steroidogenesis in male offspring during aging.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Characterization of a glutamatergic cell model derived from SHSY5Y to study glutamate excitotoxicity in Amyotrophic Lateral Sclerosis

Authors:

Julia Luiza Rodrigues de França¹ Merari de
Fátima Ramires Ferrari¹

Affiliations:

¹Institute of Biosciences, Department of Genetics and Evolutionary Biology, University of São Paulo

Keywords: Glutamate, ALS, SOD1, SH-SY5Y, neuronal differentiation

Abstract:

Glutamate is the main excitatory neurotransmitter in the central nervous system, playing a crucial role in synaptic plasticity, learning and memory. However, dysregulation of glutamatergic neurotransmission can cause excitotoxicity, a key mechanism in neurodegenerative diseases such as Amyotrophic Lateral Sclerosis (ALS). Although the majority of ALS cases are sporadic, around 10% are familial (fELA) and related to mutations in more than 40 genes, including SOD1. The G93A mutation in SOD1 is associated with neuronal dysfunction and glutamate-mediated excitotoxicity. This study investigated the impact of SOD1 mutations on glutamatergic homeostasis, by establishing a differentiation protocol to obtain glutamatergic-like neurons using SH-SY5Y cells. The cells were cultured in DMEM/F12 with 10% FBS, 100U/ml penicillin and 100µg/ml streptomycin. Neuronal differentiation was induced with 10 µM retinoic acid and 10 ng/ml BDNF, with supplementation of 5% B-27 for 6 days to promote the glutamatergic phenotype. A second 12-day protocol using dopaminergic inducing factors was also tested. Differentiation was assessed by immunocytochemistry with anti-MAP2 and anti-VGLUT antibodies. Western blot analysis showed that the 6-day protocol resulted in a significant increase in VGLUT expression, while a group of undifferentiated cells used as negative control, and the 12-day protocol maintained basal levels. The results suggest that the 6-day protocol more effectively induces a glutamatergic phenotype, establishing a reliable model for studying the interaction between glutamate and the SOD1 mutation in ALS. This study was supported by FAPESP-2024/06532-9.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Role of Osteoprotegerin from Apical Papilla Stem Cells in Monocyte Differentiation into Osteoclasts *In Vitro*: TRAP Activity and Staining Analysis

Authors:

Letícia Martins SANTOS¹

Carla Rodrigues de Almeida SILVA¹

Juliana Garuba RAHHAL¹

Mariane Cristina SLONIAK¹

Cristina Cunha VILLAR¹ Carla

Renata SIPERT¹

Affiliations:

¹ Faculdade de Odontologia da Universidade de São Paulo

Keywords: SCAP. Osteoclastogenesis. Osteoprotegerin. TRAP.

Abstract:

Introduction: The resorption of mineralized tissues is a biological event present in challenging oral pathologies such as periodontal disease, apical periodontitis, and external root resorption. The role of mesenchymal cells in modulating bone metabolism is already known; however, the role of stem cells from the apical papilla (SCAP) in this context, particularly through the effect of osteoprotegerin (OPG), remains unclear. **Objective:** To investigate the role of OPG produced by SCAP in modulating monocyte differentiation under inflammatory conditions. **Methods:** The study was approved by the local Ethics Committee. CD14⁺ monocytes were isolated using magnetic beads and cultured. They were induced to differentiate into osteoclasts by adding M-CSF and RANKL in the presence of conditioned media (CM) from SCAP-anti-OPG or SCAP, collected from pre-activated cells with LPS or control. Differentiation was assessed through histochemical analysis of tartrate-resistant acid phosphatase (TRAP) on day seven, including both qualitative analysis and quantification of multinucleated TRAP-positive cells (five image fields analyzed). The cell lysates were subjected to a TRAP activity assay at 14 and 21 days, performed in triplicate. Data were analyzed statistically, adopting a 5% significance level. **Results:** The CM SCAP groups showed reduced TRAP staining, and this reduction was also observed quantitatively, with fewer multinucleated TRAP-positive cells ($p=0,0008$). When OPG was blocked, both TRAP staining and the number of multinucleated TRAP-positive cells were similar to the positive control ($p>0.10$). TRAP activity results at both experimental time points corroborated the morphological aspect of TRAP staining in the absence of LPS. In inflammatory conditions (LPS), TRAP activity in the CM SCAP group was significantly higher than in the control group (increase $>95\%$ at both time points; $p < 0.0001$). **Conclusion:** SCAP-conditioned media inhibited the osteoclastogenic differentiation of monocytes in vitro in an OPG-dependent fashion.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Decoding the Impact of Hepatic Fibrogenesis in Gap Junction: Insights from a Multicellular Spheroid Model

Authors:

Cibele Ferreira PIMENTEL^{1,2,3,8,*},
João Marquês Duque Moreira PORTO¹,
Lucas Pires GUARNIER⁴,
Júlia Helena Oliveira BARROS¹,
Sharmila ORTIZ⁶,
Matheus Freitas ITABORAHY^{7,8},
Isadora Zhong Liang Ferreira FENG^{7,8},
Maria Eduarda FAVALESSA^{7,8},
Gabriela Oliveira Alves Moreira DE CARVALHO^{1,3},
Christina Maeda TAKIYA⁵,
Maria Fátima LEITE^{7,8},
Marlon Lemos DIAS^{1,8},
Fabio da Silva Azevedo FORTES^{2,3},
Regina Coeli dos Santos GOLDENBERG^{1,8}

Affiliations:

¹Grupo de Estudos do Fígado, Instituto de Biofísica Carlos Chagas Filho (IBCCF), Centro de Pesquisa em Medicina de Precisão (CPMP), Universidade Federal do Rio de Janeiro (UFRJ) ²Programa de Pós-graduação em Biomedicina Translacional Mestrado e Doutorado - BIOTRANS /Unigranrio/InMetro/UERJ-ZO, Rio de Janeiro ³Laboratório de Terapia e Fisiologia Celular e Molecular (LTFM), Universidade Estadual do Rio de Janeiro (UERJ-ZO), Rio de Janeiro ⁴Departamento de Genética, Escola de Medicina Ribeirão Preto, Universidade de São Paulo (USP), Ribeirão Preto, São Paulo ⁵Laboratório de Imunopatologia (LIPATO), IBCCF-UFRJ, Rio de Janeiro ⁶Laboratório de Ultraestrutura Celular Hertha Meyer, IBCCF-CPMP-UFRJ, Rio de Janeiro ⁷Departamento de Fisiologia e Biofísica, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte ⁸Instituto Nacional de Ciência e Tecnologia (INCT) - Hepatologia360°

Keywords: Hepatic fibrogenesis. Spheroids. Connexins.

Abstract:

Connexin (Cx)-formed gap junctions mediate important physiological and pathological events in the liver. However, little is known about the impact of liver fibrogenesis on gap junctions. To address this issue, a TGF- β induced in vitro fibrogenesis model was applied on multicellular spheroids (MS) obtained from HepG2, GRX (liver cell lines) and menstrual blood-derived mesenchymal cells CeSaM (n=10, Clementino Fraga Filho University Hospital Ethics Committee approval 056/09). Before, and after 7 and 14 days in culture, control and fibrotic (treated with 10 ng/mL of TGF- β) MS were submitted to volumetric ($V=4/3\pi R^3$) and phase contrast analyses. The presence of Cx26, Cx32, Cx43, albumin, α SMA, COL I and III, Ki67 and Cyp3a4 was evaluated by immunofluorescence analyses. Confocal and transmission electron microscopy were performed to detect structural alterations on MS. To evaluate functionality, MS (n=5) were treated with different concentrations of acetaminophen (1, 5, 25, 50, 60, 70, 80 mM) for 2, 5, and 24h. MS viability was accessed by PrestoBlue assay. Intracellular Ca²⁺ signalization assay was performed to investigate whether fibrogenesis impacts on MS physiology. Volumetric analysis revealed significant changes when comparing MS obtained from different cell densities (2 and 4x10⁴ cells, $p<0.01$). Fibrotic MS differed from control in size (smaller at 7 and 14d; $p<0.05$). Histological analysis revealed a spherical and uniform MS. Sirius red staining highlighted collagen distribution on fibrotic MS. Immunofluorescence analyses showed differences between Cx26, Cx32, Cx43 and COL I & III distribution between



**XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO**

**8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP**

control and fibrotic spheroids. TEM showed morphological changes between control and fibrotic MS and revealed that fibrogenesis impairs architecture of liver organelles. Dose-response assay shows that acetaminophen was metabolized and contributed to a significant decrease in viability at 2 and 24H. Sequential confocal images obtained in Fluo-4/AM loaded SM showed that fibrogenesis promoted a significant impact on Ca^{2+} signaling amplitude and ATP responsiveness when compared to control MS. Taken together, these data suggest that liver fibrogenesis can impact on gap junctions promoting structural alterations that impairs on liver physiology.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Parkin Controls K48 and K63 Ubiquitination levels During Skeletal Muscle Regeneration in Mice

Authors:

Rafael Paschini TONON¹
Isabela Aparecida DIVINO¹
Felipe Oliveira Gomes da CRUZ¹
Ana Laura Vieira da SILVA¹
Igor Luchini BAPTISTA¹

Affiliations:

¹ Laboratory of Cell and Tissue Biology (LaBCeT), School of Applied Sciences, University of Campinas, Limeira, Brazil

Keywords: Regeneration; Skeletal muscle; Parkin; Ubiquitin.

Abstract:

Some studies suggest that Parkin may have a function in the nucleus. The objective of this study is to characterize Parkin-mediated ubiquitination and its nuclear levels during skeletal muscle regeneration. The animals for this study were provided by CEMIB, CEUA 6444-1. The tibial anterior muscle (TA) of C57BL/6J (WT) and Parkin knockout (KO) mice was injured using barium chloride and was extracted at 3 and 21 days after injury (dai). Through a Subcellular Fractionation technique, nuclear and cytosolic fractions were isolated and along with the total extracts, were analyzed by Western Blot technique. To evaluate the co-localization of Parkin with myogenic proteins, histological sections slides were prepared and analyzed by Immunofluorescence. In our initial analyses, no significant difference in muscle mass was observed between the injured and control limb at 3 and 21 dai. Immunofluorescence analysis showed K48-ubiquitinated proteins accumulating around myofibrils in WT animals, while it was accumulated near the nucleus in Parkin^{-/-} animals. Meanwhile, K63-ubiquitinated proteins accumulated in WT myofiber cytoplasm, with significant reduction of this pattern in KO animals, at 3 dai. At 21 dai, ubiquitin K63 accumulates in the cytosol on both groups, however in a lower level in KO animals. Our Western Blot analysis revealed that the amount of Ubiquitin K48 3dai in the nucleus of KO animals is superior compared to the cytosol, (0.53 a.u. in WT nucleus vs 0.90 a.u. in KO). In Ubiquitin K63 21dai we have 0.10 a.u. in cytosol of WT animals vs 0.06 a.u. in KO animals. We also investigated, Pax7 21dai, and we observed 0.15 a.u. in WT nucleus vs. 0.27 a.u. in KO nucleus, indicating higher expression in KO animals. Despite this study is still in progress, we have already observed that the absence of Parkin in the skeletal muscle is able to modulate different types of ubiquitination during the regeneration process and this can be related to expression of myogenic factors.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Maternal Malnutrition Alters the Expression of lncRNAs That Regulate Interaction Networks with Targets that Contribute to the Developmental Origin of Prostate Cancer

Authors:

Luiz Marcos Frediani PORTELA¹,
Marcel Ferreira RODRIGUES²;
Jordana Inacio Nascimento OLIVEIRA³;
Gustavo Daniel VEGA-MAGDALENO⁴;
Renato MATTOS¹;
Ana Beathriz Leite LORENTE¹;
Hector Sebastian BAPTISTA¹;
Carlos Henrique SIMÕES¹;
Marcelo Augusto RIBEIRO¹;
Matheus Naia FIORETTO¹;
Isabelle Tenori RIBEIRO¹;
Guilherme Targino VALENTE²;
João Pedro de MAGALHÃES⁴;
Luis Antonio JUSTULIN¹

Affiliations:

¹Department of Structural and Functional Biology, Institute of Biosciences, São Paulo State University, Brazil.
²Experimental Research Unity, Botucatu Medical School, São Paulo State University, Brazil.
³Biology Department, Faculty of Sciences, University of Ottawa- Canada.
⁴Genomics of Ageing and Rejuvenation Lab, Institute of Inflammation and Ageing, University of Birmingham, United Kingdom

Keywords: DOHaD; lncRNAs; networks, prostate cancer

Abstract:

The DOHaD concept investigates how early-life adversities shape lifespan health. Maternal malnutrition is a possible contributor to the developmental origins of prostate cancer (PCa), though molecular mechanisms remain unclear. Pregnant rats were fed either a control (CTR, 17% protein) or a low-protein diet (GLLP, 6%) during gestation/lactation (n=15/group). On postnatal day (PND) 21, two males per litter were euthanized; the remainder were fed a standard diet until PND540. Ventral prostates were collected for RNA-seq, small RNA-seq, and proteomics (GSE180673, GSE180674) (CEUA949). We developed a pipeline to identify lncRNAs and mRNAs with RNA-Seq data. miRNAs were analysed using miRDeep2. Proteins were analysed by LC-MS/MS (MaxQuant). Differentially expressed (DE) were filtered using DESeq2 ($|\log_2FC| \geq 1$, $p < 0.05$). Co-expression networks linked lncRNAs to mRNAs; lncRNA-miRNA interactions were predicted (RNAcofold). Functional enrichment and network analysis (igraph) identified key molecules. A total of 1489 lncRNAs were identified in the prostate rat. At PND21: 14 lncRNAs, 196 DEGs, 15 miRNAs, and 289 DEPs were altered; at PND540: 35 lncRNAs, 279 DEGs, 20 miRNAs, and 68 DEPs. The PND21 network contained 259 molecules and 798 edges, while the PND540 network included 215 molecules with 1082 interactions. Functional enrichment showed that at PND21 the cell cycle and chromatin condensation were involved, while at PND540 inflammatory pathways and cellular secretion. We identified 4 molecules in common: Man2a1, Cyp2b12, Ccdc172, and Slc34a2, which was validated Cyp2b12 (up at PND21, $p=0.0123$; downregulated at PND540, $p=0.0282$). Our results show that maternal malnutrition modulate lncRNAs expressions, leading to deregulation of important signalling pathways involved in prostate developmental biology with potential long-term consequences for prostate carcinogenesis with aging.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Using therapeutic ultrasound in different cell lines exposed to Lipopolysaccharide

Authors:

Larissa Gonçalves SILVA¹ Ramony
Gonzaga VIEIRA² Ana Beatriz
Farias de SOUZA¹
Laser Antônio Machado OLIVEIRA² Frank
Silva BEZERRA¹

Affiliations:

¹Laboratory of Experimental Pathophysiology (LAFEx), Department of Biological Sciences (DECBI), Institute of Exact and Biological Sciences (ICEB), Federal University of Ouro Preto, Ouro Preto, MG, Brazil.

²Laboratory of Neurobiology and Biomaterials, Department of Biological Sciences(DECBI), Institute of Exact and Biological Sciences (ICEB), Federal University of Ouro Preto (UFOP), Ouro Preto, MG, Brazil.

Keywords: Ultrasound, Lipopolysaccharide, J774A.1 cells; MRC5 cells.

Abstract:

Therapeutic ultrasound is a non-invasive technique frequently employed for its anti-inflammatory and tissue-repairing effects. Lipopolysaccharide (LPS), a potent bacterial endotoxin, it is commonly utilized in experimental models to induce controlled inflammatory responses, mimicking pathological conditions such as sepsis and chronic inflammation. This study evaluated the effects of therapeutic ultrasound using an in vitro experimental model of lipopolysaccharide (LPS) exposure. J774A.1 cells (murine macrophages) and MRC5 cells (human fibroblasts) were used. Six groups were assessed: Control; LPS-infected (LPS) cells exposed to LPS for 3 hours; cells exposed to ultrasound for 8 minutes at a frequency of 1 MHz (T1F1); cells exposed to ultrasound for 8 minutes at a Frequency of 3 MHz, (T1F2), LPS-infected cells exposed to ultrasound for 8 minutes at a frequency of 1 MHz (LPS T1F1), LPS-infected cells exposed to ultrasound for 8 minutes at a frequency of 3 MHz (LPS T1F2). The main parameters evaluated were cell viability and metabolism through specific assays. The MTT analysis for MRC5 cells revealed reduced viability (%) in the LPS group (91.14 ± 2.78) compared to the control (100 ± 1.966) ($p < 0.0001$). However, the LPS T1F1 (93.58 ± 1.82) group displayed improved viability relative to LPS exposure alone ($p < 0.0001$), while LPS T1F2 (80.54 ± 4.42) reduced cell viability compared to the control. Regarding J774 cells, LPS exposure resulted in lower viability (85.72 ± 1.47) than the control (100 ± 1.02) ($p < 0.0001$), enhanced viability was observed in the LPS T1F1 group (100.30 ± 1.64), while T1F2 showed no significant differences compared to the LPS group. Preliminary results indicate that therapeutic ultrasound may affect cell viability in LPS-induced inflammation, suggesting its potential as a complementary treatment for inflammatory conditions. Its application could enhance current therapeutic strategies. However, more experiments are needed to prove our initial hypothesis.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Evaluation of antitumoral activity of three traditional plants in prostate cancer cells, *in vitro*

Authors:

Marina Andrade ROCHA¹
Ana Gabriela Silva OLIVEIRA¹ Carlos
Alexandre CAROLLO² Katyuze de
Souza FARIAS² Hélio Batista
SANTOS³
Ralph Gruppi THOMÉ³ Moon
Nyeo PARK⁴ Bonglee KIM⁴
Rosy Iara Maciel de Azambuja RIBEIRO¹

Affiliations:

¹Federal University of São João del Rei, Experimental Pathology Laboratory ²Federal University of Mato Grosso do Sul, Pharmacognosy Laboratory ³Federal University of São João del Rei, LAPROTEC
⁴Kyung Hee University, Department of Pathology, College of Korean Medicine

Keywords: Cytotoxic. Molecular biology. Prostate cancer. Traditional plants.

Abstract:

Prostate cancer is the second most common cancer worldwide, with an estimated 2.4 million new cases by 2040. Current treatments are often ineffective and cause significant side effects. Currently, several antitumor drugs are derived from plants, in addition to other medications. The traditional plants 27VI, 42VI and 43VI are popularly used as tonics or anti-inflammatories, however, there are few records in the literature regarding their antitumor activities. This study evaluated the antitumor activities of crude extracts from the roots of these plants *in vitro*. The extracts were characterized using LC-DAD-MS, and cell viability and selectivity (SI) were assessed by MTT assays. Additional analyses included MMP-9 activity, cell adhesion, invasion, and migration assays. Morphological changes were observed via fluorescence microscopy, and protein expression was analyzed by Western blotting. Phytochemical analysis identified ginsenosides, saponins, alkaloids, and both known and unknown compounds. The plant extracts significantly reduced the viability of the PC-3 prostate cancer cell line. The IC₅₀ values for 27VI, 42VI, 43VI, and cisplatin were 1853 µg/mL, 2863 µg/mL, 2327 µg/mL, and 150 µg/mL, respectively. The selectivity indices were 4.1, 3.4, 3.6, and 1.2 for 27VI, 42VI, 43VI, and cisplatin, respectively. Treatment with the extracts did not affect MMP-9 activity. Cell adhesion was reduced with 42VI and 43VI at 312 µg/mL but increased with 43VI at 2863 µg/mL. All three extracts significantly reduced cell invasion and migration. Morphological changes such as membrane blebbing, vacuoles, loss of membrane integrity, and DNA fragmentation were observed. No significant changes in protein expression were found with 27VI and 43VI, but 42VI reduced mTOR and Bax expression and increased N-cadherin and Snail expression. In conclusion, all three extracts effectively reduced cell viability, invasion, and migration, suggesting apoptosis and autophagy as key cell death pathways.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Assessment of hypothalamic inflammation and the blood-brain barrier in an experimental colitis model associated with high-fat diet consumption

Authors:

Catarina MICHELONE¹ Caroline
Lobo COSTA¹ Marcio Alberto
TORSONI¹

Affiliations:

¹ FCA – UNICAMP

Keywords: High-fat diet. Experimental colitis. Blood-brain barrier. Gut-brain axis.

Abstract:

Inflammatory bowel diseases (IBD) are prevalent and carry significant morbidity. These conditions have multifactorial origins, with nutrition playing a key role in their progression. Research indicates that a high-fat diet (HFD) exacerbates inflammation by reducing the expression of the alpha-7 nicotinic acetylcholine receptor ($\alpha 7nAChR$), known for its anti-inflammatory properties. The gut-microbiota-brain axis and its connection to blood-brain barrier (BBB) dysfunction are also relevant. This study aimed to investigate the effects of HFD on BBB permeability and inflammation in C57BL/6 wild-type mice with dextran sodium sulfate (DSS)-induced colitis. Male mice were divided into four groups: control, HFD, colitis with control diet, and colitis with HFD (45%). After 4 weeks, hypothalamic tissue was analyzed via Western blot and RT-qPCR. Body weight changes and colitis severity were monitored. CEUA 6342-1/2023. Results revealed that the CT+DSS group experienced greater weight loss and higher disease activity compared to the CT and HFD+DSS groups. During DSS induction, weight loss was more pronounced in CT+DSS than in HFD+DSS. Colon length was shorter in CT+DSS and HFD groups compared to control, with further shortening in HFD+DSS relative to HFD alone. Gene expression of TNF- α and IL-6 showed no significant differences, while IL1B was elevated in CT+DSS compared to control; HFD and HFD+DSS groups showed lower IL1B levels than CT+DSS. No differences were observed in the mRNA levels of tight junction proteins ZO-1, Occludin, Claudin-1, and Claudin-4, nor in the protein levels of Occludin, ZO-1, and α -Catenin. In conclusion, DSS effectively induced colitis, while HFD reduced weight loss and disease severity but negatively impacted intestinal integrity. Increased IL1B expression in the hypothalamus of CT+DSS mice suggests a central inflammatory response linked to peripheral inflammation. Further studies are needed to explore the effects of colitis and HFD on BBB integrity.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Anti-Glioma Potential of the *Dioclea violacea* Lectin

Authors:

Rudimar MARTINS JUNIOR¹
Nicole SARTORI¹
Ana Carolina SANTOS¹
Valéria BRESOLIN¹ Clara
SUAREZ²
Messias Vital de OLIVEIRA² Benildo
Sousa CAVADA²
Kyria Santiago NASCIMENTO²
Josiane MANN³
Rodrigo Bainy LEAL³

Affiliations:

¹ Universidade Federal de Santa Catarina
² Universidade Federal do Ceará
³ Universidade Federal de Santa Catarina

Keywords: Glycans. Glioblastoma. Lectins.

Abstract:

Glioblastoma is an aggressive primary brain tumor, characterized by rapid growth and resistance to conventional therapies. These features highlight the need for new therapeutic strategies. Tumor cells exhibit altered glycosylation patterns on the cell surface, which may be associated with the activation of signaling pathways involved in cell survival, proliferation, and migration. Lectins are a group of proteins with a carbohydrate recognition domain that reversibly binds to isolated glycans or glycoconjugates, representing a potential strategy for antitumor therapies. Previous studies with the leguminous lectin from *Dioclea violacea* (DvL) demonstrated its antitumor potential against glioma cell lines C6 and U87. The present study aimed to further understand the biochemical mechanisms of antitumor activity triggered by DvL. Hence, C6 glioma cells were exposed to DvL (1–100 µg/ml) for 6–12 hours. The parameters evaluated included: cell morphology, by microscopy; cell viability, using the MTT assay; induction of autophagy, through Western blot analysis of LC3II protein expression, as well as by assessment of acidic vesicle formation (using acridine orange staining followed by fluorescence microscopy). Additionally, the potential internalization of the lectin was analyzed by confocal microscopy using DvL-FITC. The results showed that DvL caused morphological changes and reduced cell viability at all tested concentrations ($p < 0.0001$). Moreover, it induced the formation of acidic vesicles and increased LC3 expression ($p < 0.05$), indicating stimulation of autophagy. Fluorescence images suggest internalization into the cells, although the mechanism remains unknown. These findings reinforce the antitumor potential of DvL in glioma cells. However, further studies will be necessary to uncover the signaling mechanisms involved in its action.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Isolation and characterization of human limbus-derived stromal/mesenchymal stem cells for regenerative medicine and ophthalmological and treatment of ocular surface diseases

Authors:

João Pedro Arantes Aun MIGUEIS¹; Caroline Nascimento BARQUILHA^{1,2}; Helga Caputo Nunes HOLZHAUSEN^{1,2}; Damiana Pedro de OLIVEIRA¹; Mariane Aparecida RISSO^{1,2}; Rafael Júnior de AZEVEDO²; Ana Carolina Migliorini FIGUEIRA²; Mônica ALVES¹

Affiliations:

¹Department of Ophthalmology, School of Medical Sciences (FCM), State University of Campinas (UNICAMP), Campinas, SP, Brazil.

²Brazilian Biosciences National Laboratory (LNBio), Brazilian Center for Research in Energy and Materials (CNPEM), Campinas, SP, Brazil.

Keywords: Cell therapy. Ocular surface diseases. Regenerative Medicine. Stem cells.

Abstract:

Severe cases of ocular surface diseases (OSDs) demand new perceptions of treatment. The use of limbal stem cells (LSCs) has emerged as a promising tool in cell therapy, once they renew the cornea and prevent conjunctivalization around the area. Despite that, tissue sources to obtain these cells are limited, and there is no consensus about the isolation process. Thus, this project aimed to standardize protocols to isolate and characterize LSCs under microbiological and quality control for pre-clinical and clinical application. Four samples of scleral-cornea ring were collected in partnership with the Biobank of the Brazilian Biosciences National Laboratory (LNBio/CNPEM, Campinas, SP, Brazil - CAAE: 82902024.3.0000.5404). Protocols for decontamination, isolation, and culture of LSCs were standardized and validated. Cells are being characterized by morphological analyses, Population Doubling Time (PDT), potential to tri-lineage differentiation, and immunophenotyping (flow cytometry). Briefly, the limbic area of the scleral-corneal rings was cut into small fragments, washed with PBS, and incubated overnight with a decontamination solution. Then, fragments were incubated overnight with Collagenase I in DMEM at 37°C. The remaining tissue was dissociated, filtered, and centrifuged. The pellet of cells was resuspended, counted, and cultured. The obtained LSC population presented fibroblastoid morphology, typical of stromal/mesenchymal stem cells. The average PDT from passages 2 to 8 was 38 hours. Furthermore, preliminary results demonstrated that LSCs were positive to CD90 (>90%) and negative to CD34/CD45 (<5%). Also, they were able to differentiate into osteogenic, adipogenic, and chondrogenic cells. Our partial results highlight the plasticity and potential of LSCs to be used in regenerative medicine to treat OSD. The implementation of standardized protocols may increase the availability of characterized LSCs for pre-clinical and clinical applications in ophthalmology.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Antitumor action of ConBr lectin on glioma cells and relationship with p38MAPK activity

Authors:

Valéria Bresolin da ROSA¹ Ana
Carolina dos SANTOS¹ Nicole
SARTORI¹
Rudimar Martins JUNIOR¹ Kyria
Santiago NASCIMENTO² Messias
Vital de OLIVEIRA² Clara SUAREZ²
Rodrigo Bainy LEAL¹

Affiliations:

¹ Universidade Federal de Santa Catarina ²
Universidade Federal do Ceará

Keywords: Glioma; lectins; oxidative stress; p38MAPK; SB203580

Abstract:

Gliomas are a type of brain tumor that originate from glial cells, with glioblastoma multiforme (GBM) being the most aggressive and lethal form, presenting an average survival of 15 months. Standard treatment involves surgical removal, chemotherapy with Temozolomide (TMZ), and radiotherapy; however, tumor cells commonly develop resistance to these therapies, highlighting the need for more effective and selective therapeutic strategies. GBM cells exhibit genetic mutations that alter signaling pathways involved in cell proliferation, survival, invasion, and angiogenesis. Additionally, tumor cells display altered activity of glycosyltransferase enzymes, leading to modifications in the glycosylation pattern of surface proteins and lipids. Lectins are proteins that selectively recognize and bind to glycans, and several studies have shown that plant lectins can exert biological effects, including antitumor properties. Our group previously demonstrated antiglioma activity of the lectin from *Canavalia brasiliensis* seeds (ConBr), potentially through inhibition of the cystine/glutamate exchanger (system Xc⁻), which is responsible for cystine uptake used in glutathione synthesis—a crucial antioxidant that protects tumor cells from oxidative stress and ferroptosis. The mitogen-activated protein kinase (MAPK) family, through phosphorylation mechanisms, regulates processes such as proliferation, differentiation, migration, and cell death. p38MAPK, in particular, plays a key role in tumor cell responses to stimuli like oxidative stress and inflammation, influencing the balance between cell survival and death. Considering ConBr's ability to inhibit system Xc⁻ and promote oxidative stress, our study aimed to investigate whether this activity is related to the activation of p38MAPK. Using glioma C6 cell culture treated with ConBr (30 and 50 µg/mL for 24h), we observed reduced cell viability via the MTT assay. ConBr also induced autophagy, evidenced by acidic vesicle staining using Acridine Orange. Additionally, Western blot analysis revealed increased p38MAPK phosphorylation after treatment with ConBr (50 µg/mL for 12h), suggesting that p38MAPK is a signaling component activated by ConBr, possibly due to increased reactive species from system Xc⁻ inhibition. Notably, this activation was not blocked by the p38MAPK inhibitor SB203580. These results emphasize the importance of further investigating ConBr's mechanism of action, including alternative pre-incubation or co-incubation protocols with SB203580, and evaluating whether antioxidant pre-treatment can block p38MAPK activation. Finally, the modulation of downstream targets of p38MAPK (e.g., Hsp27) should also be examined.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Exercise-improved muscle APLNR expression is impaired in kidney injury and restored by STAT3 inhibition

Authors:

Gabriel PEREIRA¹
Danilo Candido de ALMEIDA¹
Rafael Luiz PEREIRA²

Affiliations:

¹ Universidade Federal de São Paulo
² Universidade Federal do Paraná

Keywords: Bioinformatics. Differentially expressed genes. Resistance training. Tissue crosstalk.

Abstract:

Resistance training (RT) protects against muscle deterioration in chronic conditions, including chronic kidney disease (CKD). This study investigates how RT, combined with STAT3 inhibition, preserves muscle tissue and renal parameters in a doxorubicin-induced injury model and highlights the role of APLNR in this process. Male BALB/c mice followed two protocols: (i) sedentary (SED) or (ii) 8 weeks of RT. After training, mice received a single i.v. dose of doxorubicin (DOX, 10 mg/kg) and Stattic (ST, 2 mg/kg). Muscle parameters were assessed via histology, while renal function was evaluated by albuminuria and fibrosis. Ethics approval was granted by the Federal University of Paraná (CEUA 1184). Data from RNA sequencing of muscle samples of CKD patients (GSE157712) was analyzed. Doxorubicin-treated animals showed body weight loss, but the DOX+ST RT group had less weight reduction over time (AUC; DOX+ST RT: -50.4 ± 27.2 vs. DOX-SED: -109.0 ± 30.6 , $p=0.033$). Albuminuria was lower in DOX+ST RT than in DOX-SED (median, day 7: 0.121 vs. 1.65, $p=0.026$; day 14: 0.215 vs. 3.10, $p=0.008$). Renal histology confirmed fibrosis in DOX-treated groups. Muscle histology showed RT-induced structural changes. These changes were absent in DOX mice, although DOX+ST RT maintained exercise-induced adaptations: increased capillarity (2.27 ± 0.061 vs. 1.36 ± 0.037 , $p<0.001$), nuclei per fiber (2.84 ± 0.036 vs. 1.70 ± 0.022 , $p<0.001$), and central nuclei (3.16 ± 0.44 vs. 1.26 ± 0.179 , $p<0.001$). Enrichment analysis of 769 downregulated genes in the muscle of CKD patients highlighted roles in muscle differentiation and angiogenesis, with APLNR gene as a key component. APLNR expression was reduced in DOX RT mice ($p=0.007$ vs. control), but ST treatment restored its levels ($p=0.004$ DOX+ST vs. DOX). APLNR expression correlated with histological parameters (moderate: $R>0.5$; strong: $R>0.7$). Thus, RT combined with STAT3 inhibition protects muscle tissue in CKD, with APLNR playing a key role in maintaining muscle integrity.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Walker 256 tumor induces histomorphological alterations in the rat plantaris muscle 14 days after inoculation

Authors:

Letícia Dalla Vechia HENSCHÉL¹ Flávia
Camille FAGUNDES¹
Maria Eduarda Rizzardi de LIMA¹ Maria
Clara Chirnev da SILVA¹ Luiz Claudio
FERNANDES¹
Katya NALIWAIKO¹

Affiliations:

¹Federal University of Paraná

Keywords: Cancer cachexia. Muscle atrophy. Walker 256 tumor.

Abstract:

Walker 256 tumor (W256) is a model of cancer cachexia (CC) in rats. CC is characterized by body mass loss, associated with muscle atrophy. However, detailed descriptions of morphological, morphometric, and fiber-type changes in muscles from W256-bearing animals are still lacking. Ninety-day-old Wistar rats were divided into two groups: control (C, n=5) and W256-bearing (W, n=5). W animals were inoculated with 2×10^7 W256 cells. All procedures were approved by the Ethics Committee on Animal Use (CEUA 1474) at the Federal University of Paraná. On day 14, animals were euthanized, and the plantaris muscle was collected and cryopreserved. Histological sections were stained with NADH-TR for fiber typing and area measurement, and with hematoxylin and eosin for capillary assessment and major (dM) and minor (dm) fiber diameters. Tumor presence increased capillary number (W:30.8±8.7 vs. C:17.6±3.5, p=0.014). Conversely, dM (W:39.2±6.4 vs. C:68.8±5.1 μm , p<0.0001) and dm (W:20.1±1.4 vs. C:33.4±2.9 μm , p<0.0001) were reduced in W, along with dM-dm difference (W:19.1±5.5 vs. C:35.4±4.8 μm , p=0.0011), indicating higher fiber deformity. Type IIA (W:1620±405 vs. C:2067±232 μm^2 , p=0.041) and IIB (W:2528±268 vs. C:3301±259 μm^2 , p=0.0005) fiber areas were significantly reduced in W, but not type I fibers (W:920±207 vs. C:1001±89 μm^2 , p=0.4). W group had a higher proportion of type I fibers (W:29%±3 vs. C:21%±6, p=0.0258) and fewer type IIA fibers (W:37%±3 vs. C:47%±3, p=0.0003). In conclusion, W256 presence led to increased capillarity and muscle fiber deformity, with type I fibers showing resistance to atrophy.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Melanoma spheroid age negatively affects response to single-dose treatment

Authors:

Felipe Kennedy Carvalho TORQUATO¹
Adrielly Cintra SILVA¹
Bruno Fonseca BRANCO¹
Letícia Michelle FERNANDES¹
Tiago de Lima ERCOLIN¹
Beatriz Aires LOPES¹
Carmen Veríssima Ferreira HALDER¹

Affiliations:

¹ Department of Biochemistry and Tissue Biology, Biology Institute (IB), Universidade Estadual de Campinas (UNICAMP)

Keywords: 3D culture. Biomimetic. Melanoma.

Abstract:

The use of *in vitro* 3D culture models offers a viable alternative for obtaining results that closely resemble those found in *in vivo* studies. This important difference is taken into consideration during research that investigates the tumor microenvironment and its particularities. In this study, we aimed to understand the response of melanoma mini-tumors to single-dose treatment with different concentrations of violacein and 5-Fluorouracil (5-FU) and spheroid ages. For this, SKMEL-103 melanoma cell line (10,000 cells per well) was plated in 96 wells cell repellent plate and maintained in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% FBS (Fetal Bovine Serum), to form spheroids. Treatments were applied to spheroids of different ages, starting from an immature stage (24h old), to 5 and 10 days old. A single dose (0.5 and 1 μ M) of violacein (pigment produced by *Chromobacterium violaceum*) was used. For the treatment with 5-FU, 5, 10 and 25 μ M, with the control consisting only of supplemented DMEM medium. All spheroids underwent partial medium renewal every 4 days (100 μ L were removed from each well and replaced with the same volume of fresh medium). Images of the spheroids were captured over the course of 20 days. We noticed that 24h old spheroids treated for 24h with 1 μ M violacein showed a slower area change compared to the control. We observed that 24-hour-old spheroids treated with violacein exhibited a visible morphological change in terms of area. However, older spheroids (5 and 10 days old) challenged with the same dose showed no response. The spheroids were also treated with 5 μ M of 5-FU. Interestingly, we detected that the morphological effect on the spheroids was only visible after 48h of exposure. Spheroids displayed smaller size, but maintained their structural characteristics. This study reveals that using spheroids with different lifespans for compound screening makes the mimetic model more robust.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: The relevance of Nrf2 pathway in the progression of periodontitis and the effect of its activation/inhibition

Authors:

Lucas Nogueira RAMOS¹
Vitória Bonan COSTA¹
Mariely Araújo de GODOI¹
Angelo Constantino CAMILLI¹
Bruna Carolina da SILVA²
Isabela Rinaldi Gomes NOGUEIRA¹
Morgana Rodrigues Guimarães STABILI¹

Affiliations:

¹São Paulo State University (UNESP), School of Dentistry at Araraquara, Araraquara, Brazil.
²Federal University of São Paulo (UNIFESP), Paulista School of Medicine, São Paulo, Brazil.

Keywords: Dimethyl Fumarate. Nrf2. Periodontitis.

Abstract:

The production of antioxidant enzymes necessary to counteract the harmful effects of oxidative stress is partly regulated by the Nrf2 (nuclear factor erythroid 2-related factor 2) signaling pathway. The present study aimed to evaluate the protective role of the Nrf2 activator, dimethyl fumarate (DMF), on the progression of periodontitis in an experimental model of ligature-induced periodontitis in rats (Ethics Committee number 12/2021). DMF (50, 100 and 150 mg/kg) was administered orally for 10 days in periodontally diseased animals. To evaluate whether the effects of DMF on gingival tissue are dependent on Nrf2, a group of animals treated also received concomitant administration of the selective Nrf2 inhibitor (ML385, 15 mg/kg). The effects of Nrf2 activation and inhibition on periodontal tissues were evaluated according to the following parameters: percentage of mandibular bone volume (bone microtomography - μ CT); quantification of osteoblasts in histological sections; presence of inflammatory infiltrate (stereometry) and density of collagen fibers (generation of second harmonic); expression of oxidative stress mediators (MDA (TBARS method), catalase (RT-qPCR) and 8-OHdG (immunohistochemistry)) in gingival tissues; TNF-alpha levels (ELISA). The values were subjected to the homoscedasticity test and ANOVA with Tukey's post-test, establishing the significance level ($p < 0.05$). DMF prevented bone loss, increased osteoblasts numbers, reduced cellular infiltrate, increased density of collagen fibers, inhibited MDA and TNF levels in gingival tissues, and increased Catalase expression. Although all doses of DMF showed an anti-osteolytic effect, the lowest dose (50mg/kg) showed a greater antioxidant and anti-inflammatory effect. Effects of DMF on bone volume, MDA, TNF, Catalase levels and cell proportion were reversed by Nrf2 inhibition. The results indicate that Nrf2 activation by DMF plays a relevant role in the progression of periodontitis.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Lipid overload leads to intra- and extracellular miR-122 modulation in an *in vitro* hepatic steatosis model

Authors:

Maria Laura Delatorre MALVEZZI¹
Carolina PANZARIN¹
Marciane MILANSKI¹
Letícia Ignácio de Souza ZIMMERMANN¹
Marcio Alberto TORSONI¹
Adriana Souza TORSONI¹

Affiliations:

¹Faculty of Applied Sciences - UNICAMP.

Keywords: MASLD. miR-122. NEFA. VLDL.

Abstract:

Hepatic steatosis is defined by excessive lipid accumulation of 5% or more in hepatocytes and, when associated with systemic metabolic dysfunction, is referred to as metabolic dysfunction-associated fatty liver disease (MASLD). In the MASLD cenário, studies suggest that the liver increases VLDL secretion to compensate the increased lipids influx to the liver. However, this response is insufficient for disease remission. In addition to elevated VLDL levels, researchers have reported increased circulating miR-122 in MASLD patients compared to healthy patients. The miR-122 is a liver-specific microRNA, but it remains unclear whether VLDL can transport miR-122 under lipid overload conditions. To investigate miR-122 levels under lipid overload in hepatocytes, a time course was done with non-esterified fatty acids (NEFA, 500uM) in cells derived from mouse hepatocellular carcinoma (Hepa1c1c7) to mimic lipid overload during 6 hours, 12 hours and 24 hours and the control group (C) was maintained with NEFA vehicle, NaOH and albumin. The expression of hepatic miR-122 was greater with NEFA 6h compared to C (NEFA: 2.20 ± 0.19 vs C: 1.0 ± 0.06 ; $p < 0,05$). The expression of hepatic miR-122 was lower with NEFA 24h compared to C (NEFA: 0.84 ± 0.05 vs C: 1.0 ± 0.06 ; $p < 0,05$). The expression of miR-122 in the cellular medium was greater with NEFA 12h compared to group C (NEFA: 11.30 ± 4.64 vs C: 1.0 ± 0.13 ; $p < 0,0001$). In conclusion, these findings are consistent with previous studies showing that hepatic miR-122 levels increase in hepatocytes during the early stages of MASLD but decrease in advanced stages, such as steatohepatitis. Additionally, studies indicate that circulating miR-122 levels are higher in the context of MASLD, which we also observed *in vitro*, with greater miR-122 levels detected in the hepatocyte culture medium. The next steps will focus on identifying whether the exportation of miR-122 from hepatocytes is mediated by VLDL by inhibiting its secretion using Dimaprit.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Parkin overexpression alters myogenic factors during c2c12 differentiation in a dependent proteasomal activity manner

Authors:

Isabela DIVINO^{1,2}
Rafael Paschini TONON¹ Ana
Laura Vieira da SILVA¹
Felipe Oliveira Gomes da CRUZ¹
Michelle Barbosa ROSÁRIO¹ Åsa
GUSTAFSSON²
Igor Luchini BAPTISTA¹

Affiliations:

¹Laboratory of Cell and Tissue Biology (LaBCeT), School of Applied Sciences, University of Campinas, Limeira, Brazil

²Skaggs School of Pharmacy and Pharmaceutical Sciences, School of Medicine, University of California San Diego, San Diego, USA

Keywords: C2C12 differentiation; Parkin; Proteasomal activity.

Abstract:

C2C12 differentiation is a process regulated by multiple myogenic factors as Pax7 and Myod. During C2C12 differentiation, mitochondrial quality control is crucial for energy supply. Parkin is a E3 Ligase crucial to maintenance quality control through the Mitophagy process but Parkin also can migrate to the nucleus of the cells in stressful conditions. Therefore, the aim of this study is to understand the function of Parkin during C2C12 differentiation and analyze the effects of proteasomal inhibition on myogenic factors in Parkin overexpression. We used C2C12 cells in pre differentiation and 2, 4 and 6 days after differentiation. The cells were overexpressed with Mcherry empty (Mch), Mcherry-Parkin (WT-Pkn) and Mcherry-NLS-Parkin (NLS-Pkn). For proteasomal inhibition, we used 10 μ M of MG132 and DMSO as control. We used Western blot and Immunofluorescence techniques for analysis. Total ubiquitination levels of the C2C12 during differentiation showed an increase (Pre diff 0,5 vs 6 days diff 1,15 a.u.) In addition, We observed an increase in nucleo-positive cells for Parkin (Pre diff 48% vs 6 days diff 90%) In the Parkin Overexpression + MG132 analyses, we observed that cells overexpressed with Mch (Mch+DMSO 0,015 vs Mch+MG132 0,008 a.u.) and WT-Pkn (WT-Pkn+DMSO 0,017 vs WT-Pkn+MG132 0,011 a.u.) have a decline in Pax7 levels with MG132 treatment in pre differentiation, which did not happen with NLS-Pkn overexpression. MYOD levels at 6 days of differentiation increase with WT-Pkn overexpression (Mch+DMSO 0,022 vs WT-Pkn+DMSO 0,028 a.u.) but decline with MG132 treatment (WT-Pkn DMSO 0,028 vs WT-Pkn MG132 0,007 a.u.) which does not happen when we overexpress NLS-Pkn. In addition, Immunofluorescence images reveal that MYOD co-localizes in the nucleus of cells overexpressed with either WT-Pkn or NLS-Pkn, but with MG132 treatment, this colocalization is lost in WT-Pkn cells but not with NLS-Pkn. Our findings suggest that Parkin may regulate myogenic factors important for myoblast differentiation and that this regulation depends on proteasomal activity and its localization.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Mycoplasma Contamination Treatment Does Not Impact Murine Preadipocyte Differentiation

Authors:

Paula Araújo MONTEIRO-MAFRA¹
Rhaíssa GODOI¹
Thaís Holtz THEIZEN¹
Marcelo MORI¹

Affiliations:

¹ University of Campinas, Institute of Biology, Department of Biochemistry and Tissue Biology

Keywords: Cell culture. Differentiation. Mycoplasma. Preadipocytes. UCP-1.

Abstract:

One of the biggest concerns for cell culturists is mycoplasma contamination. This microorganism may disrupt normal cell physiology and impair the reliability of experimental results. Although decontamination protocols exist, most are time-consuming and can also interfere with normal cell growth. To reduce treatment time, we aimed to apply a high concentration of plasmocin and assess its potential impact on preadipocyte differentiation. To address this, 9W preadipocytes were seeded onto plates containing DMEM high-glucose medium, 10% FBS and 1% penicillin-streptomycin and tested for mycoplasma infection using PCR. During decontamination process, the cells were subcultured into 10 mm round dishes at maximum 70% confluence. The 9W cells contaminated with mycoplasma were treated daily with 25 and 50 µg/ml of plasmocin for 10 days. After this treatment period, mycoplasma PCR tests returned negative results. To perform a counter-test, the original cell culture was maintained in plasmocin-free medium for 7 days, after which PCR results were again negative. The decontaminated cells were then differentiated into beige adipocytes according to Rocha et al. (2021). Consecutively, Ucp1 expression, the main marker for thermogenic adipocyte differentiation, was analyzed by qPCR. The cells treated with 25 µg/ml of plasmocin exhibited a relative Ucp1 expression of 0.008 ± 0.003 , while cells treated with 50 µg/ml showed a relative expression of 0.0073 ± 0.0028 . Under these conditions, no significant changes in Ucp1 expression were observed. Therefore, although at higher doses than those traditionally used in the decontamination process, we conclude that the treatment does not appear to significantly interfere with the beige adipocyte differentiation of 9W cells.



XXXIX REUNIÃO ANUAL DA FeSBE
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XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Hypoxia-conditioned Extracellular Vesicles Restore Mitochondrial Function in Ischemia/reperfusion-induced Acute Kidney Injury

Authors:

Mayra Cristina de Souza ALVES¹,
Clara RODRIGUES-FERREIRA¹,
Jarlene Alécia LOPES¹,
Glória COSTA-SARMENTO¹,
Amaury PEREIRA-ACÁCIO¹,
Marcela ANDRADE-SOARES¹,
Adalberto VIEYRA¹

Affiliations:

¹Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

Keywords: Extracellular vesicles. Hypoxia. mesenchymal cells. mitochondria. renal ischemia/reperfusion.

Abstract: Acute kidney injury (AKI) is characterized by a sudden loss of renal function over a short period and can result from several mechanisms. When induced by ischemia/reperfusion (I/R), AKI leads to significant mitochondrial dysfunction. Due to their ability to modulate cellular responses and low immunogenicity, extracellular vesicles (EV) have emerged as promising therapeutic tools in various diseases, including AKI. This study (CEUA 081/23-UFRJ approval) aimed to evaluate the effects of EV derived from hypoxia-preconditioned mesenchymal stem cells in a rat model of I/R-induced AKI. Male Wistar rats (250–300 g) were divided into SHAM, I/R, SHAM+EV, and I/R+EV groups. The animals received subcapsular injections of EV obtained from adipose tissue-derived mesenchymal cells cultured under hypoxic conditions. The animals were subjected to bilateral renal pedicle clamping for 1 h after EV administration for 45 min, followed by 1 h of reperfusion. Kidneys were collected for histological evaluation and preparation of mitochondrial-enriched cortical fractions. The histological analysis revealed increased injury scores in I/R animals compared to SHAM (5.9 ± 0.3 vs 1.9 ± 0.1), while EV treatment significantly reduced the damage (2.6

± 0.1), bringing values close to SHAM levels. Mitochondrial respiration showed decrease in O_2 consumption coupled to ATP synthesis, rotenone-sensitive respiration, and maximal respiratory capacity in I/R animals compared to SHAM (1193 ± 174 vs 3332 ± 257 ; 2167 ± 253 vs 4276 ± 265 ; and 2173 ± 255 vs 4237 ± 262 pmol $O_2/(s \times mg)$, respectively). EV administration in I/R animals restored these parameters to levels comparable to SHAM (3545 ± 237 ; 5018 ± 369 ; and 4980 ± 369 pmol $O_2/(s \times mg)$). These findings suggest that hypoxia-conditioned EV administration represents a promising therapeutic tool for AKI, particularly by mitigating mitochondrial dysfunction.

*Honoring Professor Gerhard Malnic



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XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Heterotypic Spheroid, 3D Model for Immunotherapy Studies for Triple Negative Breast Cancer

Authors:

Samuel Iwao Maia Horita¹,
Julia Badaró Mendonça³,
Natassia Silva de Araújo³,
Rayane da Silva Abreu³,
Aline Moreira dos Santos³,
Mariana Caldas Waghbi³,
Tatiana Martins Tilli^{1,2}.

Affiliations:

¹Laboratory of Clinical and Experimental Pathophysiology - IOC/Fiocruz

²Translational Oncology Platform, Center for Technological Development in Health, Fiocruz, Rio de Janeiro, RJ, Brazil.

³Laboratory of Applied Genomics and Bioinnovations, Oswaldo Cruz Institute, Fiocruz, Rio de Janeiro, RJ, Brazil.

Keywords: Triple negative breast cancer. Advanced therapies. Immunotherapy

Abstract:

Introduction: Triple-negative breast cancer (TNBC) is an aggressive subtype lacking estrogen, progesterone, and HER2 receptors, which limits treatment options and contributes to poor prognosis and high mortality. Bispecific antibodies (BsAbs) emerge as promising immunotherapeutic agents for TNBC, as they can simultaneously target tumor antigens and activate immune cells. However, in vitro validation of BsAbs remains challenging due to the need for complex models that mimic tumor-immune interactions. **Objective:** This project aims to develop a complex heterotypic spheroid model with tumor and immune components to validate the therapeutic effects of BsAbs. **Results and Methodology:** For the initial characterization of the spheroid model, fibroblasts, endothelial cells, breast epithelial cells (non-tumor) and TNBC cells were used. The model was maintained on the magnetic apparatus in a mixed medium, prepared by combining three different culture media in a 1:1:1 ratio, for either 3 or 5 days before being processed for histological analysis. By HE staining, the spheroid showed good cohesion and viability. By immunofluorescence, it was possible to observe the presence of cytokeratin-expressing epithelial cells and endothelial cells (CD31), also the acellular portion. To incorporate immune components into the model, lymphocytes were introduced to pre-formed spheroids after 3 days of culture. The cells were added at three different concentrations (1.5×10^5 , 3×10^5 , or 1.5×10^6 cells), and the co-culture was maintained for an additional 2 days to allow for immune-tumor interactions. Anti-CD4 labeling evaluated the migration of lymphocytes into the spheroid, and the concentration of 1.5×10^6 was the one with the highest entry of lymphocytes. **Conclusion:** Heterotypic spheroids demonstrate promising characteristics for the evaluation of BsAbs: good cohesion, good cell viability, the presence of tumor cells to evaluate antitumor action, and lymphocyte migration and activation.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Evaluation of the effects of cyclic Semaglutide treatment in C57BL/6 mice with diet-induced obesity

Authors:

Nayra Fernanda de OLIVEIRA¹
Marcela Reymond SIMÕES²
Bruna BOMBASSARO²
Ana Luísa Gallo FERRAZ²
Erika Anne de Freitas Robles ROMAN²
Lício Augusto VELLOSO²

Affiliations:

¹ Paulista University (UNIP), Campinas – SP, Brazil

² Faculty of Medical Sciences (FCM), University of Campinas (UNICAMP), Campinas – SP, Brazil

Keywords: Body Composition. Cyclic Treatment. Metabolism. Obesity. Semaglutide.

Abstract:

Obesity is a multifactorial chronic disease, characterized by excessive fat, derived by an imbalance between caloric intake and energy expenditure. This state compromises individual's health by increasing the risk of developing type 2 Diabetes, cardiovascular diseases, and other metabolic complications. Overall, obesity disturbs quality of life, and influences life expectancy due to the comorbidities associated to it. Currently, we have a variety of treatments available for this condition like bariatric surgery, restricting diets, physical exercise and weight loss drugs. Among the medications GLP1 agonists are a class of medicaments that are prevailing in medical prescriptions. Semaglutide is a molecule originally designed to treat type 2 Diabetes, but it also helps in the treatment of obesity as studies demonstrate a weight loss of 15%. Nevertheless, this drug is not available on SUS and is expensive. Because of this, it is common that individuals use the medication in a discontinued way, without medical orientation. Considering that, the aim of this project is to evaluate the metabolic and physiologic impact of the cyclical treatment with Semaglutide in C57BL/6 mice, with diet-induced obesity (DIO), by measuring the caloric ingestion, ponderal evolution, body composition and glycemic profile, and comparing it to a group that receives a continuous treatment of Semaglutide and a control group in chow diet. The cyclic group presented – gradually over the four cycles – a higher caloric intake during the pauses in the treatment, followed by an increase in body weight. During the treatment stage, the glycemic profile did not differ from the controls. The body composition analysis showed a tendency in difference between lean body mass. Therefore, it's of most importance that the treatment with Semaglutide, and any other weight loss drugs is accompanied by a professional and used as it's described in the leaflet, followed by changes in life style.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Chondroitin Sulfate as a Mediator of Arthritis Severity in Chikungunya Virus Pathogenesis

Authors:

Camila de Oliveira SILVA¹
Giovane Galdino de SOUZA¹
Flávio Protásio VERAS²

Affiliations:

¹ Universidade Federal de Alfenas

² Universidade de São Paulo

Keywords: Arthritis. Chikungunya. Chondroitin sulfate. Tropism.

Abstract:

Chikungunya fever is a viral disease transmitted by *Aedes* mosquitoes, posing a growing public health challenge in tropical and subtropical regions. The Chikungunya virus (CHIKV), from the Alphavirus genus in the *Togaviridae* family, causes both acute and chronic symptoms, including fever and debilitating arthritis that often persists, severely affecting patients' quality of life. "Tropism" describes the virus's preference for specific cells and tissues, which is key to its pathogenesis. CHIKV shows a particular affinity for joint tissue cells, promoting infection and replication within connective tissues. Joint tissues comprise cells, extracellular matrix components, and glycosaminoglycans (GAGs), which may mediate the virus's tropism. Chondroitin sulfate (CS), a GAG abundant in joint tissues, is hypothesized to play a role in CHIKV pathogenesis. This study investigates how CS interacts with CHIKV and contributes to virus-induced arthritis. The experiments include *in vivo* assays using C57BL/6 mice, which received systemic injection with CHIKV and were later challenged with either CHIKV or CS. Additionally, *in vitro* analyses were performed to model the virus-CS interaction. All experiments were approved by the Ethics Committee for Animal Use (CEUA) at UNIFAL, under registration number 0027/2024. Initial results show that experimental models replicate key features of CHIKV-induced arthritis, including increased paw swelling in mice exposed to the virus or CS. Both groups exhibited edema, suggesting that CS intensifies arthritis. However, the precise mechanisms by which CS enhances these effects remain unclear. This research aims to further understand CHIKV's pathogenesis, especially how CS mediates its effects on joint tissues, potentially contributing to the development of treatments for both acute symptoms and chronic arthritis.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: The Wnt/Beta-Catenin Pathway and Cytoskeletal Filaments Are Involved in the Positioning, Size, and Function of Lysosomes during Chick Myogenesis

Authors:

Kayo Moreira BAGRI¹,
Luiz Fernando OLIVEIRA²,
Miria Gomes PEREIRA³,
José Garcia ABREU²,
Claudia MERMELSTEIN¹

Affiliations:

¹ Laboratório de Diferenciação Muscular, Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, Rio de Janeiro 21941-902, Brazil

² Laboratório de Embriologia de Vertebrados, Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, Rio de Janeiro 21941-902, Brazil

³ Laboratório de Ultraestrutura Celular Hertha Meyer, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro 21941-902, Brazil

Keywords: chicken; LAMP2; lysosome; myogenesis; Wnt/beta-catenin

Abstract:

Lysosomes are highly dynamic organelles involved in the breakdown and recycling of macromolecules, cell cycle, cell differentiation, and cell death, among many other functions in eukaryotic cells. Recently, lysosomes have been identified as cellular hubs for the modulation of intracellular signaling pathways, such as the Wnt/beta-catenin pathway. Here we analyzed morphological and functional characteristics of lysosomes in muscle and non-muscle cells during chick myogenesis, as well as their modulation by the Wnt/beta-catenin pathway. Animal procedures were approved by the Ethics Committee for Animal Use (CEUA - UFRJ) under protocol number 081/22. Vital acridine orange labeling and LAMP-2 immunolabeling show that

(i) muscle and non-muscle cells show differences in lysosomal size and its distribution, (ii) lysosomes are found in spherical structures in myoblasts and fibroblasts and tubular structures in myotubes, (iii) lysosomes are found close to the plasma membrane in fibroblasts and close to the nucleus in myoblasts and myotubes. (iv) Lysosomal distribution and size are dependent on the integrity of microtubules and microfilaments in myogenic cells, after cytoskeleton interfering drugs Taxol, Colcemid, and Cytochalasin B. (v) Alterations in lysosomal function, in the expression of LAMP2, and in Wnt/beta-catenin pathway affect the distribution and size of lysosomes in myogenic cells, (vi) the effects of the knockdown of LAMP2 on myogenesis can be rescued by the activation of the Wnt/beta-catenin pathway by cell treatment with BIO and Wnt3a conditioned media and (vii) the chloroquine Lys05 is a potent inhibitor of both the Wnt/beta-catenin pathway and lysosomal function, shown by reporter gene assay and β -catenin immunolabeling. Our data highlight the involvement of the Wnt/beta-catenin pathway in the regulation of the positioning, size, and function of lysosomes during chick myogenesis.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Generation of UHMK1 knockout in melanoma cells through CRISPR/Cas9

Authors:

Isabela David CARDOSO¹
Giovanna Gonçalves de ALMEIDA¹
Vanessa Cristina ARFELLI^{2,3}
Leticia Fröhlich ARCHANGELO¹

Affiliations:

¹ Department of Cellular and Molecular Biology and Pathogenic Bioagents, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil

² Department of Medicine III, University Hospital, LMU Munich, Munich, Germany

³ German Cancer Consortium (DKTK), partner site Munich a partnership between DKFZ and LMU University Hospital Munich (LMU Klinikum), Germany

Keywords: Cutaneous melanoma. Kinase. Splicing. UHMK1. UHM motif.

Abstract: Melanoma is very aggressive disease due to its early capability to metastasize during tumor development. Dysregulated RNA splicing produce alternative protein variants that promote tumor formation, malignancy progression, and therapeutic resistance. Splicing perturbation arise from somatic mutations or dysregulated phosphorylation of splicing factors. We demonstrated that the kinase UHMK1 regulates the phosphorylation of several splicing factors, altering global splicing patterns and affecting alternative splicing events in hundreds of transcripts. We hypothesize that UHMK1 contributes to malignant phenotypes in melanoma by dysregulating the phosphorylation of splicing factors, leading to aberrant splicing in oncogenic pathways. TCGA data analysis revealed that *UHMK1* is overexpressed in melanoma compared to nevus, with higher levels in metastases. Gene ontology analysis showed that upregulated DEGs in *UHMK1*-high melanoma samples are associated with RNA regulation, protein synthesis, and energy metabolism. To access the UHMK1 function in melanoma, A375 and WM793 cells were transfected with px458 constructs expressing Cas9 with gRNAs targeting Exon 2 of UHMK1 (KO1-4), a GFP-coding sequence, and the gRNA scaffold. Transfections were performed using lipofectamine, followed by single-cell sorting based on GFP expression. Ten knockout candidate clones were generated for each gRNA. Knockout was confirmed by western blotting and Sanger sequencing to identify deleterious alterations in the *UHMK1* locus. One clone of each monoclonal cell line was selected for further experiments. Functional assays will be conducted to characterize UHMK1 in proliferation, cell cycle, apoptosis, viability, epithelial-mesenchymal transition, and colony formation. Additionally, nanopore RNA-seq will be used to investigate *UHMK1*-regulated splicing events in melanoma. We expect the findings will shed light into UHMK1 contribution to melanoma and pave the way for new targeted therapeutic strategies.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: New Insights into the Role of the RAS Signal Transduction Pathway in Breast Cancer during Pregnancy

Authors:

Leisa LOPES-AGUIAR^{1*}
Rafaella Trevisan SCANDIUZZI¹
João Paulo Dorta MACHADO¹
Guilherme Moraes NOBREGA²
Fernanda Garanhani Castro SURITA³,
Maria Laura Costa NASCIMENTO³
André Schwambach VIEIRA¹
Maria Cristina Cintra GOMES-MARCONDES¹
Laís Rosa VIANA^{1*}

Affiliations:

¹ Department of Structural and Functional Biology, Biology Institute, University of Campinas, SP, Brazil.

² Department of Microbiology, Icahn School of Medicine at Mount Sinai, NY, USA.

³ Department of Obstetrics and Gynecology, School of Medical Sciences, University of Campinas, Campinas, SP, Brazil.

Keywords: Breast cancer, chemotherapy, placenta, pregnancy, transcriptomic.

Abstract:

Breast cancer during pregnancy (PrBC) and chemotherapy treatment can cause placental damage, impairing foetal development. These changes may have a multifactorial origin involving transcriptional, protein, and metabolic modifications. However, the molecular mechanisms underlying placental damage - particularly the transcriptomic profile - remain unknown and are the focus of this study. **Methods:** Placentas were obtained from patients diagnosed with PrBC who received chemotherapy (case group, n= 7) and from healthy participants (control group, n= 8) through the Center for Integral Attention to Women's Health biobank of University of Campinas (Ethics Committee: #65070122.0.0000.5404). Gene expression was analysed using RNA-Seq transcriptomics in the chorionic villi region of the placenta. The Differentially Expressed Genes (DEGs) were calculated by DESeq2 package and submitted to Gene Ontology enrichment analysis. *P*-value < 0.05, adjusted by Bonferroni test, was considered significant for all analyses. **Results:** Patients in the case group were older compared to the control group (34.1±4.7 vs 26.0±6.9). Conversely, gestational age (35.4±4.4 vs 38.9±1.1), placental weight (450.6±153.4 vs 682.9±138.6), and neonatal birth weight (2262.5±874.1 vs 3363.6±342.3) were lower in the case group. The Principal Component Analysis revealed an evident clustering of the samples between the groups (PC1: 26% and PC2: 16%). 919 DEGs were identified in case group compared to control group, of which 377 were downregulated and 542 were upregulated. The only significantly enriched biological process was "Ras protein signal transduction" (*P*= 0.005), involving 35 DEGs - 16 downregulated and 19 upregulated. **Conclusion:** Our results suggest that changes in placental gene expression may specifically affect pathway associated with cell growth, differentiation, and other cellular processes in patients with PrBC, which are directly related to foetal growth restriction and preterm birth.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Generation of PX458 constructs for UHMK1 knockout via CRISPR/Cas9

Authors:

Giovanna Gonçalves de ALMEIDA¹,
Maria Vitória de Rizzo GASPARINI¹,
Vanessa Cristina ARFELLI¹,
Leticia Fröhlich ARCHANGELO¹

Affiliations:

¹ Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo

Keywords: UHMK1. CRISPR/Cas9. Cloning. Melanoma. Splicing.

Abstract:

Dysregulation of splicing is an emerging hallmark of cancer. Aberrant splicing can promote cell proliferation, resistance to chemotherapy and decreased apoptosis, contributing to tumorigenesis. UHMK1 is a kinase that contains a C-terminal UHM domain that enables interaction with splicing factors such as SF1 and SF3B1. UHMK1 is overexpressed in melanoma, especially in metastatic tumors. Alternative splicing, is often dysregulated pathways in melanoma and may influence both disease progression and treatment response. Recently, we described UHMK1 as a splicing regulatory kinase. We hypothesize that UHMK1 contributes to melanoma by phosphorylating splicing factors, leading to aberrant splicing in oncogenic pathways. This study aimed to generate PX458 plasmids containing single-guide RNAs (sgRNAs) targeting exon 2 of *UHMK1*, for subsequent CRISPR/Cas9-mediated knockout in melanoma cells, allowing functional assays to be performed. Four sgRNAs (1-4) targeting *UHMK1* were designed using Benchling platform. The sense and antisense sgRNAs were annealed and one-step digestion-ligation reaction was performed to insert the annealed oligonucleotides into the PX458 vector using Bpil and T4 DNA Ligase enzymes, allowing the digestion and ligation reactions to occur simultaneously. Cloning efficiency was tested by digestion with Bpil enzyme, since the correct insertion of the oligonucleotide disrupts the FastDigest Bpil restriction site of the vector. Thus, only empty vectors could be linearized by the test digestion. Constructs were transformed into DH5- α E. coli and colonies were selected. Mini-prep DNA extraction was performed. Correct insertion of the gRNAs into the plasmid was confirmed by Sanger Sequencing. Sequences were analyzed using SnapGene software. All four PX458 constructs were successfully generated to produce *UHMK1* knockout in melanoma cells.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Morphological Analysis of a Cyclopic Fetus: Study of Possible Etiologies

Authors:

Felipe de Lara JANZ¹
Emanuelli Novossad VOLOVITZ¹
Paulo Roberto Costa de ALMEIDA¹
Gabriela Ruva FAGUNDES¹
Giovani Marino FAVERO¹

Affiliations:

¹ State University of Ponta Grossa (UEPG)

Keywords: Cyclopia. Fetal Development. Human Morphology. Trisomy 13 Syndrome.

Abstract:

Cyclopia is an alobar type of holoprosencephaly characterized by the complete or partial fusion of the eyes, the presence of a tubular nose located above the median eye, and often accompanied by postaxial polydactyly, vertebral defects, limb reduction, and transposition of the great vessels. The occurrence of this condition is approximately 1.05 per 100,000 births, including stillbirths. It is also observed that its highest incidence is in female fetuses, with the vast majority having an extremely low life expectancy, resulting in stillbirth or early death. Regarding its etiology, cyclopia is highly heterogeneous. To present a case report of a fetus with cyclopia, perform a morphological evaluation, and discuss likely etiologies of this condition. A case study was conducted on a stillborn fetus donated to the Anatomical Center of the State University of Ponta Grossa (UEPG), Paraná, Brazil. A morphological analysis was performed, including measurements of key body dimensions using a digital caliper, along with a literature review to investigate possible causes. This study was exempted from ethical review by the institution's research ethics committee. The fetus was female, in the third trimester of gestation, weighing approximately 2,800 grams. In addition to cyclopia, unilateral polydactyly, a proboscis, and a simian crease were also observed. The main measurements recorded were: head circumference: 21.3 cm; left foot length: 5.01 cm; right foot length: 5.16 cm; frontomandibular distance: 9.65 cm; and fronto-occipital distance: 5.85 cm. Given the characteristics described, one of the possible causes of the malformation is believed to be trisomy of chromosome 13 - could only be confirmed through fetal karyotyping. Other possible causes include chronic consumption of ethanol, infections such as rubella, toxoplasmosis, and herpes, as well as prolonged exposure to radiation during pregnancy. Although a rare condition, this study was able to report a case of fetal cyclopia, whose most common cause is typically trisomy of chromosome 13.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: The Role of SET7 In Cardiorenal Syndrome Type 3 Induced by Acute Renal Ischemia

Authors:

Lara Mei Honda^{1,2},
Guilherme Lunardon²,
Raquel Silva Neres dos Santos¹,
Gabriela Placoná Diniz²,
Marcela Sorelli Carneiro-Ramos^{1,2}

Affiliations:

¹Laboratory of Cardiovascular Immunology, Center for Natural and Human Sciences, UFABC, Santo André-SP

²Department of Anatomy, Institute of Biomedical Sciences, USP, São Paulo, Brazil

Keywords: Epigenetics, inflammation, renal ischemia/reperfusion

Abstract:

Background: Cardiorenal Syndrome Type 3 (CRS3) is a condition in which Acute Kidney Injury (AKI) triggers cardiac alterations. SET7 is a methyltransferase enzyme involved in epigenetic modifications across various pathologies. This study investigates the role of SET7 in Cardiorenal Syndrome Type 3 (CRS3) induced by Acute Kidney Injury (AKI). Aim: To evaluate functional and inflammatory markers in kidneys and heart following AKI induced by ischemia/reperfusion (I/R). Methods: Male C57BL/6 mice, wild-type (WT) and SET7 knockout (KO), 6–8 weeks (22–26 g), were anesthetized with xylazine and ketamine. The I/R group underwent left renal pedicle occlusion for 60 minutes followed by reperfusion. After 15 days, animals were euthanized, and blood, heart, kidneys, and tibia collected. Gene expression of *KIM-1*, *NGAL*, *vimentin*, *Cystatin C*, *IL-6*, and *IL-1 β* was assessed by RT-qPCR. Statistical analysis was performed using GraphPad Prism (ANOVA, $p < 0.05$, $n = 4/\text{group}$). Protocol approved by the CEUA ICB/USP nº 9344271022. Results: Morphometric analysis showed a significant decrease in left kidney size in the I/R group compared to Sham (WT: 41.19 ± 8.52 vs. 76.08 ± 5.86). In KO mice, I/R also caused damage (KO: 45.49 ± 9.37 vs. 84.42 ± 11.73). The contralateral right kidney significantly increased in size in the WT-I/R group compared to Sham (104.30 ± 7.80 vs. 80.64 ± 7.28), with no changes in KO mice (92.12 ± 8.93 vs. 88.08 ± 13.37). No significant changes in *KIM-1*, *NGAL*, *vimentin*, *IL-6*, or *IL-1 β* expression were observed in the heart or right kidney. In the left kidney, *KIM-1* and *NGAL* expression increased a hundredfold, and vimentin twenty fold, in I/R compared to Sham. KO animals were able to prevent inflammation and improve glomerular filtration in the left kidney by reducing the expression of *IL-6* (23.49 ± 11.22 vs. 4.82 ± 1.29) and *Cystatin C* (4.71 ± 1.35 vs. 2.26 ± 0.53). Conclusion: AKI induced by I/R causes structural and functional damage to the left kidney, and SET7 knockout can improve renal structure or inflammatory marker expression.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: The effect of hypoxia on the activation of pathways related to the control of aging in hypothalamic proopiomelanocortin neurons

Authors:

Carolina Namie Sato CORTEZÃO¹,
Joseane Morari Ricciardi de AGUIAR¹,
Lara Regina FERREIRA¹,
Julia Moreira da SILVA¹,
Lício Augusto VELLOSO¹

Affiliations:

¹ Faculty of Medical Sciences (FCM), University of Campinas (UNICAMP), Campinas-SP, Brazil

Keywords: POMC; aging; hypoxia; mitochondria.

Abstract:

Proopiomelanocortin neurons (POMC) in the hypothalamus are important components of a neural circuit that regulates aging. Previous studies have shown that the activity of the inflammatory transcription factor, NFκβ, in hypothalamic POMC neurons activates systemic pathways that accelerate the aging process. During aging, there is a progressive reduction in cerebral blood flow, resulting in a decline in neuronal oxygenation and, consequently, an increase in hypoxia-inducible factor 1-α (Hif1α). However, it is not known whether exposure to a hypoxic environment can accelerate the aging process in POMC neurons. In this exploratory study, a POMC neuronal cell line was used to evaluate the impact of exposure to hypoxia on cellular pathways that regulate aging. In a preliminary bioinformatics analysis, we found that the expression of Hif1α is low in POMC neurons, a result also confirmed by gene expression analysis. Previous studies indicate that alterations in mitochondrial genes such as Drp1 and Lonp1 are indicative of hypoxic conditions in mammalian cells. Therefore, in the second stage of the study, cultured POMC neurons were exposed to a 1% oxygen gradient and genes related to mitochondrial dynamics and stress were analyzed. The results obtained showed that exposure to low levels of oxygen significantly modulated mitochondrial genes, with emphasis on Drp1 and Lonp1, thus confirming that hypoxia occurred in POMC neural cells. The induction of acute hypoxia (45 minutes) increased genes related to mitochondrial dynamics and stress, while the induction of acute hypoxia followed by exposure to basal levels of oxygen caused significant changes in genes related to mitochondrial dynamics, suggesting that the acute induction of hypoxia generates mitochondrial damage, which is not reversed after exposure to basal levels of oxygen. In the next steps, we will search for key components of the ageing machinery that could be intervened to slow-down ageing.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Potential inflammatory effect of mineral oil on intestinal parameters in an experimental model

Authors:

Beatriz Moreira Nascimento¹
Susana Castelo Branco Ramos Akandakari²
Thaiane Da Silva Rios¹
Amanda Rocha Fujita¹
Eduardo Rochete Ropelle³
José Rodrigo Pauli³
Dennys Esper Cintra¹

Affiliations:

¹ Laboratório de Genômica Nutricional (LabGeN)- Faculdade de Ciências Aplicadas - Universidade Estadual de Campinas
² Perry Lab - Department of Cellular and Molecular Physiology Yale University ³
Laboratório de Biologia Molecular do Exercício (LabMEx)- Faculdade de Ciências Aplicadas - Universidade Estadual de Campinas

Keywords: Inflammation. Mineral oil. Placebo.

Abstract:

Mineral oil (MO) is a non-absorbable mixture of hydrocarbons derived from petroleum. This substance is commonly used to treat constipation due to its ability to increase water secretion in the intestinal lumen and promote stool softening. Additionally, it is used as a placebo in clinical trials because of its inert characteristic; however, recent studies question this inert property. Experimental models indicate increased inflammation and absorption of intestinal endotoxins. Clinical trials further support these findings, as increased inflammatory markers and lipid alterations were observed in patients who received MO. Therefore, it is necessary to define the impact of mineral oil on inflammation and its mechanism. This study aims to evaluate the effects of mineral oil and its potential inflammatory action on intestinal permeability. This study was approved by the Ethics Committee (CEUA nº 6470-1/2024) of UNICAMP. Thus, C57BL/6 mice, 6 weeks old, were randomized into two groups: control (CT) and mineral oil treated (MO), both with N=6, which received saline solution or mineral oil, via oral administration for 15 days, respectively. After euthanasia, the spleen and intestinal fragments were collected for analysis. Measurements such as body weight, ileal pH, intestinal length, and histology were carried out. For statistical analysis, the Kolmogorov-Smirnov and Shapiro-Wilk tests were applied. For parametric data, the unpaired t-test was used. For non-parametric data, the Mann-Whitney test was applied. The results suggested that mineral oil treatment did not cause significant changes in body weight (week 1: CT=21,28g $\pm$ 0,793; MO=21.15g $\pm$ 1.110; week 2: CT=22.22g $\pm$ 0.567; MO=22.53g $\pm$ 1.663), intestinal length (CT=35.08cm $\pm$ 0.584; MO=35.13cm $\pm$ 2.057), ileal pH (CT=9.008 $\pm$ 0.175; MO=8.758 $\pm$ 0.277), and spleen weight (CT=0.062g $\pm$ 0,009; MO=0.054g $\pm$ 0.007), suggesting the absence of inflammation. Therefore, it can be concluded that under the evaluated conditions, MO did not induce relevant modifications in the analyzed parameters.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Expression of HuC/D in the Early Embryogenesis of the Dorsal Root Ganglion

Authors:

Beatriz do Nascimento Barbosa¹
Chao Yun Irene Yan²

Affiliations:

¹ University of São Paulo
² University of São Paulo – Department of Cell and Developmental Biology

Keywords: DRG – dorsal root ganglion; HuC/D; neural crest; neuronal differentiation; chick embryos.

Abstract:

Neural development depends on regulation of these factors that halt proliferation and direct lineage-specific differentiation. Here, we focus on the differentiation of sensory neurons of the dorsal root ganglia (DRG). These structures arise from neural crest cells (DRG), starting at 1,5 days of incubation in chick embryos (Hamburger & Hamilton, 1951). Neural crest cells that recently arrived to the DRG express the HNK1 epitope (Rothstein et al., 2018). Thereafter, their differentiation involves transcription factors, cell interactions and molecular signaling (Masuda et al., 2012). Our main question here is to understand how neural crest cells are directed towards sensory differentiation. HuC/D plays a role in sensory neuron differentiation and maturation. It is a mRNA-binding protein, associated with stabilization, localization, and potential alternative splicing modulation (Perrone et al., 2006). This study aims to characterize the temporal and spatial expression of HuC/D in the DRG, alongside HNK1. HuC/D is expected to be most active in the DRG when the highest differentiation rate occurs between 4,5 and 8 days. *Gallus gallus* embryos (CEUA - 9506131021) were incubated, fixed, sectioned at 10–14 µm and analyzed by immunofluorescence with primary antibodies (HNK1, HuC/D), Alexa Fluor 488-conjugated secondary antibody, and DAPI. Results show that HNK1 remains expressed during DRG formation and marks cells in the periphery of the DRG. HuC/D is found throughout the DRG in earlier stages and is concentrated in the center of the structure at later stages. HNK1 and HuC/D markings partially overlap, suggesting distinct roles during neurogenesis. We also complemented our histology analysis with bioinformatic analysis using the Sharma dataset revealed coexpression of HuC/D with *Islet*, *Scrt2*, and *nhlh2*. In conclusion, we suggest that HuC/D plays an important role in the process of neural differentiation and maturation, being expressed in the DRG from early to later stages (HH20 to HH34). The specifics of this role, including alternative splicing in the embryonic context, need to be explored in future work.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Impact of Lipid Composition on Embryonic Cryotolerance: Insights from Organellar Profiling and Cryopreservation

Authors:

Kelly ANNES¹
Rogério Amorim dos REIS¹
Andressa Minozzo OLIVEIRA¹
Mateus José SUDANO¹

Affiliations:

¹Department of Genetics and Evolution, Federal University of São Carlos – UFSCar.

Keywords: Cryobiology. Embryo. Lipids. Organelles.

Abstract:

Cryopreservation is a commercially used technique that enables the storage of surplus *in vivo* or *in vitro* produced embryos (IVP) for later transfer. However, IVP embryos have lower cryotolerance compared to *in vivo* embryos, besides this event has been related to changes in embryonic lipid metabolism. Lipid profiles of whole embryos are well documented, but this approach can overlook important subcellular and metabolic details, limiting the understanding of key processes during preimplantation development. Therefore, the present study aims to elucidate differences related to the lipid composition of the cell membrane system and its relationship with cryotolerance of bovine embryos. Expanded blastocysts (XB) were subjected to the vitrification and warming protocol. After 12 hours of re-culture, the embryos were classified based on their cryotolerance: higher cryotolerance embryos (HCryo) included hatched blastocysts (EB) and XB, while lower cryotolerance embryos (LCryo) were identified as non-expanded or degenerated (DN) (n=120 embryos/group; 4 replicates). The experimental groups were subjected to cell organelle isolation: lipid droplets (LD), endoplasmic reticulum (ER), mitochondria (MIT), and nuclear membrane (NUC). Thereafter, lipid extraction from cell organelles and lipid analysis using the Multiple Reaction Monitoring (MRM) profiling method were performed. Univariate and multivariate data analyses were conducted on MetaboAnalyst 6.0. Embryos presenting higher cryosurvival were characterized by increased ($P<0.05$) abundances specific lipid species biomarkers in LD and MIT (FFA 20:1), ER (SMd16:1/16:0; FFA18:1) and NUC (SMd16:1/16:0; CE22:1NH4). This is the first work describing the involvement of organelle-specific lipid species in the embryonic cryosurvival. Such information, therefore, certainly can help to improve embryonic cryobiology and fertility preservation. CEUA 9799100322 (ID 001640). FAPESP 2020/08826-9; 2018/21496-8; 2021/01446-9.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: *In silico* characterization and role of α -glucosidases in the detoxification of heme in *Rhodnius prolixus*

Authors:

Fernanda Ferreira MAISSNER¹
Pedro Corrêa NASCIMENTO¹
Carina Azevedo Oliveira SILVA³
José Roberto da SILVA²
Flávia Borges MURY¹

Affiliations:

¹Laboratório Integrado de Biociências Translacionais (LIBT), NUPEM/UFRJ, Macaé-RJ ²Laboratório Integrado de Bioquímica Hatisaburo Masuda (LIBHM), NUPEM/UFRJ, Macaé-RJ ³Laboratório Integrado de Biologia Molecular (LIBM), NUPEM/UFRJ, Macaé-RJ

Keywords: Digestion, heme, pathogen vector interaction, perimicrovillar membranes

Abstract:

During digestion in hematophagous insects, heme is released, which in its free form, and in high concentrations, causes toxic and deleterious effects on cells. As a result, there are antioxidant mechanisms that promote the detoxification of this molecule. In *Rhodnius prolixus*, both the perimicrovillar membranes (MPMV) and their biochemical marker, the α -glucosidase enzyme, are responsible for the synthesis of the hemozoin crystal (Hz). Genome sequencing of this insect has revealed eight isoforms of the enzyme, but the contribution of each in the detoxification process of the heme molecule is still unknown. The aim is therefore to analyze the effect of each RNAi isoform on the digestion and physiology of the kissing bug. To this end,

R. prolixus are reared at temperature and humidity stabilized and periodically fed on a artificial system with defibrinated rabbit blood (CEUA Fiocruz LW-27/21). Before RNAi experiments, a tissue expression profile of the isoforms was carried out in the fat body (FB), AM, PM, rectum (R), trachea and Malpighian tubules (MT). It was found that isoforms B, C, E and G show significant gene expression in AM and PM tissues, the focus of our group's research. RpaGluB (MT: n=7; AM: n=6; PM: n=9; R: n=2; FB: n=7) (Student's t Test, PM *** $p=0,0001$). RpaGluC (MT: n=6; AM: n=6; P: n=9; R: n=3; FB: n=7) (Student's t Test, AM ** $p=0,0031$; PM ** $p=0,0042$; FB ** $p=0,0064$). RpaGluE (MT: n=6; AM: n=6; PM: n=9; R: n=3; FB: n=7) (Student's t Test, AM **** $p<0.0001$, R * $p=0.0457$). RpaGluG (MT: n=7; AM: n=7; PM: n=8; R: n=3; FB: n=7) (Student's t Test, AM *** $p=0.0001$, PM ** $p=0.0025$, R * $p=0.0305$, FB * $p=0.0331$). At the same time, *in silico* analyses are also being carried out using tools such as WolfPsort, SMART, String, SignalP and InterPro for sequence and functional prediction. Insights from

in silico will support RNAi experiments for our comprehension of blood digestion and heme detoxification in *R. prolixus*, shedding light on parasite-vector interface.



XXXIX REUNIÃO ANUAL DA FeSB
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Molecular Study of the Spike Protein Receptor-Binding Domain in SARS-CoV-2 Variants in Porto Alegre/RS Post-Omicron

Authors:

Victória Borgmann A. de SOUZA^{1,2},
Vanise Pereira de MEDEIROS^{1,2},
Henrique Leal de OLIVEIRA¹,
Sara HARTKE¹,
Joao Vitor Barboza CARDOSO,
Carolina Vaccari BATISTA,
Léo Anderson Meira MARTINS²,
Gabriela PASQUALIM^{3,4},
Ilma Simoni BRUM².

Affiliations:

¹Laboratório de Biologia Molecular, Endócrina e Tumoral, Departamento de Fisiologia - ICBS - UFRGS.

²Programa de Pós-Graduação em Ciências Biológicas: Fisiologia - UFRGS.

³Laboratório de Genética, Instituto de Ciências Biológicas - FURG.

⁴Programa de Pós- Graduação em Ciências da Saúde - FURG.

Thematic axis: Biologia Celular e Molecular

Keywords: SARS-CoV-2; Omicron variant; Receptor-Binding Domain (RBD); Mutations; Viral transmissibility.

Abstract:

The COVID-19 pandemic triggered a global health crisis, marked by high morbidity and mortality rates and the high transmissibility of SARS-CoV-2. Variants of Concern (VOCs), such as Delta and Omicron, were classified as such due to changes in disease severity and epidemiological patterns. The Omicron variant, in particular, carries several mutations in the receptor-binding domain (RBD), a critical region for the interaction between the virus and the host cell, influencing transmissibility and the clinical profile of the infection. This study aimed to investigate the genetic alterations associated with the introduction of the Omicron variant in samples collected in the city of Porto Alegre. This study presents methods that follow guidelines and regulations approved by the UFRGS Ethics Committee (CAAE No. 59815622.1.0000.5347). A total of 80 samples were analyzed, in which the replacement of the Delta variant by the Omicron subvariants BA.1 and BA.2 was observed, with a predominance of the latter, suggesting greater transmission capacity. Among the recurrent mutations identified in the Omicron subvariants, S:T478K, S:S373P, S:S375F, and S:K417N stand out. Structural modeling revealed changes in the interactions between the RBD and the ACE2 receptor, with an increased number of contacts in the BA.1 and BA.2 subvariants compared to the original strain. Mutations such as S:Q493R, S:N501Y, and S:Y505H contributed to strengthening these interactions, possibly enhancing viral transmissibility. Although the study faced limitations, especially regarding the structural modeling of the Delta variant, it highlights the importance of analyzing mutations in the SARS-CoV-2 RBD. It is concluded that the genetic alterations observed in Omicron play a key role in virus-host interactions, helping to better understand the dynamics of viral spread and supporting the development of more effective public health strategies.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Applicability of Collagen Scaffolds Derived from Tilapia Skin Associated with Mangosteen Extract for the Treatment of Premaxillary Lesions

Authors:

Carolina Chen PAURIS¹
Yggor Biloría e SILVA¹
Marcelo Rodrigues da CUNHA²

Affiliations:

¹ Master's Student at the Faculdade de Medicina de Jundiaí - SP, São Paulo, Brazil.

² Coordinator of the Bone Plasticity Laboratory at Faculdade de Medicina de Jundiaí - SP, São Paulo, Brazil.

Keywords: Bone regeneration. Collagen. Mangosteen. Premaxillary.

Abstract:

In cases of maxillofacial trauma or congenital anomalies, the bone tissue's self-repair capacity may be insufficient, requiring additional therapeutic approaches. Among the investigated biomaterials, collagen extracted from tilapia skin stands out due to its notable biocompatibility, low cost, and anti-inflammatory and healing properties. Furthermore, mangosteen peel extract has been explored for its high concentration of xanthones, bioactive compounds with antioxidant, antimicrobial, and anti-inflammatory activities. Therefore, the aim of this study was to evaluate bone neoformation in experimentally induced defects in the premaxilla of rats using tilapia collagen scaffolds in association with mangosteen extract. This project was approved by the CEUA-FMJ under protocol No. 23783125. Forty rats were divided into four groups: Group 1, with unfilled premaxillary defects (G1-C); Group 2, filled with mineralized tilapia collagen scaffolds (G2-CM, n=10); Group 3, with collagen and 10% mangosteen extract scaffolds (G3-M10%); and Group 4, with collagen and 30% mangosteen extract scaffolds (G4-M30%). Sixteen weeks after surgery, the animals were euthanized, and samples from the surgical sites were subjected to macroscopic, radiological, and histomorphometric analyses of the lesion repair process. The results showed an absence of inflammatory infiltrates in the surgical area, indicating good scaffold integration within the host site microenvironment. In groups G1 and G2, there was a predominance of reactive connective tissue, while in groups G3 and G4, bone formation occurred from the lesion margins, although insufficient for complete defect repair within the experimental period. Histomorphometric analysis revealed the average percentage of bone volume formed in the surgical area was 8.36 ± 1.21 , 11.13 ± 1.97 , 6.96 ± 0.82 , and 10.89 ± 0.80 (mean $\pm$ standard deviation) for groups G1, G2, G3, and G4, respectively. It is concluded that biomaterial-based scaffolds may represent a viable alternative for bone repair, as they are easy to handle, cost-effective, and promote new bone formation through osteoconduction.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Repair of Pre-Maxillary Injury Using Collagen Matrices Derived from Tilapia Skin and Coated with Chitosan and Curcumin Extract

Authors:

Yggor Biloría e SILVA¹

Carolina Chen PAURIS¹

Marcelo Rodrigues da CUNHA²

Affiliations:

¹Masters students at the Faculdade de Medicina de Jundiaí (FMJ) - SP, São Paulo, Brazil ²Coordinator of the Bone Plasticity Laboratory at Faculdade de Medicina de Jundiaí (FMJ) - SP, São Paulo, Brazil

Keywords: Bone regeneration; Chitosan; Collagen; Curcumin; Scaffolds

Abstract:

The labial and alveolar cleft, represents one of the most common human craniofacial malformations at birth. In this context, the present research aims to contribute an alternative for the repair of alveolar clefts, with or without associated palatal clefts, using matrices composed of collagen extracted from tilapia skin and coated with chitosan and curcumin extract. Therefore, the objective of this study is to evaluate the osteoregenerative capacity of tilapia skin-derived matrices coated with chitosan and curcumin extract for the treatment of bone lesions created in the premaxilla of rats. This project was approved by the CEUA-FMJ (Ethics Committee on the Use of Animals - FMJ) under protocol number 23650058. Forty rats were used, distributed into four groups of 10 animals each. In Group 1 (control), the bone defect was not filled with any scaffold. In Groups 2, 3, and 4, the defects were filled with a scaffold made of collagen extracted from tilapia skin, a matrix coated with chitosan, and a matrix coated with chitosan and curcumin extract, respectively. Sixteen weeks after surgery, the animals were euthanized, and samples from the surgical areas were subjected to macroscopic, radiological, histological, and morphometric analysis of the premaxillary lesion repair process. In the (G1) and (G2), there was a predominance of reactive connective tissue. In the grafted groups (G3 and G4), bone formation was observed starting from the margins of the lesion, although still insufficient for complete bone repair of the defect within the standardized experimental period of this study. Histomorphometric analysis showed that the average percentage of bone volume formed in the surgical area of groups G1, G2, G3, and G4 was 8.59 ± 2.49 ; 11.13 ± 1.97 ; 5.60 ± 1.41 ; and 16.19 ± 3.22 (mean $\pm$ standard deviation), respectively. It is concluded that these scaffolds, with the addition of natural polymers, may represent a viable alternative for bone repair, as they are easy to handle, have low production costs, and stimulate the formation of new bone through osteoconduction.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Analysis of mRNAs associated with neurodegeneration and neuroprotection in the hippocampus and hypothalamus from adult mice with type 2 diabetes and obesity

Authors:

Juliana Luiza de LEMOS¹
Isabela de Oliveira e Silva Campos ABREU²
Daniela Aparecida MASCHIO³
Helene Cristina de Lima BARBOSA⁴

Affiliations:

¹ State University of Campinas

Keywords: Diabetes type 2. Neurodegeneration. Obesity.

Abstract:

Type 2 diabetes mellitus (T2D) and obesity are chronic diseases associated with high frequency and morbidity worldwide. Although a direct relationship with Alzheimer's disease (AD) has not been established, both conditions are known to share a state of central insulin resistance and metabolic dysfunctions. Thus, the objective of this study was to identify, in the hippocampus and hypothalamus of mice with T2D, possible alterations in key molecules associated with AD. For this, we used adult mice, C57BL/6, CEUA 6069-1/2023, fed a high-fat diet for 8 weeks (HFD 8w) vs controls fed a standard diet (CTL 8w); and we investigated the mRNA levels of APP and X11α, related to neurodegeneration and neuroprotection, respectively. After treatment, HFD 8w mice became hyperglycemic, with increased blood glucose fasting (CTL 8w: 99±3, vs HFD 8w: 138±3 mg/dL, $p<0.0001$, $n=6-7$ mice/group), and postprandial (CTL 8w: 149±5, vs HFD 8w: 195.7±11 mg/dL, $p=0.0007$, $n=7$), glucose intolerant, as shown by ipGTT (CTL 8w: 774±13, vs HFD 8w: 3461±78 AUC, $p=0.005$; $n=3-5$ mice/group), and with peripheral insulin resistance, as shown by ipITT (CTL 8w: 1190±37.5, vs HFD 8w: 1348±19 AUC, $p=0.0016$; $n=6$ mice/group). Mice also became obese, with a body weight gain of 51% (CTL 8w: 5±0.6%, vs HFD 8w: 56.5±2%, $p<0.0001$, $n=13$). RT-qPCR shown that, in the hippocampus, there is a significant increase in APP mRNA (CTL 8w, 1±0 vs HFD 8w, 1.17±0.16, $p=0.014$), but there is no change in X11α (CTL 8w, 1±0 vs HFD 8w, 1.13±0.3, $p=0.29$), $n=8-4$ /group. In the hypothalamus, there was also a significant increase in APP (CTL 8w, 1±0 vs HFD 8w, 1.3±0.34; $p=0.026$), but there was no difference in X11α (CTL 8w, 1±0 vs HFD 8w, 1.07±0.39, $p=0.64$); $n=8-4$ mice/group. The $2^{-\Delta\Delta Ct}$ of the target genes in relation to the housekeeping gene HPRT was calculated. In summary, the results suggest that neurodegeneration may occur in adult male mice with type T2D and obesity. However, further studies will be necessary to prove this possibility.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Dual effects of Metformin and Resveratrol on compromising / impairing viability of Endometrial Adenocarcinoma Cells

Authors:

Henrique Leal de OLIVEIRA^{1,2},
Sara HARTKE¹,
Victória Borgmann A. de SOUZA^{1,2},
Carolina Vaccari BATISTA¹,
Gabriela PASQUALIM⁴,
Vânia Marisia Santos Fortes dos REIS^{1,3},
Edison CAPP^{1,5},
Leo Anderson Meira MARTINS^{1,2},
Ilma Simoni Brum da SILVA^{1,2}

Affiliations:

¹Laboratório de Biologia Molecular, Endócrina e Tumoral, Departamento de Fisiologia, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul ²Programa de Pós-Graduação em Ciências Biológicas: Fisiologia, Universidade Federal do Rio Grande do Sul
³Programa de Pós-Graduação em Ciências da Saúde: Ginecologia e Obstetrícia, Universidade Federal do Rio Grande do Sul
⁴Instituto de Ciências Biológicas, Universidade Federal do Rio Grande
⁵University of Heidelberg, Baden-Württemberg, Heidelberg, Germany.

Thematic axis: Cellular and Molecular Biology

Keywords: Endometrial cancer. Metformin. Ishikawa cells. PI3K/Akt-mTOR signaling pathway. Resveratrol.

Abstract:

Endometrial adenocarcinoma (EA) is the most common type of Endometrial cancer, a malignant neoplasm with increasing incidence in developed countries. Raising of mitogenic and anti-apoptotic agents – such as insulin, insulin-like growth factor type 1, and estrogen – during Obesity and Diabetes Mellitus (type 1 and 2) favors the EA development. Metformin (MF), commonly used for treating type 2 diabetes, decreases cancer cell proliferation by modulating the PI3K/Akt/mTOR pathway. Resveratrol (RSV), a natural polyphenol, may also act in this pathway. This study evaluated the effects of MF (0.1 to 50 mM) and/or RSV (0.1 to 75 µM) in a model of EA, the Ishikawa cells. *In vitro* analyses assessed cell viability, cytotoxicity, caspases activation (MTT, Sulforhodamine B, ApoTox™), mitochondrial function (Mitotracker™), cellular death (Annexin/PI), cell cycle (BD Cycletest™), and the PI3K/Akt/mTOR pathway gene expression (RT-qPCR). *In silico* analysis through Cytoscape software was conducted using the String database. MF induced dose- and time-dependent effects, triggering mitochondrial swelling, apoptosis via caspase activation, and G1 arrest at 25 mM. RSV 75 µM decreased mitochondrial mass, cytotoxicity, caspase activation, and induced G1 arrest. Concomitant treatment with highest concentrations of MF (25 mM) and RSV (75 µM) reduced mitochondrial activity, cytotoxicity, and caspase activation while maintaining high apoptosis levels and G1 arrest. RSV possibly attenuates the cellular toxic effects of MF even though it maintains high levels of apoptosis. Although *in-silico* analysis suggested potential molecular targets and interconnected mechanisms, bioavailable concentrations of MF and RSV (i.e., lower doses) did not alter key PI3K/Akt/mTOR genes. However, when critically examined, gene expression trends mirrored *in-silico* predictions, supporting a coherent biological response. Further studies are required to investigate long-term treatment effects and survival mechanisms.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Highly invasive OSCC modifies fibroblast behavior and extracellular matrix organization

Authors:

Luiza Meurer BRAND¹

Luisa DOCKHORN¹

Bibiana Franzen MATTE¹

Leonardo Francisco DIEL¹

Marcelo Lazzaron LAMERS^{1,2}

Affiliations:

¹ Basic Research Center in Dentistry, Dentistry School, Federal University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil.

² Department of Morphological Sciences, Institute of Basic Health Sciences, Federal University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil.

Keywords: Cancer-associated fibroblasts. Collagen. Extracellular matrix. Oral Squamous Cell Carcinoma. Organotypic cell culture.

Abstract:

Oral squamous cell carcinoma (OSCC) is the most common malignancy affecting the oral cavity, characterized by a complex tumor microenvironment (TME) that plays a critical role in disease progression. Within this microenvironment, normal oral fibroblasts (NOFs) undergo phenotypic reprogramming, transitioning into cancer-associated fibroblasts (CAFs), which exhibit altered proliferative behavior and changes in extracellular matrix (ECM) remodeling. This study investigates the response of NOFs to factors secreted by keratinocytes and OSCC cell lines with differing invasive levels. Primary NOFs, keratinocytes, and OSCC cells with variable invasiveness were used in *in vitro* experiments. Conditioned media and co-culture systems were employed in both two-dimensional and three-dimensional models to evaluate fibroblasts proliferation, contractility, ECM remodeling and markers demonstrating transformation into CAFs. Conditioned media from highly invasive OSCC cell lines significantly increased NOF proliferation. In three-dimensional cultures, OSCC-derived factors notably enhanced NOF contractility. An organotypic culture model revealed dynamic bidirectional interactions between cell types, closely resembling *in vivo* histological features. The most aggressive OSCC cell line strongly activated NOFs, resulting in increased collagen deposition. Moreover, exposure to OSCC-conditioned media upregulated CAF markers in NOFs. These findings highlight the impact of tumor-derived factors on shaping the TME, particularly in fibroblasts, with potential implications for OSCC progression.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Mitoquinone Reestablishes Calcium Overload and Proton Leakage after Acute Myocardial Infarction

Authors:

Geórgia Azevedo Oliveira TRAICHEL¹,
Anna Karolina Nascimento COSTA²,
Katyana Kalyne Silva FERREIRA²,
Camilla Lóren da Silva NASCIMENTO²,
Eduardo Hertel RIBEIRO²,
Lais de Oliveira TRAICHEL¹,
Marcella Porto TAVARES¹,
Sara Bianca Mendes TAUFNER¹,
Thiago Barbosa SPALENZA,
Maicon Landim VIEIRA³,
Ivanita STEFANON²,
Aurélia Araújo FERNANDES¹

Affiliations:

¹Graduate Program in Biochemistry; Federal University of Espírito Santo ²Graduate Program in Physiological Science; Federal University of Espírito Santo ³Illinois Institute of Technology

Keywords: Antioxidants. calcium overload. mitochondrial dysfunction. mitoquinone. myocardial infarction.

Abstract:

Myocardial infarction (MI) leads to mitochondrial calcium overload and oxidative stress, causing mitochondrial dysfunction. Mitoquinone (MitoQ), a mitochondria-targeted antioxidant, could be an approach for cardiac disease by reestablishing mitochondrial function. This study evaluated the effects of MitoQ on calcium overload and mitochondrial metabolism in an infarcted rat model induced by coronary occlusion. Male Wistar rats were randomized into Sham, MI and MI treated with MitoQ (MI+MitoQ). MitoQ (100µM) was administered orally for 7 days. Procedures were approved by the ethics committee (CEUA-06/2023). Mitochondria from the left ventricle (Interfibrillar – IFM and subsarcolemmal – SSM) were isolated for analysis. Oxidative phosphorylation was assessed with glutamate/malate; State 3 and 4 respirations were measured, and the State 3/4 ratio was calculated to estimate the respiratory control ratio (RCR). Calcium uptake capacity (CUC) was measured using Calcium Green fluorimetry. Results are expressed as the mean ± SEM. One-way or two-way Anova were used, followed by Tukey pos hoc test (p<0.05). MI increased CUC (extramitochondrial calcium at 330 nMol/mg: IFM: Sham (13): 7422±759; MI (5): 2111±1116*[&]; MI+MitoQ (7): 7414±519; SSM: Sham (13):6676±1123; MI (5):3124±1182*; MI+MitoQ (7):4390±1482; *vs sham; [&]vs MI+MitoQ p<0.05). MI also elevated State 4 respiration, suggesting proton leakage, which MitoQ mitigated (IFM: State 4: Sham (10) 3.88±0.2; MI (5) 6.96±1.3[&]; MI+MitoQ (7) 3.38±0.4; SSM: Sham (8) 1.83±0.2; MI (5) 2.85±0.3*; MI+MitoQ (7) 1.78±0.2; *vs sham; [&]vs MI+MitoQ p<0.05). RCR was increased in MI+MitoQ in the IFM subpopulation (IFM: Sham (10) 18.39±0.7; MI (5) 15.2±1.6[&]; MI+MitoQ (7) 21.33±1.9 [&]vs MI+MitoQ p<0.05). RCR was reduced in MI in SSM and reestablished in MI+MitoQ (SSM: Sham (8) 21.66±1.7; MI (5) 14.48±1.9*; IM+MitoQ (7) 19.6±1.2; *vs Sham p<0.05). Short-term MitoQ treatment reversed mitochondrial calcium overload and prevented proton leakage after MI.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Analysis of the Effects of Interleukin 10 (IL-10) on Inflammatory Markers and on the Etiopathogenesis of Alzheimer's Disease in HT-22 Hippocampal Cells

Authors:

Ana Beatriz Araújo BRANDÃO¹

Ana Paula MORELLI¹

Eduardo Rochete ROPELLE¹

Affiliations:

¹Exercise Molecular Biology Laboratory (LaBMEx) - Faculty of Applied Sciences (FCA), State University of Campinas (São Paulo, Brazil).

Keywords: Amyloid hypothesis. Neurodegeneration. Neuroinflammation.

Abstract:

Alzheimer's disease (AD) is the leading cause of dementia, accounting for approximately 70% of all global cases. The amyloid hypothesis, combined with Tau protein hyperphosphorylation, underlies much of the current understanding of AD pathogenesis, highlighting the accumulation of amyloid plaques and neurofibrillary tangles as central drivers of neurodegeneration. In this context, anti-inflammatory strategies have gained attention in elucidating AD etiopathogenesis. Among anti-inflammatory mediators, interleukin-10 (IL-10) is notable for its potent immunosuppressive activity. This study investigates the effects of IL-10 treatment on inflammatory and morphological parameters in mouse hippocampal HT-22 cells exposed to β -amyloid oligomers (A β Os). Cells were cultured in DMEM and differentiated in NeuroBasal medium for 0, 24, 48, and 72 hours. Optimal differentiation (48h) was confirmed by immunofluorescence and Western blotting. Cell viability was assessed using the MTT Assay, and IL-10 dose-response curves were standardized across seven concentrations. IL-10 at 25 ng/mL significantly reduced cell viability (CTL = 100.0 vs. IL-10 25 ng/mL = 81.11, $p = 0.0207$, $n = 4$), while 10 ng/mL did not (CTL = 100.0 vs. IL-10 10 ng/mL = 90.85, $p = 0.4858$), and was thus selected for further experiments. Cells were treated with 500 nM A β Os and/or 10 ng/mL IL-10 during differentiation. Western blot analysis was performed to assess levels of A β Os, phosphorylated STAT3 (Y705) and p-Tau. A β Os increased STAT3 phosphorylation, not presenting the expected effect in A β or p-Tau accumulation. Notably, IL-10 effectively prevented A β O-induced STAT3 hyperphosphorylation. This finding is relevant, as STAT3 activation is implicated in the transcription of pro-inflammatory genes.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Interference of Docosahexaenoic Acid with the response of PC3 prostate cancer cells to a PARP inhibitor

Authors:

Alana Della Torre da SILVA¹
Guilherme Henrique TAMARINDO^{2,3}
Daniele Lisboa RIBEIRO⁴
Rejane Maira GÓES^{1,2}

Affiliations:

¹ Department of Biological Sciences, Institute of Biosciences, Humanities and Exact Science, State University of São Paulo, São José do Rio Preto, São Paulo, Brazil.

² Institute of Biology, University of Campinas, Campinas, São Paulo, Brazil.

³ Brazilian Biosciences National Laboratory, Brazilian Center for Research in Energy and Materials (CNPEM), Campinas, São Paulo, Brazil.

⁴ Department of Cell Biology, Histology and Embryology, Institute of Biomedical Sciences, Federal University of Uberlândia, Uberlândia, Minas Gerais, Brazil.

Keywords: Docosahexaenoic acid. Omega-3. PARP inhibitor. PC3 cell line. Prostate cancer.

Abstract:

Prostate cancer (PCa) is one of the leading causes of cancer-related death in men, largely due to its progression to a castration-resistant stage (CRPC). Recently, new drugs such as PARP (poly[ADP-ribose] polymerase) inhibitors (iPARPs) have been approved for CRPC treatment. Although several antitumor properties of the omega-3 fatty acid docosahexaenoic acid (DHA) have been demonstrated in PCa, no clinical trials have yet addressed its specific effects and possible interference with current therapies. Here, we investigated the effect of DHA on castration-resistant prostate cancer cells (PC3) and its interference with the response to iPARP. Cells were cultured for 48 h in standard medium (**C**) or with 20 μ M of the iPARP AZD2461 (**iP**), 100 μ M of DHA (**D**), or both combined (**D/iP**). The MTS assay showed that PC3 metabolic activity was reduced by ~39% in **iP** and more markedly in **D/iP** (~55%, $p < 0.0001$). Trypan Blue and DAPI-stained nuclei counts also indicated that DHA increased the cytotoxic effect of iPARP by 16.6% ($p = 0.0439$). In the clonogenic assay, no colonies were observed in **D** and **D/iP** groups. All treatments reduced PI3K protein expression (more pronounced in **iP**, ~59%, $p = 0.0028$) and pAKT (more pronounced in **D/iP**, 80.3%, $p < 0.0001$). Glucocorticoid receptor alpha expression decreased in all treatments, particularly in **D/iP** (48%, $p = 0.0009$). Increased apoptosis (activated caspase-3 assay) and decreased antiapoptotic protein Bcl-2 expression were observed in all treatments. Mapping of other types of regulated cell death showed that DHA alone or with iPARP increased pyroptosis (~33%, cGSDMD marker) and expression of the death receptor TNF-R1. Necroptosis (pMLKL marker) also increased in all treatments, with greater levels in **D/iP** than in **iP**. In conclusion, DHA enhances the cytotoxic effects of iPARP in PC3 cells through multiple mechanisms. These findings suggest its potential use as an adjuvant in CRPC treatment.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Glucose-6-phosphate dehydrogenase inhibitor alters cell interaction and migration dynamics in oral squamous cell carcinoma cell lines

Authors:

Sérgio Edgar Campos de MATTOS¹
Julia Stela Xavier PAIM^{2,3}
Leonardo Francisco DIEL^{2*}
Pâmela Müller de Souza SILVA²
Lisiane BERNARDI³
Leonardo da Silva BITTENCOURT^{3,4}
Marcelo Lazzaron LAMERS³

Affiliations:

¹Postgraduate Program in Physiology, Institute of Basic Health Sciences (ICBS), Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, Rio Grande do Sul, Brazil.

²Basic Research Center in Dentistry, School of Dentistry, Federal University of Rio Grande of Sul, Porto Alegre, Brazil

³Department of Morphological Sciences, Institute of Basic Health Sciences, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil

⁴State Department of Education of Rio Grande do Sul – State Professional School of Health, Clinical Hospital of Porto Alegre

Keywords: 6-Aminonicotinamide, cell behavior, cell migration, oral cancer, pentose phosphate pathway.

Abstract:

Cancer progression is intimately associated with metabolic reprogramming that supports tumor growth and survival. Tumor cells adapt their metabolism to maintain redox homeostasis, primarily through the production of NADPH via the pentose phosphate pathway (PPP). This metabolic plasticity enables invasion, metastasis, and resistance to therapy. Targeting key enzymes involved in the PPP may represent a promising therapeutic strategy. 6-Aminonicotinamide (6AN) inhibits glucose-6-phosphate dehydrogenase (G6PD), a rate-limiting enzyme in the PPP. To investigate the *in vitro* effects of 6AN on oral squamous cell carcinoma (OSCC), we employed two tumor cell lines CAL27 (low invasiveness) and SCC9 (high invasiveness) alongside a non-tumoral human keratinocyte line HaCaT. The antiproliferative effect of 6AN was assessed using the sulforhodamine B (SRB) assay, through which the IC₅₀ values were determined for each cell line. We further evaluated the impact of 6AN on 3D cellular architecture using a spheroid model assay and assessed migration dynamics and directionality via time-lapse microscopy. Treatment with 6AN significantly inhibited cell proliferation while exhibiting only moderate cytotoxicity in HaCaT cells. In 3D models, 6AN induced spheroid disaggregation in a time-dependent manner, reflected by a reduction in spheroid area. Additionally, 6AN reduced cell migration speed by 74% in CAL27 and 79% in SCC9 after 48 hours at a concentration of 20 μ M. Directionality of migration was also impaired in both CAL27 and SCC9, with the effect becoming more pronounced over time; by contrast, HaCaT cells maintained directional migration. These findings suggest that 6-aminonicotinamide holds potential as a therapeutic agent for OSCC by promoting tumor disaggregation, significantly impairing migratory capacity, and disrupting directional cell movement—factors that may collectively reduce the invasive potential of tumor cells.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Wnt signaling in the regulation of glucose transporters GLUT1 and GLUT3 in the hypothalamus of C57BL/6 mice with type 2 diabetes and obese

Authors:

Isabela de Oliveira e Silva Campos ABREU¹
Juliana Luiza de LEMOS²
Helena Cristina de Lima BARBOSA³
Daniela Aparecida MASCHIO⁴

Affiliations:

¹State University of Campinas

Keywords: Neurodegeneration; Hypothalamus; Glucose Transporters; Wnt proteins.

Abstract: Alzheimer's disease (AD) is one of the most important modern epidemics, being a neurodegeneration associated with aging, but it is not yet clear whether obesity and Type 2 Diabetes Mellitus (T2D) can anticipate its onset. In AD there is central insulin resistance, which can also occur in T2D and obesity, therefore, the roles played by glucose transporters (GLUTs) are extremely important. This way, we chose to study Wnt8b, responsible for regulating the expression of GLUTs, responsible for importing glucose across the blood-brain barrier (GLUT1) and into neurons (GLUT3). Aging, obesity and T2D can affect the function of the Wnt proteins, resulting in impaired glucose uptake by the brain. Preliminary data from our research group show a significant increase at the GSK-3 β mRNA level, the main inhibitor of Wnt signaling, in the hippocampus of mice with T2D and obese. Thus, in this work we investigated the expression of GLUT1 and 3, and Wnt8b, by RT-qPCR, in the hypothalamus of C57BL/6 mice (CEUA 6069-1/2023) fed a high-fat diet (HFD 8w) vs a control group fed a standard diet (CTL 8w). After 8 weeks, the HFD mice became hyperglycaemic, glucose intolerant, and with peripheral insulin resistance, therefore with T2D, as well as obese, confirmed by the *Lee index* (CTL 8w 0.3 ± 0.001 vs, HFD 8w 0.35 ± 0.002 , $p < 0.0001$; $n = 6-7$ mice/group). RT-qPCR analysis showed no difference in expression of GLUT1 (CTL 8w 1 ± 0 vs, HFD 8w 1.03 ± 0.3 , $p = 0.88$; $n = 3$ mice/group), and a statistical trend towards an increase of Wnt8b (CTL 8w 1 ± 0 vs, HFD 8w 1.05 ± 0.36 , $p = 0.55$; $n = 3-8$ mice/group), in mice hypothalamus. But there was a significant increase in GLUT3 expression in the HFD group (CTL 8w 1 ± 0 vs, HFD 8w 1.15 ± 0.18 , $p = 0.03$; $n = 6$ mice/group). In summary, the results suggest that in a situation of central insulin resistance, there may be upregulation of GLUT3 (insulin-insensitive) in neurons as a response to maintain glucose levels in neurons. However, more studies are needed to further comprehend the matter.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: PD-L1 Blockade and MAPK Pathway Inhibition Modulate Mitochondrial Metabolism in Patients with BRAF-Mutant Melanoma

Authors:

Anderson Gabriel dos Reis Ortelan¹,
Alessandra Valéria Sousa Faria^{1,2},
André Schwambach Vieira³,
Carmen Veríssima Ferreira Halder¹

Affiliations:

¹Department of Biochemistry and Tissue Biology, InsFtute of Biology, University of Campinas (UNICAMP), Campinas, São Paulo, Brazil.

²Faculdade Israelita de Ciências da Saúde Albert Einstein, Hospital Israelita Albert Einstein, São Paulo, SP, Brazil.

³Department of Structural and FuncFonal Biology, InsFtute of Biology, University of Campinas (UNICAMP), Campinas, São Paulo, Brazil.

Keywords: Melanoma, RNA-Seq, Diagnostic, Gene

Abstract:

Two key factors contribute to the severity and limited therapeuFc efficacy of melanoma: its high metastafC potenFal and intratumoral heterogeneity. Consequently, large-scale analyses such as RNA sequencing (RNA-Seq) have proven valuable for defining gene signatures, sets of genes expressed in tumor Fssue, that influence tumor behavior in individual paFents. In other words, the gene signature is the molecular fingerprint of a tumor, being useful for diagnosis, prognosis, and personalized therapy. In melanoma, gene expression signatures hold promise for improving predicFons of tumor behavior by enabling classificaFon into molecularly defined "high-risk" categories. Within this context, the present study aimed to compare the transcriptomic profiles of biopsies from paFents with metastafC BRAF-mutant melanoma, collected before and a_er 15 days of treatment with durvalumab (anF-PD-L1), in combinaFon with dabrafenib (a BRAF inhibitor) and trameFnib (a MEK inhibitor). The goal was to idenFfy RNAs potenFally associated with therapeuFc failure. To achieve this, an exploratory and staFsFcal analysis was conducted using publicly available data from the GEO dataset (Ribas et al., 2020). The dataset comprises 95 biopsies, each with expression data for 26,334 RNAs. Samples from metastafC sites were categorized into two groups: untreated (Screening) and post-treatment (Day 15), both receiving the full pharmacological protocol. The Screening group included 18 biopsies, while the Day 15 group consisted of 11. Using R so_ware and libraries such as Fdyverse, dplyr, ggplot, and the DESeq2 package, the dataset was analyzed. InferenFal analysis idenFfied 100 RNAs with significant expression changes (either upregulaFon or downregulaFon) between the two Fme points. IntegraFng both exploratory and inferenFal approaches, 57 differenFally expressed RNAs were selected: 30 unique to the Screening group and 27 to the Day 15 group. This study presents a strategy for RNA-Seq data analysis that led to the idenFficaFon of six RNA candidates for gene signatures through interactome analysis: NDUFA4L2, NDUFB11, MT-ND6, RPP38, MRPS12, and EIF3G. The findings suggest that treatment induces changes in mitochondrial metabolism. However, whether these changes lead to tumor cell death or represent adapFve mechanisms enabling tumor survival remains an open quesFon that warrants further invesFgaFon.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: The influence of treatment with 5-FLUOROURACIL on viability and total ROS production at B16-F10, C2C12 myoblasts, and myotubes

Authors:

Paloma Resende ROMERO¹
Rosália Fernandes Fernandes Vivan¹
Fernanda Paschoal Blegniski¹
Beatriz Ticiani Vieira Pereira²
Hannah Hamada Mendonça Lens¹
Flávia A. Guarnier¹

Affiliations:

¹Laboratório de Fisiopatologia e Adaptações Musculares, Depto de Imunologia, Parasitologia e Patologia Geral, Universidade Estadual de Londrina

²Laboratório de Imunofarmacologia Experimental, Depto de Imunologia, Parasitologia e Patologia Geral, Universidade Estadual de Londrina

Keywords: Skeletal muscle, melanoma, 5-FLUOROURACIL, total ROS, viability

Abstract:

Skeletal muscle loss is a hallmark of cancer cachexia, contributing to reduced quality of life, treatment resistance, and lower survival in cancer patients. Reactive oxygen species (ROS) generation is one of the mechanisms underlying muscle atrophy. Some chemotherapeutics may aggravate muscle wasting, accelerating cachexia. However, few studies have compared the effects of the same chemotherapeutic dose on tumor cells, myoblasts, and myotubes. This study aimed to evaluate the effects of 5-Fluorouracil (5-FU), a drug used in melanoma treatment, on C2C12 myoblasts (C2C12b), myotubes (C2C12t), and B16-F10 melanoma cells. Cell viability was assessed using MTT (mitochondrial activity), resazurin (oxidative metabolism), and LDH (membrane integrity) assays. Cells were exposed to 5-FU at 0.1, 0.5, 1, 5, and 10 μM —doses within patient plasma levels. Total ROS production was also measured. Statistical analysis was performed using ANOVA with Tukey's test or Kruskal–Wallis with Dunn's test. MTT revealed reduced viability in B16-F10 cells from 0.5 μM , and in C2C12b/C2C12t from 5 μM . Resazurin showed decreased viability in C2C12b from 0.1 μM , indicating sensitivity to oxidative metabolism disruption. LDH indicated increased membrane permeability in B16-F10 cells from 0.1 μM ; no significant changes were seen in C2C12 cells. ROS levels were not significantly affected at any dose or cell type. In conclusion, 5-FU reduced cell viability in a dose- and cell type-dependent manner, independently of ROS. Myoblasts were more sensitive to oxidative disruption, while B16-F10 cells showed higher membrane damage at lower doses. These findings emphasize the importance of evaluating chemotherapy effects on muscle cells and call for further studies to clarify the mechanisms involved.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Pineal melatonin differentially regulates the skin layers and *Per2* Circadian Rhythms in skin and SCN of female mouse and rat

Authors:

Ana Flavia TOSTES¹,
Juliano Jefferson da SILVA¹,
Ruan Carlos Macedo de MORAES²,
Eliz Maria de Oliveira FURTADO³,
Maria Nathália MORAES³,
Fernanda AMARAL⁴,
Fernanda AKANE⁴,
Caroline FERRAZ¹,
Natalia KEDISHVILI⁵,
Karen GAMBLE²,
José CIPOLLA-NETTO¹

Affiliations:

¹ Department of Physiology and Biophysics, Institute of Biomedical Sciences of University of São Paulo

² Department of Psychiatry & Behavioral Neurobiology, University of Alabama at Birmingham

³ Department of Biological Sciences, Institute of Environmental, Chemical and Pharmaceutical Sciences, Federal University of São Paulo (Diadema)

⁴ Department of Physiology, Federal University of São Paulo

⁵ Department of Biochemistry and Molecular Genetics, University of Alabama at Birmingham

Keywords: Biological clock. Lumicycle. Skin. Suprachiasmatic.

Abstract:

Recent studies shown that melatonin have an antioxidant effect and promotes DNA repair on the skin. Although, the direct relationship of pineal melatonin and the skin physiology and circadian activity is still unclear. As the skin is exposed to environmental light, it also shows rhythmic expression of clock genes, such as *Per2*, which is directly regulated by melatonin. Thus, we investigated the effects of pineal melatonin (0.5mg/kg) on skin thickness and PER2 rhythms in the skin and suprachiasmatic nucleus(SCN), the master clock which synchronizes the synthesis and secretion of pineal melatonin in the body. To do this, we used two experimental models.1)Wistar rats (male/female) were divided into: Control, Pinealectomized(PINX) and Pinealectomized+Melatonin(PM)(CEUA2032191023).We found that pinealectomized females had a reduction in the thickness of the epidermis ($F(2,21)=7.475;p=0.0035$) and dermis ($F(2,22)=6.126;p=0.0077$), while melatonin replacement restored control levels. In addition, the collagen I is decreased with pinealectomy and increased with melatonin replacement ($F(2,16)=6.208;p=0.0101$). Interestingly, these effects did not show significant changes in males.2)We used *Per2::Luc^{+/-} knock in mice* in C57BL6 background (males/females) (IACUCProtocol#21600) which naturally expresses low levels of pineal melatonin and treated with melatonin in drinking water and control (untreated water). Then, we analyzed the rhythms of PER2::LUC in skin and SCN explants using LumiCycle. In females, we observed an increased damping in the skin after melatonin treatment (T-Test; $p=0.0054;t=3,454,df=1$). In contrast, melatonin increased the amplitude in male SCN(T-Test; $p=0.0016;t=4,270,df=10$). Interestingly, we did not observe effects of melatonin treatment in female SCN. Altogether, our results suggest that melatonin modulates skin layers and *Per2* circadian rhythms in a sex-dependent manner, acting as a synchronizer in the skin, especially in females, while reinforcing SCN rhythms.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: DMSO induces major morphological and physiological alterations in zebrafish embryos.

Authors:

Geyse Gomes¹
Allaina Andrade¹
Manoel Luis Costa¹
Claudia Mermelstein¹

Affiliations:

¹ Universidade Federal do Rio de Janeiro

Keywords: development, DMSO, treatment, zebrafish

Abstract:

Dimethyl sulfoxide (DMSO) is a polar aprotic solvent that dissolves both polar and nonpolar compounds and is miscible in a wide range of organic solvents as well as water. These characteristics render DMSO one of the most widely used solvents in pharmaceutical industry, as well in basic biomedical research, such as in developmental biology studies. The analysis of the effects of DMSO during the development of zebrafish larvae is particularly important since zebrafish is one of the most studied vertebrate models in developmental biology and in toxicology. It has been reported that DMSO at concentrations up to 1% are safe to be used in zebrafish embryo developmental toxicity assays, but fundamental questions such as whether and how different concentrations of DMSO affect the morphology and physiology of zebrafish have not been investigated. Therefore, our study focused on the analysis of DMSO treatment during zebrafish development using high resolution optical microscopy and real-time video-microscopy of the whole embryo, as well as focusing on some organs and tissues. Our results show that concentrations above 5% of DMSO are lethal to embryos, whereas concentrations between 1 to 4% induce different morphological and physiological alterations in embryos. The alterations observed include up-curved tail, heart beating frequency, heart edema, somite size, notochord morphology and swim bladder. These results show for the first time a detailed analysis of the major effects of the widely used solvent DMSO during zebrafish development and call for action and care in the use of DMSO in basic and applied research and in industry. The use of zebrafish embryos was approved by the Ethics Committee for Animal Care and Use in Scientific Research from the Federal University of Rio de Janeiro and received approval number: 036/21.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Autologous Fat Enhances Muscle Recovery After End-to-Side Neurorrhaphy Between the Sciatic and Femoral Nerves in Rats

Authors:

Mariana Inocência MANZANO¹
Izabele dos Santos Echague LEITE²
Felipe Stinguem BARBOSA²
Isabella Corrêa de OLIVEIRA¹
Maitê MATEUS²
Alfredo Beijamim Duarte da SILVA²
Katya NALIWAICO²
Matheus Felipe ZAZULA^{1,2}

Affiliations:

¹ Centro Universitário Cesumar – Curitiba, Paraná, Brasil.
² Universidade Federal do Paraná – Curitiba, Paraná, Brasil.

Keywords: Skeletal Muscle; Regeneration; Plasticity; Plastic Surgery

Abstract:

Irreparable peripheral nerve injuries, such as complete sciatic nerve transection, often lead to poor functional recovery and progressive muscle atrophy due to the lack of effective reinnervation strategies. End-to-side neurorrhaphy, which allows the distal stump of a severed nerve to be connected to the lateral surface of an intact donor nerve, has emerged as a potential alternative to promote collateral sprouting and partial reinnervation. In parallel, autologous fat grafting has shown promising trophic, angiogenic, and immunomodulatory effects in regenerative medicine. This study aimed to evaluate the effects of end-to-side neurorrhaphy between the transected sciatic nerve and the intact femoral nerve, with or without the addition of autologous fat, on functional recovery and muscle preservation in rats. Male Wistar rats were allocated into four groups: CTL (controls), LES (sciatic nerve transection), NEU (end-to-side neurorrhaphy), and ENX (end-to-side neurorrhaphy with autologous fat surrounding the coaptation site). Baseline functional tests were performed 72 hours before surgery and repeated weekly. On postoperative day 22, animals were euthanized and the plantar, gastrocnemius, and soleus muscles were harvested for muscle mass analysis (CEUA 1621/2024). Both NEU and ENX groups demonstrated substantial functional improvement compared to the LES group. Ladder climbing performance indicated enhanced hindlimb strength, especially in ENX. Swimming speed increased significantly in NEU and ENX animals, reflecting improved motor coordination. Additionally, the Sciatic Functional Index progressively improved in NEU and ENX, with ENX presenting the best recovery profile. Muscle analysis revealed that both NEU and ENX mitigated muscle atrophy compared to LES. The ENX group showed the most preserved muscle mass and visual maintenance of muscle density, particularly in the soleus and gastrocnemius. These findings suggest that end-to-side neurorrhaphy can induce effective functional reinnervation, and that the application of autologous fat may enhance this process by modulating the microenvironment and supporting neuromuscular integrity. This strategy holds translational potential for complex nerve injury repair in clinical settings.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Inhibition of the Oncogenic Enzyme PKM2 by Coumarin-Naphthoquinone Hybrids: Enzymatic Characterization and Autophagy-Induced Cancer Cell Death

Authors:

Afonso Thales de Sousa ARRUDA¹,
Amanda Vieira RIBEIRO²,
Anna Carolina Carvalho FONSECA³,
Gabriel OUVRENEY¹,
Lucas Rubini DIAS¹,
Vitor Francisco FERREIRA⁵,
Fernando Carvalho da SILVA⁵,
Luana da Silva FOREZI⁴, Bruno
Kaufmann ROBBES².

Affiliations:

¹ Programa de Pós-graduação em Ciências Morfológicas, Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, Fundão, RJ;
² Departamento de Ciências Básicas, Instituto de Saúde de Nova Friburgo, Universidade Federal Fluminense, Nova Friburgo, RJ;
³ Programa de Pós-Graduação Stricto Sensu em Odontologia, Instituto de Saúde de Nova Friburgo, Universidade Federal Fluminense, RJ;
⁴ Programa de Pós Graduação em Ciências Aplicadas a Produtos para Saúde, Faculdade de Farmácia, Universidade Federal Fluminense, RJ;
⁵ Departamento de Química Orgânica, Instituto de Química, Universidade Federal Fluminense, RJ;

Keywords: Autophagy. Carcinoma. Chemotherapy. Oral cancer. PKM2.

Abstract:

Oral squamous cell carcinoma (OSCC) is the most prevalent type of oral cancer, with poor prognosis often associated with overexpression of pyruvate kinase M2 (PKM2). Inhibition of PKM2 may trigger autophagic cell death via energy deprivation. This study introduces novel naphthoquinone-coumarin hybrids as potential PKM2 inhibitors with selective cytotoxic activity against OSCC. Cell viability was assessed by MTT assay across several cancer lines and fibroblasts. Autophagy was evaluated in SCC9 cells stably expressing LC3-GFP. Additional analyses included time-lapse video microscopy, in silico ADMET profiling and molecular docking. Enzymatic activity of PKM2 and LDH was kinetically measured via NADH-coupled assays and validated by PKM2-mediated ATP production. Glucose consumption and lactate production were determined using Interkit and Kovalent kits, respectively. The acute toxicity study in mice was approved by the Animal Ethics Board (CEUA:2699110419), and the hemolysis assay in human blood was approved by CEP/UFF (CAAE: 43134721.4.0000.5626). CUME6e was the most promising coumarin-naphthoquinone hybrid, demonstrating cytotoxicity in different cell lines, as well as inducing a cell death phenotype via autophagy. It was evaluated in silico and demonstrated good solubility (LogS = 6.91), fulfilling three of the four criteria of Lipinski's Rule of Five. Molecular docking analysis suggested strong binding affinity to PKM2. In vitro enzymatic assays using recombinant PKM2 confirmed that CUME6e inhibits its activity at nanomolar concentration. Additionally, CUME6e reduced glucose uptake, lactate production, and intracellular ATP levels. The compound binds to the ADP and FBP binding site, affecting enzyme activity. It wasn't hemolytic and showed an LC₅₀ of 60mg/kg in mice in the acute toxicity assay. Further studies are warranted to explore additional pharmacological properties and assess the potential of CUME6e as an anticancer therapeutic agent.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: NEGR1 Modulates Neuronal Survival Independently of mGluR5 in Excitotoxic Conditions

Authors:

Luan Alves PEREIRA¹
Jennifer Diniz Soares GUIMARÃES¹
Fabíola Mara RIBEIRO¹

Affiliations:

¹ Universidade Federal de Minas Gerais

Keywords: Excitotoxicity. mGluR5. NEGR1. Neuronal survival.

Abstract:

Glutamate is the main excitatory neurotransmitter in the central nervous system and acts on ionotropic receptors (iGluRs) and metabotropic receptors (such as mGluR5). Calcium-permeable iGluRs play a key role in synaptic plasticity and are central to excitotoxicity, a pathological process triggered by excess glutamate leading to calcium overload, cellular stress, and neuronal death. mGluR5, in turn, signals through G protein to promote calcium release from intracellular stores and activates pathways that induce neuroprotective factors, such as the ERK1/2 pathway that promotes BDNF expression. Studies demonstrate the effect of mGluR5 modulating the activity of iGluRs. NEGR1 is a cell adhesion molecule important for processes such as neuronal arborization and also activates survival pathways. It has been identified as a partner of mGluR5. This study investigated the effects of NEGR1 silencing in neuronal cultures from mGluR5^{+/+} and mGluR5^{-/-} mice (CEUA 50/2021), transduced with lentivirus containing either a scramble shRNA, which does not target any murine mRNA, or shRNA targeting NEGR1 mRNA, leading to its silencing. After 24 hours of exposure to glutamate (50 μ M), a significant increase in neuronal death was observed in the shNEGR1 condition in both mGluR5^{+/+} and mGluR5^{-/-} neurons, suggesting that NEGR1 modulates survival mechanisms independently of mGluR5. The data indicate that NEGR1 influences glutamate receptor signaling, calcium homeostasis, and the response to excitotoxicity, making it a promising target for studies on nervous system disorders with an excitotoxic component.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Neuritogenic mechanism of *Phallusia nigra* dermatan sulfate with FGF-2 a *in vitro* neurodegenerative model

Authors:

Victoria Navartovicis MARTINS^{1,2}

Taiane Barreto MEDEIROS^{1,2,3}

Cintia Monteiro de BARROS^{1,2,3}

Affiliations:

¹ Universidade Federal do Rio de Janeiro

² Instituto de Biodiversidade e Sustentabilidade ³

PMPGCF - Multicêntrico

Keywords: Neuro-2A, glycosaminoglycans, Condroitin sulfate

Abstract:

Neurodegenerative diseases are one of the main causes of death in the world and, despite their clinical relevance, remain cureless. New studies have been using molecules involved during the neuroregeneration, such as complex carbohydrates called glycosaminoglycans (GAGs). One of the mechanisms of action of GAGs already described in the literature is through the interaction with growth factors, which are known for their performance in neuroprotection and neuritogenesis. However, these events are not yet fully elucidated with all types of GAGs. GAGs are sulfated polysaccharides composed of a long chain of repeated disaccharides. They differ through the organization of their chain, size and their sulfation pattern, being divided into six main groups: heparin (HEP), heparan sulfate (HS), chondroitin sulfate (CS), dermatan sulfate (DS), keratan sulfate (KS) and hyaluronic acid (HA), in addition to being one of the main components of the extracellular matrix and presenting biological roles, such as cell growth and neuritogenesis. Dermatan 2,6 sulfated, extracted from the ascidians *Phallusia Nigra*, is a significant component in the mammalian brain and has already been shown to have neuroprotective and neuritogenic properties in *in vitro* experiments. That said, the objective of this study is to evaluate the interaction of Fibroblast Growth Factor 2 (FGF-2) along with different glycosaminoglycans and different sulfation patterns in *in vitro* experiments. Thus, experiments were carried out using murine neuroblastoma (Neuro-2A) lineage for morphological analysis. With the present study, we observed that FGF-2 enhances significantly the neuritogenic action of PnDS in Neuro-2A, helping increase the length and amount of neurites. This action was seen similarly in the CS 6 sulfated and significantly lower in the DS 4 sulfated. These results suggest that PnDS has biological properties that favor its use as a potential drug for the treatment of neurodegenerative diseases.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Exploring Heterogeneity in Magnocellular Neurons of the Supraoptic Nucleus: An Integrated Investigation of Electrical and Genetic Responses

Authors:

Julia Nogueira LIMA¹,
Karoline Martins dos SANTOS¹,
Niltra Beltrão ROSA¹,
Melina Pires da SILVA¹

Affiliations:

¹Federal University of São Paulo (UNIFESP)- Paulista School of Medicine (EPM)

Keywords: electrophysiology. gene expression. hypothalamus. osmolarity. vasopressin.

Abstract:

It is well established in the literature that hypertonicity can trigger structural, functional, and genetic changes in neurons of the supraoptic nucleus (SON). Genetic evidence also suggests the existence of neuronal subpopulations with different osmotic sensitivities; however, the electrophysiological properties and gene expression profiles associated with this heterogeneity have not yet been characterized. Thus, we aimed to evaluate the electrical properties of magnocellular neurons under basal conditions and after acute hypertonic stimulation, seeking to identify possible subpopulations. For this purpose, we used genetically modified mice (IRES-AVP/OT-CRE) aged 8 weeks, submitted to electrophysiological recordings using the patch-clamp technique in hypothalamic slices containing the SON. After recordings, the cytoplasmic content of each cell was harvested for single-cell gene expression analysis (RT-qPCR). All protocols were approved by the CEUA (approval no. 2683090524). Our results showed no significant changes in firing frequency, resting membrane potential, or input resistance following hypertonic stimulation. However, variability in neuronal response patterns was evident: 64.3% of VP neurons remained silent, 21.4% depolarized, and 14.3% hyperpolarized. Similar results were observed in oxytocinergic neurons. Input resistance, which reflects passive membrane properties, remained stable in both groups. These findings support our hypothesis of functional heterogeneity among SON neurons, with potential subpopulations displaying distinct sensitivities to osmotic changes. Next steps include increasing the sample size and performing gene expression analysis of the recorded cells, allowing for the correlation between molecular profiles and functional patterns. We expect to contribute to the understanding of osmoregulation and the identification of novel molecular markers associated with the functional variability of magnocellular neurons.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Influence of Nanoplastics on the Response of Ovarian Tumor Cells to Chemotherapy Treatment

Authors:

Geovanna Carla AMARO DA SILVA¹,
Luis Marcos Frediani PORTELA¹,
Micheli Canuto de LIMA¹,
Mirian Esgoti AAL¹,
Vanessa Aguiar ROCHA¹,
Wellerson Rodrigo SCARANO¹,
Flávia Karina DELELLA¹

Affiliations:

¹Department of Structural and Functional Biology, Institute of Biosciences, São Paulo State University, Brazil

Keywords: nanoplastics, ovarian cancer, chemoresistance, tumor microenvironment

Abstract:

Nanoplastics (NPs) from plastic degradation present an environmental and health risk due to their capacity to cross biological membranes and accumulate in tissues. Epithelial ovarian cancer (EOC) can be resistant to chemotherapeutic agents like carboplatin (CB), which remains a significant challenge. This study examined the effects of NPs, both alone and in combination with CB, on EOC cells. SKOV-3 cells were exposed to NPs (40 µg/mL) and CB (100 µM) for 24 hours, based on preliminary assays indicating viability modulation within an NP exposure range of 10-80 µg/mL. Cell viability, cell migration and expression of genes related to tumor progression were performed. Statistical analyses utilized one-way ANOVA followed by Tukey's test ($p < 0.05$). NPs (40 µg/mL) significantly increased cell viability and migration, associated with upregulation of mTOR and BCL2, indicating activation of survival pathways. An upregulation of IL-6 and a downregulation of IL-10 suggest a pro-tumor inflammatory microenvironment. Despite the downregulation of PI3K, AKT, and MAPK, suppression of CASP8 and CASP3 accounts for apoptosis resistance. When combined with CB, NPs enhanced cell viability and induced EMT through upregulation of vimentin, while inhibiting migration, likely due to disrupted PI3K/MAPK activation and CB-induced cytoskeletal damage. Interestingly, despite increased BAX, apoptosis remained inhibited through downregulation of CASP3/8, reinforcing NPs protective role against chemotherapy. At higher concentrations, NPs became cytotoxic. These results reveal that low doses of NPs-PS promote tumor aggressiveness and CB resistance mainly through PI3K/AKT-independent mechanisms involving mTOR/BCL2 activation and IL-6-mediated inflammation, while higher concentrations have the opposite effects. The findings emphasize the impact of NPs pollution on cancer progression and treatment efficacy.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Expression of adhesion molecules in melanoma microenvironment

Authors:

Rafael Lima SANTOS¹
Eduardo Ferreira dos REIS¹
Laura Rangel Oliveira MORAES¹
Ana Paula Mendes MUNIZ¹
Letícia TEODORO¹
Jair Pereira da CUNHA-JUNIOR¹

Affiliations:

¹Immunotechnology and Immunochemistry Laboratory, Federal University of Uberlândia

Keywords: Adhesion molecules. B16F10. Melanoma

Abstract:

Melanoma is a form of skin cancer known for its aggressiveness and high metastatic capacity. Cells within the tumor microenvironment are intrinsically interconnected, leading to the production of numerous factors and molecules that contribute to melanoma development and progression. Several lines of evidence have been demonstrated the role of adhesion molecules in the tumor development and progression. The aim of this study was evaluating the expression of the adhesion molecules (ICAM-1, ICAM-2, ICAM-4, ICAM-5, NCAM-1, VCAM, PECAM and MCAM) in murine and human melanomas, using public datasets available. An active search was conducted using the mRNA sequencing database available for B16F10 murine melanoma and human gene expression profiling for adhesion molecules. The *ICAM1* gene was widely expressed in murine malignant B16F10 cells, cancer associated-fibroblasts (CAFS), dendritic cells, macrophages, tumor T cells, NK cells and B cells. Otherwise, the genes *ICAM2*, *PECAM*, *MCAM* displayed a more restricted expression with predominance in malignant B16F10 cells. The gene *NCAM1* and *VCAM* were highly expressed in CAFs whereas *VCAM* gene was partially identified in subpopulations of B16F10 cells. The *ICAM4* and *ICAM5* gene expression was not observed in cellular murine melanoma microenvironment. The analysis of skin cutaneous human melanoma dataset from normal and tumor tissues revealed the *ICAM1*, *VCAM* and *MCAM* genes were significantly upregulated in tumor in comparison with normal skin. Our preliminary data indicate that some adhesion molecules (ICAM-1, VCAM and MCAM) are shared and upregulated in the murine and human tumor microenvironment. Otherwise, *ICAM2* and *PECAM* genes displayed differential expression profile between murine and human melanoma microenvironment, representing distinctive potential targets to be explored in the study of this type of cancer.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Título: Estudo sobre os possíveis impactos do aumento da temperatura em aspectos biológicos do *Aedes aegypti*

Autores:

João Victor Oliveira SANTOS¹,
Bárbara Silva SOUZA¹,
Jessica Gouveia FERREIRA¹,
Ademir MARTINS¹

Afiliações:

¹ Laboratório de Biologia, Controle e Vigilância de Insetos Vetores (LBCVIV) – Instituto Oswaldo Cruz – IOC, FIOCRUZ, RJ

Palavras-chaves: Mudanças climáticas, Mutações *kdr* e Morfometria de asa

Resumo:

As mudanças climáticas, impulsionadas pelo aquecimento global, estão afetando a saúde pública, especialmente no aumento da transmissão de arbovírus transmitidos pelo mosquito *Aedes aegypti*. O controle químico é a principal estratégia para combater esse vetor, mas o uso excessivo e indiscriminado de inseticidas tem levado à seleção de populações resistentes, representando um desafio crescente para o controle de vetores. As diferentes condições climáticas do Brasil, intensificadas pelo aquecimento global, podem impactar de forma distinta as populações de *Ae. aegypti*, influenciando características biológicas e mecanismos de resistência. Este projeto busca investigar como as populações de *Ae. aegypti* de regiões distintas do país (sul, norte e nordeste) estão adaptadas às variações de temperatura. Para isso, ovos de mosquitos foram coletados nessas regiões e estão sendo mantidos em laboratório sob diferentes regimes de temperatura. Serão realizadas análises sobre o tempo de desenvolvimento, longevidade, tamanho das asas (morfometria) e frequência de mutações *kdr* (associadas à resistência aos inseticidas piretroides). O objetivo é identificar adaptações regionais significativas, influenciadas tanto pelas condições ambientais locais quanto pelo uso de inseticidas. Os resultados podem ajudar a criar estratégias de controle mais eficazes e adequadas a cada região em um cenário com temperaturas mais altas.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Avaliação do Perfil de Resistência aos Antimicrobianos e Diversidade Genética de Bactérias Gram Negativas Isoladas em Pinguins no Ambiente Antártico

Authors:

Bruno Rocha PRIBUL¹
Bruna Guimarães MARTINS²

Affiliations:

¹ Instituto Oswaldo Cruz
² Colégio Pedro II; Programa de Vocação Científica (PROVOC)

Keywords: Pinguins antárticos. Resistência antimicrobiana. Saúde pública.

Abstract:

A resistência antimicrobiana é um crescente problema de saúde pública global. Projeções futuras indicam a urgência em combater o uso indiscriminado de antimicrobianos, já que infecções por bactérias multirresistentes, especialmente as produtoras de ESBLs (β -lactamases de espectro estendido) das famílias TEM, SHV e CTX-M, vêm aumentando. Nas últimas décadas, animais, incluindo aves silvestres, têm sido identificados como reservatórios de bactérias multirresistentes e facilitadores da transferência desses microrganismos. No ambiente antártico, os pinguins destacam-se por sua relevância ecológica e baixo contato com humanos, o que os tornam espécies sentinelas para o monitoramento de mudanças climáticas e possíveis indicadores da presença natural de genes resistentes a antimicrobianos na sua microbiota intestinal. Assim, o projeto busca investigar a diversidade microbiana e a resistência antimicrobiana, a partir da análise de amostras de 50 aves das espécies *P. antarcticus* e *P. papua* submetidas a coletas de orofaringe e de coacla, a fim de avaliar o risco de propagação de patógenos multirresistentes. Adotando a abordagem de Saúde Única, o estudo examina a interação entre saúde humana, animal e ambiental, contribuindo para a compreensão da dispersão de microrganismos e genes resistentes em ecossistemas isolados. A pesquisa também visa fornecer dados cruciais para estratégias globais de controle da resistência bacteriana e mitigação dos efeitos das mudanças climáticas sobre a saúde pública. A hipótese central é que as mudanças climáticas, as atividades humanas e o degelo nas regiões polares podem liberar microrganismos patogênicos e resistentes aos antimicrobianos, alterando a dinâmica da resistência e criando riscos à saúde pública mundial.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Avaliação do Perfil de Resistência aos Antimicrobianos e Diversidade Genética de Bactérias Gram Negativas Isoladas em Pinguins no Ambiente Antártico

Authors:

Bernardo Pastana de Sousa POLTRONIERI¹
Bruno Rocha PRIBUL²

Affiliations:

¹Instituto Oswaldo Cruz/IOC/Fiocruz; Laboratório de Bacteriologia Aplicada a Saúde Única e Resistência Antimicrobiana (LabSUR/IOC); Escola Politécnica de Saúde Joaquim Venâncio/PROVOC; Colégio Pedro II - Humaitá II

²Instituto Oswaldo Cruz/IOC/Fiocruz; Laboratório de Bacteriologia Aplicada a Saúde Única e Resistência Antimicrobiana (LabSUR/IOC);

Keywords: Pinguins. resistência Antimicrobiana. saúde única.

Abstract:

A resistência antimicrobiana vem se tornando um desafio de saúde pública. Com um número crescente de bactérias multirresistentes, o cenário previsto é de, em breve, alcançarmos um cenário alarmante quanto à essa questão, devido ao uso indevido de antimicrobianos. Diante disso, faz-se necessária a criação de políticas de saúde para que seja controlado o uso desses medicamentos. O aumento no número de infecções causadas por bactérias resistentes às principais classes de antimicrobianos já é real, e tem ocorrido predominantemente entre as ESBLs, pertencentes as famílias: TEM, SHV e CTX-M. Além da saúde humana, foram detectadas bactérias multirresistentes em aves silvestres, essas consideradas reservatórios para diversas bactérias, bem como são consideradas também transportadoras das mesmas. Essas populações servem de bioindicadores da qualidade do ecossistema marinho, sobretudo nos oceanos do sul. Dentre essas aves, os pinguins são maioria, compondo 90% da biomassa avícola dos oceanos austrais. As colônias de pinguins dividem-se entre as ilhas antárticas, subantárticas e o continente antártico em si. Esses estão entre os animais com menor contato humano, o que os torna possíveis indicadores da presença natural de genes de resistência em aves e no ambiente. O objetivo deste projeto é avaliar a presença de resistência a antimicrobianos na microbiota endógena de *P. antarcticus* e *P. papua* no ambiente antártico, encontrando assim isolados bacterianos resistentes à antimicrobianos, a partir de swabs oral e cloacal obtidos dos espécimes. A partir da abordagem de Saúde Única, esta pesquisa busca investigar a interação entre as saúdes animal, ambiental e humana, colaborando para uma maior compreensão acerca da resistência antimicrobiana. Ademais, esse estudo pode ter como resultado uma gama de dados cruciais para entender como a resistência antimicrobiana pode se ampliar e também em como as mudanças climáticas e o degelo das calotas polares pode interferir na saúde única.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Impact of miR-181a-5p Upregulation on Cell Signaling and Apoptotic Pathways in Placental Tissue of Women with Breast Cancer During Pregnancy

Authors:

Gabriela Figueiredo MICHELAZZO¹,
Leisa Lopes-AGUIAR¹,
Rafaella Trevisan SCANDIUZZI¹,
Fátima Solange PASINI²,
Cláudia Malheiros Coutinho-CAMILLO³,
Murilo Vieira GERALDO¹,
Fernanda Garanhani de Castro SURITA⁴,
Maria Laura Costa NASCIMENTO⁴,
Maria Cristina Cintra Gomes-MARCONDES¹,
Laís Rosa VIANA^{1*}

Affiliations:

¹Department of Structural and Functional Biology, Biology Institute, University of Campinas, Brazil;

²Cancer Institute of the State of Sao Paulo, Faculty of Medicine, University of Sao Paulo, Brazil;

³International Research Center, A.C. Camargo Cancer Center, Brazil;

⁴Department of Obstetrics and Gynecology, School of Medical Sciences, University of Campinas, Campinas, Brazil.

Keywords: Breast cancer during pregnancy. Chemotherapy. MiR-181a-5p. Placenta. Potential targets

Abstract:

The association between breast cancer during pregnancy (PrBC) and subsequent chemotherapy treatment may lead to molecular alterations in the placenta, potentially affecting fetal development. However, the molecular mechanisms underlying these changes remain poorly understood. It is already known that miRNAs - short non-coding RNAs - play an important role in regulating gene expression and are involved in distinct biological processes related to cancer and placenta. Among them, miR-181a-5p deserves particular attention, as it has been associated with placental dysfunction in patients with preeclampsia. Therefore, we evaluated the placental expression of miR-181a-5p in patients with PrBC undergoing chemotherapy. Placentas were obtained from patients diagnosed with PrBC who received chemotherapy (case group, n= 7) and from healthy participants (control group, n= 9) through the Centre for Integral Attention to Women's Health biobank of University of Campinas (Ethics Committee: #65070122.0.0000.5404). The miR-181a-5p expression was analysed using RT-qPCR in the chorionic villi region of the placenta. The expression was calculated by 2- $\Delta\Delta$ CT method and compared by *t*-test. The respective potential targets and their impacts on biological processes were identified by bioinformatics tools (miRWalk database and DAVID resources). For all statistical analyses, *P* < 0.05 was considered significant. The miR-181a-5p expression was upregulated in the case group compared to the control group (1.90 ± 0.49 vs 0.94 ± 0.40 , *P* = 0.0008). 776 potential targets were identified with impact in 46 biological processes, being mostly related to signaling (17%; 108 genes), development (13%; 24 genes), apoptosis (11%; 27 genes) and growth (9%; 25 genes). Our findings suggest that miR-181a-5p and its potential targets can modulate different biological processes, such as cell signaling and apoptosis, which can be associated with placental pathologies leading to impairments to pregnancy course for patients with PrBC.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Hepatic Metabolomic Profile of Male Rats Subjected to Maternal Protein Restriction

Authors:

Isabelle Tenori RIBEIRO¹;
Matheus Naia FIORETTO¹;
Pedro Menchini VITALI¹;
Sérgio Alexandre Alcantara DOS SANTOS²;
Luisa Annibal BARATA¹;
Maria Clara Caruso CORSO¹
Renato MATTOS¹;
Luiz Marcos Frediani PORTELA¹;
Hector Baptista SEBASTIAN¹;
Keila Nascimento CAVALCANTE³;
Daniel Rodrigues CARDOSO³;
Marcus Vinicius Niz ALVAREZ¹;
Luis Antonio JUSTULIN¹

Affiliations:

¹Sao Paulo State University (UNESP), Department of Structural and Functional Biology, Institute of Biosciences, Botucatu, SP, Brazil.

²Cancer Signaling and Epigenetics Program, Fox Chase Cancer Center, Philadelphia, PA 19111, USA

³University of São Paulo (USP), Institute of Chemistry of São Carlos (IQSC), São Carlos, SP, Brazil.

Keywords: DOHaD, Hepatic Morphophysiology, Maternal Malnutrition, Metabolomics, Omics Sciences

Abstract:

Epidemiological and experimental evidence shows that gestational adversities increase the incidence of metabolic diseases in offspring, supporting the concept of fetal programming and DOHaD. Maternal Protein Restriction (MPR) is associated with a reduced number of nephrons, hormonal imbalances, liver architecture alterations, and increased susceptibility to prostate carcinogenesis with aging. This project aims to investigate, through metabolomics, the hepatic metabolite profile and metabolic pathways in rats subjected to MPR at PND 90 and 540. Male Sprague Dawley rats from litters exposed to MPR were divided into two groups: CTR (normoproteic diet, 17% protein; 8 animals/group) and RPGL (hypoproteic diet, 6% protein; 8 animals/group) during gestation and lactation (CEUA nº 7376171224). The livers were then subjected to morphological analysis, zymography, and metabolomics. The Shapiro-Wilk test was used for normality, with subsequent statistical analysis using the Student's t-test (parametric) or Mann-Whitney test (non-parametric), considering significant differences when $p < 0.05$. In biometric analyses ($n=10$ /group), RPGL animals exhibited lower body weight at birth, weaning, adulthood, and aging (p -value: <0.0001 ; <0.001 ; <0.001). At PND 90 and 540, there was a significant reduction in total fat weight (p -value: <0.001). In morphological analyses ($n=6$ /group), no hepatic alterations were observed at PND 90, but at PND 540, RPGL animals showed significant inflammatory foci (p -value: <0.0001) and increased collagen levels (p -value: 0.0154). In metabolomic analysis ($n=5$ /group), PLS-DA showed distinct separation between groups at both time points. At PND 90, RPGL animals had elevated levels of amino acids, glucose, and mannose, with reduced inosine and xanthosine. At PND 540, GTP, IMP, and NADP⁺ levels were increased, and uracil and creatine levels were reduced. MPR negatively impacts body weight throughout life, with more pronounced hepatic and inflammatory alterations at PND 540. Metabolomic analysis revealed changes in amino acid and purine metabolism, affecting liver function. These findings suggest that MPR exacerbates metabolic and inflammatory dysfunctions with aging.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Functional and Molecular Study of Comorbidity between Neural Alterations, Heart Failure, and Metabolic Dysfunction: The Interaction between the Renin- Angiotensin System, Intestinal Microbiota, and Heart Disease

Authors:

Mellyely Carolina Basílio SOARES¹,
Cristiane Amorim de PAULA¹,
Nícia Pedreira SOARES¹,
Thiago VERANO-BRAGA¹

Affiliations:

¹Functional Proteomics Nucle, Department of Physiology and Biophysics,
Institute of Biological Sciences, Federal University of Minas Gerais

Keywords: Heart Disease. Intestinal Microbiota. Metabolic Dysfunction.

Abstract:

The growing evidence on the role of the intestinal microbiota in regulating behavior and metabolism has driven studies investigating the effects of dysbiosis in different pathophysiological contexts, such as comorbidities, to better understand the functional axis involving the intestinal microbiota, the renin-angiotensin system, and the heart. This study evaluated the metabolic and behavioral effects of intestinal dysbiosis induced by chronic mild and unpredictable stress (CUMS) and antibiotic therapy (ATB). Eight-week-old male C57BL6/J mice were used and monitored weekly for body weight. Dysbiosis was induced through the CUMS and/or ATB protocols. Fecal samples were collected before and after 21 days of the experiment. On the 22nd day, the animals were subjected to the elevated plus maze and open field tests to assess anxiety-like behavior. On the 23rd day, the glucose tolerance test (GTT) was performed, followed by euthanasia for further analysis (CEUA: 69/2025). Preliminary results indicated that CUMS did not significantly alter the weight of the animals CTR (23.68 g $\pm$ 0.63); CUMS (23.68g $\pm$ 0.63); ATB (23.40g $\pm$ 0.52); CUMS+ATB (23.69g $\pm$ 0.47). In contrast, the induced dysbiosis was associated with greater glucose tolerance, evidenced by the reduction in the glycemic curve in relation to the control group: CTR (308.5 mg/dL $\pm$ 69.11); CUMS (267.1 mg/dL $\pm$ 58.38); ATB (267.3 mg/dL $\pm$ 44.28); CUMS+ATB (255.1 mg/dL $\pm$ 50.46). In the elevated plus maze, a reduction in time in the open arms was observed in the ATB (79.69 s $\pm$ 8.39) and CUMS+ATB (30.04 s $\pm$ 7.44) groups, indicating anxiety-like behavior. Thus, the data suggest that induced intestinal dysbiosis improves glucose tolerance but promotes behavioral changes compatible with anxiety. Molecular, histological, and cardiac proteomic analyses are ongoing to investigate the mechanisms involved in the behavioral and metabolic changes induced by intestinal dysbiosis.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Physiological impact of multi-channel ion blockade on cardiac autonomic regulation

Authors:

Amanda Cardoso MOREIRA¹,
Rym GHIMOUZ²,
Renata MIYABARA^{1,3},
Ovidiu Constantin BALTATU^{1,3,4},
Luciana Aparecida CAMPOS^{1,3}

Affiliations:

¹ Center of Innovation, Technology, and Education (CITE) at Anhembi Morumbi University, Anima Institute,

Sao Jose dos Campos Technology Park, Sao Jose dos Campos, Brazil

² College of Natural and Health Sciences, Zayed University, Abu Dhabi, United Arab Emirates³ College of

Medicine, Alfaisal University, Saudi Arabia

³ UNINOVAFAPI University Center, Teresina, Piauí, Brazil.

⁴ College of Medicine, Alfaisal University, Saudi Arabia

Keywords: Autonomic nervous system. Heart rate variability. Ion channel blockers. Pharmacological response. Sex differences.

Abstract:

Ion channel blocker combinations are commonly used for cardiac arrhythmias and other cardiovascular disorders. In this study, we investigated how specific combinations of sodium channel blockers (mexiletine, lidocaine), calcium channel blocker (diltiazem), and IKr (rapid delayed rectifier K⁺ current) blockers (dofetilide, moxifloxacin) affect cardiac autonomic modulation, with focus on potential sex-specific differences. In a randomized, double-blind, 5-period crossover study, we analyzed ECG recordings from 19 healthy volunteers (8 women, 11 men, aged 18-35 years). Participants received placebo, diltiazem+moxifloxacin (Ca²⁺ and IKr blockers), mexiletine+dofetilide, lidocaine+dofetilide (Na⁺ and IKr blockers), and dofetilide alone. Heart rate variability was assessed using time domain, frequency domain, and nonlinear measures. Significant drug effects were observed across all parameters: time domain ($F(4,68)=10.73$, $p<0.001$), frequency domain (LF: $F(4,68)=3.76$, $p=0.008$; HF: $F(4,68)=3.30$, $p=0.016$), and nonlinear measures (SD2: $F(4,68)=3.34$, $p=0.015$). Baseline sex differences were found in time domain measures ($p=0.027$), with women showing higher heart rates and shorter RR intervals, indicating higher baseline sympathetic tone. However, no significant drug-sex interactions were observed across any parameters. Multi-channel ion blockade significantly modulates cardiac autonomic control, with consistent effects across sexes despite inherent baseline autonomic differences between men and women. Financial Support: We acknowledge research support from Anima Institute - AI (research professor fellowships to OCB and LAC) and CAPES fellowship to ACM.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Cardiac autonomic regulation and mental health: Physiological markers for predicting post-infarction psychological outcomes

Authors:

Luana Lorena MOREIRA^{1,2},
Marcelle SÁ²,
Lucas Salgado Rezende de MENDONÇA¹,
Layla RADDAOUI³,
Noor ELABD³,
Zafirah RAHMAN³,
José Antônio SILVA JR.⁴,
Luciana Aparecida CAMPOS¹,
Ovidiu Constantin BALTATU^{1,3}

Affiliations:

¹Center of Innovation, Technology, and Education (CITE), Anhembí Morumbi University, São José dos Campos, SP, Brazil

²Hospital Pio XII, São José dos Campos, SP, Brazil

³College of Medicine, Alfaisal University, Riyadh, Saudi Arabia

⁴Postgraduate Program in Medicine, UNINOVE, São Paulo, SP, Brazil

Keywords: Depression and anxiety. Heart rate variability. Machine learning. Myocardial infarction. Psychological distress

Abstract:

Psychological distress following myocardial infarction (MI) significantly impacts patient recovery and outcomes. This study investigated the relationship between post-MI depression and anxiety, identified potential predictors, and assessed machine learning models' predictive performance. In this prospective, single-center observational cohort study (n=70), we collected demographic, clinical, and psychological data using the Hospital Anxiety and Depression Scale (HADS) and heart rate variability (HRV) metrics. Results revealed high prevalence of depression (41.43%) and anxiety (44.29%) post-MI, particularly among women. For depression prediction, Logistic Regression showed high recall (0.8) and balanced accuracy (0.713). For anxiety prediction, Random Forest Classifier and GaussianNB models demonstrated optimal performance (F1 Scores: 0.546 and 0.625; Recall: 0.6 and 1.0, respectively). Our findings highlight the potential of machine learning models incorporating HRV measures for enhanced psychological distress prediction in post-MI patients, suggesting the value of objective measures for psychological assessment in this population. Financial Support: We acknowledge research support from Anima Institute - AI (research professor fellowships to OCB and LAC).

Ethics Committee Approval: CAAE 63596422.1.0000.5492.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Perivascular adipose tissue protects the thoracic aorta from isoproterenol-induced hypercontractility in female mice

Authors:

Camila Beatriz RISSO¹
Jamaira Aparecida VICTORIO²
Luciana Venturini ROSSONI³
Ana Paula DAVEL¹

Affiliations:

¹Departamento de Biologia Estrutural e Funcional, Instituto de Biologia, Universidade Estadual de Campinas- UNICAMP - Brazil
²Departamento de Ciências Fisiológicas, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina- UFSC - Brazil
³Departamento de Fisiologia e Biofísica, Instituto de Ciências Biomédicas, Universidade de São Paulo- USP - Brazil

Keywords: Beta-adrenergic receptor. female mice. isoproterenol. perivascular adipose tissue.

Abstract:

Physiological estrogen levels protect the cardiovascular system, reducing premenopausal cardiovascular risk. Nevertheless, cardiovascular diseases (CVD) cause 35% of female deaths worldwide. A key CVD mechanism is β -adrenergic receptor (β -AR) hyperactivation. Isoproterenol (ISO), a β -AR agonist, induces oxidative stress and reduces nitric oxide (NO) bioavailability in perivascular adipose tissue (PVAT), leading to PVAT and vascular dysfunction in males. However, β -AR signaling effects in female PVAT remain unclear. This study assessed the impact of β -AR hyperactivation on thoracic aorta PVAT function in female mice. Female mice (12 weeks old, CEUA#6387-1/2024) received ISO (15 μ g/g/day, s.c.) or vehicle (VEH) for 7 days. Blood pressure (BP), body weight (BW), and organ weights (heart, uterus, ovaries, adipose tissue) were measured. Aortic reactivity to phenylephrine and acetylcholine was assessed with and without PVAT. Aorta and adjacent PVAT were analyzed for reactive oxygen species (ROS) via fluorescence (DHE). ISO-treated females exhibited an increase in BW and brown adipose tissue weight, without changes in white fat, uterus, ovaries, or BP. Heart weight was higher in ISO compared with VEH group, suggesting cardiac hypertrophy. ISO increased phenylephrine-induced contraction in aortas without PVAT compared with VEH group (E_{max} PVAT-, VEH= $4,1 \pm 0,1$ vs. ISO= $2,6 \pm 0,3$ mN/mm, $P < 0.05$). However, a greater anticontractile PVAT effect in aorta from ISO group was observed, and there is no difference between ISO and VEH groups in the presence of PVAT (E_{max} PVAT+, VEH= $0,8 \pm 0,1$ vs. ISO= $0,9 \pm 0,1$ mN/mm, $P > 0.05$). ROS production was similar between groups in the aorta but lower in PVAT of the ISO group. No differences in acetylcholine induced relaxation were observed. These findings suggest that β -AR overactivation-induced aortic hypercontractility in females is counterbalanced by an enhanced PVAT anticontractile effect, linked to reduced ROS production.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Testing Deactivation of Peripheral Chemoreceptors to Lower Arterial Pressure in Spontaneously Hypertensive Rats by Chronic Intermittent Hyperoxia

Authors:

Ítalo C. Raulino¹,
Vinícius H. Ferreira¹,
Juliana R. Souza¹,
Mauro de Oliveira¹,
Daniela Accorsi- Mendonça¹,
Julian F.R. Paton²,
Benedito H. Machado¹

Affiliations:

¹Department of Physiology, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil;

²Manaaki Manawa, The Centre for Heart Research, Department of Physiology, Faculty of Health & Medical Sciences, The University of Auckland, Grafton, Auckland, New Zealand.

Keywords: Carotid body, High blood pressure, Peripheral chemoreflex, Sympathetic overactivity, Neurogenic hypertension microenvironment

Abstract:

Increased carotid body chemoreceptor activity is implicated in the sympathetic overactivity contributing to high arterial pressure in experimental models of neurogenic hypertension. Based on this concept, our study investigated whether chronic intermittent hyperoxia (CIH) could deactivate these peripheral chemoreceptors and reduce blood pressure in spontaneously hypertensive rats (SHR). Normotensive Wistar Kyoto rats and SHR were exposed to a chronic intermittent protocol—cycles elevating the fraction of inspired oxygen from 20.8% to 30% for 10 min per cycle/6 cycles per hour, 8 h daily, over 10 days (CIH)—while controls were maintained in normoxia (NOR). Systolic and mean arterial pressures were measured using tail plethysmography during the protocols and direct recordings in conscious animals at the end of the protocols. Baseline respiratory frequency was evaluated before and after the protocols. All protocols were approved by the institutional ethical committee (CEUA#1336/2024R1). The data shows that CIH produced no significant changes in the mean arterial pressure of SHR [159 ± 9.02 (n=4) vs 153 ± 7.85 mmHg (n=7)] or WKY [115 ± 6.08 (n=5) vs 111 ± 7.66 mmHg (n=6)] in relation to their respective control (NOR) as well no changes in all other cardiovascular and respiratory parameters evaluated. The overall data are indicating that CIH produced no major changes in the cardiovascular and respiratory parameters of SHR. Although increased carotid body chemoreceptor activity is a key factor in sympathetic overactivity and maintenance of high arterial pressure, our data shows that CIH did not result in a significant reduction in this cardiovascular parameter in SHR. We suggest that carotid body chemoreceptor deactivation was counteracted by hyperoxia-induced vasoconstriction, which may prevented the expected hypotensive effect. Financial support: FAPESP (2018/15957-2, 2024/09071-2 and 2024/03471-9) and CNPQ (309338/2020-4).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Meta-Analysis of Cardiac Transcriptome of Animals Treated with Doxorubicin Reveals Target Genes

Authors:

Karoline dos Santos RODRIGUES¹

João Vitor CAVALCANTE²

Gleison AZEVEDO²

Rodrigo DALMOLIN^{2,3}

Michael ANDRADES^{1,4}

Affiliations:

¹Programa de Pós-Graduação em Ciências da Saúde: Cardiologia e Cardiovascular, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.

²Bioinformatics Multidisciplinary Environment - IMD, Universidade Federal do Rio Grande do Norte, Natal, Brazil.

³Departamento de Bioquímica - CB, Universidade Federal do Rio Grande do Norte, Natal, Brazil.

⁴Hospital de Clínicas de Porto Alegre (HCPA), Porto Alegre, Brazil.

Keywords: heart, gene expression, meta-analysis, doxorubicin

Abstract:

Doxorubicin (DOX) is a widely used anthracycline in cancer treatment, but its dose-dependent cardiotoxicity limits its therapeutic use. Despite numerous studies in animal models on its mechanisms, the variability in findings hinders the identification of reliable biomarkers for predicting heart damage. This study aims to meta-analyze transcriptomic data from the myocardium of DOX-treated animals to establish a reliable gene expression profile. Our research protocol was registered on PROSPERO (CRD42020205390) and consisted of a systematic search in GEO, Google Scholar, PubMed, and EMBL-EBI to identify relevant transcriptome datasets from non-human cardiac biopsies. The meta-analysis was conducted using a random-effects model. We included 11 datasets (8 from *M. musculus*; 2 from *R. norvegicus*, and 1 from *S. scrofa*) and obtained 1,723 significantly altered transcripts (633 up-regulated and 1,090 down-regulated). Since 810 genes had $I^2 > 50\%$, we conducted a subanalysis based on the cumulative dose of DOX (low vs. high). We found great changes in gene expression when comparing low-dose datasets and high-dose datasets. The most pronounced changes were seen in *Trem1*, *Oas1f*, and *Nlrc5*, with increases of 1.8, 1.6, and 1.4 Log₂FC in high-dose, respectively. *Foxo6* showed a decrease of 1.2 Log₂FC in high-dose. We also found *Agrn* consistently suppressed both in the main analysis (Log₂FC = -0.34; CI 95%: -0.54 to -0.14; $p = 0.001$; $I^2 = 69.3$) or in subanalysis (Low-doses – Log₂FC = -0.37; CI 95%: -0.70 to -0.04; $p = 0.03$; $I^2 = 83.07$; High-doses – Log₂FC = -0.22; CI 95%: -0.37 to -0.07; $p = 0.004$; $I^2 = 0.0$). Our findings provide a statistically robust identification of gene expression profiles in the myocardium of animal models of DOX-induced heart failure, revealing potential cardio-oncology targets that may have been overlooked by low sample size and statistical power in transcriptomic studies.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Prevalence of Cardiovascular Complications in Children with Malaria at the General Hospital of Luanda/Angola

Authors:

Germana Amélia KUTASSI¹,
Luísa Esperança Soares Nsiloulou DA SILVA²,
Amílcar Bernardo Tomé DA SILVA¹

Affiliations:

¹Department of Physiology and Pharmacology of the Faculty of Medicine, Agostinho Neto University;

²Instituto Nacional de Emergências Médicas de Angola

Keywords: Cardiovascular Complications; Children; Luanda/Angola; Malaria; Prevalence.

Abstract:

Malaria is a parasitic disease with high prevalence in countries Asia and Africa, especially in the Sub-Saharan region. Caused by Plasmodium, the Falciparum species causes the most severe form and is the most prevalent in Angola. According the WHO, it is estimated that in 2023, there will be 263 million cases of malaria with around 597,000 deaths, 94% in the African Region and 76% in children under 5 years of age. Recent studies suggest that malaria can affect the cardiovascular(CV) system in adults and with greater severity when multiple episodes occur in childhood. The aim of this study is to show the prevalence of CV complications in children with malaria. Cross-sectional, observational study with 215 children, aged 7 to 144 months, both sexes, with a positive laboratory diagnosis for Plasmodium Falciparum malaria, admitted to the Pediatrics Department of the General Hospital of Luanda/Angola. Recent studies suggest that malaria can affect the CV system in adults and with greater severity when multiple episodes occur in childhood. The aim of this study is to show the prevalence of CV complications in children with malaria. Cross-sectional, observational study with 215 children, aged 7 to 144 months, both sexes, with a positive laboratory diagnosis for Plasmodium Falciparum malaria, admitted to the Pediatrics Department of the General Hospital of Luanda/Angola. Blood pressure (BP) was measured at rest with an ONROM® brand oscillometer, model HEM 742, calibrated with a cuff appropriate to the size of the arm. The identification of high BP levels was through percentiles. The project was approved by the Ethics Committee of the Faculty of Medicine of Agostinho Neto University under resolution No. 09/01-PAR/CE/FMUAN/2023. Of the total, 104 (48.4%) had high BP, of which 87.5% were at $P \geq 95$. However, 93 (43.2%) children had elevated systolic blood pressure (SBP), 88.2% at $P \geq 95$, 104 (48.4%) children had elevated diastolic blood pressure (DBP), 87.5% at $P \geq 95$ and 78 (36.3%) children had elevated both; Children with underweight 34.% and overweight/obesity 38.5%. Mean age 72.6 ± 47.6 months, birth weight 3.4 ± 1.7 kg, current weight 20.6 ± 13.7 kg, height 105.4 ± 27.5 cm, therefore the mean SBP was 101.6 ± 16 mmHg, DBP 66.0 ± 15.1 mmHg, MAP 77.9 ± 14.2 mmHg, PP 35.6 ± 12.9 mm Hg and HR 113.9 ± 21.5 bpm. The main complications found among children with malaria were a high prevalence of overweight/obesity and blood pressure levels.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Submaximal Physical Capacity in Children and Adolescents with Congenital Heart Disease: A Cross-Sectional Study Using the 3-Minute Step Test

Authors:

Priscilla MORETTO¹

Ana Inês GONZÁLES²

Anelise SONZA¹

Affiliations:

¹ Universidade do Estado de Santa Catarina

² Universidade para o Desenvolvimento do Alto Vale do Itajaí

Keywords: Congenital heart disease. Submaximal physical capacity. Three-minute step test. Muscle Oxygenation.

Abstract:

Congenital heart diseases (CHD) are the most common congenital anomalies in newborns and a leading cause of infant mortality. Advances in surgical interventions and clinical management have improved survival rates, but research on cardiac rehabilitation for children and adolescents remains scarce. This study evaluated the submaximal physical capacity of children and adolescents with CHD (7–18 years) through the 3-minute step test (3MST) and peripheral muscle oxygenation using near-infrared spectroscopy (NIRS) at triceps surae muscle, during regular follow-up visit at a hospital outpatient clinic. This cross-sectional study included patients clinically diagnosed with CHD, surgically corrected or not, treated at Joana de Gusmão Children's Hospital (HIJG). Participants were divided into four subgroups: children with corrected (CCC) and uncorrected CHD (CCNC) and adolescents with corrected (ACC) and uncorrected CHD (ACNC). Submaximal capacity was analyzed based on 3MST performance and associations with hemodynamic responses and exertion. Statistical analysis included t-tests (independent/paired), Mann-Whitney, Wilcoxon, One-Way ANOVA, or Kruskal-Wallis ($p \leq 0.05$). The study included 48 participants: 22 children (CCC: $n=13$; CCNC: $n=9$), mean ages 9.1 and 9.4 years, and 26 adolescents (ACC: $n=15$; ACNC: $n=11$), mean ages 14.5 and 13.2 years. Significant differences were observed for predicted heart rate (CCC vs. ACC $p \leq 0.000$; CCC vs. ACNC $p \leq 0.005$; CCNC vs. ACC $p \leq 0.000$; CCNC vs. ACNC $p \leq 0.011$), blood pressure (CCC vs. ACC $p \leq 0.029$; CCNC vs. ACC $p \leq 0.000$), and SpO_2 (CCNC vs. ACNC $p \leq 0.035$). Intragroups differences were found in CCC for resting HR vs. final and 1st min post-test ($p \leq 0.000$; $p \leq 0.001$) and Tissue Saturation Index (TSI) at rest vs. min 1, 2, and final ($p \leq 0.007$; 0.010; 0.012). In ACC, HR at rest vs. final and 1st min post-test differed ($p \leq 0.000$; $p \leq 0.001$). In ACNC, resting vs. final TSI differed ($p \leq 0.002$). BORG correlated with 3MST performance in the CCC group ($\rho=0,555$, $p \leq 0,049$) and for CCNC group, HR at final min ($\rho=-0,717$, $p \leq 0,030$). The findings suggest that children and adolescents with CHD, regardless of correction, have reduced submaximal capacity, evidenced by insufficient HR response and significant intra- and intergroup differences.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Short-term heat therapy promotes cardioprotection and reduces systolic blood pressure in spontaneously hypertensive rats

Authors:

Ronaldo André Castelo dos Santos de ALMEIDA^{1,2}

Jéssica da Silva SANTOS^{1,2}

Letícia de Sousa AMORIM^{1,2}

Julien PERIARD³

Kevin JOHN³

Emerson Lopes OLIVARES^{1,2}

Anderson Luiz Bezerra da SILVEIRA^{1,2}

Affiliations:

¹ Federal Rural University of Rio de Janeiro, Laboratory of Cardiovascular Physiology and Pharmacology.

² Federal Rural University of Rio de Janeiro, Laboratory of Physiology and Human Performance.

³University of Canberra Research Institute for Sports and Exercise.

Keywords: Cardioprotection. Heat therapy. Systolic blood pressure.

Abstract:

Hypertension is a disease that significantly reduces quality of life and increases the risk of death from cardiovascular complications. Heat therapy via hot water immersion (HWI) has been shown to reduce blood pressure, supporting its use as a prophylactic and non-pharmacological strategy against high blood pressure. Procedures of this study were approved by CEUA under n° 14/2022/ICBS/UFRRJ. Spontaneous hypertensive rats (SHR) were randomly assigned to heat therapy (HT) consisting of 11 consecutive hot water immersion sessions or sedentary controls (CTR). After euthanasia, the extracted hearts were subjected to the isolated heart apparatus. Following the stabilization period of 20 minutes of left ventricular developed pressure (LVDP), basal recording was done for 20 minutes. Then, hearts underwent 30 min of global ischemia (GI) followed by 60 min of reperfusion. After the isolated heart protocol, the hearts were weighed and prepared for slide mounting to assess the infarct area. Systolic blood pressure (SBP) was reduced after HT ($p = 0,014$; Cohen's $d = 0,119463$; $95\%CI_{CTR}$: 218.79 to 249.64mmHg; $95\%CI_{HT}$: 161.39 to 233.39mmHg). LVDP recovery after GI was better in HT than CTR ($p < 0.0216$; Cohen's $d = 11.8$; $95\%CI_{CTR}$: 4.99 to 5.34mmHg; $95\%CI_{HT}$: 14.21 to 15.66mmHg) as was the infarct size area ($p < 0.0001$; Cohen's $d = 3.5$; $95\%CI_{CTR}$: 52.93 to 75.97%; $95\%CI_{HT}$: 9.73 to 29.77%). In this rat strain, heat therapy reduced SBP, improved LVDP recovery, and reduced the infarct size area in the hearts after GI. HT promoted cardioprotection and reduced cardiovascular risk. As a prophylactic treatment, HT experimentally demonstrated good potential for preserving cardiac function, in addition to good potential for use as adjuvant therapy.



XXXIX REUNIÃO ANUAL DA FeSB
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: The impact of cardiovascular diseases in Brazil, 1996-2023: a retrospective study of epidemiology and impact on the unified health system

Authors:

Mellyely Carolina Basílio SOARES¹,
Nícia Pedreira SOARES¹,
Thiago VERANO-BRAGA

Affiliations:

¹Functional Proteomics Nucle, Department of Physiology and Biophysics, Institute of Biological Sciences, Federal University of Minas Gerais

Keywords: Cardiovascular diseases. DATASUS. Epidemiology. SUS.

Abstract:

Cardiovascular diseases are the main cause of mortality and morbidity in Brazil, and their epidemiology is impacted by socioeconomic factors, globalization, and urbanization. The World Health Organization defines cardiovascular diseases as being caused by a set of risk factors such as high blood pressure, high blood sugar, tobacco and alcohol use, unhealthy diets, and obesity. These factors are increasingly present in today's society, contributing to the growing rate of related diseases. Given this scenario, recording this and other pathologies is essential for the management of public and social policies. One of the current forms of measurement by the Unified Health System (SUS) is the SUS Information Technology Department (DATASUS), which manages health information. Aiming to characterize cardiovascular diseases epidemiologically and demographically in Brazil, this study collected data provided by this platform related to such diseases, classified in ICD IX, for the analysis of morbidity and mortality from 1996 to 2023 by ethnicity, age, geographic region, and sex in Brazil. The data obtained were analyzed using R (version 4.1.3) and Rstudio (version 2022.02.1 Build 461), following an adaptation of the general linear model. A growing increase in diseases such as hypertension and ischemia was observed, with their epidemiology varying according to region, sex, age, and ethnicity. Our study consists of a retrospective and detailed analysis that contributes to a better presentation of the data collected over the last few years to be a means of scientific dissemination and support for the development of public health policies.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Proteomic profiling of preeclampsia: investigating correlations with soluble endoglin and soluble fms-like tyrosine kinase

Authors:

Caroline Cristina PINTO-SOUZA¹,
Júlyane Natsumi Saito KAIHARA¹,
Bruno César ROSSINI²,
Ricardo de Carvalho CAVALLI³,
Lucilene Delazari DOS SANTOS²
Valéria Cristina SANDRIM¹

Affiliations:

¹Department of Biophysics and Pharmacology, Institute of Biosciences of Botucatu (IBB), São Paulo State University (UNESP), Botucatu, SP, Brazil;

²Biotechnology Institute (IBTEC), São Paulo State University (UNESP), Botucatu, SP, Brazil;

³Department of Gynecology and Obstetrics, Faculty of Medicine of Ribeirão Preto, University of São Paulo (USP), Ribeirão Preto, SP, Brazil;

Keywords: Antiangiogenesis factors. Preeclampsia biomarkers. Plasma proteomics. sFlt-1. Soluble endoglin.

Abstract:

Preeclampsia (PE) is a hypertensive disorder of pregnancy characterized by target organ damage, particularly in the brain, liver, lungs, and kidneys. This cardiovascular damage is closely associated with endothelial dysfunction (ED), oxidative stress (OS), and hyperlipidemia. This study aims to identify plasma proteins in 20 PE patients most strongly correlated with antiangiogenic factors, such as soluble endoglin (sEndoglin) and soluble fms-like tyrosine kinase-1 (sFlt-1) and explore whether any associations are related to the disease's pathophysiology. These findings could contribute to the identification of distinct PE phenotypes. The study was approved by the Research Ethics Committee of Ribeirão Preto Medical School, University of São Paulo (FMRP-USP, CAAE37738620.0.0000.5440). Plasma protein quantification was performed using mass spectrometry, and the data were analyzed using bioinformatics tools: UniProt, PatternLab for Proteomics, and MetaboAnalyst. Groups were categorized into higher (HL) and lower levels (LL) based on a cutoff of 50% of biomarkers' plasma concentrations. Using a fold change (FC) of 2.0, in both HL groups, pregnancy-specific beta-1-glycoprotein 1 (PSG1) was upregulated (FC: 3.1 in sFlt-1, 2.6 in sEndoglin). In the HL sFlt-1 group, complement (C4B), corticosteroid-binding globulin (SERPINA6), and complement (C4A) were more expressed (FC: 2.1, 2.1, and 2.0, respectively). In contrast, fibronectin (FN1) and paraoxonase (PON1) were downregulated in both HL groups (FC: 0.4 and 0.3, respectively). The upregulated proteins in the sFlt-1 HL group, such as SERPINA6, C4A, and C4B, suggest a potential role in the inflammatory and immune responses in PE. Additionally, the upregulation of PSG1 warrants further investigation as a biomarker for disease severity. The downregulation of FN1 and PON1 may indicate impaired tissue remodeling or ED. By understanding PE's molecular profile, we can move toward personalized treatment strategies. Financial Support: CAPES [financial code 001], CNPq [315026/2021-9 - CNPq PQ2; 308504/2021-6], and FAPESP [2021/12010-7 and 2022/07605-4].



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Integrative Physiological Study of Radiation-Induced Lung Injury: Effects on Cardiac Function and Kidney Integrity

Authors:

Lucas Rodrigues de Moraes¹
Maicon Luiz de Lima¹
Antônio Pedro Abido Ribeiro²
Nazareth de Novaes Rocha^{1,8}
Tula Celeste Wilmarth Gonçalves³ Rodrigo
Jorge Vianna Barbosa³
Klara de Souza Roque¹
Vera Luiza Capelozzi⁴
Camila Machado Baldavira⁴
Sarah Aparecida dos Santos Alves⁵
Fernanda Ferreira Cruz¹
Patrícia Rieken Macedo Rocco¹ Sergio
Augusto Lopes de Souza⁶ Lorenzo Ball⁷
Pedro Leme Silva¹

Affiliations:

¹ Laboratório de Investigação Pulmonar, Instituto de Biofísica Carlos Chagas Filho Universidade Federal do Rio de Janeiro ² Hospital Universitário Clementino Fraga Filho, Universidade Federal do Rio de Janeiro ³ CENABIO: Centro Nacional de Biologia Estrutural e Imagem, Universidade Federal do Rio de Janeiro ⁴ Departamento de Patologia, Faculdade de Medicina, Universidade de São Paulo ⁵ Laboratório de Bioquímica e Sinalização Celular, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro ⁶ Departamento de Radiologia, Faculdade de Medicina, Universidade Federal do Rio de Janeiro ⁷ Dipartimento di Scienze Chirurgiche e Diagnostiche Integrate, Universidade de Genoa, Itália ⁸ Departamento de Fisiologia e Farmacologia, Instituto Biomédico, Universidade Federal Fluminense

Keywords: Cardiopulmonary interaction. Chest computed tomography. Histology. Lung mechanics. Radiation

Abstract:

Introduction: Radiotherapy is used to treat chest tumors, but can result in radiation-induced lung injury (RILI) in 5 to 50% of patients. We sought to investigate additional changes in cardiac function and kidney integrity following the development of a RILI model. **Methods:** CEUA 009/23. Thirty adult Wistar rats (8-10 weeks old, 390±22g) were randomized into two groups: one received 15Gy of irradiation to the right lung (RLR group, n=20), while control group (CTRL group, n=10) received no irradiation. After irradiation, echocardiography and chest quantitative computed tomography (CT) were performed every 3 weeks, while respiratory mechanics and right ventricle systolic pressure (RVSP) were assessed at 12 weeks. Lung tissue was analyzed for collagen deposition and immunohistochemistry markers, including signal transducer and activator of transcription 3 (STAT-3) as well as transforming growth factor-beta (TGF-β). Kidney tissue was evaluated for tubular cell spacing and collagen deposition. **Results:** In RLR, compared to CTRL group, lung compliance reduced (CTRL: 0.29±0.03 ml/cmH₂O vs RLR: 0.24±0.02 ml/cmH₂O, p=0.039) while respiratory effort increased. CT analysis demonstrated progressive left lung volume expansion over time. Radiation exposure led to changes in lung macrophages, increased arterial wall thickness, fibroblast proliferation, and collagen deposition in peripheral and perivascular regions. Moreover, both STAT-3 and TGF-β positive cells were increased in lung parenchyma. Pulmonary hypertension developed, as detected by echocardiography and confirmed by invasive RVSP measurement (CTRL: 21±1 mmHg vs RLR: 57±8 mmHg, p<0.001). Furthermore, RLR group exhibited increased kidney collagen deposition and tubular cell thickening due to edema, suggesting vascular congestion. **Conclusion:** These findings highlight the systemic impact of radiation, emphasizing not only its effects on lung but also on cardiac function and kidney integrity.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Reactive oxygen species mediate platelet hyperreactivity induced by oxidized collagen

Authors:

Mayara Grazielly Brito ROCIO¹,
Felipe Caliani Mathias NETTO¹,
Beatriz Scardua SILVA¹,
Tatiana Alves TOLEDO¹,
Renato Simões GASPAR¹.

Affiliations:

¹Universidade Estadual de Campinas, Department of Pharmacology

Keywords: Cardiovascular disease. Collagen. Oxidation. Oxidative stress. Platelet aggregation.

Abstract:

Reactive oxygen species (ROS) leads to protein oxidation, which is associated with the progression of chronic diseases such as cardiovascular diseases. In this context, platelet hyperreactivity, observed in patients at increased risk for ischemic events, plays a central role in thrombus formation. Collagen, a key agonist in platelet activation, is present in the vascular subendothelium and has a long half-life, making it susceptible to oxidation. Therefore, we hypothesized that oxidized collagen can affect platelet function. To test this, a solution of type I fibrillar collagen from bovine Achilles tendon was oxidized using a range of H₂O₂ concentrations overnight at 4°C. Light transmittance platelet aggregation was performed in platelet-rich plasma (PRP) collected from healthy human donors. Inhibitors of NADPH oxidases (GKT137831), COX (Acetylsalicylic acid, AAS), and catalase were used. ROS was assessed using the DCFDA probe measured in a plate reader. When using suboptimal concentrations of oxidized collagen, a ~7x increase in platelet aggregation was observed compared to the control collagen. No differences between oxidized and control collagen-activated PRP was observed when using the NADPH oxidases inhibitor. In contrast, PRP was less sensitive to AAS when stimulated with oxidized collagen. Interestingly, platelets produced ~30% more ROS upon stimulation with oxidized collagen while oxidized collagen alone did not release ROS. Thus we tested whether the lack of sensitivity to AAS in oxidized collagen-aggregated platelets was due to exacerbated H₂O₂ production. Indeed, co-incubation of catalase and AAS in PRP reversed the lack of sensitivity observed when using oxidized collagen. These results indicate that oxidized collagen potentiates platelet function and this is mediated by increased ROS generation. These data could help explain why patients with chronic diseases that concur in oxidative stress experience a higher likelihood of developing thrombosis.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Effect of Electrical Activation of the Carotid Sinus Nerve after Acute Myocardial Infarction in a Cardiorenal Syndrome Type 1 Model

Authors:

Rollsman de Oliveira CAVALHEIRO¹,
Fernanda BROGNARA²,
Thais Marques da SILVA¹,
Carlos Alberto Aguiar da SILVA¹,
Jaci Airton CASTANIA¹,
Carlos Augusto Fernandes MOLINA³,
David MURPHY⁴,
Minna Moreira Dias ROMANO⁵,
Helio Cesar SALGADO¹.

Affiliations:

¹Department of Physiology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, SP, Brazil

²Department of General and Specialized Nursing, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, SP, Brazil

³Urology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, SP, Brazil

⁴Bristol Medical School, University of Bristol, Bristol, UK

⁵Cardiology - Department of Internal Medicine, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, SP, Brazil

Keywords: Attenuated the renal injury. Carotid sinus nerve stimulation. Inflammatory responses. Reduction the ischemic zone.

Abstract:

Cardiorenal Syndrome Type 1 (CRS1) involves heart-kidney dysfunction, after an acute myocardial infarction (AMI), frequently triggers renal impairment. The progression of CRS1 was evaluated three days post-AMI, focusing on cardiac and renal function alongside inflammatory responses, combined with Carotid Sinus Nerve Stimulation (CSNS). Rats were divided into four groups: Control, AMI, CSN-Stimulated, and CSN-Stimulated + AMI. CSNS was applied daily for 10 minutes over three days. All procedures were approved by the Animal Use Ethics Committee (CEUA) from FMRP-USP. Cardiac, renal, and inflammatory parameters were assessed for characterizing the progression of CRS1 and the effects of CSNS. CSNS reduced the ischemic zone compared to the AMI group not exposed to CSNS ($32.7 \pm 2.2\%$ vs. $8.0 \pm 1.8\%$). Heart rate (HR) was increased in the AMI group, showing 440 bpm at 48 hours and 428 bpm at 60 hours post-AMI. Additionally, arterial pressure was markedly increased in the AMI group at 48 hours, showing the following values: 98 mmHg (mean arterial pressure), 89 mmHg (diastolic arterial pressure), and 122 mmHg (systolic arterial pressure). In contrast, the CSN-Stimulated + AMI group showed significant reductions of these parameters, exhibiting mean, diastolic, and systolic arterial pressures of 79 mmHg, 66 mmHg, and 99 mmHg, respectively. Renal injury was confirmed by increased apoptosis in the AMI group. A significant increase was observed in TNF- α in both cardiac and renal tissues in the AMI group, and reduced IL-6 and IL-1 β levels in the CSN-Stimulated + AMI group, indicating an attenuation of the inflammatory responses by the CSNS. This study demonstrates early cardiac and renal dysfunction in CRS1 following AMI, associated with elevated inflammatory markers (TNF- α , IL-6, IL-1 β) and renal apoptosis. CSNS mitigated the inflammatory responses, reduced the infarct size, and attenuated the renal injury, suggesting a promising electroceutical approach for dealing with CRS1.



XXXIX REUNIÃO ANUAL DA FeSB
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Different doses of Bisphenol S alter the renin-angiotensin system and promote cardiac remodeling in adult male mice

Authors:

Guilherme dos Santos REIS^{1,2}
Luiza Mazzali FERRAZ^{1,2}
Maria Eduarda Lima da SILVA^{1,2}
Gabriel Ximenes de OLIVEIRA^{1,2}
D'Angelo Carlo MAGLIANO²
Eliete Dalla Corte FRANTZ^{1,2}
Beatriz ALEXANDRE-SANTOS^{1,2}

Affiliations:

¹ Laboratory of Exercise Sciences, Biomedical Institute, Fluminense Federal University, Niteroi, RJ, Brazil

² Research Center on Morphology and Metabolism, Biomedical Institute, Fluminense Federal University, Niteroi, RJ, Brazil

Keywords: Bisphenol S; endocrine disruptor; renin-angiotensin system; cardiac remodeling

Abstract:

Bisphenol S (BPS) is linked to cardiovascular diseases (CVD), but its effect on the renin-angiotensin system (RAS), a key mechanism in CVD, remains unclear. Adult male mice were divided in C (control), B4 (4µg/Kg/day), B25 (25µg/Kg/day), and B50 (50µg/Kg/day). BPS exposure was through drinking water for 12 weeks. Left ventricle (LV) mass and wall thickness, cardiac risk ratio, RAS components, and cardiac remodeling mediators were assessed. Ethical approval n. 1929240521. Statistical analyses were performed by one-way ANOVA and Holm-Sidak post hoc. B4 and B25 had higher cardiac risk ratio (B4: +44,1%, $p < 0,05$ vs C; +34,3%, $p < 0,05$ vs B50; B25: +89,6%, $p < 0,001$ vs C; +76,7%, $p < 0,001$ vs B50), LV mass (B4: +21,8%, $p < 0,001$ vs C; +14,4%, $p < 0,05$ vs B50; B25: +19,1%, $p < 0,001$ vs C; +12,0%, $p < 0,05$ vs B50) and wall thickness (B4: +20,4%, $p < 0,05$ vs C; +25,0%, $p < 0,01$ vs B50; B25: +24,6%, $p < 0,05$ vs C; +29,2%, $p < 0,01$ vs B50) than to C and B50. ACE activity (B4: +15,1%, $p < 0,05$; B25: +13,3%, $p < 0,05$; B50: +13,2%, $p < 0,05$) was higher in all groups than C. All groups showed higher AT1R protein expression (B4: +55,9%, $p < 0,05$; B25: +73,6% $p < 0,05$; B50: +61,8%, $p < 0,05$), as supported by immunostaining, along with increased ACE protein expression (B4: +88,1%, $p < 0,001$; B25: +66,1%, $p < 0,05$; B50: +66,3%, $p < 0,05$) compared to C. MasR protein expression was reduced than to the C group (B4: -58.4%, $p < 0.05$; B25: -31.6%, $p < 0.05$; B50: -30.4%, $p < 0.05$), which was supported by immunostaining. ACE2 protein expression was lower than C (B4: -56.8%, $p < 0.05$; B25: -70.9%, $p < 0.001$; B50: -53.5%, $p < 0.05$). All groups had higher TGFβ (B4: +31,5%, $p < 0,05$; B25: +29,5%, $p < 0,05$; B50: +30,2% $p < 0,05$), ANP (B4: +58,8%, $p < 0,05$; B25: +61,6%, $p < 0,05$; B50: +78,3%, $p < 0,05$) and TNF-α (B4: +55,7%, $p < 0,05$; B25: +54,3%, $p < 0,05$; B50: +58,4%, $p < 0,05$) protein expression than to C. BPS exposure overactivated the classical RAS axis and downregulated the counterregulatory axis, contributing to cardiac remodeling at different doses.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Effects of CETP Expression on Endothelial Modulated Relaxation and Perivascular Adipose Tissue Function in male and female mice with Cardiac Hypertrophy

Authors:

Carolina Martins LAZARO¹
Camila Beatriz Risso VITÓRIA¹
Teresa M. D. SILVA¹
Ana Paula DAVEL¹
Helena Coutinho Franco de OLIVEIRA¹

Affiliations:

¹ Departamento de Biologia Estrutural e Funcional, Instituto de Biologia, Universidade Estadual de Campinas (UNICAMP), Campinas, SP, Brasil.

Keywords: CETP. Cardiac hypertrophy. PVAT. Endothelial dysfunction. Sex dimorphism.

Abstract:

Cholesteryl ester transfer protein (CETP) lowers plasma high-density lipoprotein (HDL) cholesterol levels, which are cardioprotective. Recent studies show that CETP impairs endothelial and perivascular adipose tissue (PVAT) function in the aorta of male mice but not females. However, its vascular role in cardiovascular disease, such as cardiac hypertrophy, remains unclear. This study examined transgenic male and female mice expressing human CETP and non-transgenic controls (NTg), aged 4–6 months (CEUA #5353-1/2019), treated or not with isoproterenol (ISO, 15 $\mu\text{g g}^{-1} \text{ day}^{-1}$, s.c.) for 7 days. Cardiac hypertrophy was confirmed in all treated groups. Thoracic aortas were isolated with (+) or without (-) PVAT to assess vasodilation (reactivity to acetylcholine, ACh) and vasocontraction (phenylephrine, PE). Data are expressed as mean $\pm$ SEM, with * indicating $p < 0.05$ vs. NTg control for maximal relaxation or vs. PVAT- rings of the same group in contraction analyses. In ISO-treated females, CETP preserved PVAT anti-contractile function (-LogEC50: NTg control PVAT- 7.08 $\pm$ 0.14 and PVAT+ 6.19 $\pm$ 0.14*; NTg ISO PVAT- 7.03 $\pm$ 0.04 and PVAT+ 6.63 $\pm$ 0.07, ns; CETP ISO PVAT- 6.88 $\pm$ 0.11 and PVAT+ 6.12 $\pm$ 0.15*) and the endothelial-mediated relaxation (NTg control 93.7 $\pm$ 4.2%; NTg ISO 68.4 $\pm$ 5.2%*; CETP ISO 85.4 $\pm$ 4.6%), suggesting vascular protection against ISO-induced hypercontractility and endothelial dysfunction. In contrast, CETP did not confer protective effects in males. Both NTg and CETP males treated with ISO showed vascular dysfunction, characterized by reduced maximal relaxation to ACh (NTg control 95.3 $\pm$ 2.2%; NTg ISO 68.2 $\pm$ 6.0%*; CETP ISO 74.1 $\pm$ 2.8%*), aortic hypercontractility and PVAT dysfunction (-LogEC50 NTg control PVAT- 6.77 $\pm$ 0.06 and PVAT+ 5.84 $\pm$ 0.21*; NTg ISO PVAT- 6.95 $\pm$ 0.16 and PVAT+ 6.65 $\pm$ 0.21, ns; CETP ISO PVAT- 6.96 $\pm$ 0.08 and PVAT+ 6.82 $\pm$ 0.28, ns). These findings indicate that CETP plays a sex-specific role in modulating vascular and endothelial function in cardiac hypertrophy.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Cardiac and Renal Benefits of Empagliflozin in Heart Failure: Exploring Direct and Indirect Effects

Authors:

Larissa FERREIRA-SANTOS¹
Livia C. SIMONETE¹
Jennifer N. COELHO¹
Andreia BOARO¹
Danúbia SILVA DOS SANTOS¹
Bruno CARAMELLI¹
Adriana C. C. GIRARDI¹

Affiliations:

¹ Heart Institute (InCor), Clinical Hospital, University of Sao Paulo Medical School, Sao Paulo, Brazil

Keywords: Heart Failure. Empagliflozin. Angiogenesis. Cardiac Remodeling.

Abstract:

Background: Empagliflozin provides cardiovascular and renal benefits in heart failure (HF), regardless of type 2 diabetes status. This study examined the effects of empagliflozin on renal neurohormonal activation, cardiac remodeling, and capillary rarefaction in HF rats, alongside its direct effects on isolated rat cardiomyocytes and human coronary artery endothelial cells (HCAECs). Methods: Male rats with myocardial infarction-induced HF and sham controls received empagliflozin (300 mg/kg chow) or no treatment for four weeks (CEUA:941/2018). Histological analyses assessed cardiac hypertrophy, fibrosis, inflammation, and capillary density. ELISA was used to determine renal cortical angiotensin II (Ang II) and urinary norepinephrine levels. Rat cardiomyocytes were treated with endothelin-1, and HCAECs were exposed to chemical hypoxia (CoCl₂) with or without empagliflozin (1μM). Results: Compared to untreated HF rats, empagliflozin-treated HF rats displayed lower cardiomyocyte hypertrophy (81.44±4.56, 85.55±5.96, 151.9±5.26, and 101.6± 8.22 to Sham, Sham+EMPA, HF, and HF+EMPA, respectively, p<0.001), interstitial fibrosis (p<0.001), urinary norepinephrine concentration (p<0.001), along with increased cardiac and renal capillary density (p<0.05). Renal cortical Ang II concentration and macrophage counts were unchanged with empagliflozin treatment (p>0.05). However, empagliflozin downregulated M1 (TNF-α, iNOS, and CCR2, p<0.05) and upregulated M2 (Arg1, CD206, and CD163, p<0.05) macrophage markers in the kidney. The cardiomyocyte area increased with endothelin-1 exposure but was smaller with empagliflozin co-treatment (5138±547.5, 5575±319.8, 7620±605.0, and 4975±396.6 to control, control+EMPA, ET-1, and ET-1+EMPA, respectively, p<0.05). CoCl₂ reduced EdU-positive HCAECs, while empagliflozin prevented this reduction and increased EdU-positive cells (24.34±0.9344, 27.53±0.4316, 16.80±0.3456, and 21.44±0.4522 to control, control+EMPA, CoCl₂, and CoCl₂+EMPA, respectively, p<0.05). Conclusion: Together, these findings suggest that empagliflozin mitigates cardiac remodeling and enhances capillary density in HF, likely through direct protective effects on cardiomyocytes and endothelial cells. However, in the kidney, empagliflozin does not directly influence macrophage counts or Ang II levels.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Impact of uremic toxin indoxyl sulfate on cardiac cell viability and renal: type 3 cardiorenal syndrome model

Authors:

GIOVANNA PRIMO DE FREITAS
BEATRIZ FAVERO BEDIN
RAQUEL SILVA NERES DOS SANTOS
MARCELA SORELLI CARNEIRO-RAMOS

Affiliations:

Center for Natural and Human Sciences, Federal University of ABC (UFABC), Santo André/SP.

Keywords: Cardiac cells. Cardiorenal syndrome. Renal cells. Uremic toxin

Abstract:

Under kidney diseases, because of renal excretory impairment, uremic toxins accumulate in the bloodstream, among them the indoxyl sulphate (IS), a protein-bound uremic toxin difficult to eliminate. The accumulation of IS is associated with multi-organ complications, special in the kidneys and heart, through oxidative stress and inflammation. The pathological cross-talk between the kidney and heart is called cardiorenal syndrome, which involves different players, such as the immune and sympathetic nervous systems. However, it is still elusive the role of uremic toxins, specifically IS, in the pathophysiology of CRS. We aimed to evaluate whether the secretome of renal cells exposed to IS would affect cardiac cell viability. HEK-293 (human epithelial kidney cells) and H9c2 (embryonic rat heart tissue) cell lines were placed in 96 well plates for the IS treatment at doses of 0.5 mM, 1 mM, and 1.5 mM for 6h, 12h, and 24h to assess the cell viability of both lines by MTT assay. Subsequently, H9C2 cells were exposed for 24 hours to the conditioned medium (CM) derived from HEK-293 cells that were pretreated with IS (1 mM) for 6 hours. At the end of the CM treatment, H9c2 cell viability was also analyzed by MTT assay. This experiment was run three times. Data were expressed as Mean $\pm$ SD and p values < 0.05 were considered significant. A significant decrease in cell viability was observed in both cell lines at all treatment time points. For the HEK-293, a more intense effect was demonstrated at all doses at the 6h time point (0,5 mM: 76,29 $\pm$ 1,21, 1 mM: 68,20 $\pm$ 4,63, 1,5 mM: 59,63 $\pm$ 4,38 vs control: 100 $\pm$ 6,93). For the H9c2, no statistically significant differences were observed between treatment times and doses. When evaluating the H9c2 cells viability treated with conditioned medium from the HEK-293 cells, a significant decrease was observed at the 24h time point when compared to the control group and the 12h group (H9c2: IS: 71,82 $\pm$ 3,50, conditioned medium: 75,48 $\pm$ 2,31, conditioned medium + IS: 64,65 $\pm$ 3,63 vs control: 100 $\pm$ 7,26 vs H9c2 12h - IS: 86,24 $\pm$ 2,85 and conditioned medium + IS: 102,53 $\pm$ 1,24). Therefore, indoxyl sulfate decreases the viability of both cell lines, regardless of the dose and treatment time.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Does the disruption of the intestinal barrier promote the development of pre-eclampsia?

Authors:

Deyse Corrêa de AZEVEDO¹
Claudio Gabriel Aguiar De OLIVEIRA¹
Renata Maria LATARO¹

Affiliations:

¹Department of Physiological Sciences, Center of Biological Sciences, Federal University of Santa Catarina, Florianópolis, Santa Catarina, Brazil

Keywords: Hypertensive disorders of pregnancy. Inflammation. Intestinal microbiota.

Abstract:

This study was designed to test the hypothesis that disruption of the gut barrier promotes a pro-inflammatory profile and the development of pre-eclampsia (PE). All experimental procedures were approved by the Committee of Ethics in Animal Research (CEUA/UFSC 680720042). Intestinal barrier disruption was induced in female Wistar rats by dextran sodium sulfate (DSS) administered in drinking water (5%) for 6 days. Data are presented as mean $\pm$ SD (control vs DSS group). Compared to the control group (n=9), rats treated with DSS (n=9) displayed higher scores in the intestinal disease activity index (0 ± 0 vs 0.88 ± 1.5 , $p=0.0014$) and an increase in the colon histological damage score (2.57 ± 0.9 vs 22 ± 5 , $p<0.0001$), showing intestinal barrier break. Interleukin 6 was reduced in the DSS group in mesenteric lymph nodes (649 ± 105.9 vs 499.6 ± 75.02 pg/mL, $p=0.0057$) and placenta (799.4 ± 31.28 vs 735.7 ± 64 pg/mL, $p=0.016$). No significant difference was found in the number of fetuses (11.1 ± 1.05 vs 10.8 ± 2.08 , $p=0.78$), fetal weight (1.8 ± 0.3 vs 1.6 ± 0.4 g, $p=0.49$), and size (27.5 ± 1.8 vs 25.8 ± 2.9 mm, $p=0.19$), or the number of reabsorptions (0 ± 0 vs 0.1 ± 0.3 , $p=0.33$). Placenta histological analysis demonstrated a decrease in the labyrinth zone in DSS group (853.4 ± 114.6 vs 703 ± 97.5 μ m, $p=0.02$), with no changes in the decidua and junctional zone. On gestacional day 18, systolic blood pressure (SBP) was higher in the DSS group (109.6 ± 7.4 vs 116.8 ± 3.4 mmHg, $p=0.018$), but the values were not characteristic of PE (>140 mmHg). We did not find a significant change in proteinuria (0.28 ± 0.07 vs 0.33 ± 0.14 g/L, $p=0.43$). In conclusion, the disruption of the gut barrier did not produce symptoms of PE, such as arterial hypertension and proteinuria, however, it induced an increase in SBP at the end of gestation and a decrease in the labyrinth zone of the placenta. Financial support: Capes, CNPq and FAPESC.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Short-term mitoquinone therapy prevents aorta elastin injury after myocardial infarction in rats

Authors:

Sara Bianca Oliveira Mendes TAUFNER¹
Tadeu Ériton Caliman ZANARDO²
Georgia Azevedo de Oliveira TRAICHEL¹
Lais de Oliveira TRAICHEL¹
Thiago SPALENZA¹
Anna Karolina NASCIMENTO COSTA²
Katyana Kalyne Silva FERREIRA²
Eduardo Hertel RIBEIRO²
Ivanita STEFANON²
Maicon Landim-VIEIRA^{2,3}
Aurélia Araújo FERNANDES¹

Affiliations:

¹ Department of Biochemistry, Federal University of Espírito Santo, Vitória, Espírito Santo, Brazil
² Department of Physiological Sciences, Federal University of Espírito Santo, Vitória, Espírito Santo, Brazil
³ Department of Biology, Illinois Institute of Technology, Chicago, IL, USA.

Keywords: Myocardial infarction, vascular remodeling, Mitoquinone.

Abstract:

Mitochondria are a major source of oxidative stress and reactive oxygen species (ROS) following myocardial infarction (MI). Mitoquinone (MitoQ), a mitochondria-targeted antioxidant, has been used to mitigate oxidative damage. This study investigated vascular remodeling and the effects of MitoQ on the aorta of rats 7 days post-MI. Male Wistar rats (10–12 weeks old) were divided into three groups: Sham, MI, and MI+MitoQ. MI was surgically induced, and shortly after recovery, rats were treated with MitoQ (100 µM/kg/day) in drinking water. On day 7, aortic Doppler echocardiography was performed, and on day 8, the thoracic aorta was collected for histological and scanning electron microscopy (SEM) analyses. All procedures were approved by the local ethics committee (CEUA-06/2023). Aortic distension (%) showed no significant differences among groups (Sham=19.21±2.10; MI=5.58±0.71; MI+MitoQ=9.07±1.79; $P < 0.05$). Similarly, lumen thickness and area did not differ significantly (Sham: 178.9 ± 8.4 µm², N=4; MI: 116 ± 43.1 µm², N=4; MI+MitoQ: 162.5 ± 12.7 µm², N=4). Elastin injury (a.u.) was significantly increased in the MI group but was not observed in MI+MitoQ (Sham: 6.5 ± 1.6, N=4; MI: 13.5 ± 1.6*, N=4; MI+MitoQ: 8.18 ± 1.7, N=4; vs. Sham, $P < 0.05$). Collagen content (%) was elevated in the MI group, and MitoQ treatment did not alter this increase (Sham: 13.6 ± 0.9, N=4; MI: 24.7 ± 3.0, N=4; MI+MitoQ: 25.7 ± 1.9, N=4; vs. Sham, $P < 0.05$). SEM analysis revealed significant endothelial denudation in the MI group, whereas this damage was markedly reduced in the MI+MitoQ group (Sham: 0.2 ± 0.1, N=4; MI: 2.3 ± 0.1, N=4; MI+MitoQ: 1.3 ± 0.2, N=4; *vs. Sham, &vs. MI, $P < 0.05$). Data are presented as mean ± SE. Statistical analysis was performed using one-way ANOVA followed by Fisher's post hoc test, with significance set at $P < 0.05$. In conclusion, acute MitoQ therapy effectively prevented maladaptive aortic remodeling 7 days after myocardial infarction in rats.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Aerobic training improvements in cardiac dysfunction associated to type 2 diabetes are related to advanced glycation end products pathway

Authors:

Milena Gomes RODRIGUES¹,
Karine Lino RODRIGUES¹,
Vivian Vieira Dias Da SILVA¹,
Daniel Olindo de Castro LINHARES¹,
Evelyn Nunes Goulart da Silva PEREIRA¹,
Raquel Rangel SILVARES¹,
Beatriz Peres de ARAUJO¹,
Juliana Magalhães Chaves BARBOSA²
Anissa DALIRY¹

Affiliations:

¹Laboratory of Clinical and Experimental Physiopathology, Oswaldo Cruz Institution, Oswaldo Cruz Foundation,

²Cellular Biology Laboratory, Oswaldo Cruz Institute, Rio de Janeiro, Brazil

Keywords: Advanced glycation end products. Diabetes type 2. Physical training. Cardiac dysfunction

Abstract:

In patients with type 2 diabetes, hyperglycemia is the main factor responsible for diabetic complications, including cardiovascular disease. Advanced glycation end products (AGEs) are considered key elements in the progression of diabetes and also contribute to structural remodeling of the heart. Regular aerobic exercise at moderate intensity has a positive effect on the control of T2D and consequently on cardiovascular health. Considering that the AGE-RAGE signaling pathway plays a central role in the pathophysiology of diabetes, this study investigated whether modulation of this pathway by exercise could explain the improvement of cardiac dysfunction in diabetic mice. Eight-week-old C57BL/6 mice (Fiocruz, Brazil) were divided into three groups: CTL (control diet, sedentary), T2D (high-carbohydrate/high-fat diet, sedentary) and T2D EX (same diet, with exercise). The training protocol included treadmill training three times a week (30 minutes/session) for 12 weeks. After 36 weeks, blood and organ samples were harvested for analysis. The study followed international guidelines and was approved by the Fiocruz Animal Welfare Committee (license L-0012/18 A1). In the AGE pathway, the exercised group showed a decrease in cardiac RAGE protein expression of approximately 27% compared to the T2D group, while the reduction in CML protein expression was 22.8%. Physical exercise also reduced the gene expression of galectin (T2D EX: 0.59 ± 0.04 vs. T2D: 1.23 ± 0.13 ; $p < 0.001$) and CD36 (T2D EX: 1.34 ± 0.13 vs. T2D: 2.15 ± 0.21 ; $p < 0.001$). No significant changes were observed in the gene expression of DIAPH1 and GLO-1. Thus, by reducing the expression of proteins and genes associated with the AGE-RAGE pathway, exercise disrupts pathological processes and offers a promising non-pharmacologic strategy to mitigate cardiovascular complications in T2D, providing an effective approach to combat AGE-induced cardiac injury.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Structural and Molecular Aspects of Collagen upon Oxidation with Hydrogen Peroxide and its effect in platelet adhesiveness

Authors:

Beatriz Scardua da SILVA¹,
Felipe Caliani Mathias NETTO¹,
Mayara Grazielly Brito ROCIO¹,
Tatiana Alves TOLEDO¹,
Renato Simões GASPAR¹.

Affiliations:

¹Universidade Estadual de Campinas

Keywords: Collagen. Oxidation. Oxidative stress. Platelet adhesion. Cardiovascular disease.

Abstract:

The production of reactive oxygen species (ROS) is a physiological response; however, it can lead to protein oxidation, which in turn may contribute to chronic diseases, particularly cardiovascular diseases. Conditions of excessive oxidants result in platelet hyperactivity, which promotes thrombus formation. In parallel, fibrillar type I collagen is a long-lived protein that is a potent platelet agonist. It is therefore possible that collagen undergo oxidation in chronic diseases given its long half-life. However, it is unclear whether oxidation of collagen alters its structure and interaction with platelets. Therefore, we evaluated whether oxidative modifications alter the structural properties of collagen and how these changes influence platelet adhesiveness. To investigate this, collagen fibrillogenesis assays and SDS-PAGE were performed using both native and oxidized collagen at different collagen:H₂O₂ ratios. The results showed that at low hydrogen peroxide concentrations (1:100; 1:300; 1:1000), no significant structural changes were observed. Nevertheless, at 1:3000 and 1:10000 ratios, considerable alterations were detected. Notably, SDS-PAGE revealed an increase in the 100 kDa chain and stronger smear bands, indicating a loss of the molecule's triple-helix structure. Additionally, platelet adhesion assays were conducted using native and oxidized collagen, as well as human platelets obtained from healthy adult volunteers in accordance with the guidelines approved by the institutional ethics committee (CEP), CAAE:(77219624.5.0000.5404). Preliminary data indicate increased platelet adhesiveness onto collagen oxidized at a low H₂O₂ concentration (1:100). Collectively our data indicate that H₂O₂ oxidizes type I fibrillar collagen *in vitro*, and that structural changes are observed at concentrations far greater than those that promote increased platelet adhesiveness. This indicates that oxidized collagen potentiates platelet function and could be identified as a novel mechanism in thrombotic diseases.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: The effects of Losartan on the memory of Hypertensive rats treated with the high-fat-diet

Authors:

Andressa BETAT¹
Lais ALFLEN¹
Willyan Joseph DA SILVA¹
Claudio Gabriel Aguiar DE OLIVEIRA¹
Geison Souza IZÍDIO¹
Renata Maria LATARO¹

Affiliations:

¹ Universidade Federal de Santa Catarina

Keywords: Hypertension. Hypercholesterolemia. Cognitive function.

Abstract:

Considering that a high-fat diet (HFD) and hypertension are associated with cognitive impairment, we tested the hypothesis that HFD will impair memory in spontaneously hypertensive rats (SHR) and that the treatment with losartan will reduce the systolic blood pressure (SBP) and reverse the memory dysfunction. The study was approved by The Committee of Ethics in Animal Research (CEUA/UFSC: 3064221121). The experiments were carried out in adult male SHR (14 weeks old), distributed into 2 experimental groups: i) SHR fed with HFD for 7 weeks followed by standard diet for 7 weeks (n=9) and ii) SHR fed with HFD for 7 weeks followed for HFD + LOSARTAN (10 mg/kg) for 7 weeks (n=10). The object recognition protocol and the T Maze Test were performed to evaluate memory after 7 and 14 intervention weeks. The results are presented as mean±SD. One-way ANOVA for repeated measures was used to compare the groups. The HFD did not produce memory impairment in SHR (Discrimination index: before -0.2 ± 0.3 vs. 7 weeks -0.02 ± 0.3 ; $p > 0.05$; New object exploration time: before 54 ± 16 vs. 7 weeks 72 ± 24 ; $p > 0.05$; Spontaneous alternation in the T-Maze test: before 7 ± 5 vs. 7 weeks 10 ± 11 ; $p > 0.05$). The HFD replacement by standard diet for 7 weeks did not affect the SHR performance in memory tests (Discrimination index: 7 weeks -0.02 ± 0.3 vs. 14 weeks 0.01 ± 0.2 ; $p > 0.05$; New object exploration time: 7 weeks 73 ± 24 vs. 14 weeks 75 ± 18 , $p > 0.05$; and Spontaneous alternation: 7 weeks 10 ± 11 vs. 14 weeks 6 ± 4 ; $p > 0.05$), as well as, the treatment with Losartan (Discrimination index: 7 weeks -0.1 ± 0.3 vs. 14 weeks -0.01 ± 0.2 ; $p > 0.05$; New object exploration time: 7 weeks 85 ± 25 vs. 14 weeks 75 ± 14 ; $p > 0.05$; Spontaneous alternation: 7 weeks 6 ± 5 vs. 14 weeks 6 ± 4 ; $p > 0.05$). In conclusion, the memory in SHR was not affected by the chronic administration of HFD or Losartan.

Financial Support: Capes and CNPq.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Increased platelet aggregation induced by oxidized collagen is potentially mediated by CD36

Authors:

Felipe Caliani MATHIAS-NETTO¹,
Beatriz Scardua SILVA¹,
Mayara Grazielly Brito ROCIO¹,
Tatiana Alves TOLEDO¹,
Renato Simões GASPAR¹.

Affiliations:

¹Departament of Pharmacology, Faculty of Medical Sciences, Universidade Estadual de Campinas

Keywords: CD36. Collagen.Oxidation. Platelets. Thrombosis.

Abstract:

Collagen is a major protein in the human body, being present in several tissues. It is subject to interactions with different substances produced by physiological and pathological processes, such as hydrogen peroxide (H₂O₂), an abundant reactive oxygen species (ROS). ROS can cause protein oxidation, leading to structural and functional changes. This study evaluates how platelets respond to collagen upon its oxidation and identifies potential underlying mechanisms. Collagen was oxidized using a range of H₂O₂ concentrations (collagen:H₂O₂ molecular proportions of 1:100; 1:300; 1:1000; 1:3000 and 1:10.000). Oxidized collagen was compared to control collagen to evaluate platelet aggregation and signalling. For platelet aggregation experiments, blood was collected from healthy individuals (Ethics Committee Approval Number 77219624.5.0000.5404). To obtain platelet-rich plasma (PRP), whole blood was centrifuged at 100G for 20 minutes. To obtain washed platelets (WP), the PRP was centrifuged at 400G for 20 minutes, with the supernatant discarded and the pellet resuspended in Tyrod's Buffer. PRP was used in traditional aggregometry and a plate-based assay. WP stimulated with control and oxidized collagen (1:100) were used for immunoblotting. Platelet aggregation experiments showed an increase of approximately 7x in platelet response to oxidized collagen (1:100 and 1:300, n=8, p<0.05). Experiments with inhibitors of cyclooxygenases (AAS) and FAK (Inhibitor 14) showed decreased sensitivity to these inhibitors when platelets were stimulated with oxidized collagen (n=7, p<0.05). Inhibition of CD36 by Sulfosuccinimidyl oleate (SSO) was potentiated when platelets were stimulated with oxidized collagen. Immunoblotting experiments showed increased SRC phosphorylation (n=4) in oxidized collagen-stimulated platelets. To further assess changes in collagen structure, scanning electron microscopy and high-performance liquid chromatography experiments are being conducted. These results point to increased platelet response to oxidized collagen; an effect potentially mediated by CD36. These findings could translate to chronic patients that undergo oxidative stress and are at an increased risk of developing thrombosis.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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b

Title: Collagen glycation reduces endothelial cell migration in vitro

Authors:

Samuel Duarte MAIA¹,
Renato Simões GASPAR²

Affiliations:

¹ Departamento de Farmacologia, Faculdade de Ciências Médicas, Universidade Estadual de Campinas.

² Departamento de Farmacologia, Faculdade de Ciências Médicas, Universidade Estadual de Campinas.

Keywords: Collagen; Glycation; Diabetes; receptor for advanced glycation end products; Endothelial Cells.

Abstract:

Diabetes is a disease defined by a faulty glucose metabolism that leads to hyperglycemia and is an important and growing cause of death worldwide. This scenario is concerning since diabetics are at an increased risk of developing cardiovascular diseases mainly due to endothelial dysfunction caused by chronic hyperglycemia. We hypothesize that chronic hyperglycemia occurring before diagnosis causes glycation of long-lived macromolecules in the vascular matrix, and that such alterations lead to endothelial dysfunction and thrombus formation. Thus, we sought to evaluate whether glycation of type I collagen affects endothelial cell function. *In vitro* experiments were conducted exposing collagen to different molar proportions of methylglyoxal (collagen:methylglyoxal of 1:0, 1:0.9, 1:9, and 1:90, respectively) for 7 days at 37 °C. Human umbilical vein endothelial cells (HUVECs) were left to adhere onto glycated or non-glycated collagen surfaces for 24h at 37 °C, upon which we conducted survival (MTT) and migration (Wound Healing) assays. We also evaluated the contribution of the receptor for advanced glycation endproducts (RAGE) by using a well-established RAGE inhibitor, Azeliragon. We found no significant changes in cell viability upon adherence to glycated collagen vs control collagen. Azeliragon was not toxic to HUVECs up to 1 μ M (1 μ M vs. 0 μ M; Mean Dif. 0,01300; $p > 0.05$; $n = 3$). Interestingly, endothelial cells migrated ~ 7 x less when adhered onto glycated collagen, when compared to those adhered onto control collagen (1:90 proportion $11,57 \pm 3,034$ vs. $79,91 \pm 6,911$ $n = 4$, % wound closure, $p < 0.05$). The collagen glycation effect on cell migration was reverted by 1 μ M Azeliragon (12.83% variation in wound closure, $n = 4$, $p < 0.05$). We conclude that collagen glycation impairs endothelial cell migration through RAGE activation. Further *in vivo* and *in vitro* experiments are being performed to ascertain these findings.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Angiotensin-(1-7) and emotional regulation in allergic asthma: a new therapeutic approach

Authors:

Sthéfanie Chaves de Almeida GONÇALVES¹,
Juliana Fabiana GREGÓRIO¹,
Kamylle Silva FERRAZ¹,
Giselle Santos MAGALHÃES¹,
Sílvia Amaral ZEBRAL¹,
Lucas Miranda KANGUSSU²,
Marco Antônio Peliky FONTES¹,
Maria da Glória RODRIGUES-MACHADO³,
Ruben Dario Sinisterra MILLÁN⁴,
Andrea Siqueira HAIBARA¹,
Robson Augusto Souza dos SANTOS¹,
Maria José CAMPAGNOLE-SANTOS¹

Affiliations:

¹Department of Physiology and Biophysics - UFMG

²Department of Morphology -

UFMG ³Department of

Chemistry - UFMG ⁴Program in

Medical Science – FCMMG

Keywords: Asthma. Inflammation. Angiotensin-(1-7). Anxiety. Depression

Abstract:

Allergic asthma is a chronic respiratory disease characterized by airway inflammation and hyperresponsiveness, often associated with anxiety and depression, which make disease control difficult. Angiotensin-(1-7) [Ang-(1-7)], a component of the renin-angiotensin system, has been shown to attenuate airway remodeling, hyperresponsiveness, and inflammation in chronic asthma models. Furthermore, when administered into the central nervous system, Ang-(1-7) also exhibits anxiolytic and antidepressant effects. In this study, we investigated whether Ang-(1-7) could not only attenuate lung inflammation in asthmatic mice but also improve related emotional behaviors. Balb/c mice (8-10 weeks) were divided into control, asthmatic, and Ang-(1-7)-treated groups via oral, intranasal, or intracerebroventricular (ICV) injection. Pulmonary inflammation was assessed by histology, while anxiety- and depression-like behaviors were analyzed by elevated plus maze, open field, and tail suspension tests. We also evaluated c-Fos in the hippocampus and PVN, in addition to TNF- α , IL-6, and IL-10 in the cortex of the animals. Treatment with Ang-(1-7), by oral, intranasal, or ICV routes, improved anxiety- and depression-like behaviors and pulmonary inflammation in asthmatic mice. Oral and intranasal administration decreased the infiltration of inflammatory cells into the lungs, while only intranasal administration prevented the increase in TNF- α and IL-6 in the central nervous system, without altering IL-10 levels. In addition, asthmatic animals showed a significant increase in c-Fos expression in the hippocampus, cortex, and also in the PVN, which was attenuated by oral administration of Ang-(1-7). The results suggest that Ang-(1-7), administered by different routes, exerts anti-inflammatory, anxiolytic and antidepressant effects, representing a potential therapeutic approach for emotional comorbidities associated with asthma. (CEUA #290/2018). Financial support: Capes, Cnpq, Fapemig, e INCT Nanobiofar.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Fluid-electrolyte balance and anxiety-like behavior in spontaneously hypertensive rats

Authors:

Emilson Donizete PEREIRA JR¹
Aline Aparecida ZENATTI¹
Lucas BARRETTO-DE-SOUZA²
Bruna DIAS¹
Isabela Mendes PAZELLO¹
Carlos C. CRESTANI²
Marcelo Tadeu MARIN²
José Vanderlei MENANI¹
Laurival Antonio DE LUCA JR¹
Carina Aparecida Fabrício ANDRADE¹

Affiliations:

¹Department of Physiology and Pathology, School of Dentistry, São Paulo State University – UNESP, Araraquara – SP, Brazil

²Department of Drugs and Medicines, School of Pharmaceutical Sciences, São Paulo State University (UNESP), Araraquara, SP, Brazil.

Keywords: Anxiety, Behavior, Dehydration, Hypertension

Abstract:

Spontaneously hypertensive rats (SHRs), an animal model for the study of primary hypertension, ingest more NaCl and exhibit less anxiety-like behavior than normotensive strains. Here we investigated whether disturbances in the hydromineral balance (such as during dehydration) would change locomotor activity and anxiety-like behavior in SHRs compared to normotensive rats (CEUA-FOAr 24-2016;15-2020/CEUA-FCFAr 07/2021). Behavioral responses of male SHRs (n = 11-12 per group) and normotensive Holtzman rats (n=13-14 per group) were evaluated in the elevated plus-maze (EPM) and in the open field (OF) test under the following conditions: a) euhydrated; b) after 24 h water deprivation (WD); c) WD followed by 2 h access to water for partial rehydration (PH); and d) after WD-PH followed by 2 h access to both water and 0.3 M NaCl (sodium appetite test). Under euhydrated condition, SHRs had increased time spent in the EPM open arms ($49.5 \pm 4\%$, vs. normotensive: $33 \pm 5.4\%$) and increased central locomotor activity (23.2 ± 2.6 , vs. normotensive: 7.8 ± 1.8 min) in the OF. After WD and WD-PH, the behavioral responses in EPM and OF were not different from euhydrated condition when compared within the same strain. Interestingly, 0.3 M NaCl intake changed behavioral responses compared to euhydrated condition only in SHRs. After 0.3 M NaCl intake, SHRs showed a decreased time spent in the EPM open arms (32 ± 5 , vs. euhydrated: $49 \pm 4\%$), a decreased peripheral locomotor activity (78.7 ± 8.9 , vs. euhydrated: 91 ± 5.5 min), decreased central locomotor activity (14.4 ± 1.5 , vs. euhydrated: 23.2 ± 2.6 min), and time spent in the center in the OF (25 ± 2 , vs. euhydrated: 46 ± 7 min). The results suggest that WD or WD-PH produced no change in the decreased anxiety-like behavior pattern and increased locomotor activity in SHRs, whereas 0.3 M NaCl intake after WD-PH may attenuate the increased locomotor activity and increase anxiety-like behavior in SHRs. Financial support: CAPES; CNPq; FAPESP.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Role of Annexin A1 in Cardiac Remodeling and Dysfunction in a Murine Model of Type 1 Diabetes

Authors:

Sarah Bezerra Brum Da SILVA¹,
Diego Dias dos SANTOS¹,
Renata Ramos VIEIRA¹,
Rafael André da SILVA²,
Luis Philipe de Souza FERREIRA¹,
Kátia de Vasconcelos¹,
Cristiane Damas GIL^{1, 2}

Affiliations:

¹ Structural and Functional Biology Graduate Program, Department of Morphology and Genetics, Universidade Federal de São Paulo - UNIFESP, SP, Brazil.

² Biosciences Graduate Program, Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista (UNESP), São José do Rio Preto, Brazil.

Keywords: Echocardiography. Inflammation. Myocardium. Oxidative stress. Streptozotocin.

Abstract:

Diabetes mellitus (DM) is one of the main causes of cardiomyopathy. In murine experimental models, the protein annexin A1 (AnxA1) is known to regulate inflammatory responses and has therapeutic potential in myocardial remodeling. However, its role in cardiac function regulation under diabetic conditions remains unclear. This study investigated the role of AnxA1 in cardiac remodeling and dysfunction in a streptozotocin (STZ)-induced DM model in mice (CEUA 7257110624). Wild-type (AnxA1^{+/+}) and AnxA1 knockout (AnxA1^{-/-}) male C57BL/6 mice received STZ injections for five consecutive days and were analyzed 90 days post-induction. Prior to euthanasia, the animals underwent echocardiography, and hearts were collected for histological and oxidative stress analyses. Echocardiographic parameters showed that diabetic AnxA1^{+/+} mice exhibited significantly prolonged ejection time, compensatory bradycardia, and reduced A' and S' waves compared to both control AnxA1^{+/+} and diabetic AnxA1^{-/-} groups, indicating systolic dysfunction without effective ventricular adaptation. Superoxide dismutase activity was lower in control AnxA1^{-/-} mice compared to control AnxA1^{+/+}, suggesting impaired antioxidant defense. Histological analysis showed that cardiomyocytes in diabetic mice of both genotypes had reduced area and perimeter relative to their controls. Moreover, cardiomyocytes from control AnxA1^{-/-} mice had smaller area and perimeter compared to control AnxA1^{+/+} mice. These findings highlight the essential role of AnxA1 in cardiac homeostasis, reinforcing its function as a modulator of ventricular activity and adaptive responses in DM. Furthermore, the absence of AnxA1 appears to be associated with more adverse cardiac remodeling, increasing heart vulnerability to DM-induced dysfunction.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Endothelial-derived catecholamines profile and its association with serum parathormone and blood pressure in a model of chronic kidney disease

Authors:

Marilia Escrivani de Almeida PETRILLO¹
Flaviano LORENZON²
Gilberto Quirino dos Santos JUNIOR²
Tiago BALDASSO³
Pedro Bonifácio CHITECULO¹
Lauter Eston PELEPENKO¹
Jessica Martins CAMARGO¹
Bruna Lourençoni ALVES²
Alfio José TINCANI³
Gilberto De NUCCI²
Rodrigo Bueno de OLIVEIRA^{1,4}

Affiliations:

¹ Laboratory for Evaluation of Mineral and Bone Disorders in Nephrology (LEMON), School of Medical Sciences, University of Campinas – UNICAMP, Campinas, Brazil.

² Department of Pharmacology, School of Medical Sciences, University of Campinas (UNICAMP), Campinas, São Paulo, Brazil.

³ Department of Surgery (Head and Neck), School of Medical Sciences, University of Campinas (UNICAMP), Campinas, São Paulo, Brazil.

⁴ Department of Internal Medicine (Nephrology), School of Medical Sciences, University of Campinas UNICAMP, Campinas Brazil.

Keywords: Blood pressure. Chronic kidney disease. Endothelial-derived catecholamines.

Abstract:

Introduction: Recent evidence points to a major role of endothelium and epithelium-derived catecholamines (EDC) in hemodynamics. 6-cyano-dopamine (6-CyDA) and 6-bromo-dopamine (6-ND) were previously detected in plasma from chronic kidney disease (CKD) patients. The relationship between EDC and hemodynamics is unknown in CKD settings. We evaluated the profiles of serum, kidney, and heart EDC release in association with serum parathormone (PTH) and blood pressure in Wistar rats with adenine-induced chronic kidney disease (CKD). Methods: 36 animals were divided into control and CKD groups (CEUA_6239-1/2023). The CKD group received a diet containing 0.75% adenine (2 wks) and 0.5% (2 wks). An additional 5-wks period was used for CKD progression. In the control group, 8 animals underwent parathyroidectomy (PTX), and 9 in CKD. Blood pressure analyses were performed (Vistech BP-2000 system). Blood general biochemistry was performed; 6-CyDA, 6-ND, 6-nitroDOPA, 6-BromoDopamina, 6-Nitronoradrenaline, and 6-NAdr were measured in the serum, kidney, and heart by mass spectrometry. Data was expressed as mean/median/log. Means comparisons and correlations were analyzed; $p < 0.05$ was considered. Results: serum 6-CyDA levels were higher in CKD than in control [25.4 (21.7-36.7) ng/mL vs. [20.7 (17.9-27.1); $p = 0.04$]; heart 6-NAdr levels were higher in CKD than in control [0.01 (0.01-6.0) ng/mL vs. [0.01 (0.01-0.01); $p = 0.07$]; 6-CyDA was correlated with blood urea ($r = 0.37$; $p = 0.03$), blood osmolarity ($r = 0.34$; $p = 0.05$), and mean arterial pressure (MAP) ($r = 0.39$; $p = 0.002$). Other EDCs did not differ between groups. There was no correlation between serum PTH and EDC (within and between groups). Conclusion: A differential EDC profile was observed among CKD animals, regardless of PTH. The observed positive relation between 6-CyDA and MAP suggests a role of 6-CyDA in the hemodynamic regulation of CKD.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Effects of probiotic administration on inflammation and cardiac and intestinal remodeling in spontaneously hypertensive rats

Authors:

Cláudio Gabriel Aguiar De OLIVEIRA¹
Deyse Corrêa de AZEVEDO¹
Juliano Custódio VIEIRA JUNIOR²
Helio Cesar SALGADO³
Renata Maria LATARO¹

Affiliations:

¹ Department of Physiological Sciences, Center of Biological Sciences, Federal University of Santa Catarina, Florianópolis, Santa Catarina, Brazil

² Department of Pharmacology, Center of Biological Sciences, Federal University of Santa Catarina, Florianópolis, Santa Catarina, Brazil

³ Department of Physiology, Ribeirão Preto Medical School, University of São Paulo, São Paulo, Brazil

Keywords: Hypertension treatment. Intestinal microbiota. *Lactobacillus Plantarum*

Abstract:

The use of probiotics reduces blood pressure (BP) in hypertensive humans and animals. Therefore, the objective of this study was to evaluate, in spontaneously hypertensive rats (SHR), the effects of chronic probiotic administration on inflammatory cytokines, and cardiac and intestinal remodeling. The study was approved by The Committee of Ethics in Animal Research (CEUA/UFSC; Protocol No. 8400060918). *Lactobacillus Plantarum* was administered orally (1×10^9 CFU/day), for 10 weeks. Data are presented as mean $\pm$ SD (SHR+Probiotic vs SHR+Vehicle). Systolic blood BP was lower in SHR+Probiotic (n=9) compared to SHR+Vehicle (n=8; 166.6 ± 7.074 vs 178.5 ± 9.574 mmHg, $p=0.002$). Probiotic treatment reduced interleukin (IL)-6 in mesenteric lymph nodes (903.4 ± 158.8 vs 1718 ± 283.0 pg/mL, $p=0.0001$) and tumor necrosis factor alpha (TNF- α) in the spleen (118.3 ± 14.13 vs 182 ± 11.15 pg/mL, $p=0.0001$). However, SHR treated with probiotic did not show changes in IL-6 and TNF- α in the heart (IL-6: 793.9 ± 62.47 vs 786.1 ± 52.13 pg/mL, $p=0.98$; TNF- α : 79.19 ± 8.34 vs 77.28 ± 7.87 pg/mL, $p=0.94$); IL-6 in the spleen (369.1 ± 115.1 vs 494.9 ± 158.9 pg/mL); TNF- α in the colon (53.2 ± 2.1 vs 54 ± 4.8 pg/mL, $p=0.79$) and mesenteric lymph nodes (50.54 ± 4.7 vs 53.78 ± 4.3 pg/mL, $p=0.2624$). Histological analyses showed a reduction in cardiac hypertrophy in SHR+probiotic (25.63 ± 0.8326 vs 27.17 ± 0.8961 μ m, $p=0.0188$). In the colon, the treatment did not affect tunica muscularis layer (146.7 ± 14.97 vs 148.6 ± 21.75 μ m, $p=0.9797$), the crypts depth (184.3 ± 10.19 vs 172.1 ± 19.01 μ m, $p=0.3388$) and the number of goblet cells (2.1 ± 0.1732 vs 1.986 ± 0.3625 %, $p=0.8817$) in SHR, but reduced the intestinal fibrotic area (3.248 ± 0.4052 vs 4.93 ± 0.5158 %, $p=0.0023$). In conclusion, chronic treatment with probiotic mitigated the progression of arterial hypertension in SHR, in addition to attenuating inflammation and adverse cardiac and intestinal remodeling. These data indicate that probiotics may have positive effects on hypertension treatment. Financial Support: CNPq and FAPESP.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Effects of Different Concentrations of Magnesium on Vascular Reactivity in Wistar and SHR Rats

Authors:

Livia Seif EDDINE^{1,2}
Livia Suzano de Paula dos SANTOS^{1,2}
Paula dos Santos ATHAYDE¹
Michelle Rossana Martins HORTELAN¹
Katyana Kaline Silva FERREIRA¹
Aurélia Araújo FERNANDES³
Eduardo Hertel RIBEIRO¹
Ivanita STEFANON^{1,2}

Affiliations:

¹ Federal University of Espírito Santo, Department of Physiological Sciences, Health Sciences Center, Vitória, ES, Brazil

² Centro Universitario Multivix, Vitória, ES, Brazil

³ Federal University of Espírito Santo, Department of Morphology, Health Sciences Center, Vitória, ES, Brazil

Keywords: Hypertension. Magnesium. Blood Pressure.

Abstract: Magnesium (Mg) is a mineral that plays a critical role in blood pressure regulation by modulating vascular reactivity. While a diet rich in magnesium has been associated with antihypertensive effects, its acute impact on vascular tone and reactivity remains unclear. This study aimed to investigate the acute effects of different magnesium concentrations on vascular reactivity. Aortic rings from Spontaneously Hypertensive Rats (SHR) and Wistar rats were isolated and assessed for vascular reactivity in response to increasing concentrations of phenylephrine (10^{-9} - 10^{-3} M PHE, % KCl 75 mM) under different $MgCl_2$ concentrations in the bath solution: Normal ($N=Mg^{2+}$ 1.2 mM), Low ($L=Mg^{2+}$ 0.3 mM), and High ($H=Mg^{2+}$ 3.0 mM). Data were analyzed using one-way ANOVA and Tukey post-hoc (Local Animal Ethics Committee: 16-2020). Potassium chloride-induced contraction was significantly different between SHR and Wistar rats without specific treatment (KCl: SHR: 1.88 ± 0.10 , $N=25$; Wistar: 2.4 ± 0.11 g, $N=25$, % KCl 75 mM, $p < 0.05$). The maximum response (R_{max}) to phenylephrine was significantly higher in the low [Mg] SHR group compared to the normal [Mg] SHR group (L-Mg-SHR: 129.2 ± 9.8 , $N=10$; N-Mg-SHR: 97.4 ± 9.9 , $N=9$, % KCl 75 mM, $p < 0.05$). No significant differences were observed between Wistar groups (L-Mg-Wistar: 110.9 ± 7.5 , $N=8$; N-Mg-Wistar: 94.50 ± 4.1 , $N=11$, % KCl 75 mM, $p > 0.05$). The EC_{50} values were significantly different between SHR and Wistar rats in both low- and high-magnesium bath solutions (L-Mg-SHR: -6.07 ± 0.28 , $N=9$; L-Mg-Wistar: -6.93 ± 0.17 , $N=8$, % KCl 75 mM, $p < 0.05$; H-Mg-SHR: -5.89 ± 0.12 , $N=8$; H-Mg-Wistar: -6.58 ± 0.12 , $N=8$, % KCl 75 mM, $p < 0.05$). These findings suggest that a lower magnesium environment enhances the vasoconstrictor response in aortic rings of SHR, indicating a potential role for magnesium in modulating vascular reactivity under hypertensive conditions. Financial Support: FAPES, UFES



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Apocynin prevents the effect of Losartan on the contractile response to phenylephrine in aortas of treated SHR

Authors:

Amanda Coelho de LIMA^{1,2}

Mariana Santana Quirino da SILVA^{1,2}

Cristina ANTONIALLI^{1,2}

Affiliations:

¹Multicenter Postgraduate Program in Physiological Sciences

²UNESP, School of Dentistry, Araçatuba, São Paulo

Keywords: Apocynin. AT1 receptor. Losartan. SHR

Abstract:

Apocynin reduces blood pressure and prevents endothelial dysfunction in SHR. In the aortas and mesenteric beds of SHR treated with Apocynin, AT1 receptor expression is decreased, whereas AT2 receptor expression in the aorta is increased compared to untreated SHR aortas. In this study, we evaluated whether Apocynin treatment would alter the effect of Losartan, an AT1 receptor antagonist of angiotensin II, on the contractile response to phenylephrine (Phe). Wistar and SHR rats, both untreated and treated with Apocynin (30 mg/kg/day, p.o.) from the 4th to the 10th week of age, were studied. Aortic ring reactivity to Phe (10^{-7} M) was assessed in the absence and presence of Losartan (10^{-5} M, incubated for 30 min). Results were compared between treated and untreated groups using an unpaired Student's t-test, and differences were considered significant when $p < 0.05$. The results showed that Losartan reduces the contractile response to Phe in both Wistar rats treated with Apocynin and untreated SHR. However, in the aortas of SHR treated with Apocynin, the contractile response to Phe after incubation with Losartan was not different from that obtained in the absence of the antagonist. The findings suggest that, unlike the other groups, Losartan does not reduce the contractile response to Phe in the aortas of SHR treated with Apocynin, possibly due to the lower AT1 receptor expression previously observed in these animals. These results reinforce the potential therapeutic use of Apocynin as an antihypertensive agent. CEUA-FOA nº 886/2023



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Acute Exposure Effects of Trivalent Sodium Antimoniate and Pentavalent Sodium Antimoniate on Isolated Mitochondria from Heart of Mice

Authors:

Cesar Francisco Maricato da ROSA¹,
Caroline da Silva Moraes²,
Laís Eduardo Marinho²,
Maria Eduarda Maciel Fernandes Pavarino²,
Itanna Isis Araújo de Souza²,
Leonardo Maciel De Oliveira Pinto²

Affiliations:

¹Federal Rural University of Rio de Janeiro

²Federal University of Rio de Janeiro, Carlos Chagas Filho Institute of Biophysics - Antônio Paes de Carvalho Cardiac Electrophysiology Laboratory

Keywords: Mitochondria. Pentavalent Sodium Antimoniate. Trivalent Sodium Antimoniate. Neglected diseases. Heart.

Abstract:

Introduction: Pentavalent sodium antimoniate (Sb(V)) has been a primary leishmaniasis treatment for over 50 years. As a prodrug, it requires conversion to trivalent antimoniate (Sb(III)) for activation. In the intramacrophagic medium, Sb(V) is metabolized into Sb(III), which exhibits leishmanicidal activity by disrupting fatty acid β -oxidation and glycolysis, impairing the parasite's energy metabolism. However, its effects on host mitochondria remain unknown. Objective: This study aimed to examine the effects of Sb(V) and Sb(III) on isolated mitochondria from mouse hearts. Methodology: Seven 4-week-old male C57BL/6 mice (25–30 g) were used (CEUA protocol 119/21), euthanized by cervical dislocation. Hearts were removed for mitochondrial isolation via differential centrifugation. Mitochondrial function was assessed by measuring O₂ consumption, ATP and ROS production, and transmembrane potential. Mitochondria were exposed to 1 ng/ml, 1 μ g/ml, and 1 mg/ml of Sb(V) or Sb(III). Results are expressed as mean $\pm$ standard error of the mean (SEM). One-way ANOVA was used for comparisons, followed by Bonferroni post- tests when significant differences were detected ($p < 0.05$). Results: Oxygen consumption in isolated mitochondria decreased in respiratory states 2 and 3 of complex I at 1 μ g/ml and 1 ng/ml of Sb(V), respectively, compared to the control. Sb(III) showed no difference in complex I state 2 respiration but reduced state 3 respiration at 1 ng/ml. No significant ROS production differences were observed. ATP production decreased with 1 ng/ml and 1 μ g/ml of Sb(V), while Sb(III) reduced ATP at 1 ng/ml. Transmembrane potential remained unchanged across all Sb(V) and Sb(III) doses. Conclusion: The findings suggest a direct action of Sb(V) and Sb(III) on isolated mitochondria, impairing mitochondrial functions and reducing ATP production.



XXXIX REUNIÃO ANUAL DA FeSB
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Functional Study of a *TNNC1*-Encoded Variant of Cardiac Troponin C, E135A, in a Multigeneration Family with Autosomal Dominant Dilated Cardiomyopathy

Authors:

Maicon Landim-VIEIRA¹,
Robin M. PERELLI^{2,3},
Ronnie CHASTAIN¹,
Michelle Rodriguez-GARCIA¹,
Vivek P. JANI⁴,
Joshua H. LAMAR¹,
Ellen MINES³,
Aurelia Araujo FERNANDES⁵,
Vitold E. GALKIN⁶,
Bjorn KNOLLMANN⁷,
Michael P. CARBONI⁴,
Stephen P. CHELKO^{1,8},
P. Bryant CHASE⁹,
Andrew P. LANDSTROM^{2,3},
Jose Renato PINTO¹

Affiliations:

¹ Department of Biomedical Sciences, College of Medicine, Florida State University, Tallahassee, FL, USA ² Department of Cell Biology, Duke University School of Medicine, Durham, NC, USA ³ Department of Pediatrics, Division of Pediatric Cardiology, Duke University School of Medicine, Durham, NC, USA ⁴ Division of Cardiology, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA ⁵ Department of Physiological Sciences of the Federal University of Espírito Santo, Vitória, Espírito Santo, Brazil ⁶ Department of Physiological Sciences, Eastern Virginia Medical School, Norfolk, VA, USA ⁷ Department of Medicine, Vanderbilt University Medical Center, Nashville, TN, USA ⁸ Department of Medicine, School of Medicine, Johns Hopkins University, Baltimore, MD, USA ⁹ Department of Biological Science, Florida State University, Tallahassee, FL, USA

Keywords: Dilated cardiomyopathy. cardiac troponin C. genetic variants. cross-bridge kinetics

Abstract:

Disease-associated variants of *TNNC1*, which encodes cardiac troponin C (cTnC), are linked to dilated cardiomyopathy (DCM). Individuals carrying the novel *TNNC1*-E135A variant, along with their family members, were identified and consented under Institutional Review Board approval from the Duke University Health System. Left ventricular muscle strips were dissected into muscle bundles. steady-state isometric force (SSIF) and cross-bridge kinetics were measured in the presence or absence of PKA after troponin exchange protocol. Statistical significance was assessed using paired or unpaired Student's t-tests, with significance to $P < 0.05$ (*) and $P < 0.001$ (**) for comparisons within the same group, and $P < 0.05$ (#) and $P < 0.001$ (###) for comparisons between different groups. Permeabilized myocardial strips were depleted of endogenous cTnC and reconstituted with either exogenous wild-type cTnC (100% cTnC-WT) or a 1:1 mixture of cTnC-WT and cTnC-E135A. No significant differences were observed in the cooperativity of thin filament activation between cTnC-WT ($n_{Hill} = 1.83$



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

± 0.03) and cTnC-E135A ($n_{\text{Hill}} = 1.81 \pm 0.18$). SSIF was reduced in cTnC-E135A compared to cTnC-WT (22.92 ± 1.83 vs. 22.54 ± 2.48 mN/mm², respectively). PKA treatment of cTnC-WT resulted in a 0.21 pCa unit decrease in myofilament Ca²⁺ sensitivity ($p\text{Ca}_{50} = 5.51 \pm 0.12$). cTnC-E135A exhibited a slight 0.03 pCa unit increase ($p\text{Ca}_{50} = 5.65 \pm 0.07$). PKA treatment reduced the cooperativity of thin filament activation in cTnC-WT ($n_{\text{Hill}} = 1.43 \pm 0.12$ vs. 1.83 ± 0.03 pre-PKA). No significant change was observed in cTnC-E135A ($n_{\text{Hill}} = 1.63 \pm 0.03$ vs. 1.81 ± 0.18 pre-PKA). PKA-mediated phosphorylation decreased SSIF in cTnC-WT (22.92 ± 1.83 vs. 17.22 ± 2.08 mN/mm² post-PKA). No significant reduction was observed in cTnC-E135A (22.54 ± 2.48 vs. 20.47 ± 2.34 mN/mm² post-PKA). TNNC1-E135A variant attenuates the effects of PKA-mediated phosphorylation, suggesting a potential mechanism underlying its pathogenicity in DCM.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Intracranial and cerebral perfusion pressure changes induced by increases in intracerebroventricular volume in male and female Dahl Salt-Sensitive rats

Authors:

Fernanda Campos de MEDEIROS¹,
Eduardo COLOMBARI¹,
Richard David WAINFORD²,
Debora Simões de Almeida COLOMBARI¹

Affiliations:

¹School of Dentistry, Department of Physiology and Pathology, Sao Paulo State University,
²School of Medicine, Emory University

Keywords: Intracranial pressure, intracranial perfusion, Dahl Salt Sensitive, hypertension

Abstract:

The primary mechanism of intracranial pressure (ICP) adaptation to acute increases in arterial pressure (AP) is through autoregulation to maintain cerebral perfusion pressure (CPP). However, this mechanism is impaired in hypertension. We previously demonstrated that male rats with renovascular hypertension, which presents high renin-angiotensin system (RAS) activity, showed increased baseline levels of ICP and CPP compared to normotensive rats. Therefore, in the present study we aimed to evaluate the baseline levels of ICP and CPP in Dahl Salt-Sensitive (DSS) rats, a hypertensive model independent of high RAS activity and to assess the effects of increasing intracranial volume with intracerebroventricular (ICV) infusion of saline (0.15 M NaCl) on ICP and CPP. Male and female DSS rats (3-4 months of age) were used and randomly assigned to be fed with a normal salt diet (DSS-NS; 0.6% NaCl) or high salt diet (DSS-HS; 4% NaCl) for three weeks. After this period, rats were anaesthetized with (urethane, 1.4 g/Kg, i.p.), tracheostomized, and one catheter was inserted in the femoral artery for mean AP (MAP) recording and other catheter was inserted in the right lateral ventricle (LV) for ICP recording. A needle was placed in the left LV to infuse 100 µl of saline for 10 min (10 µL/min rate). CPP was calculated by subtracting MAP and ICP. Male and female DSS-HS rats presented higher baseline MAP compared to their NS pairs (Male: 124 ± 9, vs NS: 90 ± 10 and Female: 134 ± 11, vs NS: 100 ± 8 mmHg, respectively; $p < 0.05$). While MAP was not changed during ICV infusion in males DSS-HS and NS, it was decreased in female DSS-HS compared to the baseline and DSS-NS (-13 ± 5 mmHg at 10 min of infusion, $p < 0.05$). Baseline ICP was comparable in male DSS-HS and NS (7.1 ± 2.3, vs NS: 8.1 ± 0.6 mmHg; $p > 0.05$), as well as the increase in ICP during the ICV infusion. Conversely, in the females, baseline ICP was greater in DSS-HS compared to NS (5.6 ± 2, vs NS: 1.7 ± 1.9 mmHg; $p < 0.05$), but ICP increased similarly in HS and NS during the ICV infusion. Baseline CPP was higher in male and female DSS-HS compared to their NS pairs (Male: 117 ± 5, vs NS: 82 ± 4 and Female: 128 ± 4, vs NS: 98 ± 5 mmHg, respectively; $p < 0.05$). During the ICV infusion, there was a decrease in CPP in male DSS-HS (-8.0 ± 4 at 10 min of infusion; $p < 0.05$) and in the female DSS-HS (-22 ± 5 mmHg at 10 min of infusion; $p < 0.05$) compared to their baseline and to the NS pairs. DSS-NS male and female did not present any change in CPP during ICV infusion. Although there are sex-specific differences in baseline ICP and in MAP responses to increased ICV infusion, an HS diet affects the ability to control CPP in both male and female DSS rats adequately. Institutional Animal Care and Use Committee of the Emory University protocol number IPROTO20230000021. Support or Funding Information: CNPq and CAPES Print(88887.194785/2018-00).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Mitochondrial antioxidant Mitoquinone prevents cardiac dysfunction 7 days after myocardial infarction in rats

Authors:

Carolina Falcão XIMENES¹
Carmen CASTARDELI¹
Pietra Zava LORENCINI¹
Marlon Ramos Rosado MACHADO¹
Katyana Kaline Silva FERREIRA¹
Marcos Eliezeck dos SANTOS²
Sérgio Ricardo Aluotto Scalzo JÚNIOR³
Mário de Moraes e SILVA²
Sílvia Guatimosim²
Eduardo Hertel RIBEIRO¹
Aurélia Araújo FERNANDES¹
Ivanita STEFANON¹

Affiliations:

¹ Federal University of Espírito Santo
² University of Minas Gerais
³ Harvard Medical School

Keywords: Myocardial Contractility. Mitoquinone Mesylate. Oxidative Stress. Acute Myocardial Infarction. Isolated Papillary Muscle. Isolated Cardiomyocyte.

Abstract:

Introduction: The role of mitochondrial ROS (ROSm) in early myocardial infarction (MI) dysfunction remains unclear. Objective: Analyze Mitoquinone (MitoQ)'s effect, for 7 days, on acute contractile dysfunction post-MI in rats. Methods: Wistar males rats (12 weeks) were divided into Sham (S), MI (I) and MI+MitoQ (MI). MitoQ (100 μ M), in drinking water, was offered for 7 days. Hemodynamic *in vivo*, papillary muscle contractility, cardiomyocyte function, and Ca^{2+} transiente were assessed. Superoxide anion ($\text{O}_2^{\bullet-}$) production was quantified using Dihydroethidium (DHE) and mitochondrial $\text{O}_2^{\bullet-}$ ($\text{O}_2^{\bullet-m}$) using MitoSox Red. ANOVA two-way and test *t* student ($p < 0,05$) (CEUA 16/2021). Results: MitoQ prevented weight loss and hemodynamic/contactile dysfuction: systolic blood pressure (P) (SBP) S: 113 ± 3 ; I: 93 ± 4 ; MI: $108 \pm 3^*$; diastolic blood P (DBP) S: 84 ± 3 ; I: 68 ± 3 ; MI: $108 \pm 3^*$; left ventricular systole P (LVSP) (S: 116 ± 4.5 ; I: 79 ± 2.7 ; MI: $100 \pm 4.4^*$); left ventricular end-diastolic P (LVDP) (S: 5 ± 0.4 ; Infarto: 10 ± 1 ; MI: $5 \pm 0.5^*$), (mmHg); first derivate of maximum P ($dP/dt_{\text{máx}}$) (S: 3993 ± 199 ; I: 2480 ± 58 ; MI: $2738 \pm 103^*$) and first derivate of minimum ($dP/dt_{\text{mín}}$) (S: -3273 ± 227 ; I: -1486 ± 41 ; MI: $-2083 \pm 88^*$), (mmHg/s); Isometric force (S: 0.56 ± 0.06 ; I: 0.29 ± 0.05 ; MI: $0.73 \pm 0.08^*$ g/mg), maximum positive derivative of force ($+dF/dt_{\text{máx}}$) (S: 25 ± 2.18 ; I: 14.43 ± 2.20 ; MI: 24.17 ± 1.75 g/g/s) and extracellular Ca^{2+} influx (1,25 mM-S: 444 ± 49.86 ; I: 229.83 ± 68.28 ; MI: $640.17 \pm 60.9^*$ g/g). Cell area (S: 3846 ± 105 ; I: 4309 ± 107 ; MI: $3782 \pm 116^*$ μm^2) and cell length (S: 141 ± 2.8 ; I: 153 ± 1.95 ; MI: $137 \pm 2.25^*$ μm). Cardiomyocytes contractility (S: 610 ± 26 ; I: 815 ± 30 ; MI: $553,6 \pm 30^*$ μm^2). $[\text{Ca}^{2+}]_i$ transient amplitude (S: 2.58 ± 0.04 ; I: 3.15 ± 0.07 ; MI: $2.30 \pm 0.05^*$ F/F₀). (* $p < 0.05$). MitoQ restored redox homeostasis ($\text{O}_2^{\bullet-}$ and $\text{O}_2^{\bullet-m}$, $p < 0.01$). Conclusion: MitoQ prevented contractile dysfunction, confirming ROSm's role in post-MI. Mitochondrial modulation is a promising therapeutic target. Financial support: FAPES, CNPq, UFES.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Effect of resistance training on cardiac function of spontaneously hypertensive rats (SHR)

Authors:

Jéssica da Silva SANTOS^{1,2}

Ronaldo André Castelo dos Santos de ALMEIDA^{1,2}

Letícia de Sousa AMORIM^{1,2}

Anderson Luiz Bezerra da SILVEIRA^{1,2}

Emerson Lopes OLIVARES^{1,2}

Affiliations:

¹ Federal Rural University of Rio de Janeiro, Laboratory of Cardiovascular Physiology and Pharmacology.

² Federal Rural University of Rio de Janeiro, Laboratory of Physiology and Human Performance.

Keywords: Resistance training. Cardiac function. Heart rate. Systolic blood pressure.

Abstract:

Hypertension (HT) is one of the main cardiovascular risk factors, in addition to being considered a serious public health problem due to its chronicity and high costs. HT results in myocardial hypertrophy and increases the risk of ischemia and infarction. The pharmacological treatment of HT has adverse effects, especially when used for long periods, which is a limiting factor in quality of life. Resistance training (RT) effectively reduces BP and improves myocardial function. Procedures of this study were approved by CEUA under nº 14/2022/ICBS/UFRRJ. This study aimed to assess the effect of RT on Systolic blood pressure (SBP) and cardioprotection. Eighteen male spontaneously hypertensive rats were randomly divided into a control group (CTR, n=9) and a group trained 3x/week/10 weeks (RT, n=9). SBP was assessed by tail plethysmography. Left ventricular function (LVF) was assessed by the isolated heart method. The assessment of cardioprotection was based on LVF in the 60 minutes after global ischemia (GI = 30 min). T-test was performed for the comparison of means. At the end of the experimental protocol, SBP was lower in RT compared to CTR (RT: 206.4±8.9 mmHg vs CTR: 235.4±8.8 mmHg, p=0.0392, 95% CI=-61.00 to -1.000). The ECG was used to assess the heart rate (HR). It was observed that RT had a lower HR in the post-experimental period ((CTR vs RT, p=0.0003) 95% CI=-116.4 to -42.62). LVF was not different between groups in the basal period. In the ischemia period, RT delayed the complete stopping of beats in the first 5 min (RT vs CTR: p=0.0324), suggesting cellular energy savings. In the reperfusion period, there was no difference between groups. RT was effective in reducing SBP and heart rate, however it was not able to generate cardioprotection.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: SGLT2 inhibitors attenuate iNOS activation and H₂O₂ production in endothelial cells exposed to doxorubicin

Authors:

Beatriz R. GOES-SANTOS¹
Adriana GIRARDI²
Ana Paula DAVEL³
Ligia M. ANTUNES-CORREA¹

Affiliations:

¹Faculdade de Educação Física, Universidade Estadual de Campinas (FEF/UNICAMP), Campinas
²Instituto do Coração do Hospital das Clínicas da Faculdade de Medicina, Universidade de São Paulo Clinical (InCor-HCFMUSP)
³Instituto de Biologia, Universidade Estadual de Campinas (IB-UNICAMP), Campinas

Keywords: Doxorubicin. Endothelial cells. Hydrogen Peroxide. iNOS. SGLT2 inhibitors.

Abstract:

Doxorubicin (DOXO) is widely used as a chemotherapy treatment for breast cancer, leukemia and other cancers. However, DOXO is cardiotoxic and can lead to vascular dysfunction. Endothelial cells (EC) play a key role in regulating vascular tone, cell migration and angiogenesis. DOXO impairs EC function, increases cell death, produces reactive oxygen species (ROS) and inflammatory factors. Conversely, sodium-glucose co-transporter 2 inhibitors (SGLT2i), such as dapagliflozin (DAPA) and empagliflozin (EMPA), may improve EC function by attenuating oxidative stress and inflammation. We hypothesized that DAPA and EMPA can attenuate DOXO-induced EC injury. Our aim was evaluating the effect of SGLT2i attenuating DOXO-induced oxidative stress and reduction on cell viability. Human umbilical vein endothelial cells (HUVECs) were treated for 24h with 0.5 μ M DOXO and/or 1.0 μ M DAPA or EMPA. Cell viability was evaluated by MTT assay, NO production was assessed by fluorescence to DAF-2DA, and hydrogen peroxide (H₂O₂) by AmplexRed. DOXO reduced EC viability (-55.5% $\pm$ 7.2%, $p < 0.001$) and both SGLT2i did not prevent this effect. DOXO increased H₂O₂ (+29.3% $\pm$ 3.2%, $p < 0.001$) and NO production (+31.7% $\pm$ 6.8%, $p = 0.001$), which were normalized by DAPA and EMPA. Next, we investigated a possible role of iNOS by using the iNOS inhibitor 1400w (10 μ M, 24h). Similarly to DAPA and EMPA, 1400w significantly reduced NO production in DOXO-treated EC to control levels (-62.6% $\pm$ 6.6%, $p < 0.001$), suggesting iNOS participation. In conclusion, DAPA and EMPA did not attenuate the cytotoxic effects of DOXO in cell viability; however, both SGLT2i reduced H₂O₂ and iNOS-mediated NO overproduction induced by DOXO. These data suggest that SGLT2i can attenuate oxidative stress and inflammatory signaling in EC exposed to this chemotherapeutic.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Impacts of Hypoxia and the Uremic Toxin p-Cresyl Sulfate on Cardiorenal Syndrome Type 3

Authors:

Beatriz Favero BEDIN¹

Marcela Sorelli CARNEIRO-RAMOS¹.

Affiliations:

¹ Federal University of ABC (UFABC), Center for Natural and Human Sciences.

Keywords: Cardiorenal. Hypoxia. p-Cresyl Sulfate. Cell viability.

Abstract: Cardiorenal syndrome type 3 (CRS3) occurs when an acute kidney injury (AKI) leads to acute heart failure. Hypoxic injury happens when there is a decrease in the partial pressure of oxygen reaching the tissues, leading to AKI. Uremic toxins are residues of organic compounds that, in some cases, such as AKI, cannot be eliminated from the body and affect various organs, including the kidneys and heart. Specifically, the uremic toxin p-Cresyl Sulfate (pCS) is bound to albumin in the bloodstream, making its excretion more difficult, and is known to negatively affect biological systems. This study aimed to assess the viability of renal and cardiac cells after exposure to hypoxia and pCS, mimicking CRS3. HEK293 (kidney) and H9c2 (cardiomyocyte) cells were cultivated and stimulated with 1.5 mM of pCS for 24 h under hypoxia (21 h) followed by reoxygenation (2 h). Also, the conditioned medium from HEK293 with the same conditions was transferred to H9c2 and evaluated for 6 h and 24 h. Cell viability was measured using the MTT assay. Data were presented as mean $\pm$ SEM and analyzed with one-way ANOVA ($p < 0.05$). pCS seems to decrease cell viability in all cell types and conditioned medium exposure times, when comparing pCS to the control (from a $26.72\% \pm 1.30\%$ to $35.13\% \pm 2.35\%$ decrease) and Hypoxia+pCS to Hypoxia (from a $26.64\% \pm 1.21\%$ to $28.98\% \pm 1.52\%$ decrease). When analyzing the influence of hypoxia alone, only the conditioned medium from 6 h showed a decrease ($4.32\% \pm 1.59\%$) compared to the control group. pCS can significantly reduce cell viability across different cell types and exposure times to conditioned medium. Additionally, hypoxia alone showed a slight effect on cell viability, with a minimal decrease observed only after 6 h of conditioned medium exposure. These findings highlight the cytotoxic effects of pCS, associated with hypoxic injury, and suggest its potential role in contributing to cellular dysfunction in cardiorenal syndrome type 3. Funding: FAPESP 2023/17588-2, 2022/00153-0



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Chronic Apocynin Treatment Preserves Caveolar Integrity and Mitigates Endothelial Dysfunction in Spontaneously Hypertensive Rats

Authors:

Mariana SANTANA QUIRINO¹;
Amanda COELHO DE LIMA¹;
Simone R. POTJE²;
Daniel RODRIGUES³;
Rita TOSTES³;
Cristina ANTONIALI¹.

Affiliations:

¹Departamento de Ciências Básicas, Faculdade de Odontologia, Campus de Araçatuba, UNESP;
²Departamento de Ciências Médias, Campus de Passos, UEMG; ³Departamento de Farmacologia, Faculdade de Medicina de Ribeirão Preto, USP.

Keywords: Apocynin, SHR, hypertension, endothelial cells, caveolae

Abstract:

Apocynin, an antioxidant, prevents high blood pressure in spontaneously hypertensive rats (SHR) by improving endothelial function and upregulating endothelial nitric oxide synthase (eNOS) expression. Since eNOS is anchored in caveolae, specialized endothelial membrane microdomains, we investigated whether apocynin's chronic treatment modulates caveolar expression. Wistar and SHR received either apocynin (30 mg/kg in drinking water, from the 4th to the 10th week of life) or water (control). At the 10th week, thoracic aorta rings were incubated with or without methyl- β -cyclodextrin, a caveolae disruptor (10 mmol/L, 60 min), and concentration-response curves to acetylcholine (ACh) were assessed. NO_x concentration was measured (Griess method) in the presence and absence of dextrin. eNOS and caveolin-1 (Cav-1) expressions were evaluated in human umbilical vein endothelial cells (HUVECs) and aortic tissue from rats treated or not with apocynin. Apocynin reduced SHR systolic blood pressure (untreated: 173 ± 6 mmHg, $n=5$; treated: 137 ± 5 mmHg, $n=5$; $p<0.05$). Dextrin impaired endothelium-dependent vasodilation in Wistar ($pD_2 = 7.01 \pm 0.1$, $n=9$ vs 6.2 ± 0.2 , $n=9$, $p<0.05$) and SHR aorta ($pD_2 = 7.2 \pm 0.1$, $n=6$ vs 6.3 ± 0.1 , $n=6$, $p<0.05$). Apocynin improved SHR endothelial function (ACh E_{Max}: untreated $60.16 \pm 7.17\%$, $n=7$; treated $94.23 \pm 2.92\%$ mN, $n=10$; pD_2 : untreated 7.17 ± 0.1 ; treated 7.36 ± 0.11 , $p<0.05$). Notably, dextrin did not impair ACh responses in treated SHR (E_{Max} = 92.7 ± 3.9 mN; $pD_2 = 6.9 \pm 0.1$, $n=5$). NO_x levels were higher in treated SHR (untreated = 0.073 vs treated = 0.365, $p<0.05$), with no reduction after dextrin incubation (0.377). Apocynin increased eNOS and Cav-1 expression in HUVECs (absence = 0.403 vs presence = 0.735 for eNOS; absence = 0.451 vs presence = 0.922 for Cav-1) and Cav-1 in SHR aorta (treated = 1.306 vs untreated = 0.463, $p<0.05$). Apocynin enhances endothelial function in SHR by promoting caveolar integrity and function. CEUA: FOA-CEUA 0097-2021



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Sex-dependent changes in intracranial pressure and intracranial compliance in a rat model of renovascular hypertension

Authors:

Fernanda Campos de MEDEIROS¹,
Pedro Lourenço KATAYAMA¹,
José Vanderlei MENANI¹,
Eduardo COLOMBARI¹,
Debora Simões de Almeida COLOMBARI¹

Affiliations:

¹Department of Physiology and Pathology, School of Dentistry of Araraquara, UNESP, Araraquara, SP, Brazil

Keywords: Intracranial compliance, intracranial pressure, renovascular hypertension, sexual dimorphism

Abstract:

Intracranial pressure (ICP) reflects the pressure within the cranium, while intracranial compliance (ICC) indicates the brain's capacity to accommodate changes in intracranial volume without substantial increases in ICP. ICC serves as a critical marker for understanding the brain's adaptive mechanisms, particularly in pathological conditions such as hypertension. This study evaluated ICC during a 10-minute artificial cerebrospinal fluid (aCSF) infusion to incrementally increase ICP in male and female two-kidney, one-clip (2K1C) renovascular hypertensive rats. Male and female Holtzman rats (180–210 g) underwent either sham surgery (normotensive, NT) or partial left renal artery occlusion to induce 2K1C hypertension. Six weeks post-surgery, rats were anesthetized with urethane (1.4 g/kg i.v.), tracheostomized, and artificially ventilated. Arterial blood pressure was recorded via femoral artery catheterization, and ICP was monitored using a sensor in the lateral ventricle, where aCSF was infused (100 μ L over 10 minutes at 10 μ L/min). ICC was calculated as the change in volume (ΔV) per unit change in pressure (ΔP). Baseline mean arterial pressure (MAP) was significantly higher in 2K1C rats compared to their NT counterparts (Male: 149 ± 10 vs. 96 ± 14 mmHg; Female: 147 ± 15 vs. 87 ± 11 mmHg, $p < 0.05$). Male 2K1C rats exhibited elevated baseline ICP compared to NT (8.5 ± 3.1 vs. 4.3 ± 1.2 mmHg, $p < 0.05$), while no significant baseline ICP differences were observed between female 2K1C and NT rats (7.9 ± 4.2 vs. 6.4 ± 2.9 mmHg, $p > 0.05$). During aCSF infusion, ICP increases were more pronounced in hypertensive rats (Male: 26.1 ± 7.3 vs. 15.7 ± 3.4 mmHg; Female: 19.5 ± 7.4 vs. 14.1 ± 1.8 mmHg at 10 minutes, $p < 0.05$). ICC was significantly reduced in 2K1C rats relative to NT controls (Male: 5.9 ± 1.2 vs. 9.3 ± 2.4 μ L/mmHg; Female: 9.0 ± 2.4 vs. 13.2 ± 2.9 μ L/mmHg, $p < 0.05$). Furthermore, male 2K1C rats exhibited lower ICC than female 2K1C rats (5.9 ± 1.2 vs. 9.0 ± 2.4 μ L/mmHg, $p < 0.05$). These findings indicate that renovascular hypertension impairs ICC in both sexes, with a more pronounced effect in males. This highlights both the pathological impact of hypertension on brain compliance and sex-specific variability in its severity. Ceua: 16/2023. Funding: FAPESP, CAPESPRINT-UNESP, and CNPq.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Mito-TEMPO treatment prevents progression of aortic PVAT dysfunction in spontaneously hypertensive rats

Authors:

Beatriz Monteiro CURCIO¹
Luciana Venturini ROSSONI¹

Affiliations:

¹ Institute of Biomedical Sciences, University of Sao Paulo

Keywords: Hypertension. Perivascular adipose tissue. Mito-TEMPO.

Abstract:

Perivascular adipose tissue (PVAT) exerts an anticontractile effect under physiological conditions. Our group demonstrated that the loss of this effect in thoracic aortae PVAT (tPVAT) is associated with oxidative stress, impairment of mitochondrial antioxidant defense and reduced nitric oxide bioavailability in adult spontaneously hypertensive rats (SHR). Therefore, the aim of the present study was to evaluate the effect of an early treatment with a mitochondrial antioxidant on tPVAT function in SHR. Wistar rats and SHR (6, 10 and 13 weeks old; CEUA No. 3041240723) were used. A subgroup of rats at 10 weeks of age was treated with the antioxidant Mito-TEMPO (0.7 mg/kg, ip, 21 days) or with vehicle. Systolic blood pressure (SBP) was measured by tail-cuff plethysmography and vascular reactivity was assessed by concentration-response curves to phenylephrine (PHE) in aortic rings, with (+) or without (-) tPVAT. Statistical analysis: ANOVA or t-test; data are expressed as mean±SEM. The SBP or tPVAT anticontractile function did not differ between SHR and Wistar rats at 6 weeks; but at 10 weeks, SHR presented higher SBP (+39%) and lower tPVAT anticontractile effect than Wistar (difference in the area under the curve (dAUC) induced by PHE between PVAT- and PVAT+ rings, Wistar: 107±17.4 vs. SHR: 49±10.4* AU). In addition, at 13 weeks of age, the anticontractile effect of tPVAT was abolished in SHR. Although Mito-TEMPO treatment did not alter the SBP, it prevented the loss of the anticontractile effect of tPVAT observed in SHR at 13 weeks of age (dAUC induced by PHE between PVAT- and PVAT+ rings, Wistar: 145±28.9 vs. SHR+vehicle: -7.5±21.6* vs. SHR+Mito-TEMPO: 106±28.2[#] AU). As has been demonstrated, the complete loss of the anticontractile effect of tPVAT in SHR occurs between the 10th and 13th weeks of age, and the early Mito-TEMPO treatment prevents the progression of tPVAT dysfunction, without modifying SBP.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Protective Role of Crp3 Cysteine and glycine-rich protein 3 deletion against Age-Related Cardiac Dysfunction

Authors:

Kawane Fabricio MOURA¹;
Renato Crajoinas¹;
Gustavo José Justo da Silva²;
Eduardo Moacyr Krieger¹,
José Eduardo Krieger¹;
Ayumi Aurea MIYAKAWA¹.

Affiliations:

¹Laboratório de Genética e Cardiologia Molecular, Instituto do Coração (InCor), Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil.

²Department of Molecular Medicine, Institute of Basic Medical Sciences, University of Oslo, Norway

Keywords: ICrp3; aging; cardiac function

Abstract:

Cysteine and glycine-rich protein 3 (Crp3) play a role in the architecture and mechanical function of cardiac myocytes. Here, we investigated the role of Crp3 in modulating cardiac function during aging. Crp3-knockout (KO) rats and wild-type (WT) at 3 months (young) and 18 months (old) were evaluated. As expected, increased Systolic Blood Pressure (SBP) was observed in aged animals in both WT and KO. However, Crp3-KO showed lower values of SBP than WT either in young or old ages (WT young: $112.6 \pm 1.8(9)$ vs Old: $125.9 \pm 2.5^*(10)$; KO young: $102.5 \pm 2.2(10)$ vs Old: $116.53 \pm 2.1^{**}(10)$). Aged animals showed increased LV posterior wall regardless of the genotype (WT-young: $0.8 \pm 1.03(11)$ vs WT-Old: $1.1 \pm 0.01^*(8)$; KO-young: $0.8 \pm 0.001(10)$ vs KO-Old: $1.2 \pm 0.008^{**}(10)$). As LV mass was similar in WT and KO independently of age, the increase of LV wall thickness in aged animals is not associated with cardiac hypertrophy. Otherwise, LV internal diameter (systole) was increased in aged WT but not in KO compared to young animals (WT young: $5.6 \pm 0.1(9)$ vs Old: $8.2 \pm 0.3^*(10)$; KO young: $5.7 \pm 0.1(10)$ vs Old: $5.9 \pm 0.2^{**}(10)$) indicating that absence of Crp3 protects against aging LV dilatation. In accordance, ejection fraction is decreased in aged WT compared to young animals but not in KO (WT young: $62.4 \pm 1.4(11)$ vs Old: $51.7 \pm 1.9^*(8)$, KO young: $64.9 \pm 2.0(10)$ vs Old: $63.5 \pm 1.9^*(8)$) indicating impaired systolic function only in WT-Old animals. Decreased expression of MyHC alpha (Myh6) and increased expression of MyHC beta (MyHC7) indicates a reduction in contractile velocity of the old WT heart. Interestingly, the levels of Myh6 and Myh7 in KO young animals were similar to those in aged WT suggesting that KO-young have similar molecular expression to WT-Old without cardiac dysfunction. In conclusion, elderly WT animals demonstrated impaired LV systolic function, associated with increased SBP, indicating isolated systolic hypertension. The absence of Crp3 showed to be protective on the parameters analyzed.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Contribution of the Timd4 gene in heart rate regulation in mice

Authors:

Barbara SGANGA¹,
Samantha Kuwada TEIXEIRA²

Affiliations:

¹ Instituto de Ciências Biomédicas da Universidade de São Paulo

² Instituto do Coração do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo

Keywords: Cardiovascular diseases. Genetically modified mice. Heart rate. Timd4

Abstract:

Heart rate is a modifiable factor for cardiovascular diseases, a leading cause of death worldwide. As a complex phenotype, HR is influenced by multiple environmental, genetic and endogenous factors. Recently, our group used N-ethyl-N-nitrosourea-induced germline mutagenesis combined with automatic meiotic mapping to identify new genes involved in HR regulation. Among the new candidates, we identified the Timd4 gene, whose mutation associated with phenotype alteration occurs in the protein domain, affecting its conformation and function according to computational analysis in both *Mus musculus* and *Homo sapiens*. Thus, the present project aimed to evaluate whether the absence of the Timd4 in genetically modified mice alters the cardiac phenotype, leading to decreased HR and arrhythmia. To this end, knockout animals were obtained and a colony was established in our laboratory. Cardiac function was evaluated under baseline conditions and under stress by plethysmography and electrocardiogram (ECG), followed by assessments of sympathetic and parasympathetic tonus, under the approval of the ethical committee CEUA 2224/2025. Data differences between genotypes were assessed using one-way ANOVA. To date, 39 animals have been evaluated through plethysmography, of which 19 underwent ECG analysis of HR, and 11 had their sympathetic and parasympathetic tonus assessed. No significant differences were observed in basal (545 vs. 578 and 600 bpm, from homozygous KO vs. WT and heterozygous animals) or intrinsic HR (481 (Homo KO) vs. 481 (WT) and 546 (Het) bpm), sympathetic (125 (Homo KO) vs. 82 (WT) and 162 (Het) bpm) or parasympathetic tone (4.7 (Homo KO) vs. -8,2 (WT) and 1,5 (Het) bpm), RR (0.11 (Homo KO) vs. 0.10 (WT) and 0,12 (Het) seconds) or QRS intervals (0.0105 (Homo KO) vs. 0.0105 (WT) and 0.008 (Het) seconds) of different genotypes. Given that Timd4 is involved in efferocytosis, a physiologically important pathway, its absence may be compensated by other proteins in the "eat-me" signaling pathway. However, an increased sample size will be necessary to validate the role of Timd4 in HR regulation.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Dengue Infection in Female Mice: Cardiovascular Disturbances Beyond the Usual Symptoms

Authors:

Marina ANDRADE¹
Naiara ASSIS¹
Jaqueline SOUZA¹
Viviane Lima BATISTA²
Vivian COSTA³
Daniella BONAVENTURA¹

Affiliations:

¹Laboratório de Farmacologia Vascular, Departamento de Farmacologia - Universidade Federal de Minas Gerais, Brasil.

²Departamento de Morfologia - Universidade Federal de Minas Gerais, Brasil.

³Grupo de Pesquisa em Arbovírus, Departamento de Morfologia - Universidade Federal de Minas Gerais, Brasil.

Keywords: Dengue virus, vascular reactivity, aorta, vascular system.

Abstract:

Introduction: Dengue, an arboviral disease transmitted by the *Aedes aegypti* mosquito, represents one of the greatest global public health challenges. In addition to classical symptoms, cardiovascular manifestations have been observed. Thus, understanding the cardiovascular mechanisms associated with DENV infection is essential to identify therapeutic targets and mitigate observed complications. Objectives: To evaluate, during the acute phase of dengue virus infection, the vascular effects in female A129 mice, which are knockout for the type I interferon receptor. Methods: Female A129 mice (IFN α / β R^{-/-}) were used and divided into four experimental groups: two control groups (MOCK 3D and MOCK 5D) and two infected groups (DENV 3D and DENV 5D), exposed to 2×10^4 PFU of DENV-2 (humanized strain) via intravenous injection. Body weight and systolic blood pressure were assessed using tail-cuff plethysmography at 3 and 5 days post-infection (dpi). Ex vivo vascular reactivity experiments using aortic rings were conducted through concentration-response curves to phenylephrine (PE, 10^{-10} to 10^{-4} mol/L). Statistical significance was defined as $p < 0.05$ (one-way ANOVA). Results: DENV infection reduced the body weight of animals at 3 and 5 dpi (%) (MOCK 3D: 102.2 ± 1.3 ; DENV 3D: 92.8 ± 1.3 ; MOCK 5D: 102.9 ± 1.8 ; DENV 5D: 89.6 ± 1.6) and caused hypotension at 3 dpi (mmHg) (MOCK 3D: 91.8 ± 1.7 ; DENV 3D: 84.7 ± 1.7). However, vascular reactivity analysis of aortic rings showed no significant differences among the groups in response to phenylephrine. Conclusion: DENV infection caused weight loss and hypotension in infected mice, indicating cardiovascular impairments associated with infection. However, no changes in vascular reactivity were observed, possibly due to the protective effect of female sex hormones in this experimental model. Ethics Committee Approval Protocol: CEUA – UFMG: 101/2024. Financial support: CAPES, CNPq, FAPEMIG.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Endothelial-dependent vascular relaxation is mediated by NO in the stimulation of $\alpha 1$ adrenergic receptors, but not in the stimulation of serotonin receptors

Authors:

Rafael Dias de Brito OLIVEIRA¹;
Marcos Paulo Pimenta da CRUZ¹;
Steyner de França CORTES¹;
Virgínia Soares LEMOS¹

Affiliations:

¹Universidade Federal de Minas Gerais - ICB/UFMG

Keywords: Cardiovascular physiology. Isometric contraction. Nitric oxide. 5-hydroxytryptamine. Non-linear regression.

Abstract:

Activation of smooth muscle cell (SMC) Gq protein-coupled $\alpha 1$ adrenergic ($\alpha 1$ ARS) receptors invokes endothelial cell vasodilatory signalling. The underlying mechanism may be NO- or EDH-dependent. Whether the mechanism is determined by the artery model or receptor activation remains unknown. This study compared the regulatory endothelial-dependent vasodilatory effect of contractions induced by the $\alpha 1$ ARS agonist phenylephrine (PE) or serotonin (5HT). We used C57Bl/6 mice, 10-12 weeks old (CEUA 111/22). Popliteal arteries were mounted on a wire myograph in physiological saline solution (PSS). The presence of functional endothelium was confirmed by acetylcholine-induced vasodilation (ACh; 10 μ M) of over 80%. Removal of the endothelium was attested by a response to ACh<10%. Concentration-response curves for PE and 5HT were compared by F-test, and by the maximum effect values (E_{max} ; mN/mm) and the -log of the effective concentration 50% (pCE50). Data are presented as mean $\pm$ standard error ($p<0.05$ indicates difference). In the presence of a functional endothelium, PE ($E_{max}=2.51\pm 0.13$; pCE50=5.90 $\pm$ 0.10) and 5HT ($E_{max}=0.237\pm 0.08$; pCE50=6.99 $\pm$ 0.07) induced a concentration-dependent contraction. Removal of the endothelium increased the E_{max} (3.12 $\pm$ 0.16 and 3.06 $\pm$ 0.07; $p<0.01$) and pCE50 (6.48 $\pm$ 0.13 and 7.16 $\pm$ 0.07; $p<0.01$) values for PE and 5HT, respectively. L-NAME (100 μ M) increased ($p<0.01$) the E_{max} (3.50 $\pm$ 0.10) and pCE50 (6.16 $\pm$ 0.06) to the levels of endothelial removal for PE. However, L-NAME ($E_{max}=2.35\pm 0.10$; pCE50=7.05 $\pm$ 0.10) and/or indomethacin ($E_{max}=2.32\pm 0.07$ pCE50=7.10 $\pm$ 0.07; $p=0.15$) did not alter the concentration-response curve of 5HT. When the arteries were pre-incubated in a 15Mm KCl PSS, which inhibits K⁺ channels, there was an increase in the concentration-response curves of 5HT. Finally, we show that in the popliteal artery, the mechanism of endothelial-dependent vasodilation in response to SMC $\alpha 1$ ARS stimulation is NO-dependent, and for 5HT receptors appears to be EDH-dependent.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Phenylalanine combined with mean blood pressure as a potential predictive biomarker for early-onset preeclampsia

Authors:

Julyane N. S. KAIHARA¹
Ricardo Carvalho CAVALLI²
Valeria Cristina SANDRIM¹

Affiliations:

¹ Department of Biophysics and Pharmacology, Institute of Biosciences of Botucatu, Sao Paulo State University, Botucatu, Brazil.

² Department of Gynecology and Obstetrics, Ribeirao Preto Medical School, University of Sao Paulo, Ribeirao Preto, Sao Paulo, Brazil.

Keywords: Biomarker. Early-onset preeclampsia. Hypertension. Phenylalanine. Serum metabolomics.

Abstract:

Preeclampsia (PE) is a pregnancy-specific hypertensive syndrome associated with end-organ damage. Its pathophysiology involves an imbalance between pro-angiogenic factors, such as placental growth factor (PlGF), and anti-angiogenic factors, including soluble fms-like tyrosine kinase-1 (sFlt-1). Globally, PE ranks among the leading causes of maternal and perinatal morbidity and mortality, especially when symptoms appear before 34 weeks of gestation. Given that, we aimed to identify potential novel metabolomic biomarkers during the second trimester in conjunction with established PE biomarkers. This study was approved by the Institutional Review Board of the University Hospital of the Ribeirao Preto Medical School, University of Sao Paulo (HCFMRP-USP), under protocol #4116/2008. Among 1,400 pregnant women evaluated between 20–25 weeks of gestation, 460 delivered elsewhere, and 940 completed the study. Of these, 30 developed PE, including 14 early-onset PE cases, with 13 included in this study (CASE). From the 910 normotensive participants, 28 were randomly selected as controls (CTRL). Maternal serum metabolites were obtained by untargeted mass spectrometry and were analyzed by metabolomic methods. The predictive value of significant metabolites, combined with clinical data, was assessed using the area under the receiver operating characteristic curve (AUC). Phenylalanine emerged as a distinguishing metabolite between CASE and CTRL (VIP score > 3.0, $p = 0.0001$), and showed a relevant predictive value (AUC = 0.82, 95% CI = 0.62–0.96). This predictive value was amplified when combined with mean blood pressure (AUC = 0.91, 95% CI = 0.81–1.00). Conversely, phenylalanine + sFlt-1 yielded an AUC of 0.84 (95% CI = 0.46–0.98), and phenylalanine + sFlt-1/PlGF ratio yielded an AUC of 0.80 (95% CI = 0.05–1.00). These findings highlight phenylalanine, especially combined with mean blood pressure, as a potential biomarker for early-onset PE, particularly in the second trimester. Ethics Committee Number: reference 4116/2008 – Institutional Review Board of the University Hospital of the Ribeirao Preto Medical School, University of São Paulo (HCFMRP-USP), Ribeirao Preto, Brazil Financial support: *Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP)* – processos 21/12010-7 e 23/08897-1, *Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)* – 308504/2021-6



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Effect of ad libitum 0.3 M NaCl intake in renovascular hypertensive rats on the expression of microglia in brain areas involved in cardiovascular regulation

Authors:

Gabriela Maria Lucera¹,
José Vanderlei MENANI¹,
Eduardo COLOMBARI¹,
Débora Simões de Almeida COLOMBARI¹

Affiliations:

¹UNESP, Departamento de Fisiologia e Patologia, Faculdade de Odontologia de Araraquara/ SP.

Keywords: Insira aqui de três a cinco palavras-chave, ordenadas em ordem alfabética. Primeira letra maiúscula, restante minúscula e separadas por ponto. *Fonte: Verdana, tamanho 9, cor: preto*

Abstract:

Two-kidney, one-clip (2K1C) renovascular hypertensive rats exhibit a sodium appetite. 2K1C and sodium intake also induce inflammation, which in turn increases sympathetic drive. In addition, previously unpublished data demonstrated that 2K1C rats with ad libitum access to 0.3% NaCl, exhibited a greater sympathetic drive. Therefore, we sought to examine whether ad libitum intake of 0.3 M NaCl, in addition to water, would increase microglial activation brain areas, such as the subfornical organ (SFO) in the forebrain, the area postrema (AP) and the nucleus of the solitary tract (NTS) in the brainstem. CEUA 03/2023: Male Holtzman rats (160-180g) underwent sham or 2K1C surgery. All rats had ad libitum water and food. Sham and part of the 2K1C rats had also available ad libitum 0.3 M NaCl, performing the following groups, sham-Na, 2K1C-Na, and 2K1C. Six weeks after, rats were under deep anesthesia and transcardially perfused with 4% paraformaldehyde. Microglia were labeled by immunohistochemistry, and the images were captured using a confocal microscope and analyzed in ImageJ software. The number of IBA-positive cells in the SFO was approximately the same in all groups. In AP, there was a significant increase in microglia number in the 2K1C-Na group compared to 2K1C and sham-Na (198 ± 9 vs $2K1C 133 \pm 14$ and vs sham-Na: 103 ± 8 ; $p < 0.05$), not observed when comparing 2K1C animals with Sham-Na (133 ± 14 vs. SHAM: 103 ± 8 ; $p > 0.05$). In the commissural NTS, the microglial number was similar in 2K1C-Na and 2K1C rats (99 ± 4 and $2K1C 94 \pm 3$; $p > 0.05$), and both were greater than in sham-Na rats (94 ± 3 and 99 ± 4 vs sham-Na: 63 ± 5 ; $p < 0.05$). Also, in intermediate NTS, the number of microglia was more significant in 2K1C-Na rats compared to 2K1C (94 ± 4 vs 73 ± 2 ; $p < 0.05$) and to sham-Na (94 ± 4 vs sham-Na: 67 ± 7 ; $p < 0.05$). The data indicate that a 0.3 M NaCl intake in 2K1C rats can exacerbate neuroinflammation in a region-specific manner, potentially influencing the increased sympathetic drive.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Effect of the uremic toxin indoxyl sulfate on renal and cardiac tissue after ischemia-induced acute kidney injury

Authors:

Fernanda FOGAÇA-RUIZ¹
Marcela Sorelli Carneiro-Ramos¹

Affiliations:

¹Universidade Federal do ABC

Keywords: Acute kidney injury. cardiorenal syndrome. indoxyl sulfate. uremia.

Abstract:

In healthy individuals, uremic toxins are excreted by kidneys. If not, uremia harms kidneys, heart, and other organs. Indoxyl Sulfate (IS), a protein-bound toxin, is not cleared by dialysis and its mechanisms remain unclear. Heart-kidney interaction is termed cardiorenal syndrome (CRS), divided into 5 types. CRS type 3 refers to acute kidney injury (AKI) causing cardiac changes. This study investigates IS effects in a CRS 3 model induced by AKI. All surgical procedures and protocols were approved by the Ethics Committee on Animal Use of the Federal University of ABC (no. 5250040123). Mice C57BL/6 (between 6 and 8 weeks of age) were submitted to the left kidney ischemia and reperfusion (IR) protocol for 60 minutes. Then, divided into two groups: 1) Injected with IS (100 mg/Kg/day) during 15 days intraperitoneally; 2) Control: treated with saline 0,9% for the same period. The Sham group went through the same procedures but didn't have the renal pedicle clipped. Animals were euthanized; blood, heart, kidneys, and tibia collected for analysis. As a result, it was observed that the IR protocol was effective, as evidenced by the increase in right kidney mass and decrease in left kidney mass, increased gene expression of renal injury molecules in the left kidney: KIM-1, Cystatin-C, and NGAL; additionally, there was an increase in the inflammatory cytokines IL-6, IL-1 β , TGF- β , and TNF- α . The expression of organic anion transporters (OATs) 1 and 3 was also analyzed. The right kidney showed no difference between the groups for both OATs, while the left kidney showed a decrease only in the expression of OAT3 in the ischemia group compared to the Sham group. Plasma IS levels revealed a significant increase in the IR+IS group compared to the Sham, while the p-cresyl sulfate (PCS) levels remained undetectable in all groups. No alterations were observed in cardiac morphometric and molecular patterns.



XXXIX REUNIÃO ANUAL DA FeSBE
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XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Functional Effects of Autoantibodies from Chagas Disease Patients on the Electrophysiology of Cardiomyocytes Derived from Human Induced Pluripotent Stem Cells

Authors:

Keyla Cristiny da Silva COUTINHO¹,
Maria Luiza Gonçalves de LIMA¹,
Cibele Ferreira PIMENTE^{1,5,6},
Dahienne Ferreira de OLIVEIRA¹,
Roberto Coury PEDROSA⁴,
Tais Hanae Kasai- BRUNSWICK^{1,2,3},
Jose Hamilton Matheus NASCIMENTO^{1,2},
Antonio Carlos Campos de CARVALHO^{1,2,3}.

Affiliations:

¹Biophysics Institute Carlos Chagas Filho, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil;

²National Center for Structural Biology and Bioimaging - CENABIO, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

³National Institute for Science and Technology in Regenerative Medicine

⁴Pedro Ernesto University Hospital-Rio de Janeiro State University, Brazil

⁵Posgraduate Program in Translational Biomedicine - Unigranrio/BIOTRANS/InMetro/UERJ-ZO, Rio de Janeiro, Brazil

⁶National Institute of Science and Technology - Hepatology360°

Keywords: Chagas disease, Arrhythmias, Autoantibodies, Cardiac Electrophysiology

Abstract:

Background: Chagas disease, caused by *Trypanosoma cruzi*, can result in cardiac dysfunction, including arrhythmias and heart failure. Autoantibodies targeting G protein-coupled receptors (GPCRs), such as β -adrenergic and muscarinic receptors, are involved in its pathophysiology. This study evaluated whether sera from chronic chagasic patients, known to activate β -adrenergic receptors in animal models, also activate these receptors in human cardiomyocytes. Methods: Cardiomyocytes (CM-hiPSCs) were derived from human induced pluripotent stem cells (hiPSCs). Electrophysiological properties were analysed using Multielectrode Array (MEA) and intracellular microelectrodes. Sera from three chagasic patients with cardiomyopathy and anti- β -adrenergic autoantibodies (A β 1, A β 82, A β 301) were tested for effects on beat rate (BR), field potential amplitude (FPA), and duration (FPD), as well as action potential duration (APD) and arrhythmogenicity. Cells were perfused with Tyrode's solution containing diluted serum (1:100) or purified IgG (80 ng/mL). The study was approved by the Research Ethics Committee (CAAE: 34571120.9.0000.5272). Results: All three sera increased BR (110%, 107%, 119%) and altered FPD (-16.77%, +20.90%, -24.5%). IgG's also increased BR (57%, 85%, 91%). Arrhythmic events, including ectopic beats, early depolarizations, and pauses, occurred in 66%, 50%, and 66% of the samples. The effects of the sera on BR, FPD, and arrhythmias were blocked by the β 1-adrenergic antagonist atenolol (10 μ M). Conclusion: CM-hiPSCs are a valuable model for studying Chagas cardiomyopathy. Anti-GPCR autoantibodies in patient sera activate β -adrenergic receptors and promote arrhythmias, supporting their role in disease pathogenesis.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Comparison between different experimental protocols to induce hypothyroidism in mice: effects on the heart

Authors:

Aline Cristina PARLETTA^{1,2}

Marcela Sorelli CARNEIRO-RAMOS²

Maria Luiza Moraes BARRETO-CHAVES¹

Affiliations:

¹ Department of Anatomy, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil.

² Center for Natural and Human Sciences, Federal University of ABC, Santo Andre, São Paulo, Brazil.

Keywords: Cardiac dysfunction. Cardiac remodeling. Hypothyroidism. Thyroid hormones.

Abstract:

Hypothyroidism is a disease with a high global incidence that affects the entire organism, particularly the heart. To study the impact of hypothyroidism on the heart using mouse models, the first challenge is selecting a protocol that replicates the classic cardiac changes associated with this condition: atrophy, diastolic dysfunction, and myocardial fibrosis. In this context, we compared two protocols for inducing hypothyroidism in mice at different time points aiming to evaluate the effects on cardiac morphology and function. Male C57BL6J mice were divided into control, treatment A (methimazole 0.05%), and treatment B (methimazole 0.1% + sodium perchlorate 1%) groups, N=4-7 per group (CEUA 9961140323). Hormonal replacement with T4 (1 µg/ml) was administered after 3 weeks of treatment B. The drugs were added to the drinking water. Data are presented as mean±standard deviation. Regarding treatment A, no changes in heart weight, cardiomyocyte area and fibrosis were observed at 6 and 12 weeks. Diastolic function was impaired at 12 weeks (E/A ratio control:2.04±0.31 treatment A:2.67±0.48). The increased TSH levels confirmed the hypothyroid state of these animals at 6 (control:42±19 treatment A:295±163 pg/ml) and 12 weeks (control:54±28 treatment A:312±160 pg/ml). With treatment B, after 4 weeks the hearts already displayed atrophy (heart weight/tibial length (HW/TL) control:7.6±0.9 treatment B:5.5±0.3 mg/mm). After 8 weeks, the hearts exhibited atrophy (HW/TL control:8.0±1.1 treatment B:5.8±1.0 mg/mm) and diastolic dysfunction (A'/E' ratio control:1.25±0.23 treatment B:0.72±0.26). The atrophy at 8 weeks was mitigated by T4 replacement (HW/TL treatment B +T4:8.9±1.1). In summary, our data show that treatment A, replicated just cardiac dysfunction associated with hypothyroidism. In contrast, treatment B led to atrophy and diastolic dysfunction, consistent with clinical observations, suggesting it is an effective protocol. Further analyses of fibrosis are ongoing.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Effects of high intensity interval training on neuro-cardiovascular dynamic changes and mitochondrial dysfunction induced by high-fat diet in rats

Authors:

Silvio Rodrigues MARQUES NETO^{1,2}
Raquel Carvalho CASTIGLIONE¹
Carla Caldas ADE^{1,2}
Giovanna de Andrade de Barros e VASCONCELLOS¹
Ana Beatriz Castro RIBEIRO¹
Iasmim Bento LEAL¹
Rayana Guagliardi BRITO¹
Eliete BOUSKELA¹

Affiliations:

¹Universidade do Estado do Rio de Janeiro

²Universidade Salgado de Oliveira

Keywords: Obesity. Mitochondrial swelling. High-fat diet. High-intensity interval training. Cerebral microcirculation.

Abstract:

Mitochondrial swelling is involved in the pathogenesis of many human diseases associated with obesity. One of the strategies for prevention of deleterious effects related to obesity is engaging in regular physical activity, of which high intensity interval training (HIIT) is efficient in promoting biogenesis and improving the function of mitochondria. Our aims were to investigate the effects of HIIT on metabolic and neuro-cardiovascular dynamic control and mitochondrial swelling induced by highfat diet (HFD). Twenty-three male Wistar rats (60 – 80g) were divided into 4 subgroups: control (C), HIIT, HFD and HFD+HIIT (CEUA 060/2012). The whole experimentation period lasted for 22 weeks and HIIT sessions were performed 5 days a week during the last 4 weeks. At the end of the experiments, fasting glucose and insulin tolerance tests were performed. Cerebral microcirculation was analyzed using cortical intravital microscopy for capillary diameter and functional density. Cardiac function and ergoespiometric parameters were also investigated. Mitochondrial swelling was evaluated on brain and heart extracts. HFD promoted an increase on body adiposity ($p<0.001$), fasting glucose levels ($p<0.001$), insulin resistance index ($p<0.05$), cardiac hypertrophy index ($p<0.05$) and diastolic blood pressure ($p<0.05$), along with worsened cardiac function ($p<0.05$), reduced functional cerebral capillary density ($p<0.05$) and its diameter ($p<0.01$), and heart and brain mitochondrial function ($p<0.001$). After 4 weeks of training, HIIT was able to improve cardiac hypertrophy index, diastolic blood pressure, cerebral functional capillary density ($p<0.01$) and heart and brain mitochondrial swelling ($p<0.001$). In animals subjected to HFD, HIIT ameliorated both cerebral mitochondrial swelling and functional capillary density, but it did not improve cardiovascular function suggesting that the cardiovascular dysfunction elicited by HFD was not due to heart mitochondrial swelling.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: MrgD receptor deficiency induces cardiomyocyte atrophic response in adult male mice

Authors:

Maria Eduarda Lima da SILVA^{1,2}
Gabriel Ximenes de OLIVEIRA^{1,2}
Nícia Pedreira SOARES³
Luiza Mazzali FERRAZ^{1,2}
Ana Beatriz PROENÇA^{1,2}
Guilherme dos Santos REIS^{1,2}
Gabriel Ferreira LIMA⁴
Fernanda Carla Ferreira BRITO⁴
Natalia Galito Rocha AYRES¹
Antonio Claudio Lucas da NÓBREGA¹
Eliete Dalla Corte FRANTZ^{1,2}
Robson Augusto Souza SANTOS³
Beatriz ALEXANDRE-SANTOS^{1,2}

Affiliations:

¹ Laboratory of Exercise Sciences, Biomedical Institute, Fluminense Federal University, Niteroi, RJ, Brazil.

² Research Center on Morphology and Metabolism, Biomedical Institute, Fluminense Federal University, Niteroi, RJ, Brazil.

³ National Institute of Science and Technology in Nanobiopharmaceutics, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil.

⁴ Laboratory of Experimental Pharmacology, Department of Physiology and Pharmacology, Biomedical Institute, Fluminense Federal University, Niteroi, RJ, Brazil.

Keywords: Renin-angiotensin system; MrgD receptor; cardiac atrophy.

Abstract:

The Renin-Angiotensin System (RAS) is composed by the classical and the counterregulatory axis, which usually exhibit opposing effects. While overactivation of the classical axis is related the development of several cardiovascular diseases, the counterregulatory axis traditionally has cardioprotective effects. The MrgD receptor is a novel component of the counterregulatory axis of the RAS. 16-week-old Wild Type (WT) and MrgD knockout (KO) C57BL/6J mice were evaluated for body mass (g), triglycerides levels (mg/dL), systolic blood pressure (SPB, mmHg), heart mass (g) and left ventricle (LV) wall thickness (mm), cardiomyocyte morphology, malondialdehyde (MDA) concentration (nmol/mg), and molecular markers of atrophy, oxidative stress, and endoplasmic reticulum (ER) stress. Ethical approval n. 2132023. Statistical analyses were performed by unpaired t test with Welch's correction. MrgD deficiency led to increased body mass (+14,2%, $p < 0,001$ vs WT) and triglycerides levels (+14.1%, $p < 0,05$ vs WT), although SBP and heart mass did not show statistical differences to the WT group. The KO group had thinner LV wall (-13.2%, $p < 0,05$), reduced cardiomyocyte cross-sectional area (-26.1%, $p < 0,05$ vs WT), and increased cardiomyocyte profile by area (+36,5%, $p < 0,05$ vs WT). This atrophic response was accompanied by increased protein degradation (Atrogin: +50.1%, $p < 0,05$; Murf-1: +59.5%, $p < 0,05$) and ER stress markers (GRP78: +46.2%, $p < 0,05$; CHOP: +49.0%, $p < 0,05$). While KO group had increased cardiac expression of proteins related to oxidative stress (NOX4: +71.7%, $p < 0,05$; NOX2: +125.0%, $p < 0,05$), MDA levels remained unaltered ($p > 0,05$). MrgD deficiency may lead to cardiac atrophy, pro- oxidative response, and ER stress.



XXXIX REUNIÃO ANUAL DA FeSB
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: The consumption of *Dipteryx alata* Vog. and goat whey during aging improves metabolism, reduces hepatic oxidative damage, and decreases atherogenic and coronary risk in aged rats

Authors:

Rita de Cássia Araújo BIDÔ¹
Diego Elias PEREIRA²
Camila Maria Sousa de Andrade Nascimento²
Thalia Amannara Melo da Costa²
Marcelo Wesley da Silva Barbosa²
Januse Míllia Dantas De Araújo¹
Renally de Lima MOURA¹
Vanessa Bordin VIERA²
Juliana Késsia Barbosa SOARES¹

Affiliations:

¹ Federal University of Paraíba
² Universidade Federal de Campina Grande

Keywords: Cardiovascular health. Degenerative diseases. Functional foods. Senescence.

Abstract:

Aging is a dynamic phenomenon, accompanied by significant biological, physiological, functional, and psychosocial changes that affect the health and quality of life of elderly individuals. These changes contribute to the onset of metabolic alterations, degenerative diseases, and functional decline. However, there are strategies that can mitigate such changes, including a healthy diet enriched with foods containing bioactive compounds, which act in disease prevention and health promotion. The baru almond (*Dipteryx alata* Vog.) and goat whey are foods rich in nutrients with various protective biological activities in the body. Aged rats were treated by gavage for 10 weeks (n=10/group) and divided into the following experimental groups: Control (CT) – received distilled water; Baru Almond (BA) – received 2000 mg of baru/kg body weight; and Baru + Whey (BW) – received 2000 mg of baru + 2000 mg of goat whey/kg body weight. This study was approved by the Animal Ethics Committee of UFCG under certification No. 62/2020. Physical and biochemical parameters, atherogenic index, and coronary risk index were evaluated. No significant differences were observed in murinometric measurements, body fat, or relative organ weights in the BA and BW groups. Renal function was preserved in these animals. The BA group showed reductions in glucose, total cholesterol, triglycerides, and HDL-c levels. Furthermore, the atherogenic and coronary risk indices, along with hepatic enzyme levels, were reduced in the BA and BW groups. These results demonstrate that the consumption of baru almonds and the almond-whey combination is promising for improving metabolism and reducing hepatic oxidative damage, as well as being an excellent ally in promoting cardiovascular health and preventing age-related diseases.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Chronic Magnesium Supplementation Attenuates Hypertensive Vascular Dysfunction via NO Enhancement and NOX Modulation

Authors:

Livia Suzano de Paula DOS SANTOS^{1,2,3}
Livia Seif EDDINE^{1,2,3}
Paula dos Santos ATHAYDE^{2,3}
Yandra Rissi NEGRI^{2,3}
Michelle Rossana Martins HORTELAN^{1,2,3}
Aurélia Araújo FERNANDES^{2,3}
Katyana Kaline Silva FERREIRA^{2,3}
Eduardo Hertel RIBEIRO^{2,3}
Ivanita STEFANON^{1,2,3}

Affiliations:

¹Multivix Brazilian College of Vitória, Vitória, ES, Brazil
²Federal University of Espírito Santo, Vitória, ES, Brazil
³Federal University of Espírito Santo, Department of Morphology, Health Sciences Center, Vitória, ES, Brazil

Keywords: Adipose Tissue. Hypertension. Magnesium. Oxidative Stress

Abstract:

Magnesium (Mg) is essential for intracellular enzymatic regulation and benefits arterial hypertension, but its effects on vascular function, especially endothelial and perivascular adipose tissue (PVAT) are unclear. This study evaluated Mg's impact on vascular reactivity of PVAT rings and inflammatory and antioxidant pathways, in non PVAT. Male spontaneously hypertensive rats (SHR, 6-week-old) were divided into control and Mg-treated, with the latter receiving MgCl₂ (50 mg/kg/day) for 2 months. Aortic rings were incubated with NS398 (COX-2 inhibitor) and ML171 (NOX inhibitor), and some had PVAT for phenylephrine (PHE)- induced contraction. NO levels were measured by DAF-FM fluorescence, and protein expression (SOD1, SOD2, NOX2, NOX4, COX-2) by Western blot (UFES - Local Animal Ethics Committee: 16-2020). PVAT significantly reduced the contractile response to PHE in controls (Rmax: CT= 139.6 ± 9.6% vs. CT+PVAT= 84.5 ± 6.6%, p<0.05), but not in Mg-treated rats (Mg= 115.5 ± 6.2% vs. Mg+PVAT= 96.0 ± 8.9%, p>0.05). There was also no difference between the treated and untreated groups with the PVAT (Mg PVAT: 96.0 ± 8.9 %KCL vs. CT- PVAT: 84.5 ± 6.6 %KCL, p>0.05). When the NOX pathway was analyzed in non PVAT rings using ML171 (NADPH Oxidase inhibitor), the NO response was lower in the control group (Mg-ML171: 124.0 ± 8.2 %KCL vs. CT-ML171: 89.1 ± 9.9 %KCL, p<0.05), analyzed by DAF protocol (Mg 144.7 ± 24.0 vs. CT: 7.55 ± 1.24, p<0.05). Similarly, the chronic administration of Mg increased NO production (CT: 7.55 ± 1.24 vs. Mg: 144.7 ± 24.06, P<0.05). COX2 inhibition was not significant in both protocols and Mg and ML171 combined had no additive effect. The protein expression analysis showed no alterations (SOD1, SOD2, NOX2, NOX4, and COX2). In conclusion, Mg treatment improves vascular function in SHRs (instead of hypertension). Financial Support: Espírito Santo Research Foundation (FAPES), National Council for Scientific and Technological Development (CNPq).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Maternal unsaturated fatty acid supplementation modulates cardiovascular and morphometric parameters in Wistar rat offspring

Authors:

Hellen Yukari Ito BEIRAUT¹
Matheus Felipe ZAZULA¹
Luiz Claudio FERNANDES²
Katya NALIWAICO¹

Affiliations:

¹ Laboratório de Plasticidade Morfofuncional, Departamento de Biologia Celular, Universidade Federal do Paraná - Curitiba, Paraná, Brasil.

² Laboratório de Metabolismo Celular, Departamento de Fisiologia, Universidade Federal do Paraná - Curitiba, Paraná, Brasil.

Keywords: Cardiac Muscle. Fetal Programming. Heart Structure.

Abstract:

Maternal nutritional status plays a crucial role in fetal development, and alterations in intrauterine conditions can predispose offspring to cardiovascular disorders. Dietary modifications in maternal fatty acid intake during gestation and lactation may induce structural and functional changes in offspring cardiac tissue. This study evaluated the effects of maternal unsaturated fatty acid supplementation on body composition and cardiac morphology in Wistar rat offspring. Thirty-two Wistar rats were divided into four groups: control (CTLE), fish oil-supplemented (SPL3, rich in n-3), soybean oil-supplemented (SPL6, rich in n-6), and olive oil-supplemented (SPL9, rich in n-9). Oral supplementation began 20 days before mating and continued throughout gestation and lactation. On postnatal day 21 (DPN21), offspring were euthanized for morphometric assessment and cardiac tissue collection (CEUA 1459/2022). SPL6 offspring exhibited lower body weight compared to all other groups, particularly in relation to SPL3. SPL3, SPL6, and SPL9 presented reduced nasoanal length, body circumference, and adiposity relative to CTLE. While no differences were observed in overall cardiac volume, offspring from supplemented mothers displayed significantly higher heart weights than the CTLE group. SPL3 and SPL6 groups showed increased cardiac tissue density, indicating potential myocardial structural adaptations. Histomorphometric analysis of the left ventricle revealed distinct remodeling effects in SPL9 compared to both CTLE and SPL6, suggesting specific alterations associated with n-9 supplementation. These findings suggest that maternal supplementation with high doses of unsaturated fatty acids induces metabolic programming, affecting offspring body composition and cardiovascular structure. Long-term implications of these adaptations warrant further investigation.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Secondary hypertension alters urinary biochemical mediators induced by DOCA-salt treatment

Authors:

Edina da Luz ABREU¹
Ana Caroline MARREIROS¹
Rafael Santos CARVALHAL¹
Diego Dias dos SANTOS¹
Cristiane DAMAS GIL¹
Cássia de Toledo BERGAMASCHI¹
Ruy Ribeiro DE CAMPOS¹

Affiliations:

¹Postgraduate Program in Pharmacology, Department of Physiology – Federal University of São Paulo (UNIFESP/EPM)

Keywords: Kidney damage. hypertension. urinary biomarkers.

Abstract:

Secondary hypertension presents higher and sustained pressure values that lead to greater cardiovascular risk and impacts on target organs. Investigate the possible changes in urinary biochemical mediators in DOCA-salt rats. Male *Wistar* (7 weeks; $\pm$ 180 g) were divided and treated for 5 consecutive weeks in: UNx (unilateral uninephrectomy) and DOCA (unilateral uninephrectomy + acetate and deoxycorticosterone (DOCA) (1st, 2nd- 3rd and 4th- 5th week: 20, 12 and 6 mg/kg respectively). DOCA rats receive daily drinking water with a NaCl solution (1%) + KCl (0.2%), and UNx animals drank only water. The rats were placed in metabolic cages; all procedures were approved by the Institutional Ethical Committee (No. 381226082). Biochemical analyses were performed on 24-hour urine samples. Lactate dehydrogenase (LDH) and gamma-glutamyl transpeptidase (GGT) activity and total protein, albumine and creatinine were evaluated. For histological evaluation, renal tissue sections were stained with periodic acid-Schiff (PAS). The results are expressed as mean $\pm$ SEM, compared by two-way ANOVA considering $p < 0.05$ and Bonferroni *post hoc*. Urinary LDH, a marker of renal cell death-related injury, especially glomerular damage, and GGT activity, a marker of proximal tubular injury, were significantly higher in DOCA-Sal compared to normotensive (UNx) rats (DOCA- 3rd and 5th, $297.5 \pm 18.5^*$, $250.2 \pm 15.6^*$ vs UNx- 3rd and 5th, 20.33 ± 12.4 and 65.81 ± 8.7) In the 3rd week of the experimental period, LDH showed statistically higher levels, however, GGT remained elevated in both the 3rd and 5th experimental weeks (DOCA: 23.9 ± 3.1 and 35.7 ± 3.8 vs UNx: 9.3 ± 7.5 and 20.8 ± 4.5). In addition to urinary markers, histological evaluation of renal showed differences in the total glomerular area in animals under DOCA-Sal induction when compared to UNx. Urinary markers of hypertensive rats were modified, denoting possible renal alterations, preferentially tubular ones, which may be therapeutic targets in future analyses.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Undernutrition as a Risk Factor for Cardiovascular-Kidney-Metabolic Syndrome: Role of Endogenous Cardiotonic Steroids*

Authors:

João Pedro Moreira VELOSO-SANTOS¹,
Amaury PEREIRA-ACÁCIO²,
Camile Oliveira SILVA- RODRIGUES³,
Debora MELLO⁴,
Danilo dos Santos ALVES-BEZERRA²,
Glória COSTA- SARMENTO⁵,
Humberto MUZI-FILHO⁵,
Carlla Assis ARAÚJO-SILVA⁵,
Jarlene Alécia LOPES⁵,
Christina Maeda TAKIYA⁵,
Sergian Vianna CARDOZO^{2,3},
Adalberto VIEYRA^{1,2,4,5}

Affiliations:

¹Leopoldo de Meis Institute of Medical Biochemistry, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

²Graduate Program of Translational Biomedicine, Grande Rio University/UNIGRANRIO, Duque de Caxias, Brazil

³Grande Rio University/UNIGRANRIO, Duque de Caxias, Brazil

⁴National Center for Structural Biology and Bioimaging/CENABIO, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

⁵Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

Keywords: Cardiac sodium ATPases. Chronic undernutrition. Endogenous cardiotonic steroids. Hypertension. Rostafuroxin

Abstract:

Cardiovascular-kidney-metabolic syndrome (CKM) is a rising global health concern. While undernutrition is linked to kidney and heart diseases, its association with CKM remains unexplored. We hypothesize that undernutrition contributes to CKM via endogenous cardiotonic steroids (ECTS). The experimental procedures were approved by CEUA-UFRJ (A03/23-066-21). Male Wistar rats of 28 days were assigned to either a control (CTRL) or a multideficient regional basic diet (RBD). Both groups had water available ad libitum. At 60 days, each group was split into 2 subgroups: one received Rostafuroxin (Rosta: 1 mg/kg body mass in 99% ethanol), and the other received water, forming 4 groups (CTRL, CTRL+Rosta, RBD, RBD+Rosta). On day 90, systolic blood pressure (SBP), heart rate (HR), and cardiac output (CO) were measured by tail-cuff plethysmography and echocardiography immediately before euthanasia. Cardiomyocyte plasma membranes were isolated by differential centrifugation. (Na⁺⁺K⁺) ATPase (NaKAct) and Na⁺-ATPase activities (NaAct) of cardiomyocytes were assessed by measuring the ATP hydrolysis. Cardiomyocyte size (CMS) was quantified by immunofluorescence. Collagen deposition (CD) was visualized using a modified Picrosirius red staining. RBD exhibited elevated (vs CTRL) SBP (145±1 vs 129±1 mmHg, n= 4-9), HR (411±5 vs 350±1 bpm, n= 4-7), and decreased CO (42±3 vs 107±3 ml/min, n= 5-8), CMS (762±16 vs 1100±17 µm², n=4-6) with an intense CD (14,2±0,4 vs 5,5±0,1%, n=4). The NaKAct decreased (133±3 vs 207±11, n=5) and NaAct increased (227±6 vs 149±4, n=4-6). RBD+Rosta exhibited increased (vs RBD) CO (57±2 vs 42±3 ml/min, n= 8), CMS (909±17 vs 762±16 µm², n=6), and NaKAct (253±7 vs 133±3, n= 5), and decreased SBP (130±2 vs 145±2 mmHg, n= 8-9), CD (7,1±0,2 vs 14,2±0,4 %, n=4), and NaAct (113±5 vs 227±6, n=4-5). These findings suggest an undescribed type of CKM and that the blockage of ECTS prevented or delayed the worsening of the syndrome.

*Honoring Professor Gerhard Malnic.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Cardiovascular and neuroendocrine effects of LVV-H7 in hypertensive and normotensive rats

Authors:

Camila Nunes da SILVA¹,
Hanniely Thais Menezes SANTOS¹,
Eloisa Alves de Souza QUEIROZ¹,
Gustavo Rodrigues PEDRINO¹,
Carlos Henrique XAVIER¹;
Stefanne Madalena MARQUES¹

Affiliations:

¹ Universidade Federal de Goiás

Keywords: Angiotensin IV receptor; Oxytocinergic system; Hypertension-associated anxiety

Abstract:

LVV-H7 is a hemomorphin derived from the β chain of hemoglobin with anxiolytic potential through its modulation of angiotensin IV receptors (AT₄/IRAP), which influence oxytocin activity. Given the role of oxytocin in social and emotional regulation, its dysregulation has been implicated in neurobehavioral disorders, such as anxiety and depression. This study investigated the cardiovascular effects of LVV-H7 in hypertensive (SHR) and normotensive (Wistar) rats, as well as its impact on oxytocin levels. Male rats (350–400 g) were anesthetized with urethane (1.2 g/kg) and submitted to surgical procedures to record mean arterial pressure (MAP), heart rate (HR), aortic blood flow (ABF), renal blood flow (RBF), renal vascular resistance (RVR), and aortic vascular resistance (AVR). After baseline recordings, an intravenous infusion of LVV-H7 ($0.1 \text{ mg} \cdot \text{kg}^{-1}$) was administered. All procedures were approved by the Animal Use Ethics Committee (CEUA) of the Federal University of Goiás (protocol no. 80/23). In anesthetized rats (Wistar; $n=5$ and SHR; $n=5$), vehicle infusion (NaCl 0.9%; $0.1 \text{ mg} \cdot \text{kg}^{-1}$) did not promote changes in cardiovascular parameters in either group MAP (Wistar: -1 ± 0.2 vs. SHR: -3.1 ± 1.1 mmHg), HR (Wistar: -2 ± 0.8 vs. 8 ± 13 bpm), RBF (Wistar: 5.6 ± 1.0 vs. SHR: $-1.3 \pm 0.8\%$ of baseline) and ABF (Wistar: 3 ± 0.5 vs. $9 \pm 2.5\%$ of baseline). Intravenous infusion of LVV-H7 was also not able to alter these responses. These findings suggest that acute administration does not impact cardiovascular parameters in either group, implying a selective action on neuroendocrine mechanisms. Serum oxytocin levels, measured by ELISA, were elevated in Wistar rats but reduced in SHR rats, suggesting a dysfunction in oxytocinergic regulation associated with the pathophysiology of anxiety and hypertension. LVV-H7 influenced hormonal feedback loops, promoting positive feedback in normotensive rats and negative feedback in hypertensive rats, highlighting its potential as a therapeutic agent for neurobehavioral disorders.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: KDEL receptor blocker induces decrease in cardioprotection Induced by Human Plasma from Volunteers Undergoing Remote Ischemic Preconditioning

Authors:

Maria Eduarda Maciel Fernandes PAVARINO¹,
Antônio Carlos Campos de CARVALHO¹,
José Hamilton Matheus NASCIMENTO¹,
Leonardo Maciel de Oliveira PINTO¹

Affiliations:

¹Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brasil.

Keywords: Heart. Ischemia. KDEL. KTEL. Reperfusion.

Abstract:

Introduction: Ischemic heart diseases are the leading cause of death worldwide. In myocardial infarction, reduced blood flow disrupts oxygen supply, inducing tissue damage. Immediate reperfusion is crucial but can also induce tissue injury. Brief ischemia/reperfusion (I/R) periods induce resistance to infarction, termed ischemic preconditioning (IPC). IPC can be induced in the heart or remotely in non-cardiac tissues (RIPC). However, the cardioprotective pathways of RIPC remain unclear. Here, we assess whether KDEL receptor activation contributes to this protection. **Methods:** Isolated hearts underwent an I/R protocol: 30 minutes ischemia, 120 minutes reperfusion (CEUA:154/21). Human plasma was categorized as placebo or conditioned. Placebo plasma was collected pre-RIPC, while conditioned plasma was obtained post-RIPC (three cycles of upper limb occlusion at 200 mmHg for 5 minutes, with 5-minute deflation). Plasma was ultrafiltered (RIPC_10 kDa) and perfused for 5 minutes pre-ischemia. A KDEL blocker (3 μ M) was perfused with RIPC_10 kDa before the I/R. **Results:** The I/R group showed $\approx 65\%$ infarct size, $\approx 28\%$ Left Ventricular Developed Pressure (LVDP) recovery, and ≈ 61 mmHg Left Ventricular End-Diastolic Pressure (LVEDP). The IPC group showed $\approx 22\%$ infarct size, $\approx 36\%$ LVDP recovery, and ≈ 49 mmHg LVEDP. Placebo plasma perfusion showed $\approx 47\%$ infarct size, $\approx 17\%$ LVDP recovery, and ≈ 54.5 mmHg LVEDP. The RIPC_10 kDa plasma group had $\approx 34\%$ infarct size, $\approx 46\%$ LVDP recovery, and ≈ 46 mmHg LVEDP. In the KDEL antagonist + RIPC_10 kDa plasma group, infarct size was $\approx 53\%$, LVDP recovery was $\approx 15\%$, and LVEDP was ≈ 62.5 mmHg. The KTEL antagonist + RIPC_10 kDa plasma group had $\approx 56\%$ infarct size, $\approx 4\%$ LVDP recovery, and ≈ 89 mmHg LVEDP. **Conclusion:** Small peptides of less than 10 kDa from preconditioned human plasma can protect rat hearts against I/R injury, and KDEL receptor activation play a role in cardioprotection.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Effect of altered miRNA processing on the anticontractile function of perivascular adipose tissue in the aorta of males and females

Authors:

Geiza Rafaela BOBATO¹
Letícia de Souza FIGUEIREDO²
Natália Ribeiro Cabacinha NOBREGA¹
Israelle Netto FREITAS¹
Jamaira Aparecido VICTORIO³
Marcelo Alves da Silva MORI²
Ana Paula DAVEL¹

Affiliations:

¹ Departamento de Biologia Estrutural e Funcional, Instituto de Biologia -IB, UNICAMP, Campinas, SP, Brasil.
² Departamento de Bioquímica e Biologia Tecidual, IB, UNICAMP, Campinas, SP, Brasil.
³ Departamento de Ciências Fisiológicas, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, Florianópolis, SC, Brasil.

Keywords: Dicer. MicroRNAs. Perivascular adipose tissue. Sex differences.

Abstract:

MicroRNAs (miRNAs) are essential regulators of gene expression and play a crucial role in adipose tissue by controlling adipogenesis and secretory functions. Alterations in miRNA processing induced by Dicer enzyme deletion in adipose tissue (ADicerKO) lead to lipodystrophy and insulin resistance. However, the impact of Dicer deletion on ectopic fat depots, such as perivascular adipose tissue (PVAT), remains unclear. We investigated the effects of altered miRNA processing on systolic blood pressure (SBP) and aortic PVAT anticontractile function of male and female mice with intact Dicer (Lox) or ADicerKO (6248-1/2023). SBP in the KO group was significantly higher compared to the Lox group in both sexes (male: Lox=94 ± 1.4 vs. KO=104 ± 2.7*; female: Lox=98 ± 1.5 vs. KO=110 ± 2.8* mmHg, *p<0.05). In males and females, relaxation responses to acetylcholine or sodiumnitroprusside were not affected by the presence of PVAT in Lox or KO. In males, the anticontractile effect of PVAT by reducing phenylephrine-induced contraction was the same in aorta from Lox (maximal response, Rmax: PVAT-=3.16 ± 0.3 vs. PVAT+=2.18 ± 0.2* mN/mm) and KO (Rmax: PVAT-=3.01 ± 0.3 vs. PVAT+=1.97 ± 0.2* mN/mm). Surprisingly, in females, the presence of PVAT did not reduce the phenylephrine-induced contraction in Lox or KO groups. Therefore, we assessed the contraction induced by serotonin and found that the presence of PVAT reduced the potency to this vasoconstrictor in both Lox and KO females. The results suggest that the deletion of Dicer in adipocytes did not impact the anticontractile function of aortic PVAT in males and females.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Reactive oxygen species drive the preganglionic sympathetic activity in experimental renovascular hypertension

Authors:

Fernanda Mano Tagliapietra da SILVA¹;
Ana Caroline MARREIROS¹;
Rafael Santos CARVALHAL¹;
Edina da Luz ABREU¹;
Cássia de Toledo BERGAMASCHI¹;
Maycon Igor de Oliveira MILANEZ¹;
Ruy Ribeiro de CAMPOS¹

Affiliations:

Departamento de Fisiologia Cardiovascular e Respiratória, ¹Universidade Federal de São Paulo, Escola Paulista de Medicina, UNIFESP/EPM, São Paulo/SP.

Keywords: Oxidative stress. Angiotensin II. Renovascular arterial hypertension

Abstract:

Background: The oxidative stress in the brain increases the sympathetic vasomotor activity in the two-kidney, one-clip (2K1C) model. Aim: To evaluate the role of spinal reactive oxygen species (ROS) in regulating the activity of sympathetic preganglionic neurons in the 2K1C hypertension model. Methods: Male Wistar rats were divided into control (n=29) and 2K1C (n=26) groups (CEUA: 5900160321/2021 and 8757070223/2023). 2K1C was induced by inserting a silver clip around the left renal artery (gap of 0.2mm). Animals were catheterized to record mean arterial pressure and heart rate. Intrathecal (i.t.) administrations of Tempol (5nmol) and Apocynin (1nmol) into the subarachnoid space under urethane anesthesia (1.2mg/kg,iv). The splanchnic and renal nerves to determine their activity (sSNA and rSNA) before and after the administration of Tempol and Apocynin. Experiments were conducted to investigate the gene expression of enzymes associated with oxidative stress, the renin-angiotensin system, and inflammation in spinal segments. In another series, the presence of ROS in preganglionic sympathetic neurons was quantitatively evaluated by fluorescence. Results: i.t. administration of Tempol caused a significant reduction in rSNA only in the 2K1C group (60 min: Control vs. 2K1C: 14 ± 6 vs. $-24 \pm 10^* \Delta \text{peaks/second}$), compared with normotensive rats. Conversely, a significant increase in sSNA was observed at 60 min ($13 \pm 6^* \Delta \text{peaks/second}$) after i.t. administration of Tempol in both groups. Apocynin administration induced hypotensive effects in both groups (120 min: $-23 \pm 4 \Delta \text{mmHg}$). Furthermore, ROS production was higher in the thoracic region compared with the lumbar region in hypertensive rats. Furthermore, increased gene expression of specific NADPH oxidase subunits was observed in the lumbar region of 2K1C rats. Data were expressed as SEM ($*p \leq 0.05$). Conclusions: The results suggest that hypertension and sympatho excitation in 2K1C rats are associated with oxidative stress in preganglionic neurons of the sympathetic nervous system. Ethics committee approval: CEUA - UNIFESP (numbers: 5900160321/2021 and 8757070223/2023)



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Role of the afferent renal nerves in the cardiovascular responses to acute renal ischemia-reperfusion in rats

Authors:

Ana Caroline MARREIROS¹.
Rafael Santos CARVALHAL¹.
Maycon Igor de Oliveira MILANEZ¹
Edina da Luz ABREU¹
Fernanda Mano Tagliapietra da SILVA¹.
Rodrigo Lantyer Marques DANTAS¹
Diego Dias dos SANTOS²
Cristiane Damas GIL².
Erika Emy NISHI¹.
Mark KNUEPFER³
Cassia Marta de Toledo BERGAMASCHI¹
Ruy Ribeiro de CAMPOS¹.

Affiliations:

¹ Department of Physiology, Universidade Federal de São Paulo - Escola Paulista de Medicina, São Paulo - Brazil.

² Department of Morphology and Genetic, Universidade Federal de São Paulo - Escola Paulista de Medicina, São Paulo - Brazil.

³ Department of Pharmacological and Physiological Science, Saint Louis University (SLU) - School of Medicine - USA

Keywords: Afferent Neurons. Ischemia/Reperfusion. Sympathetic nervous system.

Abstract:

Introduction: Renal nerve activity is composed of afferent (sensory) and efferent (sympathetic) nerve activity. Ischemia/ reperfusion (IR) of the kidney increases renal sympathetic nerve activity (rSNA) and depresses renal function. As the role of renal afferent fibers in acute renal IR is unclear, we tested the hypothesis that renal IR increases rSNA triggered by renal afferent nerves responding to acute ischemia. Methodology: Two experimental series were performed in adult male Wistar rats, ethics committee No. 1567290421. IR was induced by total obstruction of blood flow to the left kidney by clamping the renal artery for 60 min and reperfusion for 120 min. We recorded MAP, HR, rSNA, and splanchnic sympathetic vasomotor activity (sSNA) in 8 normal IR rats and 6 left kidney deafferented IR rats (IR ARD). Renal deafferentation was performed using capsaicin administration to the left renal nerve 2 weeks before the experiments. Blood samples were collected before ischemia and at the end of reperfusion for total and differential leukocyte counts. Results: Renal ischemia significantly increased rSNA 23 % (20 min: $0,07 \pm 0,04\text{mVs}$ $P < 0.05$) but not sSNA. The increase in rSNA was triggered by activation of renal afferent fibers, since IR significantly reduced rSNA in the IR ARD group maximal decrease in frequency 22 % (180 min: $62 \pm 29\Delta\text{spikes/s}$) and in amplitude 41 % ($0,29 \pm 0,12\text{mVs}$, $P < 0.05$) and induced hypotension and bradycardia. However, no significant difference was observed between groups in blood leukocyte profile, but a significant reduction in renal IL-6 was found in IR ARD, suggesting a reduction in renal inflammation in deafferented IR rats. Conclusions: The results show that renal afferent nerves trigger a preferential increase in rSNA and inflammation in the kidney during acute IR.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Afferent renal nerves promote differential cardiovascular responses in response to furosemide in normotensive and hypertensive rats

Authors:

Rafael Santos CARVALHAL¹
Ana Caroline MARREIROS¹,
Maycon Igor de Oliveira MILANEZ¹
Edina da Luz ABREU¹
Erika Emy NISHI¹
Fernanda Mano Tagliapietra da SILVA¹
Rodrigo Lantyer Marques DANTAS¹
Diego Dias dos SANTOS²
Cristiane Damas GIL²
Mark KNUEPFER³
Cassia Marta de Toledo BERGAMASCHI¹
Ruy Ribeiro de CAMPOS¹.

Affiliations:

¹ Department of Physiology, Universidade Federal de São Paulo - Escola Paulista de Medicina, São Paulo - Brazil.

² Department of Morphology and Genetic, Universidade Federal de São Paulo - Escola Paulista de Medicina, São Paulo - Brazil.

³ Department of Pharmacological and Physiological Science, Saint Louis University (SLU) – School of Medicine - USA

Keywords: Afferent neurons. Autonomic nervous system. Renovascular Hypertension. Sympathetic nervous system. Visceral afferent fibers.

Abstract:

Introduction: We hypothesized that renal sympathetic nerve activity would increase mediated by renal sensory neurons in response to furosemide infusion (i.v, subtherapeutic dose). The role of capsaicin-sensitive afferent renal nerves(positive for TRPV1) was evaluated in control(CONT) and renovascular hypertensive rats(2K1C).**Objective:** to investigate whether furosemide modulates sympathetic nervous activity in normotensive and hypertensive rats and the participation of renal afferents in this reflex.**Methodology:** All experimental procedures were approved by the Ethics Committee of the Paulista School of Medicine – Federal University of São Paulo (process No. 9214060819). In male Wistar rats, three groups of experiments were performed: control (CONT, N = 5), deafferented animals (DEAF, N = 5) with capsaicin (33 mM), renovascular hypertensive rats (2K1C, n = 6). Cardiovascular parameters (MAP and HR), renal sympathetic nervous activity (rSNA) and splanchnic sympathetic nervous activity (sSNA) were evaluated before and after intravenous infusion of furosemide (Lasix®, intravenous, 1 mg/kg/h). **Results:** furosemide induced a reduction in MAP in the DEAF group vs CONT(maximum reduction of -23 ± 4 VS -5 ± 9 Δ mmHg at 120 min, *P<0.05). A significant reduction in rSNA (spikes/s) was found in the DEAF group compared to the CONT group (maximum reduction of -21 ± 7 vs 27 ± 13 Δ spikes/s at 120 min, *P<0.05). A reduction in MAP was found in the 2K1C group compared to CONT (2K1C vs CONT: 110 min: -26 ± 6 vs -4 ± 7 mmHg*); A reduction in rSNA was found in 2K1C rats (2K1C vs CONT: 110 min: -34 ± 13 vs 21 ± 9 vs spikes/s*). **Conclusions:** In normotensive rats, TRPV1-positive renal sensory fibers participate in renal sympathetic activation induced by acute infusion of furosemide at a subtherapeutic dose. In Goldblatt rats, however, renal afferents trigger sympathoinhibition in response to furosemide.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Unraveling the role of muscarinic autoantibodies in the development of arrhythmias in Chagas Disease in a model of human cardiomyocytes derived from induced pluripotent stem cells (CM-hiPSCs)

Authors:

Maria Luiza Gonçalves de LIMA¹
Keyla Cristiny da Silva COUTINHO¹
Tais Hanae Kasai BRUNSWICK^{1,2,3}
Antonio Carlos Campos de CARVALHO^{1,2,3}

Affiliations:

¹ Biophysics Institute Carlos Chagas Filho, Federal University of Rio de Janeiro, Brazil
² National Center for Structural Biology and Bioimaging (CENABIO), Federal University of Rio de Janeiro, Brazil
³ National Institute for Science and Technology in Regenerative Medicine, Brazil

Keywords: Arrhythmias. Autoantibodies. Chagas Disease. Human cardiomyocytes derived from human induced pluripotent stem cells.

Abstract:

Introduction: Chagas disease, caused by *Trypanosoma cruzi*, leads to chronic cardiac dysfunction, including arrhythmias and heart failure. Our group previously demonstrated that IgG antibodies from chronic chagasic patients (CCP) can bind to M2 muscarinic receptors via molecular mimicry, triggering electrophysiological changes. However, these effects were only reported in animal models, highlighting the need for studies in human cardiomyocytes. Objective: To evaluate whether muscarinic sera from CCP can modulate the electrophysiological profile of human cardiomyocytes derived from induced pluripotent stem cells (CM-hiPSCs). Methods: CM-hiPSCs were generated from healthy donors and characterized by cardiac troponin T expression. Electrophysiological parameters such as beat frequency (bpm), field potential duration (FPD), and action potential duration (APD) were assessed. RT-PCR was used to confirm the expression of genes related to ion channels, contractile proteins, and receptors. Sera and isolated IgGs from CCP (M32, M81, M111) were tested with or without atropine (10 μ M). Study approved by the Ethics Committee (CAAE: 34571120.9.0000.5272). Results: CM-hiPSCs expressed key cardiac genes (CACNA1C, SCN5A, HCN4, KCNH2, MYH7, M2 receptor). Acetylcholine reduced bpm and increased FPD; atropine had no effect. Serum M32 reduced bpm (−21.8%), FPD (−14.1%), and caused 50% early after depolarization (EADs). M81 reduced bpm (−25.8%), increased FPD (+17.1%), with 16% EADs. M111 reduced bpm (−4.8%) and FPD (−24.6%), inducing 60% EADs plus ectopic beats. APD decreased with M32 and M111 and increased with M81. IgG-M81 reduced bpm (−24.5%) and increased FPD (+24.2%), while IgG-M111 reduced bpm (−16.0%) and FPD (−24.6%). All effects were blocked by atropine. CM-hiPSCs are a reliable model for electrophysiological studies. CCP sera and IgG modulate cardiac activity via M2 receptors, inducing arrhythmias with varying effects that may reflect clinical heterogeneity in Chagas disease.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: The stress-inducible protein (STI1) modulates physiological and pathological hypertrophy in neonatal cardiomyocytes treated with different biomolecules

Authors:

Iago Nascimento PINHEIRO¹
Fernando Araújo ESPANHOL¹
Victor Moura Vidal COSTA¹
Sílvia GUATIMOSIM¹

Affiliations:

¹ Universidade Federal de Minas Gerais.

Keywords: STI1, Cardiomyocyte, Proteostasis, Hypertrophy, Cardioprotection.

Abstract:

Introduction: Cardiovascular diseases are the leading cause of death worldwide, with approximately 18 million fatalities annually (WHO). Understanding these diseases is crucial for prevention and improving quality of life. Our research investigates cardiac protein quality control, focusing on the cardioprotective role of the co-chaperone Stress-induced phosphoprotein 1 (STI1). Although extensively studied in the nervous system, STI1 remains largely unexplored in the heart. Our group was the first to investigate its role, demonstrating its involvement in pathological hypertrophy—an adaptive stress response of cardiac tissue. This study examines the differential hypertrophic responses of neonatal rat ventricular cardiomyocytes (NRVM) treated with pro-hypertrophic molecules, comparing cells that express STI1 with those that do not. **Methods:** NRVMs were cultured in M199 medium with 10% fetal bovine serum in 96-well plates, (CEUA 97/2022) and transfected with an siRNA directed to STI1 (STI1-siRNA) using Lipofectamine RNAiMAX. Cells were fixed with 4% paraformaldehyde, permeabilized with PBS/Triton (0.5%), and blocked with PBS, 5% BSA, 0.5% Triton, and 5% goat serum. Treatments (48h) included angiotensin II (AngII, 10 μ M), phenylephrine (PHE, 20 μ M), triiodothyronine (T3, 10 nM), and IGF-1 (20 nM) for 48hrs. For immunofluorescence, cells were incubated overnight at 4°C with anti- α -actinin (1:150) and then with Alexa Fluor 633-conjugated anti-mouse antibody. Images were acquired using the Cytation 5 microscope (Biotek). Cell area was analyzed using ImageJ, and statistical analysis was performed with Kruskal-Wallis followed by post hoc testing ($p < 0.05$). **Results and Conclusion:** STI1-siRNA effectively reduced STI1 expression in neonatal rat cardiomyocytes (NRCMs). As expected, AngII and PHE treatment led to an increase in NRCM area. Notably, in STI1-depleted cells, these hypertrophic stimuli failed to alter cell size, indicating that STI1 is essential for cardiomyocyte hypertrophy in response to these agents. Similarly, T3-induced physiological hypertrophy was blocked in STI1-depleted cells. In contrast, IGF-1-induced hypertrophy remained unaffected by STI1 depletion. These findings highlight a specific context in which STI1 is required for cardiomyocyte hypertrophy, suggesting a differential dependency on hypertrophic signaling pathways. Future studies will investigate STI1's molecular role in both physiological and pathological hypertrophy, as well as its apparent dispensability in IGF-1-mediated pathways. We aim to assess STI1 promoter modulation (puromycin/luciferase assays), biosynthesis (Western blot, RT-qPCR), and interactions with HIF-1 α and PrPc using RNA interference. **Funding:** CNPq, CAPES, FAPEMIG, and Pró-reitoria de Pesquisa-UFGM.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Hypoxia-Induced Upregulation and Vesicular Localization of STI1 in Cardiac Fibroblasts

Authors:

Fernando Araujo ESPANHOL¹
Iago Nascimento PINHEIRO¹
Victor Moura Vidal COSTA¹
Samuel Tadeu ROCHA³
Cristina GUATIMOSIM²
Sílvia GUATIMOSIM¹

Affiliations:

¹Departamento de Fisiologia e Biofísica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brasil
²Departamento de Morfologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brasil
³Centro de Aquisição e Processamento de Imagens – CAPI, Universidade Federal de Minas Gerais, Belo Horizonte, Brasil

Keywords: Cardiac Fibroblasts; Proteostasis; Secretion; STI1; Vesicles;

Abstract:

Introduction: Cardiovascular diseases remain the leading cause of death worldwide, as reported by the World Health Organization. Our research focuses on the protective role of the co-chaperone STI1 in the heart—a stress-induced phosphoprotein widely studied in the central nervous system for its involvement in proteostasis. Previously, we were the first to establish its role in regulating pathological hypertrophy in cardiomyocytes. In this study, we investigated STI1's presence, localization, and mobilization in neonatal cardiac fibroblast cell cultures (CEUA 97/2022). **Methods:** STI1 expression in cardiac fibroblasts was assessed using immunofluorescence and qPCR under both normal conditions and chemical hypoxia induced by CoCl₂ (500 µM) treatment for 3h, 6h, and 12h. To examine its localization, fibroblasts were transfected with a plasmid encoding STI1-GFP, while the control group received the GN1 plasmid, followed by exposure to hypoxic conditions. Additionally, FM1-43FX staining was employed to identify exocytotic vesicles, and TIRF microscopy was utilized to track the co-chaperone's trafficking along the cell membrane. **Results:** Our results confirmed the presence of STI1 in fibroblasts and demonstrated a significant increase in its expression following CoCl₂ (500 µM) treatment for 3h and 6h (n=3, =p<0.05). Under control conditions, STI1 was uniformly distributed throughout the cell, whereas after 3h of hypoxic treatment, it exhibited a vesicular pattern, as indicated by punctate GFP fluorescence. FM1-43 probe staining and immunolabeling with an anti-STI1 antibody revealed co-localization of STI1 with exocytotic recycling vesicles, suggesting its involvement in these structures. Additionally, immunofluorescence analysis showed a relative reduction in nuclear fluorescence compared to the cytoplasm in the treated group, implying STI1 mobilization and potential secretion (Mann Whitney test, n=3, =p<0.0001). Furthermore, TIRF microscopy (250 nm) revealed a significant increase in fluorescence per area (Mann Whitney test, n=3, =p<0.0109) near the plasma membrane after CoCl₂ treatment, reinforcing STI1's proximity to the cell surface and supporting its possible secretion. **Conclusion:** Hypoxia induces an increase in STI1 expression and modifies its distribution in cardiac fibroblasts. Evidence suggests its presence in vesicles approaching the cell membrane, indicating potential involvement in vesicular transport. Future studies will focus on investigating STI1 secretion into the culture medium and identifying the factors that regulate this process. **Funding agencies:** CNPq, CAPES, FAPEMIG, and Pró-reitoria de Pesquisa-UFMG.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Outside the Brain: Cardiovascular Consequences of Zika Virus (ZIKV)

Authors:

Naiara de Assis Rabelo RIBEIRO¹;
Natália Ferreira de ARAÚJO¹;
Jaqueline Aparecia SOUZA¹;
Marina ANDRADE¹;
Alexandre COSTA³;
Karla de OLIVEIRA⁴;
Natália NÓBREGA²;
Felipe SANTOS²;
Raquel FERREIRA³;
Ivan LOBO⁵;
Jader CRUZ³;
Stéfany CAU⁴;
Artur MIRANDA⁵;
Vivian COSTA²;
Daniella BONAVENTURA¹

Affiliations:

¹Vascular Pharmacology Laboratory, Department of Pharmacology.

²Arbovirus Research Group, Department of Morphology.

³Excitable Membrane Laboratory, Department of Biochemistry and Immunology.

⁴Cardiovascular Pharmacology and Inflammation Laboratory, Department of Pharmacology.

⁵Cellular Electrophysiology Laboratory, Department of Physiology and Biophysics.

¹⁻⁵ Universidade Federal de Minas Gerais, Brazil.

Keywords: Aorta. Cardiovascular system. Prostacyclin. Vascular reactivity. Zika virus.

Abstract:

Introduction: ZIKV infection causes significant neurological complications, but its cardiovascular impacts remain poorly explored. This study evaluated cardiovascular alterations and their mechanisms in ZIKV-infected mice. Methods: Male A129 (IFN α / β R^{-/-}) mice were divided into control (MOCK) and infected groups (4×10^3 PFU, intravenous). Data were collected at 3- and 5-days post-infection (dpi), including complete blood count, viral titer, cytokines by ELISA, electrocardiogram, cardiac contractility, calcium transients, and vascular reactivity. Statistical significance was considered at $p < 0.05$. CEUA-UFGM: 07/2022. Results: ZIKV reduced body weight at 3 and 5 dpi and caused hypotension at 3 dpi (mmHg). Infected mice presented systemic inflammation, splenic hypertrophy, and leukocytosis (granulocytosis). ZIKV was detected in plasma, heart, and aorta at both 3 and 5 dpi, with increased CCL5 in the aorta and in plasma. The electrocardiogram revealed conduction block at 5 dpi, with prolonged P wave (ms) and PR interval. Cardiac contractility was enhanced, as indicated by increased LVDP, dP/dT, cardiomyocyte shortening fraction, and calcium transients, suggesting impaired SERCA function. In the vascular system, infection induced hyporesponsiveness to phenylephrine (5 dpi), reversed by antagonism of CCL5 receptor (Maraviroc) (MOCK5D: 4.8 ± 0.2 ; ZIKV5D: 3.8 ± 0.2 ; MOCK 5D+Maraviroc: 5.6 ± 0.7 ; ZIKV 5D+Maraviroc: 5.6 ± 0.4), COX-1 and COX-2 inhibitors (Mofezolac and Celecoxib) (MOCK5D+Mofezolac: 4.8 ± 0.3 ; ZIKV 5D+Mofezolac: 4.6 ± 0.2 ; MOCK 5D+Celecoxibe: 4.6 ± 0.4 ; ZIKV5D+Celecoxibe: 4.5 ± 0.3 , prostacyclin receptor (RO1138452) (MOCK5D+RO1138452: 4.4 ± 0.3 ; ZIKV 5D + RO1138452: 4.5 ± 0.3), and PKA inhibitor (KT-5720) (MOCK+KT-5720: 5.79 ± 0.6 ; ZIKV+KT-5720: 5.49 ± 0.4). Conclusion: ZIKV causes acute cardiovascular effects such as hypotension, leukocytosis, cardiac electrical dysfunction, increased basal cardiac contractility, and vascular dysfunction mediated by CCL5, COX, prostacyclin, and PKA.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Brain–spleen–kidney interaction in the evolution of hypertension in spontaneously hypertensive rats

Authors:

Stephanie Furtado GEROLIN¹
Gustavo dos Reis MARTINS¹
Cristiane Damas GIL²
Ruy Ribeiro de CAMPOS JR¹
Cássia de Toledo BERGAMASCHI¹

Affiliations:

¹Laboratório de Fisiologia Cardiovascular Integrativa e Molecular, Escola Paulista de Medicina (EPM/Unifesp), São Paulo/SP.

²Laboratório de Ciências Morfológicas e Inflamação (LACIMI), Escola Paulista de Medicina (EPM/Unifesp), São Paulo (SP)

Keywords: Hypertension, sympathetic nerve activity, spleen, neuroinflammation

Abstract:

Background: Several studies indicate an interaction between the activation of the autonomic nervous system and the elevation of blood pressure. However, the exact mechanisms and timing of this influence are not yet fully understood. Aim: We aim to investigate the temporal relationship between the progression of hypertension (AH) with neuroinflammation and sympathetic vasomotor activity in the renal (rSNA) and splenic (sSNA) regions in young and adult spontaneously hypertensive rats (SHRs), compared to control Wistar rats. Methods: Adult SHRs (SHRa-10 to 15 weeks) and young SHRs (SHRy-5 to 7 weeks), along with corresponding age Wistar rats (WA and WY), were used for direct measurements of blood pressure, heart rate, rSNA, and sSNA. Blood samples, as well as splenic and brain tissues, were collected for analysis of serum and tissue cytokines in regions involved in cardiovascular control, such as the RVLM and PVN. Results: The baseline mean blood pressure value was 109 ± 7 mmHg in young SHRs and 163 ± 9 mmHg in adults, as expected. The baseline rSNA levels of young SHRs were 126.6 ± 27.6 spikes/sec, and for adult SHRs, it was 138.5 ± 32.7 spikes/sec. The basal sSNA for SHRy was 144.0 ± 30.3 spikes/sec, and for SHRa, it was 130.7 ± 26.3 spikes/sec. In the control groups, WY had rSNA values of 94 ± 19 spikes/sec and WA had 87.2 ± 5 spikes/sec. Regarding sSNA, WY showed values of 97 ± 28 spikes/sec and WA had 102.8 ± 18.1 spikes/sec for sSNA. In the RVLM, we observed an increase in TNF- α in the SHRa group (0.28 ± 0.05 pg/mg) compared to both SHRy (0.17 ± 0.47 pg/mg) and WA (0.18 ± 0.02 pg/mg). The data obtained so far suggest that sympathetic hyperactivity occurs before the development of hypertension in SHRs. The increase in cytokines in the RVLM and PVN regions indicates the presence of neuroinflammation, which may be correlated with the sympathoexcitation observed and the subsequent increase in blood pressure in this experimental model. (CEUA-Unifesp nº 835908322)



XXXIX REUNIÃO ANUAL DA FeSBE
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XVI CONGRESSO DA SBBN
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8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Bk Channel Activation Induces Mitochondrial Protection Against Hypoxia/Reoxygenation Injury

Authors:

Domenique Kali Marconi Pereira RABOEIRA¹
Itanna Isis Araujo DE SOUZA¹
Thais BARENCO¹
José Hamilton Matheus NASCIMENTO¹
Cristiano PONTES¹
Leonardo MACIEL¹

Affiliations:

¹ Laboratório de Eletrofisiologia Cardíaca Antônio Paes de Carvalho, Centro de Pesquisa em Medicina de Precisão, Universidade Federal do Rio de Janeiro

Keywords: BK channel. Cardioprotection. Mitochondria. Reperfusion injury.

Abstract:

Ischemic heart disease is a significant cause of mortality globally. When a myocardial infarction occurs, the reduction or interruption of blood flow leads to an imbalance between oxygen supply and demand in the affected area. Restoring blood flow through reperfusion is crucial for the heart survival. However, reperfusion itself can cause myocardium injury. Recent findings show the cardioprotective potential of an agonist of the large-conductance calcium-activated potassium channel (BK channel). The BK channel is involved in regulating vascular tone, cardiac contractility and ischemic preconditioning, which are important factors in myocardial protection. This study aims to investigate the mechanisms of cardioprotection of BK channel activation, focusing in the effects of mitochondrial function. 4-week-old male C57BL/6 mice were used (CEUA #119/21). Hearts were quickly removed and mitochondria were isolated by a differential centrifugation method. Immediately after isolation, mitochondrial function was evaluated by measuring O₂ consumption in different respiratory states, ATP production, ROS production and transmembrane potential. The mitochondria isolated from fresh hearts were incubated directly with BK channel agonist and subjected to hypoxia/reoxygenation in vitro. Incubation of BK channel agonist in mitochondria subjected to hypoxia and reoxygenation prevented reduction in mitochondrial respiratory rate (102 ± 8 nmol O₂/min/mg protein), ATP production (211 ± 4 μ mol ATP / 300ug protein) and reduced mitochondrial ROS production (278 ± 11 nmol/100 μ g protein) compared to the group subjected to hypoxia/reoxygenation (43 ± 3 nmol O₂/min/mg protein; 167 ± 41 μ mol ATP/300ug protein; 341 ± 4 nmol/100 μ g; $p < 0.001$, respectively). BK channel activation protects mitochondrial function against hypoxia/reoxygenation injuries, putatively through modulation of the mitochondrial membrane potential.



XXXIX REUNIÃO ANUAL DA FeSBE
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XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Cardiac remodeling in 6-OHDA Parkinsonism rat model: impact of estradiol treatment in ovariectomized rats

Authors:

Fernando Henrique BORGES^b
Deborah ARIZA^b
Blenda Hyedra DE CAMPOS^a
Andrea Beatriz GONÇALVES^b
Ana Isabel Martins Novais Padrão^b
Hélder Fonseca^b
Carlos Cesar CRESTANI^c
Marli Cardoso MARTINS-PINGE^a

Affiliations:

a Departamento de Ciências Fisiológicas, Centro de Ciências Biológicas, Universidade Estadual de Londrina – Londrina, Paraná, Brasil
b Faculdade de Desporto, Universidade do Porto, UP, Porto, Portugal
c Departamento de Princípios Ativos Naturais e Toxicologia, Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista – Araraquara, São Paulo, Brasil

Keywords: Parkinson's disease, Estradiol, Apoptosis, Collagen, Elastin

Abstract:

Parkinson's disease (PD) is a neurodegenerative disorder due to progressive loss of dopaminergic neurons in substantia nigra pars compacta (SNpc). PD patients present cardiovascular symptoms, as orthostatic hypotension and blood pressure instability. The incidence and prevalence of PD is higher in men, suggesting Woman neuroprotection. Male rats present cardiovascular dysfunction not observed in females. That way, we evaluate cardiac morphology, presence of apoptosis and amount of collagen and elastin of ovariectomized rats induced parkinsonism and treated with estrogen. Ethics Approved (CEUA 001.2021). Wistar rats underwent bilateral ovariectomy. After 24 hours, treatment with estradiol initiated. Seven days after, parkinsonism was induced by bilateral microinjection of 6-hydroxydopamine (6-OHDA, 6mg/mL) into the SNpc. Seven days after parkinsonism induction, organs were collected: striatum for dopamine quantification; uterus and tibia for morphometric parameters; and right (RV) and left (LV) ventricles for histological analyses. The data were subjected to 2-way ANOVA followed by Tukey's post-test and significance of $p \leq 0,05$. Morphological analysis of the RV showed thickness reduction of cardiac tissue in the 6-OHDA group ($p \leq 0,05$). In the LV, parkinsonism induction also caused a reduction in thickness, and estradiol treatment promoted wall thickening ($p \leq 0,05$). Assessment of apoptosis resulted in an increase in cell death, and estradiol treatment reduced it ($p \leq 0,05$). The 6-OHDA groups showed reduction in collagen compared to their respective controls ($p \leq 0,05$). Estradiol increased elastin in both groups. Data showed that parkinsonism caused cardiac apoptosis, in addition to changes in collagen and elastin. Estrogen treatment promoted a reduction in cell death and caused thickening of the ventricular wall. We conclude that parkinsonism by 6-OHDA in females led to morphological alterations that may compromise cardiac function and estradiol treatment induced protection.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Does chronic exposure to air pollution influence cardiac lipofuscin deposition?

Authors:

Gabriel Henrique Rodella GUEDES¹,
Mario Costa CRUZ¹,
Paulo Hilário Nascimento SALDIVA²,
Mariana Matera VERAS³,
Ana Paula Cremasco TAKANO¹

Affiliations:

¹Institute of Biomedical Sciences, Universidade de Sao Paulo;

²Faculdade de Medicina FMUSP, Universidade de Sao Paulo;

³Hospital das Clínicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo

Keywords: Lipofuscin. Cardiac Remodeling. Senescence. Cardiac aging.

Abstract:

Inhalation of particulate matter from air pollution is a potential factor influencing age-related cardiac diseases. This study aims to assess the association between chronic air pollution exposure, estimated by anthracosis deposition, and cardiac aging, measured by lipofuscin accumulation, in individuals who lived in Sao Paulo. We also propose a new automated method for lipofuscin quantification using machine learning. Data collection included information obtained from interviews with relatives of non-smoking deceased individuals and biological samples (lung images and myocardial fragments) from autopsies conducted at the Death Verification Service of the Capital, with prior approval from the Research Ethics Committee. Lung anthracosis was calculated using ImageJ software. For lipofuscin quantification, images were taken using a confocal microscope based on autofluorescence of the pigment. Lipofuscin was quantified by three approaches: a manual method, an automated machine learning method, and the same automated method using bright-field images stained with Hematoxylin and Eosin (HE). The methods were compared using one-way ANOVA with repeated measures. The association between anthracosis and lipofuscin was evaluated by Spearman's correlation and multiple linear regression. There was no significant difference between traditional and proposed methods ($p < 0.05$), then the automated HE method was chosen. The study population ($n = 94$) ranged in age from 39 to 104 years (mean = 74), with an average residency of 52 years in São Paulo and a mean anthracosis deposition of 0.17. No significant relationship was found between lung anthracosis and heart lipofuscin, both in correlation ($p = 0,35$) and linear regression ($p=0,724$). Additional samples are currently under analysis to refine these findings.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Investigation of the involvement of platelet-activating factor in the control of hypertension by aerobic training. a randomized controlled trial

Authors:

João Paulo PRADO¹

Rayner Ribeiro CARDOSO¹

Giovane Galdino de SOUZA¹

Affiliations:

¹Federal University of Alfenas, Alfenas, MG, Brazil.

Keywords: Cytokines. Exercise. Hypertension. Quality of life. Respiratory muscle strength

Abstract:

Abstract: Although studies have demonstrated the effectiveness of exercise in controlling systemic arterial hypertension (SAH), the mechanisms involved in this effect are still poorly understood. Thus, this study investigated the impact of aerobic training on the relationship between platelet-activating factor (PAF) circulating levels and blood pressure in hypertensives. Seventy-seven hypertensive subjects were enrolled in this randomized controlled trial (age 66.51 ± 7.53 years, body mass 76.17 ± 14.19 kg). Participants were randomized to two groups: the intervention group (IG, $n = 36$), composed of hypertensive individuals submitted to an aerobic training protocol, and the control group (CG, $n = 41$), composed of non-exercised hypertensives. Body mass index, arterial blood pressure, quality of life, respiratory muscle strength, and functional capacity were assessed before and after 12 weeks. PAF and plasma cytokine levels were also evaluated respectively by liquid chromatography coupled with mass spectrometry and enzyme-linked immunosorbent assay. Aerobic training promoted a significant reduction in blood pressure [124.72 ± 1.66 vs. 133.05 ± 2.38 (95% CI 2.93–13.72, $p = 0.048$) for SBP and 76.94 ± 1.48 vs. 82.22 ± 1.44 (95% CI 4.82–5.73, $p = 0.034$) for DBP] in trained subjects compared to baseline while functional capacity, expiratory muscle strength, and quality of life physical aspects [72.19 ± 5.53 vs. 50.02 ± 6.05 (95% CI 3.16–6.43, $p = 0.006$, $d = 2.50$)] in emotional aspects [60.48 ± 6.75 vs. 40.65 ± 6.21 (95% CI 3.16–6.43, $p = 0.01$, $d = 3.06$)] compared to baseline, PAFC16:0 and PAFC18:1 plasma levels were increased in comparison to the CG Multiple linear regression analysis indicated that MEP was the strongest predictor ($F [3.19] = 6.322$; $p = 0.001$; R^2 adjusted = 0.499) for PAFC16:0 plasma levels in subjects undergoing aerobic training ($p < 0.05$). In addition, multiple correlation analysis indicated a positive correlation [$F (3.19) = 6.322$; $p = 0.001$; R^2 adjusted = 0.499] between PAFC16:0 levels and expiratory muscle strength after aerobic training. Taken together, our findings indicate that PAF may be involved in the indirect mechanisms that control SAH, being mainly associated with increased respiratory muscle strength in hypertensive subjects undergoing aerobic training.

Study approved by the ethics and research committee of the Federal University of Alfenas–MG (protocol nº. 3.262.183).



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XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Gq Protein Activation In Cardiomyocytes Modulates The Population Of Resident Macrophages Through In Situ Proliferation

Authors:

Henrique ABRAMO ¹,
Giovane PIRES¹,
Mário Morais SILVA¹,
Sílvia GUATIMOSIM¹,
Cibele ROCHA-RESENDE¹

Affiliations:

¹Departamento de Fisiologia e Biofísica, Instituto de Ciências Biológicas,
Universidade Federal de Minas Gerais.

Keywords: Cardiomyocytes. Inflammation. Macrophages. Proliferation. Signal transduction.

Abstract:

Although previous studies have shown the cardioprotective role of resident embryonic macrophages, the mechanisms involved in the maintenance of these cells are still unknown. Previously, by using a chemogenetic model of Gq protein activation exclusively on cardiomyocytes (DREADD-hM3Dq), we observed an increase in the number of cardiac macrophages. The aim of this study is to investigate whether activation of Gq protein in cardiomyocytes leads to changes in cardiac macrophage subsets. We treated 5-week-old DREADD-hM3Dq and control (CT) mice with clozapine (CNO; IP, 0.5 mg/kg) for seven days and performed immunofluorescence and flow cytometry experiments. There was an increased number of CCR2⁻ resident macrophages in the hearts of DREADD-hM3Dq mice compared to CT ($116.0 \pm 8.1; n=11$ vs $89.6 \pm 7.9; n=8$). Notably, the macrophage expansion was due to an increase in the number of CCR2-Tim4⁺ embryonic cells (66.8 ± 4.8 vs 48.4 ± 5.4). These findings were corroborated by immunofluorescence staining of CD68⁺Lyve1⁺ embryonic cells ($10.1 \pm 1.1; n=4$ vs $4.4 \pm 0.4; n=4$). Given that Tim4+Lyve1+ macrophages are seeded in the heart during the embryonic life and are kept through in situ proliferation, we asked ourselves whether cardiomyocytes, when submitted to activation of the Gq protein signaling pathway, could stimulate proliferation of macrophages. In fact, proliferative CD68⁺Ki67⁺ cells were more abundant in DREADD-hM3Dq hearts ($3.3 \pm 0.3; n=6$ vs $2.0 \pm 0.2; n=8$). Confirming these findings, there was a significant increase in BrdU incorporation by DREADD-hM3Dq macrophages in comparison to CT ($6.6 \pm 0.7; n=6$ vs $1.0 \pm 0.1; n=9$). In conclusion, Gq protein activation in cardiomyocytes promotes resident macrophage expansion via in situ proliferation. These findings suggest a cross-talk between cardiomyocytes and macrophages, potentially relevant in pathophysiological conditions. Further studies are needed to explore the mechanisms of this interaction and the effects of macrophage depletion in this model. APOIO: FAPEMIG e Instituto Serapilheira CEUA 331_2023_aprovado



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Cardioprotection by transfer of human plasma after remote ischemic preconditioning: identification of cardioprotective humoral factors through omics techniques

Authors:

Rodrigo Veras da Silva MIRANDA¹
Fábio NOGUEIRA²
Antonio Carlos Campos de CARVALHO¹
Gilberto DOMONT²
Jose HAMILTON¹
Dahienne de OLIVEIRA¹
Leonardo MACIEL¹

Affiliations:

¹ Instituto de Biofísica Carlos Chagas Filho (Federal University of Rio de Janeiro)
² Instituto de Química (Federal University of Rio de Janeiro)

Keywords: Cardioprotection. Ischemia-Reperfusion. Myocardial infarction. Remote ischemic preconditioning

Abstract:

Remote ischemic preconditioning (RIPC) provides myocardial resistance against ischemia/reperfusion (I/R) injury. It is suggested that the protective mechanism of RIPC is mediated by humoral factors. However, the identity of these humoral factors remains enigmatic. We aimed to characterize and identify the humoral factors responsible for cardioprotection induced by RIPC. For this study, a total of 74 male rats, Wistar variety, were used. The experimental protocol was approved by the Committee on Ethics in the Use of Animals (CEUA) in Scientific Experimentation, CCS/UFRJ (protocol 154/21). All volunteers were provided with an informed consent form to participate in the study. The study is submitted to the Ethics Committee of the National Institute of Cardiology and this project is registered under Ethics Committee in Research in Human Beings CEP 28998819.9.0000.5272. The volunteers were submitted to the RIPC protocol. Venous blood was collected before (Placebo plasma) and after RIPC (RIPC plasma). Human plasmas were fractionated into different molecular weight ranges and cardioprotection was evaluated in isolated rat hearts submitted to 30 minutes of ischemia and 120 minutes of reperfusion in an isolated heart apparatus. Mass spectrometry (MS) was performed on placebo plasma and RIPC plasma. The fraction of less than 10kDa of RIPC plasma reduced infarct size by 50% and induced hemodynamic recovery compared to I/R group. The fraction less than 10kDa of placebo plasma, fraction greater than 10kDa and total RIPC plasma did not induced cardioprotection. MS showed differences in protein content and the presence of 15 putatively cardioprotective proteins in qualitative analysis in RIPC plasma compared to placebo. Cardioprotective humoral factors are in the fraction less than 10kDa of RIPC plasma. Furthermore, cardioprotection by RIPC can be transferred between species. Adenosine, kininogen and 15 other proteins may be responsible for the RIPC cardioprotection.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Impact of chemotherapy on uterine artery reactivity during cancer in pregnancy

Authors:

Israelle Netto Freitas¹,
Daniele Mendes Guizoni¹,
Fernanda Aparecida Marqueto²,
Beatriz Cassaniga Talassi²,
Beatrice Candido Alves²,
Rogério Willians dos Santos²,
Maria Cristina Gomes-Marcondes²,
Laís Rosa Viana²,
Ana Paula Davel¹

Affiliations:

¹Laboratory of Vascular Biology

²Nutrition and Cancer Laboratory

Department of Structural and Functional Biology, Institute of Biology,
Universidade Estadual de Campinas-UNICAMP, Campinas, SP, Brazil.

Keywords: Chemotherapy, Walker 256 tumors, Pregnancy and Uterine Artery.

Abstract:

Maternal uterine artery (u.a) plays a key role in maintaining placental function and fetal oxygen supply. Cancer during pregnancy presents a challenge, as both the tumor and chemotherapy can impact maternal and fetal health. Although cancer and chemotherapy affect vasculature, their impact on the u.a remains unclear. This study assessed functional changes in the uterine artery (u.a.) of pregnant rats bearing Walker 256 tumors (W group), treated with chemotherapy (WQ group), and compared them to a control group (C group) or a chemotherapy-treated control group (Q group). On day 2 of pregnancy, 3×10^6 Walker 256 tumor cells were implanted subcutaneously in the W and WQ groups (CEUA: 6336-1/2023). Chemotherapy (doxorubicin 0.1 mg/kg and cyclophosphamide 1 mg/kg, i.p.) was administered to the Q and WQ groups twice during the last week of pregnancy (days 14 and 17). On day 20 of pregnancy, the rats were weighted and euthanized, and the u.a was collected for vascular reactivity. Statistical analysis: 2-way ANOVA, $*p < 0.05$ vs C. Body weight and blood pressure were similar among groups. There was an impairment in the endothelium-dependent relaxation to acetylcholine of the u.a in rats with tumor treated or not with chemotherapy ($-\text{LogEC}_{50}$: C=7.2 $\pm$ 0.2; W=6.6 $\pm$ 0.3*; Q=7.7 $\pm$ 0.05; WQ=6.5 $\pm$ 0.3*). There was no difference in the maximum contraction to 80mM KCl. The u.a internal diameter was reduced only in the pregnant rats with tumor and chemotherapy (C= 461 $\pm$ 21; W=450 $\pm$ 18; Q=441 $\pm$ 31; WQ=389 $\pm$ 34* μm). Our findings suggest endothelial dysfunction of u.a during cancer during pregnancy. The combination of tumor and chemotherapy induced additional vascular damage by reducing u.a internal diameter. Financial Support: CNPq- 173353/2023-1 e FAPESP- 2024/03039-0, 2021/08931-0, 2023/01743-9, 2023/01811-4, 2022/02114-2, 2021/08188-5, 2021/08931-0, 2017/02739-4.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Collagen matricryptin improves cardiac function by mediating scar formation

Authors:

Gabriel Araujo GRILO^{1,2}

Leonardo MACIEL¹

Jose Hamilton Matheus NASCIMENTO¹

Antonio Carlos Campos de CARVALHO¹

Affiliations:

¹ Universidade Federal do Rio de Janeiro

² East Carolina University

Keywords: Cardiac remodeling. Fibrotic scar. Matricryptin.

Abstract:

Introduction: Adverse cardiac remodeling post-myocardial infarction (MI) triggers a compensatory maladaptive response that contributes to heart failure. The infarcted area is replaced by a fibrotic scar and its formation depends on the interaction between cardiac cells, particularly fibroblasts and the extracellular matrix (ECM). Matricryptins are biologically active peptides able to regulate cell function and survival. A peptide mimetic of a collagen-derived matricryptin (p1159) was shown to stimulate left ventricular (LV) fibroblast migration *in vitro*, and reduce LV dilation and fibrosis after 7 days delivery in a mouse model of MI. This suggested p1159 long-term treatment post-MI could have beneficial effects in LV post-MI. Methodology: Using a permanent occlusion MI mouse model, animals received p1159 or vehicle solution up to 28 days (USDA 55-R-0010). We assessed peptide treatment effects on scar composition and on systolic function. To assess effects on scar vascularization, a cohort of mice were injected with isolectin-B4. To investigate p1159 mode-of-action, LV fibroblasts from naïve animals were treated with increasing doses of p1159. Results: Matricryptin p1159 significantly improved systolic function post-MI at D28 (p1159=28±14, saline=14±7, $p<0.05$) and reduce LV dilation (end systolic volume, p1159=85±42, saline=150±74, $p<0.05$) by inducing the formation of a compliant scar with reduced levels of cross-linking (p1159=1.4±0.2, saline=0.5±0.4, $p<0.05$, $n=6/\text{group}$), and improved tissue perfusion (p1159=0.5±0.1, saline=0.25±0.1, $p<0.05$, $n=8/\text{group}$). In addition, we found that p1159 increases cardiac fibroblast migration (p1159 vs. SFM 48h, $p<0.05$) by activating RhoA pathways via the membrane receptor integrin $\alpha 4$ (p1159=3±0.5, saline=1±0.4, $n=4/\text{group}$, $p<0.05$). Conclusion: Our data indicate p1159 treatment reduced adverse LV remodeling post-MI by modulating the deposition, arrangement, and perfusion of the fibrotic scar.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Targeted Gi signaling activation exclusively in cardiomyocytes reverses cardiac damage induced by adrenergic overload

Authors:

Marcos Eliezeck¹
Sérgio Scalzo¹
Mateus Chaves
Victor Moura¹
Thais Marques da Silva²
Carlos Alberto Aguiar Silva²
Cibele Rocha-Resende¹
Vânia F Prado³
Rubens Fazan Jr²
Hélio César Salgado²
Marco Antônio Máximo Prado³
Sílvia Guatimosim¹

Affiliations:

¹Department of Physiology and Biophysics, Institute of Biological Sciences, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.

²Department of Physiology, Ribeirão Preto Medical School, Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil.

³Department of Physiology and Pharmacology, Robarts Research Institute, The University of Western Ontario, London, Ontario, Canada.

Keywords: Cardioprotection; GPCRs; Gi-signaling; DREADD.

Abstract:

GPCRs are critical regulators of cardiac function, while hyperactive Gs/Gq signaling contributes to heart disease, Gi activation exerts protective effects. Here, we used the DREADD technology to generate Myh6- Cre/hM4Di mice, enabling Gi activation exclusively in cardiomyocytes (CMs) to assess its therapeutic potential under adrenergic stress. Gi activation in CMs was achieved by CNO treatment. CEUA:102/2022. To confirm Gi activation by the hM4Di receptor, we isolated CMs, treated them with ACh or CNO (100 nM) and recorded the contraction. The Myh6-Cre/hM4Di CMs showed reduced myocyte shortening after CNO, mirroring the effect observed with ACh (μm^2 : CTR 565 ± 34 vs CNO 342 ± 33 vs ACh 320 ± 30 ; $n=40$). Littermate controls showed no response, confirming specificity. To evaluate cardioprotection in vivo, mice received ISO (25 mg/kg) with or without CNO (0.5 mg/kg) for 7 days. CNO prevented ISO-induced remodeling, including hypertrophy (HW/TL mg/cm: CTR 78 ± 3 vs ISO 95 ± 1 vs CNO+ISO 77 ± 3 ; $n=9$). Analyses of isolated CM area confirmed these data. Additionally, CNO reduced CM death, inflammation, fibroblast activation, and collagen upregulation. To test the therapeutic potential of CM-Gi activation, we determined that 3 ISO doses induced hypertrophy, inflammation, and CM death. With this in mind, mice received three ISO doses, then CNO was administered from day 4 to 7. Strikingly, late CNO administration reversed ISO damage, including hypertrophy (HW/TL in mg/cm: CTR 79 ± 2 vs ISO 97 ± 2 vs CNO+ISO 82 ± 2 ; $n=17$), increased CD68+ staining, and collagen upregulation. Late CNO treatment also improved the cardiac function of ISO-mice (Ejection fraction %: CTR 54 ± 2 vs ISO 44 ± 1 vs CNO+ISO 51 ± 2 ; $n=9$). Flow cytometry revealed fewer inflammatory cells, particularly CCR2+ cells in the hearts from ISO mice subjected to late CNO treatment. This study provides the first evidence that CM-targeted Gi activation protects against adrenergic injury, highlighting its role in cardiac repair.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Vascular Effects of Perivascular Adipose Tissue are Preserved after Long-Term Androgen Depletion

Authors:

Layra Ramos LUGÃO^{1,2}
Andressa Damasceno MARCELINO^{1,2}
Anna Ruthe Santos JACOB^{1,2}
Anna Karolina Nascimento COSTA^{1,3}
Paula dos Santos ATHAYDE¹
Michelle Rossana Martins HORTELAN³
Eduardo Hertel RIBEIRO¹
Aurélia Araújo FERNANDES¹
Ivanita STEFANON^{1,2,4}

Affiliations:

¹Universidade Federal do Espírito Santo
²Faculdade Multivix Vitoria
³Faculdade Multivix Vila Velha ⁴Faculdade Multivix Cariacica

Keywords: Male Sex Hormones. Perivascular adipose tissue. Testosterone. Vascular reactivity.

Abstract:

Androgen deprivation therapy (ADT) increases cardiometabolic risk and may impair vascular function. Perivascular adipose tissue (PVAT) surrounds most arteries and exerts vasomodulatory effects by releasing vasoactive substances. This study tested whether long-term testosterone depletion alters PVAT-dependent modulation of vascular reactivity and if pharmacological blockade of renin-angiotensin and oxidative pathways interfere with this modulation. Twelve-week-old male Wistar rats were assigned to SHAM or orchiectomized (OQT) groups, treated for 3 months with losartan (15 mg/kg), spironolactone (80 mg/kg), or apocynin (30 mg/kg). Aortic rings with and without PVAT were tested in organ baths for vasoconstriction to phenylephrine (10^{-11} – 10^{-3} M). Maximal response (R_{max}) and sensitivity (log EC₅₀) were analyzed. CEUA UFES 17/2020. PVAT reduced R_{max} and increased sensitivity in both SHAM and OQT. SHAM: R_{max} without PVAT=104±10% vs. with PVAT= 45.8±6.4%, *p<0.05; log EC₅₀=-6.35±0.12% vs. -5.38±0.09%, *p<0.05. OQT: R_{max} without PVAT=107±10.8% vs. with PVAT=55.8±6.3%, *p<0.05; log EC₅₀ = - 6.26±0.13% vs -5.37±0.11%, *p<0.05. Similar PVAT-dependent reductions in R_{max} and log EC₅₀ were observed in OQT animals treated with apocynin, losartan or spironolactone (OQT+SPI: R_{max} without PVAT= 109.4±12.2% vs. with PVAT= 60.7±6.5%, *p<0.05; log EC₅₀= -6.50±0.07% vs. -5.61±0.16%, *p<0.05). Testosterone deprivation does not alter the vasodilatory influence of PVAT. Moreover, the PVAT-dependent attenuation of vasoconstriction is preserved under chronic blockade of angiotensin II or oxidative stress pathways, highlighting a robust and testosterone-independent regulatory role of PVAT in vascular function.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Influence of obesity on cardioprotection induced by remote ischemic preconditioning

Authors:

Marcella Borges COUTINHO ¹

Alberto Barreto GRIMALDI ¹

Leonardo MACIEL ¹

Affiliations:

¹ Universidade Federal do Rio de Janeiro

Keywords: Obesity; Cardioprotection; Ischemia; Reperfusion

Abstract:

Introduction: Ischemic heart disease is a leading cause of mortality worldwide, imposing a significant economic burden. Myocardial infarction results from reduced or blocked blood flow, creating an oxygen supply-demand imbalance. Although reperfusion is crucial for myocardial survival, it can paradoxically cause further damage. Remote ischemic preconditioning (RIPC) has been proposed as a non-invasive cardioprotective strategy, but its efficacy in clinical trials has been inconsistent, likely due to comorbidities and polypharmacy. Objective: To evaluate the cardioprotective effects of conditioned human plasma transfusion in obese rat hearts. Methodology: Hearts from healthy and obese male Wistar rats (cafeteria diet-induced) (CEUA: 154/21) were isolated and perfused using the Langendorff system with Krebs solution. They underwent 30 minutes of ischemia followed by 120 minutes of reperfusion (I/R). Human plasma was categorized as placebo or conditioned. Placebo plasma was obtained before the RIPC maneuver, while conditioned plasma was collected after RIPC (three cycles of upper limb occlusion at 200 mmHg for 5 minutes, interspersed with 5-minute deflation periods). Plasma fractions (<10 kDa) were ultrafiltered and perfused for 5 minutes before ischemia to assess cardioprotection. Results: The cafeteria diet induced obesity, increasing body weight by 161.1%, $83.27 \pm 11.47\%$ higher than controls. Blood glucose levels significantly increased ($p < 0.0001$), with differences between control post and diet post ($p = 0.0086$) and diet pre ($p = 0.0076$). The lipid profile remained unchanged. RIPC plasma (<10 kDa) significantly reduced infarct size in healthy rats ($p = 0.0058$) but not in obese ones, indicating abolished cardioprotection. Conclusion: Obesity impairs RIPC efficacy, underscoring the need for further research into its molecular mechanisms and adaptations for comorbid conditions.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Mitochondrial imbalance drives persistent cardiac hypertrophy following adrenergic overload in estrogen-deficient mice

Authors:

Bruno de Lima SANCHES¹
Fernanda TUPINI¹
Kiany MIRANDA¹
Victor Moura VIDAL¹
Maria LUIZA-DIAS¹
Fernando SOUZA-NETO²
Julia REZENDE¹
Marcos Eliezeck dos SANTOS¹
Sergio SCALZO¹
Raphael Escorsim SZAWKA¹
Thiago VERANO-BRAGA¹
Jop H. van BERLO²
Sílvia GUATIMOSIM¹

Affiliations:

¹Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas ²University of Minnesota, Lillehei Heart Institute

Keywords: Estrogen. Hypertrophy. Isoproterenol. Mitochondria. Takotsubo cardiomyopathy.

Abstract:

Takotsubo cardiomyopathy (TC) predominantly affects postmenopausal women, with reduced estrogen levels increasing cardiovascular risk. While the connection between estrogen decline and menopause is well established, the mechanisms driving elevated TC incidence in this population remain poorly understood. To address this problem, we used female C57BL/6J mice, 8 weeks old, divided into four groups: Sham/CTR (sham-operated treated with saline); Sham/ISO (treated with a single intraperitoneal (I.P.) 200 mg/Kg dose of isoproterenol (ISO)); OVX/CTR (subjected to bilateral ovariectomy) and OVX/ISO. Thirty days after OVX, mice were treated with ISO and euthanized ten days later. Echocardiography, proteomics, seahorse assay, and immunofluorescence were performed in cardiac tissue. CEUA: 182/2024. Remarkably, ten days post-ISO only OVX/ISO mice showed pronounced cardiomyocyte hypertrophy (OVX/ISO: $272.5 \pm 3.1 \mu\text{m}^2$ vs Sham/ISO $204.7 \pm 2.3 \mu\text{m}^2$, $n=9$) and increased left ventricle posterior wall thickness in diastole (LVPW;d, OVX/ISO: $1.0 \text{mm} \pm 0.04$ vs Sham/ISO: 0.9 ± 0.04 , $n=8$). Proteomics analysis showed enhanced processes related to metabolic dysfunction in OVX/ISO hearts compared to Sham/ISO. Confirming that, seahorse assay showed increased basal oxygen consumption rate in OVX/ISO hearts (OVX/ISO: $8.3 \text{pmol/min} \pm 1.9$ vs Sham/ISO: $1.3 \text{pmol/min} \pm 0.8$, $n=6$), and impaired mitochondrial complex I activity (OVX/ISO: $23.1 \text{pmol/min} \pm 1.0$ vs Sham/ISO: $41.2 \text{pmol/min} \pm 3.3$), indicating that a mitochondrial imbalance was occurring in OVX mice post-ISO. To test this hypothesis, OVX mice were treated with MitoTEMPO, a mitochondrial ROS scavenger. MitoTEMPO treatment prevented both cardiomyocyte hypertrophy (OVX/ISO+MitoTEMPO: $216.9 \mu\text{m}^2 \pm 3.8$ vs OVX/ISO: $283.7 \mu\text{m}^2 \pm 4.6$, $n=8$) and LVPW;d augment (OVX/ISO+MitoTEMPO: $1.1 \text{mm} \pm 0.07$ vs OVX/ISO: 1.3 ± 0.06 , $n=8$) seen in OVX/ISO mice. These findings indicate that mitochondrial dysfunction plays a pivotal role in sustaining the cardiac remodeling in response to ISO-induced TC.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Evaluation of cardiovascular reactivity to acute stress following systemic GABA administration

Authors:

Ana Clara Rocha VIANA¹
Ana Flávia Palmieri BORGES¹
Carina Cunha SILVA¹
Erick Bryan de Sousa LIMA¹
Ícaro Santos NOGUEIRA¹
Marco Antônio Peliky FONTES¹

Affiliations:

¹Hypertension Laboratory, Department of Physiology and Biophysics, Universidade Federal de Minas Gerais

Keywords: Cardiovascular; GABA; Hypertension; Stress

Abstract:

Introduction: Evidence indicates that there is a positive association between comorbid anxiety and hypertension. Sympathetic activation is a key component of the physiological response to stress and anxiety, often leading to cardiovascular alterations. Gamma-aminobutyric acid (GABA), known for its central nervous system effects, is also present in peripheral circulation. However, the effects of systemically administered GABA on cardiovascular reactivity to acute stress remain unclear. Objective: To evaluate the effects of systemic GABA administration on cardiovascular reactivity to acute stress, as well as its influence on anxiety-like behavior and motor activity. Methods: (CEUA UFMG nº219/24). Wistar rats (250–300g) were anesthetized (ketamine 0.8 mg/kg + xylazine 0.1 mg/kg, i.p.) and underwent femoral artery and vein cannulation. Animals received GABA (1 or 10 mg/kg, i.v.) or saline (control, 0.1 ml/100g), followed by exposure to cage switch stress (CSS). Heart rate (HR), mean arterial pressure (MAP), anxiety-like behavior (elevated plus maze – EPM), locomotor activity (open field), and muscle strength (wirehang test) were evaluated. Saline: 0.1ml/100g (n=6); GABA: 1mg/Kg (n=6); GABA 10mg/Kg (n=6) Results: CSS induced a tachycardic response ($\Delta 70 \pm 8$ bpm) and increased MAP ($\Delta 30 \pm 5$ mmHg) in control animals. GABA administration not only abolished but reversed the CSS-induced tachycardia (1 mg/kg: -38 ± 5 bpm; 10 mg/kg: -28 ± 10 bpm; $P < 0.0001$) without affecting the pressor response. GABA significantly increased locomotor activity (294 ± 66 s vs. 67 ± 12 s; $P < 0.006$) and reduced anxiety-like behavior (open arm time: 73 ± 24 s vs. 5 ± 3 s; $P < 0.01$), though it slightly reduced muscle strength (14 ± 2 s vs. 23 ± 3 s; $P < 0.01$). Conclusion: Systemic GABA administration effectively attenuates the tachycardia induced by acute emotional stress and dampens anxiety without apparent somatomotor effects. Financial Support: CNPq: MAPF 308923/2021-9, ACVG 165774/2021-5; MAPF FAPEMIG: APQ-01128-21.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Ovariectomized female mice are more susceptible to cardiac remodeling induced by Gq pathway activation

Authors:

Tamires MARINHO¹,
Naomi Alves REZENDE¹,
Vitoria Estanislau GANDRA¹,
Marcos ELIEZECK¹,
André MONTEIRO¹,
Mário de MORAIS¹,
Kiany de OLIVEIRA¹,
Sílvia GUATIMOSIM¹

Affiliations:

¹Department of Physiology and Biophysics, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Brazil.

Keywords: Calcium. Cardiomyocyte. Estrogen. Gq. DREADD.

Abstract:

G protein-coupled receptors (GPCRs) are highly expressed in cardiomyocytes (CMs) and, when coupled to Gq, mediate responses to hormones such as AngII and endothelin. Their hyperactivation induces hypertrophy, fibrosis, and inflammation, contributing to pathological cardiac remodeling. Although estrogen attenuates these effects, the underlying mechanisms remain unclear. We investigated the impact of Gq activation in cardiomyocytes (CMs) from intact (SHAM) and ovariectomized (OVX) female mice using DREADD-hM3Dq receptors (activated by CNO), specifically expressed in CMs via the Cre-Lox system (Myh6 promoter). All procedures were approved by the Institutional Animal Care and Use Committee (CEUA-UFGM) Protocol No. 182/2024. Seven days post-ovariectomy, mice received CNO or saline for three days. While heart weight did not differ between groups, CM cross-sectional area was larger in OVX-CNO ($289.1 \pm 15.5 \mu\text{m}^2$) vs SHAM-CNO ($240 \pm 10.5 \mu\text{m}^2$; $p=0.03$). CD68+ macrophage staining increased in SHAM-CNO (15.0 ± 0.94) vs SHAM-saline (9.5 ± 0.7 ; $p=0.003$). Likewise, OVX-CNO hearts showed more pronounced CD68+ staining (15.9 ± 1.0) vs OVX-saline (11.3 ± 0.9 ; $p=0.008$). We further addressed this issue by using AAV9-TnT-Cre to infect ~50% of CMs from DREADD-hM3Dq mice, allowing the comparison of Gq+ (hM3Dq) and Gq- cells within the same heart. Interestingly, Gq+ CMs from OVX mice showed enhanced shortening ($55.6 \pm 5.4 \mu\text{m}^2$) compared to Gq- ($37.0 \pm 4.6 \mu\text{m}^2$). Our results demonstrate that estrogen deficiency turns CMs more susceptible to Gq signaling effects. CNPq, CAPES, FAPEMIG, PRPq e INCT NanoBioFar.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Effects of Dapagliflozin, a Sodium-Glucose Cotransporter 2 Inhibitor, on Mitochondrial Function

Authors:

Caroline da Silva MORAES¹
Cesar Francisco Maricato da ROSA¹
Itanna Isis Araújo de SOUZA²
Leonardo MACIEL²

Affiliations:

¹ Federal University of Rio de Janeiro
² Carlos Chagas Filho Institute of Biophysics
³ Antônio Paes de Carvalho Cardiac Electrophysiology Laboratory

Keywords: Mitochondria. Sodium-glucose cotransporter 2 inhibitors. Diabetes mellitus.

Abstract:

Introduction: Dapagliflozin is a sodium-glucose cotransporter type 2 (SGLT2) inhibitor, commonly used as an antidiabetic drug, that has been shown to provide cardiovascular benefits, including protection against ischemic injury and prevention of heart failure. However, there is no data on the effects of dapagliflozin on cardiomyocyte mitochondria. Here, we evaluate the effects of Dapagliflozin on cardiac isolated mitochondria function. Methodology: For this study, a total of 10 four-week-old Wistar rats (*Rattus norvegicus*) were used. The hearts were rapidly excised, and mitochondria were isolated using differential centrifugation. Immediately after isolation, mitochondrial function was evaluated by measuring oxygen (O₂) consumption at complexes I and II across states 1, 2, and 3 (phosphorylative), following exposure to dapagliflozin at concentrations of 1 nM and 10 nM. Results: Dapagliflozin at a concentration of 10 nM significantly increased mitochondrial respiration in treated at the complex I, specifically in state 2 and phosphorylative conditions. Additionally, complex II respiration in state 3 was also enhanced following 10 nM dapagliflozin exposure. No differences were observed between groups in maximal uncoupled respiration or complex IV activity, indicating comparable mitochondrial viability and consistent loading control across experiments, respectively. Conclusion: These findings suggest that dapagliflozin enhances mitochondrial oxidative capacity primarily through the stimulation of complexes I and II, without compromising mitochondrial integrity or experimental consistency. This mitochondrial activation may underlie, at least in part, the cardioprotective effects attributed to dapagliflozin. Financial Support: FAPERJ, CNPq and CAPES. Ethics Committee Code: Ceua-UFRJ (154-21)



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Alamandine-(1-5): A Novel Antihypertensive Peptide of the Renin-Angiotensin System

Authors:

Kamylle Silva FERRAZ¹
Adelson Heric Alves MONTEIRO¹
Sthéfanie Chaves de Almeida GONÇALVES¹
Nícia Pedreira SOARES¹
Carolina Fonseca de BARROS¹
Robson Augusto Souza dos SANTOS¹
Maria Jose CAMPAGNOLE-SANTOS¹

Affiliations:

¹Department of Physiology and Biophysics, Biological Sciences Institute, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil

Keywords: Alamandine-(1-5). Antihypertensive. Peptide. Renin-angiotensin system.

Abstract:

The renin-angiotensin system (RAS) plays a key role in cardiovascular control and fluid balance. Angiotensin-(1-7) and Alamandine are components of the hypotensive axis of the RAS, with anti-hypertrophic and anti-inflammatory effects. Alamandine can be hydrolyzed to form Alamandine-(1-5), a peptide recently identified in the circulation of humans and rodents. In this study, we evaluated the cardiovascular effects of Ala-(1-5) in male and female Wistar normotensive and hypertensive rats (SHR; n=6–10). Animals were anesthetized with ketamine (50 mg/kg) and xylazine (10 mg/kg) and subjected to catheters implantation into the abdominal aorta and femoral vein for arterial pressure recording and intravenous drug administration, respectively. After 24h, arterial pressure (AP) was continuously monitored in conscious animals. Ala-(1-5) (30 or 100 µg/kg) or sterile saline (0.1 ml/100 g) was injected after a period of 1h for AP stabilization and was monitored for the next 6h. In another protocol, male SHR (n=6) was subjected to 14 days of continuous sc infusion of Ala-(1-5) (6 µg/kg/h; ALZET mini-pumps) or saline (0,25 µl/h) and systolic AP (SAP) was monitored by tail-cuff plethysmography. In male SHR, Ala-(1-5), 30 µg/kg, caused a progressive reduction in mean AP (MAP) up to -28±7 mmHg after 6h (baseline: 168±6 mmHg), with no change in heart rate (HR; 348±16 bpm). In female SHR, there was a significant fall in MAP only with 100 µg/kg (-15±5 mmHg) accompanied by an increase in HR of 21±10 bpm. There was a progressive fall in SAP in the 14 days of continuous Ala-(1-5) infusion in male SHR, reaching -75±8 mmHg at 14 days of infusion. No significant alteration in SAP in saline infused rats was observed. These data identify Ala-(1-5) as a novel antihypertensive agent within the RAS and a potential pharmacological target for hypertension treatment. Approved by the Animal Ethics Committee: 188/2023. Funding: CNPq, CAPES, FAPEMIG, INCT-Nanobiofar.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Efficacy of two treatment protocols for denture stomatitis: impact on inflammation, *Candida* load, and blood pressure

Authors:

Adriana Barbosa RIBEIRO^{1,5,6}
Lorena Mosconi CLEMENTE¹
Aline Barbosa RIBEIRO²
Helio Cesar SALGADO³
Rubens FAZAN-JUNIOR³
Evandro WATANABE⁴
Nicola MONTANO^{5,6}
Cláudia Helena SILVA-LOVATO¹

Affiliations:

¹ Departamento de Materiais Dentários e Prótese, Faculdade de Odontologia de Ribeirão Preto, São Paulo, Brasil.

² Centro Universitário Barão de Mauá. Ribeirão Preto, São Paulo, Brasil.

³ Departamento de Fisiologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Brasil.

⁴ Departamento de Odontologia Restauradora, Faculdade de Odontologia de Ribeirão Preto, São Paulo, Brasil.

⁵ Scienze Cliniche e Salute di Comunità, University of Milan, Milan, Lombardia, Itália.

⁶ Fondazione IRCCS Ca' Granda, Ospedale Policlinico, Milan, Lombardia, Itália.

Keywords: *Candida* spp.. Cardiovascular System. Inflammation. Pressure. Stomatitis.

Abstract

Denture stomatitis is a common condition among denture wearers. Treatment improves endothelial function and reduces systolic blood pressure, as well as inflammatory biomarkers such as IL-6, IL-2, IL-10, and IFN- γ . This study compared the effects of two protocols on stomatitis remission, *Candida* microbial load, inflammatory biomarkers, and blood pressure. Measurements were taken at baseline (T0), 15 days (T1), and 45 days (T2). The participants in this double-blind, randomized clinical trial (CAAE: 93712418.1.0000.5419) were assigned to either G1 (nystatin 100,000 IU) or G2 (0.25% sodium hypochlorite). Denture stomatitis was assessed using the modified Newton classification. *Candida* spp. the load was measured in colony-forming units. IL-6 and IL-4 levels in saliva and plasma. Blood pressure was recorded after a 10–15-minute rest. The Mann-Whitney test was used for group comparisons, and the Friedman test for temporal changes ($p < 0.05$). 47 patients (G1: 25, G2: 22), with an average age of 68 years (± 6.2). No significant differences in sociodemographic, behavioral, or medication use were found. Both treatments significantly reduced stomatitis severity at T1 ($p < 0.001$) and maintained improvements at T2. *Candida* load decreased in both groups ($p < 0.001$). Both protocols reduced salivary IL-6 and plasma IL-4 levels at T1 and T2. Systolic blood pressure decreased at T1 ($p = 0.002$) and T2 ($p = 0.013$), and diastolic pressure remained unchanged ($p = 0.654$). Both treatments reduced denture stomatitis, *Candida* load, inflammatory biomarkers, and systolic blood pressure, with notable benefits for oral and systemic health in elderly patients. Considering the potential adverse effects of nystatin, 0.25% sodium hypochlorite may be a viable alternative for managing entire stomatitis.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: The Role of Cholinergic Signaling in Postnatal Cardiomyocyte Maturation

Authors:

Rayssa Lima RODRIGUES¹,
Marcos ELIEZECK¹,
Mateus Chaves da COSTA¹,
Victor MOURA¹,
Fernando ESPANHOL¹,
Iago PINHEIRO¹,
Sérgio Ricardo Aluotto SCALZO¹,
Sílvia GUATIMOSIM¹.

Affiliations:

¹Department of Physiology and Biophysics, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Brazil

Keywords: Cardiomyocytes. Cardiac development. Maturation. Cell proliferation. Autonomic nervous system.

Abstract:

Cardiomyocyte (CMs) maturation involves significant changes in gene expression, metabolic profile, and cellular structure. We previously demonstrated that adrenergic signaling plays a crucial role in CM maturation, with its components developing up to the 21st postnatal day (Eliezeck and Jesus et al., 2024). Considering the opposing roles of adrenergic and cholinergic pathways, this study aims to investigate the influence of cholinergic signaling on postnatal cardiac development. We used male and female C57bl6J and Chat-ChR2 mice (1–21 days old) and neonatal Wistar rats for CM culture. Three protocols were conducted: 1 - In vivo treatment with the beta-blocker Propranolol (PRO) (5 mg/kg/day, P11–P20), with euthanasia at P21; 2 - Euthanasia of Chat-ChR2 mice and their controls (CTR) at P8; 3 - In vitro treatment of neonatal rat CM cultures with ACh and Pyridostigmine (Pyr), 10 μ M each. Statistical analysis: *t*-Student. CEUA: 29/2021 and 32/2025. At P21, PRO-treated mice showed a reduction in the heart weight/tibia length ratio (HW/TL) compared to CTR (mg/mm: CTR 3.74 ± 0.1 vs PRO 3.30 ± 0.09 ; $n=5$), corroborated by a smaller cross-sectional area of isolated CMs (μm^2 : CTR 171 ± 3 vs PRO 152 ± 2 ; $n=241$ cells from 4 animals). The frequency of binucleated CMs was unaltered; however, the proportion of mononucleated cells increased, while tetranucleated cells decreased compared to CTRs. When assessing the proliferative window (P7–P10), Chat-ChR2 mice exhibited a higher number of proliferating CMs at P8 compared to CTRs (Ki67+ cells per field: CTR 9.0 ± 1.1 vs Chat-ChR2 18.4 ± 1.3 ; $n=5$ hearts), suggesting an immature phenotype. In a hypercholinergic environment in vitro, treatment with ACh or Pyr reduced CM area (μm^2 : CTR 803.9 ± 31.8 vs ACh+Pyr 666.4 ± 19.3 ; $n=4$ cultures). In conclusion, the balance between adrenergic and cholinergic signaling is essential for proper CM maturation, directly impacting the structure and function of the developing myocardium.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: LEAP2, the endogenous inverse agonist of ghrelin receptor, modulates beta adrenergic-mediated cardiac effects in a GHSR-dependent manner

Authors:

¹Sixta Isabel Atencio Berrio;
¹Jhulle Horrane Moreira Carvalho;
¹João Batista Rodrigues Dutra;
²Marcos Eliezeck;
²Sérgio Scalzo;
¹Juliana Vila Verde Ribeiro;
¹Michelle M. Mendonça;
¹Rodrigo Mello Gomes;
¹Gustavo Rodrigues Pedrino;
³Eduardo Colombari;
⁴Daniel Castrogiovanni;
²Silvia Guatimosim;
^{4,7}Mario Perelló;
¹Carlos Henrique de Castro;
¹Carlos Henrique Xavier

Affiliations:

¹ Departamento de Ciências Fisiológicas. Instituto de Ciências Biológicas. Universidade Federal de Goiás. Goiânia – GO. Brazil ² Departamento de Fisiologia e Biofísica. Instituto de Ciências Biológicas. Universidade Federal de Minas Gerais – MG. Brazil ³ Departamento de Fisiologia e Patologia. Faculdade de Odontologia. Universidade Estadual de São Paulo. Araraquara – SP. Brazil ⁴ Instituto Multidisciplinario de Biología Celular (Universidad Nacional de La Plata (UNLP) – Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC-PBA) - Consejo Nacional de Investigaciones Científicas y técnicas (CONICET)). La Plata, Argentina. ⁶ Centro de Investigaciones Cardiovasculares, CONICET, UNLP, La Plata, Argentina ⁷ Functional Pharmacology and Neuroscience, Uppsala University, Sweden.

Keywords: LEAP2, Ghrelin, GHSR, β -adrenergic; cardiac physiology, hypertension.

Abstract:

GHSR is the receptor for the gastric peptide hormone ghrelin that displays an intrinsic activity and modulates cardiac β -adrenergic function. Liver-expressed antimicrobial peptide 2 (LEAP-2) was recently identified as GHSR endogenous inverse agonist. To understand whether LEAP2 modulates β -adrenergic function in hypertensive hearts, we evaluated (in vivo - CEUA-UFG 16/2022) Wistar (WT) and spontaneously hypertensive (SHR) rats, isolated hearts (ex vivo) and cardiomyocytes (in vitro). LEAP2 intravenous injection (0.1 or 1 mg/kg) caused greater blood pressure reductions in SHR (WT: -6.4 ± 2.2 vs. SHR: -43.7 ± 6 mmHg, $P < 0.05$). LEAP2 also produced a greater negative inotropy in SHR (WT: -281 ± 131 vs. SHR: -787 ± 362 mmHg/s, $P < 0.05$), whereas negative chronotropy was observed only in SHR (-33 ± 8.6 bpm vs. basal, $P < 0.05$). LEAP2 perfusion attenuated the response to isoproterenol in coronary flow (column factor 16,69%; $P < 0.05$) and ventricular pressure (column factor 43,88%; $P < 0.05$) of isolated SHR hearts, whereas they were almost unaffected by acetylcholine. In cardiomyocytes, LEAP2 or the synthetic inverse agonist PF04628935 caused



**XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO**

**8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP**

equipotent reduction in maximum contraction velocity (49 ± 36 and 39.4 ± 5.5 vs. 76.36 ± 4.7 $\mu\text{m/s}$; $P < 0.05$) and reduced the shortening area (403 ± 34.48 and 371.4 ± 51.35 vs 606.2 ± 33.7 μm^2 ; $P < 0.05$), in addition to attenuating responses to isoproterenol, but not to acetylcholine. While HPLC analyses of plasma ghrelin and LEAP2 revealed no differences between strains, Western blot analyses showed GHSR overexpression in SHR hearts ($132\% > \text{WT}$, $P < 0.05$). In conclusion, LEAP2 modulates arterial pressure and cardiac function during hypertension. The potential GHSR involvement in SHR cardiac abnormalities is unrelated to the circulating levels of its endogenous ligands and seems to rely on GHSR expression and function. LEAP2 attenuates the effects triggered from cardiac β -adrenergic receptors via GHSR, raising a great cardioprotective potential of this peptide.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Parasympathetic Control and Beyond: Insights into Heart Rate Fragmentation from Experimental Animals

Authors:

Thaís Marques da SILVA¹,
Carlos Alberto A. SILVA¹,
Sílvia GUATIMOSIM²,
Marcos ELIEZECK²,
Fernando ESPANHOL²,
Costanza SCATÀ³,
Francesca MAGNA³,
Greta SALÁFIA³,
Angelica CARANDINA³,
Eleonora TOBALDINI³,
Nicola MONTANO³,
Luiz Eduardo Virgílio SILVA⁴,
Helio C. SALGADO¹,
Rubens FAZAN JR¹

Affiliations:

¹Department of Physiology, Ribeirão Preto Medical School, University of São Paulo, São Paulo, Brazil.

²Department of Physiology and Biophysics, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.

³Department of Clinical Sciences and Community Health, University of Milan and Fondazione IRCCS Ca' Granda, Policlinico Hospital, Milan.

⁴Department of Biomedical and Health Informatics, Children's Hospital of Philadelphia, PA, USA.

Keywords: Autonomic nervous system. Diabetes *mellitus*. Heart rate fragmentation. Parasympathetic cardiac modulation.

Abstract:

Heart rate fragmentation (HRF) is a recently introduced heart rate variability (HRV) metric that quantifies erratic fluctuations in heart rate. HRF has emerged as a potential biomarker of aging and cardiovascular disease, with reported associations with increased mortality and cardiovascular risk. However, the role of cardiac autonomic modulation in HRF remains to be determined. Accordingly, this study aimed to assess the influence of parasympathetic drive on HRF, using complementary experimental models that allow manipulation of vagal cardiac modulation. ECG recordings were obtained from conscious animals to assess the percentage of inflection points (PIP), the primary index of HRF, across three experimental models: (1) Pharmacological blockade of parasympathetic cardiac receptors using methylatropine (2 mg/kg, i.v.) in Wistar rats (Protocol N° 016/2013-1); (2) Transgenic mice overexpressing the vesicular acetylcholine transporter (VACht+), reflecting enhanced parasympathetic tone (Protocol N° 026/2020); (3) Streptozotocin (STZ)-induced diabetic Sprague-Dawley rats (50 mg/kg, i.p.), evaluated 4 weeks after induction, at baseline and following parasympathetic blockade (Protocol N° 206/2019). In Wistar rats, PIP significantly decreased after parasympathetic blockade compared to baseline (73.9 ± 1.0 vs. $44.3 \pm 3.4\%$). VACht+ mice exhibited higher PIP than wild-type controls (70.4 ± 3.8 vs. $54.8 \pm 5.1\%$). Diabetic rats displayed increased PIP at baseline (81.9 ± 0.9 vs. $75.5 \pm 0.7\%$) and also after parasympathetic blockade (75.7 ± 2.6 vs. $56.0 \pm 3.2\%$), when compared to controls. Under health conditions, HRF is influenced by parasympathetic activity. In contrast, in diabetes, elevated HRF appears to be less dependent on vagal influence, as PIP keeps high after its blockade. These findings suggest that intrinsic cardiac alterations may contribute to HRF in pathological states. We conclude that PIP is a sensitive marker of both autonomic and non-autonomic mechanisms, capturing the predominant modulatory influences according to the underlying condition.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Myocardial Infarction-Induced Skeletal Muscle Remodeling Is Modulated by Orchiectomy

Authors:

Julia Riguetti VITALI^{1,4}

Mayara Laiz Fromholz SANTOS^{1,4}

Anna Karolina Nascimento COSTA^{1,5}

Sara Bianca Oliveira Mendes TAUFNER²

Maicon Landim-VIEIRA^{1,3}

Eduardo Hertel RIBEIRO¹

Aurélia Araújo FERNANDES²

Ivanita STEFANON^{1,4}

Affiliations:

¹Graduate Program in Physiological Science, Federal University of Espírito Santo, Vitória, ES, Brazil

²Graduate Program in Biochemistry, Federal University of Espírito Santo, Vitória, ES, Brazil

³Illinois Institute of Technology, IL, United States of America ⁴Multivix Brazilian College of Vitória, Vitória, ES, Brazil ⁵Multivix Brazilian College of Vila Velha, Vila Velha, ES, Brazil

Keywords: Histology. Myocardial infarction. Orchiectomy. Skeletal muscle. Testosterone.

Abstract:

Skeletal muscle dysfunction is a known consequence of myocardial infarction (MI), contributing to reduced functional capacity and poor prognosis. This condition is marked by muscle atrophy, fiber-type shifting, and reduced oxidative capacity, especially in fast-twitch muscles. Testosterone influences muscle homeostasis, but its role after MI is debated. While testosterone deficiency is linked to muscle wasting and higher cardiovascular risk, some evidence suggests that testosterone may exacerbate post-MI damage via pro-inflammatory and oxidative mechanisms. Thus, the effects of testosterone on skeletal muscle after MI remain controversial. To evaluate the effects of orchiectomy on the histological structure of the gastrocnemius, soleus, and vastus lateralis muscles sixty days after MI. Male Wistar rats were divided into: Sham-operated (SHAM, n=4), infarcted (MI, n=4), and infarcted with orchiectomy (MI-OCT, n=4). MI and MI-OCT groups were infarcted sixty days before orchiectomy. Two months later, skeletal muscles were collected for histological analysis (hematoxylin-eosin staining), and cross-sectional fiber area was measured (mean $\pm$ SEM). One-way ANOVA with Tukey's post hoc test was used (Animal Ethics Committee UFES 16-2020). Orchiectomy increased fiber area in gastrocnemius and vastus lateralis muscles. Gastrocnemius: MI ($1291 \pm 26.6 \mu\text{m}^2$) vs. SHAM ($1328 \pm 40.5 \mu\text{m}^2$, $p > 0.05$); MI-OCT ($1650 \pm 82.7 \mu\text{m}^2$) was greater than SHAM ($p = 0.0002$) and MI ($p < 0.0001$). Vastus lateralis: MI ($1294 \pm 26.7 \mu\text{m}^2$) vs. SHAM ($2043 \pm 65.7 \mu\text{m}^2$, $p = 0.0001$); MI-OCT ($1546 \pm 50.0 \mu\text{m}^2$) was greater than SHAM ($p = 0.0001$) and similar to MI ($p > 0.05$). Soleus showed no significant differences ($p > 0.05$). Orchiectomy performed after MI not only prevented muscle atrophy but also increased muscle fiber area beyond baseline levels observed in sham-operated animals. These findings suggest a potential modulatory role of testosterone withdrawal in skeletal muscle remodeling following cardiac injury. Financial Support: FAPES, CNPq e UFES.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Mitochondrial Antioxidant Therapy with Mitoquinone does not Prevent Skeletal Muscle Atrophy Following Myocardial Infarction

Authors:

Mayara Laiz Fromholz SANTOS^{1,4}
Julia Rigueti VITALI^{1,4}
Anna Karolina Nascimento COSTA^{1,5}
Sara Bianca Oliveira Mendes TAUFNER²
Maicon Landim-VIEIRA^{1,3}
Eduardo Hertel RIBEIRO¹
Aurélia Araújo FERNANDES²
Ivanita STEFANON^{1,4}

Affiliations:

¹ Graduate Program in Physiological Science, Federal University of Espírito Santo, Vitória, ES, Brazil

² Graduate Program in Biochemistry, Federal University of Espírito Santo, Vitória, ES, Brazil

³ Illinois Institute of Technology, IL, United States of America

⁴ Multivix Brazilian College of Vitória, Vitória, ES, Brazil

⁵ Multivix Brazilian College of Vila Velha, Vila Velha, ES, Brazil

Keywords: Acute myocardial infarction. Antioxidant Mitochondrial. Histology. Skeletal muscle.

Abstract:

Acute myocardial infarction (AMI) promotes not only cardiac dysfunction but also peripheral consequences, including skeletal muscle atrophy. This atrophic response is particularly evident in fast-twitch muscles and is associated with metabolic disturbances, inflammation, and mitochondrial dysfunction. Mitoquinone (MitoQ) is a mitochondria-targeted antioxidant proposed to mitigate oxidative stress-related damage in various tissues. However, its role in modulating skeletal muscle remodeling in the early phase post-AMI remains unclear. To investigate the effects of MitoQ on the histological structure of the gastrocnemius, vastus lateralis, and soleus muscles in rats seven days after AMI. Male Wistar rats were assigned to: sham-operated (SHAM, n=4), infarcted (MI, n=4), and infarcted treated with MitoQ (MI-MitoQ, n=4; 8 mg/kg/day in drinking water for 7 days). Muscles were collected for histological analysis (hematoxylin-eosin staining), and cross-sectional fiber area was quantified (mean $\pm$ SEM). Data were analyzed using one-way ANOVA with Tukey's post hoc test (UFES Ethics Committee #16-2020). AMI induced a significant reduction in muscle fiber area in the gastrocnemius and vastus lateralis. Gastrocnemius: MI ($1,958 \pm 102.8 \mu\text{m}^2$) vs. SHAM ($2,395 \pm 135.2 \mu\text{m}^2$, $p=0.0140$); MI-MitoQ ($1,840 \pm 70.9 \mu\text{m}^2$) was smaller than SHAM ($p=0.0008$) and similar to MI ($p>0.05$). Vastus lateralis: MI ($1,889 \pm 142.8 \mu\text{m}^2$) vs. SHAM ($2,539 \pm 207.7 \mu\text{m}^2$, $p=0.0039$); MI-MitoQ ($1,698 \pm 69.9 \mu\text{m}^2$) was smaller than SHAM ($p=0.0001$) and similar to MI ($p>0.05$). In the soleus, MI ($1,731 \pm 88.9 \mu\text{m}^2$) did not differ from SHAM ($1,822 \pm 39.6 \mu\text{m}^2$), but MI-MitoQ ($1,346 \pm 94.4 \mu\text{m}^2$) was significantly reduced (vs. SHAM, $p<0.0001$; vs. MI, $p=0.0017$). AMI led to atrophy in fast-twitch muscles, while the soleus was relatively preserved. MitoQ treatment did not prevent fiber atrophy and unexpectedly worsened soleus morphology, suggesting that mitochondrial oxidative stress may not be the primary driver of early muscle wasting in this context. Financial Support: FAPES, CNPq e UFES.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Selective Gi Activation in Cardiomyocytes Confers Protection Against Isoproterenol-Induced Cardiomyopathy

Authors:

Naomi Alves REZENDE¹
Marcos Eliezeck¹
Mateus Chaves¹
Tamires Amorim MARINHO¹
Cibele Rocha-RESENDE¹
Sílvia GUATIMOSIM¹

Affiliations:

¹Department of Physiology and Biophysics, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Brazil.

Keywords: Cardiomyocytes (CMs). Cardiomyopathy. Cardioprotection. DREADD. Gi signaling.

Abstract:

G-protein-coupled receptors (GPCRs) are crucial regulators of cardiac function, with chronic catecholamine stimulation activating the Gs pathway, contributing to cardiomyopathy. In contrast, Gi signaling has been linked to cardioprotection. This study investigates whether Gi activation in 30% of cardiomyocytes (CMs) mitigates isoproterenol (ISO)-induced cardiomyopathy using DREADD technology with AAV9 vectors. All procedures were approved by the Institutional Animal Care and Use Committee (CEUA-UFMG) Protocol No. 102/2022. Neonatal hM4Di mice received AAV9-CRE under a CM-specific troponin-T promoter, achieving mosaic expression (~30%) of hM4Di in CMs. At 8 weeks, mice were treated with ISO (25 mg/kg/day, 7 days) and CNO (0.5 mg/kg/day, 30 min before ISO) to activate Gi signaling. On day 8, animals were euthanized. Analysis confirmed 30% hM4Di expression in CMs, with Gi activation preventing cardiac hypertrophy (heart/tibia ratio, mg/cm: CTR 86.5±1.7 vs ISO 96.7±3.3 vs CNO+ISO 86.2±1.1; n=4 hearts/group). Gi+ CMs were protected from ISO-induced hypertrophy (Gi+ 1604±65µm² vs Gi- 1946±55µm²; p=0.0001; n=45 cells/group, 4 hearts). Notably, ISO treatment increased CD68+ immune cell presence near Gi- CMs, but not Gi+ CMs, suggesting localized cardioprotection. These findings demonstrate that selective Gi activation in a minority of CMs confers significant protection against ISO-induced cardiomyopathy, highlighting potential therapeutic avenues for cardiac dysfunction. Funding: CNPq/CAPES/FAPEMI/PRPq/INCT-NanoBioFar.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Sex difference in progesterone action on the coronary bed in gonadectomized SHR rats

Authors:

Caio Pinheiro da SILVA¹
Jocimar José PITOL¹
Roger Lyrio dos SANTOS¹

Affiliations:

¹ Universidade Federal do Espírito Santo

Keywords: Coronary artery bed. Gonadectomy. Hypertension. Progesterone

Abstract:

Endothelial integrity is essential for the functions performed by this important vascular layer, such as the regulation of vascular tone. The loss of this integrity, as occurs in endothelial dysfunction, is closely related to cardiovascular diseases (CVDs), such as coronary artery disease and hypertension. Hypertension, in turn, is more prevalent in men and postmenopausal women when compared to premenopausal women, suggesting a protective role of female sex hormones, including progesterone (P4). In fact, previous studies from our group have demonstrated that P4 was able to promote vasodilation in the coronary bed of normotensive rats in a similar manner between the sexes. This similarity was maintained in gonadectomized normotensive rats. However, the actions of P4 in the coronary bed of gonadectomized spontaneously hypertensive rats (SHR) of both sexes are still unknown. Therefore, this study aimed to investigate whether the similarity in the P4 vasodilatory response is maintained in gonadectomized SHR rats. In the present study, SHR rats of both sexes, aged 12 weeks (CEUA 24/2021), were used. Rats were gonadectomized and after 15 days of hormone deficiency, the heart was removed and analyzed using the Langendorff method. After stabilization and recording of coronary perfusion pressure (CPP), dose-response curves for P4 (1, 3, 5, 10, 30 and 50 μ M) were performed. Data were expressed as mean $\pm$ SEM, and analyzed by two-way ANOVA, followed by Tukey *post hoc* test. To date, P4 has promoted similar vasodilation between sexes (Female: $29.1 \pm 5.1\%$, $n = 9$; Male: $23.2\% \pm 6.1\%$, $n = 7$). Our study corroborates the hypothesis that the action of P4 in the coronary bed is not influenced by sex. As future perspectives, we will analyze whether similarity is maintained in the pathways that mediate vasodilation induced by P4, such as the nitric oxide and prostanoid pathways.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Vascular effects of exposure to accidental doses of cadmium in female rats - Protective effect of Red Quinoa Hydrolysate: A plant-based therapeutic alternative

Authors:

Samia Hassan Husein KANAAN¹,
Paola Zambelli MORAES¹,
Katy Yasmin de Souza de OLIVEIRA¹,
José Eudes Gomes PINHEIRO JR¹,
Franck Maciel PEÇANHA¹,
Dalton Valentim VASSALLO²,
Marta MIGUEL-CASTRO³,
Giulia Alessandra WIGGERS¹

Affiliations:

¹Laboratory of Cardiovascular Physiology / Multicentric Postgraduate Program in Physiological Sciences, Federal University of Pampa (Unipampa), Brazil-RS. ²Laboratory of Electrophysiology and Vascular Reactivity, Federal University of Espírito Santo, Brazil-ES. ³Instituto de Investigación en Ciencias de la Alimentación, CIAL/CSIC, Madrid, Spain

Keywords: Alternative therapeutic. Cadmium. Cardiovascular. Female. Plant-based

Abstract:

Cadmium (Cd) exposure causes vascular dysfunction, with scarce studies in females. Natural plant-based therapeutic alternatives should be encouraged to mitigate Cd-induced damage. A red quinoa hydrolysate (RQH) with antihypertensive and antioxidant properties could be protective. We investigated whether RQH protects the aorta from Cd-induced damage in females. Wistar female rats (12 weeks, $\pm$ 300g) were treated for 14 days in: Control (Ct): intraperitoneal (i.p.) distilled water + tap water by gavage; Cadmium (Cd): 1 mg/kg/day - CdCl₂ i.p. + tap water by gavage; RQH: 1 g/kg/day - RQH by gavage + distilled water i.p.; and CdRQH: both treatments combined. (CEUA/Unipampa: 010/2023). Systolic blood pressure (SBP) was recorded weekly. Aorta reactivity was analyzed in an isolated organ bath. Aorta immunofluorescence was assessed for NOX-1, COX-2, and TNF α . Data are expressed as mean $\pm$ SEM (ANOVA-2way, $p < 0.05$). SBP was not altered by treatments, but local aortic vascular damage was evident. RQH: a) reduced the Cd-induced increase in Phe contraction (Rmax: Ct: 51.4 ± 2.2 ; RQH: 47.9 ± 2.7 ; Cd: $71.8 \pm 3.0^*$; CdRQH: $53.5 \pm 1.7^\#$; % KCl, * vs Ct and # vs Cd); b) prevented endothelial dysfunction and restored nitric oxide bioavailability; c) reduced COX-2 pathway participation; d) prevented Cd-induced ROS increase; e) decreased aorta fluorescence intensity of NOX-1, COX-2, and TNF α (NOX-1: Ct: 0.33 ± 0.03 ; RQH: 0.27 ± 0.01 ; Cd: $0.82 \pm 0.08^*$; CdRQH: $0.26 \pm 0.0^\#$ / COX-2: Ct: 0.44 ± 0.03 ; RQH: 0.26 ± 0.01 ; Cd: $0.99 \pm 0.01^*$; CdRQH: $0.32 \pm 0.04^\#$; FU). In conclusion, Cd exposure induces vascular damage in females, and RQH shows potential as a plant-based therapeutic option.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: The effects of Losartan on the memory of Hypertensive rats fed with the high-fat-diet

Authors:

Andressa BETAT¹
Lais ALFLEN¹
Willyan Joseph DA SILVA¹
Claudio Gabriel Aguiar DE OLIVEIRA¹
Geison Souza IZÍDIO¹
Renata Maria LATARO¹

Affiliations:

¹ Universidade Federal de Santa Catarina

Keywords: Hypertension. Hypercholesterolemia. Cognitive function.

Abstract:

Considering that a high-fat diet (HFD) and hypertension are associated with cognitive impairment, we tested the hypothesis that HFD will impair memory in spontaneously hypertensive rats (SHR) and that the treatment with losartan will reduce the systolic blood pressure (SBP) and reverse the memory dysfunction. The study was approved by The Committee of Ethics in Animal Research (CEUA/UFSC: 3064221121). The experiments were carried out in adult male SHR (14 weeks old), distributed into 2 experimental groups: i) SHR fed with HFD for 7 weeks followed by standard diet for 7 weeks (n=9) and ii) SHR fed with HFD for 7 weeks followed for HFD + LOSARTAN (10 mg/kg) for 7 weeks (n=10). The object recognition protocol and the T Maze Test were performed to evaluate memory after 7 and 14 intervention weeks. The results are presented as mean±SD. One-way ANOVA for repeated measures was used to compare the groups. The HFD did not produce memory impairment in SHR (Discrimination index: before -0.2 ± 0.3 vs. 7 weeks -0.02 ± 0.3 ; $p > 0.05$; New object exploration time: before 54 ± 16 vs. 7 weeks 72 ± 24 ; $p > 0.05$; Spontaneous alternation in the T-Maze test: before 7 ± 5 vs. 7 weeks 10 ± 11 ; $p > 0.05$). The HFD replacement by standard diet for 7 weeks did not affect the SHR performance in memory tests (Discrimination index: 7 weeks -0.02 ± 0.3 vs. 14 weeks 0.01 ± 0.2 ; $p > 0.05$; New object exploration time: 7 weeks 73 ± 24 vs. 14 weeks 75 ± 18 , $p > 0.05$; and Spontaneous alternation: 7 weeks 10 ± 11 vs. 14 weeks 6 ± 4 ; $p > 0.05$), as well as, the treatment with Losartan (Discrimination index: 7 weeks -0.1 ± 0.3 vs. 14 weeks -0.01 ± 0.2 ; $p > 0.05$; New object exploration time: 7 weeks 85 ± 25 vs. 14 weeks 75 ± 14 ; $p > 0.05$; Spontaneous alternation: 7 weeks 6 ± 5 vs. 14 weeks 6 ± 4 ; $p > 0.05$). In conclusion, the memory in SHR was not affected by the chronic administration of HFD or Losartan. Financial Support: Capes and CNPq.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Ellagic Acid Reduces Oxidative Stress and Attenuates Endothelial Dysfunction in Aortas of Iron-Overloaded Rats

Authors:

Vinícius Bermond MARQUES¹
Mônica GOLDNER¹
Mateus José DEFANTE²
Larissa Lorete BARBOSA³
Julia Martins VIEIRA¹
Brenda Santos LEITE²
Sabrina Rodrigues BERTOLI³
Andressa Bolsoni LOPES¹
Leonardo DOS SANTOS¹
Alessandra Simão PADILHA¹

Affiliations:

¹Programa de Pós-Graduação em Ciências Fisiológicas, CCS, UFES, Vitória/ES

²Faculdade Multivix, Cariacica/ES

³Faculdade Novo Milênio, Vila Velha/ES

Keywords: Antioxidant. Aorta. Ellagic acid. Endothelial dysfunction. Iron.

Abstract:

Introduction: Iron overload induces vasculopathy, highlighting the need for protective therapies. Ellagic acid (EA) is a polyphenol with antioxidant and anti-inflammatory actions, holding potential benefits in this context. Objective: To assess whether EA reduces endothelial dysfunction caused by iron overload. Methods: Wistar rats (~200g) were allocated into: control (Ct), control+EA (CtEA, 30 mg/kg/day, v.o.), iron (Fe, 100 mg/kg/day, i.p.), and iron+EA (FeEA). Vehicles were administered to groups not receiving Fe or EA. After 4 weeks, serum/aortic iron, vascular reactivity, endothelial morphology, reactive species production, eNOS gene expression, AOPP/MDA levels, and SOD activity were evaluated (N=4–15). Data were analyzed using two-way ANOVA and Fisher's post-test ($P<0.05$). Study approved by CEUA-UFES 18/2023. Results: EA did not alter serum iron (Ct: 169 ± 8 ; CtEA: 171 ± 13 ; Fe: 459 ± 26 vs FeEA: 505 ± 38 $\mu\text{g/dL}$) or aortic iron content (Ct: 0.013 ± 0.004 ; CtEA: 0.04 ± 0.04 ; Fe: 0.155 ± 0.06 vs FeEA: 0.08 ± 0.01 % of normalized mass), but attenuated the loss of endothelial confluence induced by iron. EA reduced vasoconstrictor hyperreactivity in FeEA (-logEC50 Ct: 6.66 ± 0.27 ; CtEA: 6.85 ± 0.28 ; Fe: 7.12 ± 0.31 vs FeEA: 6.77 ± 0.23) and preserved endothelial (dASC Fe: 272 ± 33 vs FeEA: 407 ± 41 %) and nitroergic (dASC Fe: 123 ± 77 vs FeEA: 249 ± 141 %) modulation after endothelial removal and use of L-NAME (100 μM), respectively. VAS2870 (10 μM), SOD (150 U/mL), and DMSO (100 μM) reduced reactivity only in Fe, while catalase (1000 U/mL) had no effect in Fe but increased vasoconstriction in FeEA. EA decreased superoxide (DHE) and hydroxyl/peroxynitrite radicals (HPF), increased SOD activity, and prevented AOPP and MDA elevation. EA doubled eNOS mRNA expression in FeEA. Conclusion: EA attenuated iron-induced endothelial dysfunction, possibly through preservation of endothelial/nitroergic modulation, eNOS upregulation, NOX inhibition, and antioxidant activity. Financial support: CNPq, CAPES e FAPES.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: New Rho-kinase Inhibitor Reduces Diastolic Dysfunction Induced by Estrogen Depletion in Spontaneously Hypertensive Rats

Authors:

Bruno DEMATTÉ¹
Juliana Nicolau Aranha CARVALHO¹
Tadeu MONTAGNOLI¹
Heber TOLOMEU¹
Gisele Zapata-SUDO¹

Affiliations:

¹ Universidade Federal do Rio de Janeiro

Keywords: Aging. Biomarkers. Diastolic function. Estrogen. Heart failure.

Abstract:

Heart failure with preserved ejection fraction (HFpEF), commonly found in women experiencing estrogen depletion, results from diminished cardioprotective effects and endothelial dysfunction associated with aging. The Rho-kinase (ROCK) signaling pathway has been implicated in cardiac impairment linked to female senescence. This study aimed to evaluate the effects of a novel synthetic ROCK inhibitor (iROCK), administered orally, in spontaneously hypertensive rats (SHR) subjected to bilateral oophorectomy (OVX). All experimental procedures were approved by the Animal Care and Use Committee of the Federal University of Rio de Janeiro. Under ketamine (80 mg/kg, i.p.) and xylazine (15 mg/kg, i.p.) anesthesia, cardiac function was assessed in female SHR eight weeks post-OVX and compared with SHR- Sham animals. Subsequently, OVX-SHR were randomly assigned to receive either vehicle (DMSO) or iROCK (10 mg/kg orally) for two weeks. The compound exhibited potent inhibitory activity toward ROCK1 and ROCK2 isoforms, with IC₅₀ values of 1.3 μ M and 1.7 μ M, respectively. At the end of treatment, hemodynamic parameters were evaluated via echocardiography and left ventricular (LV) catheterization. Cardiac tissue was collected for protein analysis of p38 mitogen-activated protein kinase (p38MAPK) and extracellular signal-regulated kinases (ERK1/2) using Western blot. After eight weeks of estrogen deprivation, SHR developed HFpEF, maintaining a preserved ejection fraction (EF) of $85.2 \pm 0.9\%$; a marked decline in EF (from 83.0 ± 2.7 to $64.9 \pm 0.9\%$) occurred only after 12 weeks post-OVX. Diastolic dysfunction was confirmed by elevated LV filling pressure, with the E/e' ratio increasing from 12.1 ± 1.3 (SHR-Sham) to 19.3 ± 0.5 (OVX), and LV end-diastolic pressure (LVEDP) rising from 13.4 ± 1.3 to 21.1 ± 4.9 mmHg. iROCK treatment significantly reduced E/e' to 16.2 ± 0.5 and LVEDP to 6.4 ± 0.7 mmHg. OVX induced an increase in cardiac expression of p38MAPK and ERK1/2 by 2.3- and 1.3-fold, respectively, compared to SHR- Sham. iROCK treatment attenuated p38MAPK overexpression without affecting ERK1/2 levels. Since pro-inflammatory cytokines and oxidative stress are known to activate p38MAPK, the reversal of its expression suggests that iROCK may attenuate the inflammatory component of HFpEF. In conclusion, the new iROCK compound restored LV diastolic function impaired by estrogen depletion in SHR, likely by reducing myocardial inflammation.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Angiotensin-(1-7) attenuates cardiac hypertrophy and inflammation following adrenergic overload

Authors:

Maria Luiza DIAS-PINTO^{1, 2}
Bruno de Lima SANCHES^{1, 2}
Robson Augusto DOS SANTOS^{1, 2}
Cibele ROCHA-RESENDE^{1,2}
Silvia GUATMOSIM^{1,2}
Thiago VERANO-BRAGA^{1, 2}

Affiliations:

¹ UNIVERSIDADE FEDERAL DE MINAS GERAIS
² DEPARTAMENTO DE FISILOGIA E BIOFÍSICA-ICB-UFMG

Keywords: Angiotensin-(1-7). Cardiac hypertrophy. Inflammation. Isoproterenol. Takotsubo cardiomyopathy

Abstract:

Takotsubo cardiomyopathy (TC) is a cardiovascular disease that is triggered by physical or emotional stress, causing excessive release of catecholamines and overstimulation of cardiac beta-adrenergic receptors. This results in cardiac hypertrophy, inflammation, and cell death. Angiotensin-(1-7) [Ang-(1-7)], a component of the renin-angiotensin system (RAS), has shown anti-hypertrophic and anti-inflammatory effects in several cardiovascular models, but its role in TC remains unclear. To evaluate the anti-hypertrophic and anti-inflammatory effects of Ang-(1-7) in an experimental model of TC, female C57BL/6 mice (8–12 weeks old) were divided into four groups: CTRL-CD (mice daily treated with cyclodextrin (CD) and a single intraperitoneal (IP) dose of 0.9% saline); CTRL-Ang-(1-7)(treated with Ang-(1-7)-CD and 0.9% saline IP); ISO+CD(treated with CD and a single IP dose of isoproterenol (ISO, 300 mg/kg)); and ISO+Ang -(1-7)(treated with Ang-(1-7)-CD and ISO). Oral treatment began one day before ISO and continued for four days. Following three days of ISO treatment, animals were euthanized, and the cardiac tissue was collected for analysis (CEUA: 223/2023). Remarkably, ISO induced a cardiac hypertrophy, shown by increased heartweight-by-tibia-length ratio, which was reduced by Ang-(1-7)(ISO+CD: 6.4 ± 0.63 mg/mm vs ISO+Ang-(1-7): 5.7 ± 0.36 mg/mm, $n=13$). The oral treatment with Ang -(1-7) also decreased the cardiomyocyte cross sectional area increased by ISO (ISO+CD: $246.4 \pm 61.44 \mu\text{m}^2$ vs ISO+Ang-(1-7): $201.2 \pm 48.27 \mu\text{m}^2$, $n=6$). In the inflammation scenario, flow cytometry showed reduced in cardiac infiltration of CCR2+ monocytes (ISO+CD: 229.37 ± 53.26 cells/mg vs ISO+Ang -(1-7): 69.23 ± 12.88 cells/mg, $n=6$), key players in inflammation and related to cardiomyocyte hypertrophy and cell death. These findings highlight Ang-(1-7) preventive role in ISO-induced cardiac hypertrophy and inflammation. Further studies will explore its effects on cell death and the underlying cardioprotective mechanisms.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Influence of glucose and oxygen deprivation on the function of isolated aortic arteries

Authors:

Paola Quevedo DA COSTA^{1,2};
Karen Martirena Monks DA SILVA^{1,2},
Tais Kopp DA SILVEIRA^{1,2},
Fernando Diógenes Teixeira MEYER^{1,2},
Paulo Cavalheiro SCHENKEL^{1,2}

Affiliations:

¹Programa de Pós-Graduação Multicêntrico em Ciências Fisiológicas

²Universidade Federal de Pelotas

Keywords: Endothelium. Ischemia. Nitric oxide. Reperfusion. Vascular reactivity.

Abstract:

The modulation of vascular tone is determinant for the regulation of blood flow, with the endothelium playing a pivotal role. Endothelial dysfunction has been implicated in a wide range of pathologies, and numerous experimental models have been developed to study its mechanisms. Among these, glucose and oxygen deprivation is commonly employed to mimic ischemia-reperfusion syndrome—a classical and widely used experimental model. To evaluate the effects of glucose and oxygen deprivation on the function of isolated aortic arteries. Aortic rings were obtained from male and female Wistar rats, prepared, and mounted in organ baths under a resting tension of 1.0 g/f for a stabilization period of 30 minutes in Krebs-Henseleit solution (CEUA 047712/2023-45). Vessels were then assessed for their contractile response to potassium chloride (80 mM KCl) and endothelium-dependent relaxation in response to cumulative concentrations of acetylcholine (10^{-9} to 10^{-4} M, ACh). After re-stabilization, the rings were subjected to glucose and oxygen deprivation for either one or three hours, followed by reassessment of ACh-induced relaxation. Subsequently, the vessels were re-exposed to Krebs-Henseleit solution for 30 minutes and tested again with ACh and sodium nitroprusside. Exposure to KCl increased the vascular tension to 2.14 g/f. Endothelium-dependent relaxation to ACh was similar among all rings prior to deprivation. A significant impairment in ACh-induced vasodilation was observed following three hours of glucose and oxygen deprivation, an effect not seen after only one hour of deprivation. Notably, the vasodilatory response was fully restored following a 30-minute re-exposure to Krebs-Henseleit solution. Our findings suggest that three hours of simultaneous glucose and oxygen deprivation impairs endothelium-dependent vasodilation in isolated aortic rings. This dysfunction appears to be reversible, as reintroduction of glucose and oxygen fully restored vascular responsiveness.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Melatonin Therapy on Vascular Insulin Resistance in Males and Females with Pulmonary Arterial Hypertension

Authors:

Taís KÖPP^{1,2}

Rogério Nogueira SOARES³

Paulo Cavalheiro SCHENKEL^{1,2}

Affiliations:

¹Programa de Pós-Graduação Multicêntrico em Ciências Fisiológicas

²Universidade Federal de Pelotas

³Wayne State University

Keywords: Insulin. Melatonin. Pulmonary Hypertension. Sex-difference. Vascular function.

Abstract:

Pulmonary arterial hypertension (PAH) is a progressive cardiopulmonary disorder characterized by vascular remodeling, elevated pulmonary vascular resistance, and right ventricular failure, with documented sexual dimorphism in disease prevalence and outcomes. This study was approved by Wayne State University's IACUC (IACUC-24-02-6618) and investigated sex-specific responses to melatonin therapy in monocrotaline-induced PAH using 38 Wistar rats (18 males/20 females) divided into CTRL, PAH (60 mg/kg MCT), and PAH+MEL (MCT + 30 mg/kg/day melatonin) groups. Vascular function assessments revealed striking sexual dimorphism: Insulin-mediated vasodilation was severely impaired in PAH males ($p < 0.001$ vs CTRL), showing opposite-direction response in vasoconstriction at 10^{-7} M insulin, while melatonin partially restored responses ($p < 0.05$ vs PAH). Females maintained normal insulin sensitivity ($p = 0.87$ vs CTRL); Melatonin treatment did not improve the harmful effects of PAH on pulmonary artery dilation in response to acetylcholine (ACh) in males, but in females, only the MCT group had a significant reduction, while the MCT+MEL group did not differ from the control, suggesting a possible protective effect of melatonin in females; Sodium nitroprusside (SNP) showed impaired endothelium-independent vasodilation only in MCT males ($p < 0.01$), while females maintained preserved responses across all groups. PAH male group exhibited an attenuation in body weight gain, effect partially reversed by melatonin. This parameter did not change in females throughout the study ($p = 0.47$). These findings demonstrate that PAH-related insulin resistance is profoundly sex-dependent; melatonin shows greater therapeutic efficacy in males likely due to females' inherent hormonal protection, and endothelial preservation correlates with metabolic maintenance.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Similarities and Differences in Carotid Artery and Hemodynamic Reactivities Induced by Mental or Cold Stress: A Randomized Crossover Study

Authors:

Francisco Johann FENSTERSEIFER¹
Francine STEIN¹
Iara Gonçalves TEIXEIRA¹
Pablo Antonio Bertasso de ARAUJO¹
Richard Emanuel FILIPINI¹
Guilherme Fleury Fina SPERETTA¹

Affiliations:

¹ Federal University of Santa Catarina, Department of Physiological Sciences, Center for Biological Sciences, Florianópolis, Brazil.

Keywords: Cold pressor test. Physical stress. Stroop test. Vascular function.

Abstract:

The Cold Pressor Test (CPT) is a well-established method for assessing carotid artery reactivity (CAR) and hemodynamic responses. The Stroop test, a tool to induce mental stress, in turn, has been employed to evaluate hemodynamic responses, although its effects on CAR remain unclear. This study aimed to compare the CAR and hemodynamic responses induced by mental and cold stress. Twenty adults (10 women; 25.7±5.2 years) were evaluated. Assessments were conducted in the morning, following fasting and abstention from exercise, alcohol, and caffeine. During the visit, anthropometric assessments were conducted, followed by randomized, crossover Stroop and CPT tests (3 min each) evaluation. CAR was evaluated using ultrasound. Blood pressure (BP) and heart rate (HR) were evaluated using an oscillometric device and a Polar H10, respectively. A paired Student's T-Test was used to compare the outcomes between interventions. The study was approved by the UFSC Research Ethics Committee (No. 68282923.1.0000.0121). The results indicated a similar CAR between Stroop and CPT (Stroop: 2.8±3.6; CPT: 4.1±4.2 %; p=0.236). However, the time to peak was shorter in Stroop (Stroop: 66.5±56.5; CPT 99.5±35.1 s; p=0.020). Regarding the hemodynamic responses, CPT promoted a higher increase in mean arterial pressure (Stroop: 8.4±4.2; CPT: 13.5±8.5 mmHg; p=0.023), while Stroop caused a higher increase in HR (Stroop: 20.5 (10.2-27.7); CPT: 11.0 (5.2-14.7) bpm; p=0.013). In conclusion, we suggest that mental stress evokes a similar CAR magnitude to cold stress; however, CAR peaks earlier in response to mental stress. Further studies involving individuals with chronic diseases or higher cardiovascular risk are warranted to confirm the potential and to explore CAR as a predictive marker.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: The Role of STI1 in the Regulation of Cardiac Hypertrophy

Authors:

Graciene F. A. C. FONSECA¹
Victor Moura Vidal COSTA¹
Fernando Araujo ESPANHOL¹
Iago Nascimento PINHEIRO¹
Sílvia GUATIMOSIM¹

Affiliations:

¹Department of Physiology and Biophysics, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Brazil

Keywords: Cardiac hypertrophy; STI1; cardiomyocyte

Abstract:

Cardiac hypertrophy is an adaptive response to physiological or pathological stimuli. The pathological form is linked to cardiomyocyte loss, fibrosis, cardiac dysfunction, and an increased risk of heart failure and sudden death. It also involves fetal gene reactivation, metabolic alterations, and impaired angiogenesis. Our group previously demonstrated that the co-chaperone STI1, part of the Hsp70/Hsp90 complex, is essential for adrenergic-induced hypertrophy. The present study investigates the impact of reduced STI1 protein expression in the heart under cardiac stress caused by isoproterenol. Methods: Wild-type (WT) and genetically modified C57BL/6J mice with reduced STI1 levels (STI1^{+/-}) were treated in vivo for 7 days with saline (n=7) or ISO (25 mg/kg, intraperitoneal, n=7) and euthanized on day 8. Hearts were collected, weighed, and analyzed via immunohistochemistry; Cardiomyocytes were isolated for area measurement. Results: ISO-treated WT and STI1^{+/-} mice exhibited an increase in the heart/tibia length ratio, confirming cardiac remodeling. However, when cardiomyocyte hypertrophy was assessed, only WT cells showed a significant size increase (p<0.001), whereas STI1^{+/-} cardiomyocytes failed to enlarge. Conclusion: These results show that adequate levels of STI1 are necessary for CMs to hypertrophy in response to ISO. (CEUA 97/2022)



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Cardioprotective Effect of SGLT2 Inhibition in Stress-Induced Cardiomyopathy

Authors:

Anna Clara de Paula PIRICO¹,
Giovane PIRES¹,
Henrique ABRAMO¹,
Sílvia GUATIMOSIM¹,
Cibele ROCHA-RESENDE¹

Affiliations:

¹Departamento de Fisiologia e Biofísica, Instituto de Ciências Biológicas Universidade Federal de Minas Gerais

Keywords: Dapagliflozin. Heart. Immunomodulation. Macrophages. Takotsubo syndrome

Abstract:

Stress-induced cardiomyopathy, or Takotsubo syndrome, is a form of acute heart failure triggered by physical or emotional stress. High doses of the β -adrenergic agonist isoproterenol (ISO) induce a similar condition, leading to cardiac dysfunction, cardiomyocyte damage, and macrophage infiltration. This non-surgical model is used to study myocardial injury, as it mimics excessive adrenergic stimulation observed in stress-induced cardiac events. Sodium-glucose cotransporter 2 (SGLT2) inhibitors, such as dapagliflozin (DAPA), have been shown to exert cardioprotective effects beyond glycemic control, including anti-hypertrophic and anti-inflammatory actions. The aim of this study is to investigate whether DAPA administration prevents the hypertrophic and inflammatory effects of ISO-induced injury. Male and female mice (6–8 weeks old) were treated with DAPA (1.5 mg/kg, oral gavage, daily) from Day 0 to Day 3. Mice were treated with ISO (200 mg/kg, IP) on Day 0. Control mice were given saline. Seventy-two hours post-ISO injection, mice were euthanized, and hearts were collected for immunofluorescence analysis of inflammatory infiltration and cardiac hypertrophy. The study was approved by the Animal Ethics Committee (CEUA/UFMG, No. 62/2025). As expected, ISO treatment induced cardiomyocyte hypertrophy, along with increased CD68+ macrophage infiltration. DAPA treatment partially prevented these alterations, with reduced cardiomyocyte cross-sectional area in the DAPA/ISO group compared to the Saline/ISO group (322.6 ± 108.3 ; $n=10$ vs. 353.9 ± 128 ; $n=13$), as well as decreased CD68+ macrophage infiltration (75.2 ± 82.5 ; $n=11$ vs. 102.1 ± 92.2 ; $n=11$). These findings suggest that dapagliflozin exerts a cardioprotective effect in stress-induced cardiomyopathy, possibly through anti-inflammatory pathways. Further studies are needed to clarify the molecular mechanisms involved. Apoio financeiro: FAPEMIG e Instituto Serrapilheira.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Evaluation of the interaction between Cerebral Dopamine Neurotrophic Factor (CDNF) and KDEL receptor at the cell membrane

Authors:

Ana Paula Oliveira CAVALCANTE²
Dahienne OLIVEIRA¹
Fernando PALHANO¹
Marcelo SANTIAGO²
Leonardo MACIEL²
Débora FOGUEL¹

Affiliations:

¹Institute of Medical Biochemistry Leopoldo de Meis, Universidade Federal do Rio de Janeiro,
²Institute de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro.

Keywords: Cardiomyokine. Cardioprotection. CDNF.

Abstract:

CDNF (Cerebral Dopamine Neurotrophic Factor) is a recently identified neurotrophic factor. Its role in the cardiac environment is not fully understood, though it has been shown to promote cardioprotection. This effect is mediated through its interaction with the KDEL receptor (KDEL-R), leading to activation of the PI3K/AKT signaling pathway. There is no evidence suggesting that the KDEL-R, which is typically localized in the endoplasmic reticulum (ER), translocates to the plasma membrane (PM) during ER stress or associates with exogenous CDNF (exoCDNF). Additionally, the mechanism by which this complex is internalized by cells is still unclear. H9C2 cells were plated in 24-well plates, treated with TG for 3 hours, and then incubated with fluorescein isothiocyanate (FITC) (1 μ M) for the last 15 minutes for confocal microscopy imaging. For the evaluation, H9C2 cardiomyocytes were treated with TG during 3 hours for time-lapse experiments. CDNF or its isolated domains were applied after the first reading. Our data show that fluorescein-labeled exoCDNF binds to the KDEL-R at the membrane of TG-treated H9C2 cardiomyocytes. Regarding the time-lapse experiment, CDNF is internalized within approximately 5 to 15 minutes. Our data show CDNF's cardioprotective effect through binding to the KDEL receptor and internalization. This study suggests that CDNF interacts with KDEL1 leading to the complex internalization, although the downstream mechanism remains uncertain.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: The Cerebral Dopamine Neurotrophic Factor promotes cardioprotection via activation of the PI3K/AKT pathway and plasma membrane KDEL receptor

Authors:

Juliana Almeida FERREIRA²
Dahienne OLIVEIRA¹
Leonardo MACIEL²
Debora FOGUEL¹

Affiliations:

¹ Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro ² Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro.

Keywords: Cardioprotection. CDNF. Via PI3K/AKT.

Abstract:

(CEUA approved 100/16 and 015/17) Ischemic heart disease is the biggest cause of death in the world. Recently, the Cerebral Dopamine Neurotrophic Factor (CDNF) has been described as a neuroprotection agent. However, little is known about its role in the cardiac context. To evaluate the effects of CDNF under endoplasmic reticulum (ER) stress induced by thapsigargin (TG) and by ischemia and reperfusion (I/R), identify the possible receptor and the signaling pathway that leads to cardioprotection, cardiomyocytes derived from induced pluripotent stem cells and from CD-1 mice or H9C2 cardiomyocytes were used. To investigate CDNF secretion and cardioprotection mechanisms, cells were induced to ER stress with TG (1 $\mu\text{mol/L}$) for 3-20h. The isolated hearts from Wistar rats were submitted to I/R in the presence of CDNF before or after I/R. To evaluate the signaling pathways involved, PI3K/AKT, PKC and JAK-STAT3 inhibitors were used. To identify the cellular CDNF receptor, heptapeptides from the C-terminal domain of CDNF, a version of CDNF with deletion of the last four amino acids and the anti-CDNF antibody were used. The presence of the KDEL receptor (KDELr) on the membrane and its interaction with CDNF was monitored by immunocytochemistry. Results show that CDNF is secreted under stress in cardiomyocytes and in isolated I/R hearts. Recombinant CDNF (exoCDNF) protected cardiomyocytes against induced ER stress. In isolated hearts, exoCDNF protected against I/R damage. This protection was inhibited by the PI3K/AKT antagonist. Regarding the CDNF receptor, results point to the plasma membrane KDELr, since heptapeptides containing the KTEL sequence, the anti-CDNF antibody and the construct of CDNF without the last four amino acids inhibited the protective effects. Moreover, labeled exoCDNF binds to the KDELr on the membrane of cardiomyocytes treated with TG. In conclusion, CDNF showed a cardioprotective activity, mediated by the KDELr, leading to the PI3K/AKT pathway's activation.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Spontaneously hypertensive rats seek calories and a sweet taste after water deprivation

Authors:

Mariana Alves MORAES¹,
Eduardo COLOMBARI¹,
José Vanderlei MENANI¹,
Débora Simões Almeida COLOMBARI¹,
Milede Hanner Saraiva PAES¹

Affiliations:

¹ Faculdade de Odontologia, Universidade Estadual Paulista "Júlio de Mesquita Filho", Araraquara, SP.

Keywords: Hypertension. Energy intake. Sweet taste.

Abstract:

Different from normotensive Holtzman rats (NT), water deprivation (WD) can induce sucrose (SUC) intake in spontaneously hypertensive rats (SHR) even after 2 h of rehydration. It's unknown whether this intake is related to sweet taste or energy intake, as WD reduces food intake. We aimed to evaluate the intake of SUC, saccharin (SAC) and maltodextrin (MALTO) in NT and SHR. CEUA: 14/2023. Adult male NT and SHR rats (systolic blood pressure: 160-200 mmHg) had free access to water, food and one of the solutions: 2% SUC, 0.1% SAC or 2.08% MALTO, measuring daily consumption for preference test. The intake of water and the 3 solutions were measured in euhydration (EH), 24-h water deprivation (WD; food available), and 24-h food restriction (FR, free water and a food reduction to equate to WD consumption), in the same rats randomly distributed (n = 7-11/group). The protocol consisted of 2 h of water intake (thirst test) followed by 2 h of water and one of the 3 solutions intake to avoid ingestion of the solutions due to thirst. In preference test, NT and SHRs preferred to ingest any of the 3 solutions (all p < 0.05 vs. water) rather than water (NT: water ~ 5- 6 ml/24 h/group, vs. SUC 43 ± 6, SAC 41 ± 3 and MALTO 56 ± 11 mL/24h and SHR: water ~ 1-6 mL/24h/group, vs. SUC 92 ± 14, SAC 97 ± 7 and MALTO 152 ± 10 mL/24 h). In WD, during the thirst test, NT drank more water than SHR (p < 0.05). Almost no water intake was observed after the FR protocol. After WD and FR in the last 2 h of the test, compared to EH, SHR drank more (p < 0.05) SUC (EH: 0.3 ± 0.1, WD 5.0 ± 1.1, FR 11.6 ± 3.2 mL/2 h), SAC (EH: 2.2 ± 0.7, WD 6.2 ± 0.9 mL, FR 8.5 ± 1.7 mL/2h) and MALTO (EH: 2.3 ± 0.9; WD 9.2 ± 1.6; FR 17.7 ± 3.2 mL/2 h), while NT drank none of the solutions. The data suggest that after WD, SHR ingests SUC for its sweet taste and energy, which differs from NT rats, who drink mainly water, and could ultimately worsen the condition of the SHRs. Support: CAPES PrINT, FAPESP, CNPq.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: New acetophenones reduce oxidative stress in blood vessels

Authors:

Samuel Alexandre Melo CAMPOS¹,
Júlia Maria do Nascimento ARRUDA¹,
Sofia Santana RAMOS¹,
Mariana Santana QUIRINO²,
André Luís Batista da ROCHA³,
Cristina ANTONIALI²,
Ricardo MENEGATTI³,
Simone Regina POTJE¹.

Affiliations:

¹Department of Biomedical Sciences and Health, Minas Gerais State University – UEMG; ²Department of Basic Sciences, São Paulo State University – UNESP; ³Faculty of Pharmacy, Federal University of Goiás – UFG.

Keywords: Acetophenone derivative. Apocynin. Oxidative stress.

Abstract:

High concentrations of free radicals generate oxidative stress, a deleterious process that can damage cellular structures. Antioxidants play an important role in neutralizing free radicals in cells, then we evaluated the antioxidant capacity of the compounds LQFM355 and LQFM189. Aortic rings without endothelium from male Wistar rats (10 weeks of age) were used, which were incubated (30 min), or not (control) with apocynin, LQFM189 or with LQFM355 (100 $\mu\text{mol/L}$). The rings were stimulated with increasing and cumulative concentrations of sodium nitroprusside (SNP, 0.1 nmol/L to 0.1 mmol/L). The reduction of oxidative damage was evaluated by the TBARS (thiobarbituric acid reactive substances) method. The antioxidant capacity was determined by the FRAP (ferric reducing antioxidant power) assay. The results were expressed as mean $\pm$ standard deviation and compared between groups (ANOVA, $p < 0.05$). (Animal Use Ethics Committee: UNESP 865-2023; UEMG 003/2023). The concentration-response curve stimulated by SNP in rings incubated with the compounds was similar to the control curve. By TBARS analysis, we can observe that apocynin (0.2887 ± 0.07 , $n=10$, $\mu\text{mol/mg protein}$), LQFM355 (0.2642 ± 0.05 , $n=10$, $\mu\text{mol/mg protein}$) and LQFM189 (0.3111 ± 0.03 , $n=12$, $\mu\text{mol/mg protein}$) reduced the concentration of malondialdehyde in relation to the control group (0.4921 ± 0.06 , $n=11$, $\mu\text{mol/mg protein}$) ($p < 0.0001$). By FRAP analysis, we observed that apocynin (0.1 mmol/L: 0.254 ± 0.04 , $n=4$; 1 mmol/L: 1.035 ± 0.09 , $n=4$, FeSO_4) and LQFM355 (0.1 mmol/L: 0.167 ± 0.01 , $n=4$; 1 mmol/L: 0.177 ± 0.02 , $n=4$, FeSO_4) present antioxidant capacity at concentrations of 0.1 mmol/L and 1 mmol/L, while the compound LQFM189 (0.1 mmol/L: 0.122 ± 0.01 , $n=4$; 1 mmol/L: 0.135 ± 0.01 , $n=4$, FeSO_4) did not present antioxidant capacity at the same concentrations tested. The three compounds tested reduced oxidative damage, although only apocynin and LQFM355 showed antioxidant potential. These compounds appear promising for reducing oxidative stress.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: When Sex Matters: The Differentiated Role of the Nitric Oxide Pathway in Coronary Vasodilation of Diabetic Rats

Authors:

Nikollas Silva MEIRELLES¹;
Nathalie Tristão Banhos DELGADO¹;
Wendrea Kaylaine Burgarelli Danielli MUSINI¹;
Jéssyca Aparecida Soares GIESEN¹
Roger Lyrio dos SANTOS¹.

Affiliations:

¹ Universidade Federal do Espírito Santo

Keywords: Diabetes mellitus. Endothelial dysfunction. Coronary circulation. Sex differences. Nitric oxide.

Abstract:

Diabetes Mellitus (DM) is a metabolic disease characterized by persistent hyperglycemia, which leads to endothelial dysfunction, impairing vascular relaxation and increasing the risk of cardiovascular diseases. Evidence suggests that there are sex differences in vascular function, influenced by hormonal and metabolic factors. Based on this, we hypothesized that rats with DM exhibit reduced endothelial relaxation in the coronary bed compared to non-diabetic rats, with possible differences between sexes. The aim of this study was to investigate the impact of DM on endothelial-dependent relaxation in the coronary bed and to analyze sex differences in this response. Wistar rats of both sexes, aged 8 to 9 weeks (CEUA no. 12/2024), were used and divided into 4 experimental groups: diabetic (DM) and non-diabetic (C) females and males. Diabetes was induced by a single dose of alloxan (150 mg/kg, i.p.). After 30 days, hearts were perfused using the Langendorff system. Vasodilation was assessed by the response to bradykinin (BK, 0.1-1000 nM) after stabilization of the basal coronary perfusion pressure (CPP), before and after nitric oxide synthase (NOS) inhibition with L-NAME (100 μ M). Data were expressed as mean $\pm$ SEM and analyzed by two-way ANOVA, followed by the Sidak *post hoc* test ($P < 0.05$). Basal CPP was higher in females, regardless of DM (Female C: 90 ± 6 and DM: 89 ± 3 ; Male C: 55 ± 2 and DM: 64 ± 6 mmHg). BK induced similar vasodilation across groups (Female C: 27 ± 13 and DM: 22 ± 10 ; Male C: 22 ± 15 and DM: 26 ± 7 %). However, NO pathway inhibition impaired relaxation only in female DM rats (Female C: 27 ± 13 ; DM: 22 ± 10 ; Male C: 22 ± 15 ; DM: 26 ± 7 %). These findings suggest that female DM rats have a greater dependence on the NO pathway for coronary vasodilation, while males exhibit mechanisms that need to be further evaluated.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Evaluation of the Functional Profile of Mitochondria in Rats with Post-Infarction Heart Failure

Authors:

Itanna Isis Araújo de Souza¹²,
Maria Eduarda Maciel Fernandes PAVARINO ¹²,
Marcella Borges COUTINHO ¹,
Rodrigo Veras MIRANDA ¹²,
José Hamilton Matheus NASCIMENTO¹²,
Leonardo MACIEL ¹²

Affiliations:

¹Universidade Federal do Rio de Janeiro
²Instituto de Biofísica Carlos Chagas Filho

Keywords: Heart Failure, Mitochondrial dysfunction, Bioenergetic dysfunction

Abstract:

Introduction: Heart failure (HF) affects ~60 million people worldwide, with 30% mortality in one year and 70% in five. Beyond its fatality, it greatly impairs quality of life. Its progression remains complex and not fully understood. Mitochondrial dysfunction is implicated in HF onset and progression. Here, we characterize mitochondrial function in distinct regions of a post-ischemic failing heart. Methods: Male Wistar rats were submitted to left anterior descending coronary artery ligation (HF induced by infarction) HF group or placebo surgery (SHAM). Ninety days later, animals were euthanized, and hearts dissected into four regions: affected area, border zone, septum, and right ventricle (RV). Mitochondria were isolated by differential centrifugation of each zone. Function was assessed by measuring O₂ consumption in complexes I, II, and IV under states I, III, IV, and maximal uncoupling. ATP production, ROS generation, membrane potential, and calcium retention capacity. Results: In complex I state II, O₂ consumption was lower in the border zone and RV when compared to the affected area and septum. In complex II state III, O₂ consumption was higher in the affected area and septum compared to the other areas. ATP production was reduced in the affected area when compared to the septum and the RV ($p < 0.01$) and between the SHAM and HF groups in the affected area ($p = 0.016$). ROS production was higher in the affected area vs RV ($p = 0.01$) and in the septum vs RV ($p = 0.02$). ROS was also elevated in HF hearts (affected area vs SHAM, $p = 0.036$; RV vs SHAM, $p = 0.041$). The ATP/O₂ ratio was higher in the RV and septum vs the affected area ($p > 0.01$; $p = 0.21$). Regarding calcium retention capacity, the septal region showed a higher capacity when compared to all the other areas ($p = 0.001$). Conclusion: Mitochondria show regional phenotypes in heart failure: the affected area has inefficient O₂ use-ATP conversion, while the septum mounts a compensatory but harmful response. Financial Support: CAPES, Faperj, CNPq



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Modulation of endothelial glycocalyx by the pregnancy process in a model of essential hypertension

Authors:

Sofia S. RAMOS¹
Isabela J. OLIVEIRA¹
Antonio J.C. BEZERRA¹
Mariana S. QUIRINO²
Daniel RODRIGUES³
Mirele R. MACHADO³
Cristina ANTONIALI²
Rita C. TOSTES³
Simone R. POTJE¹

Affiliations:

¹Department of Biomedical Sciences and Health, Minas Gerais State University – UEMG;
²Department of Basic Sciences, São Paulo State University – UNESP;
³Department of Pharmacology, University of São Paulo – USP.

Keywords: Endothelial glycocalyx. Hypertension. Pregnancy.

Abstract:

The endothelial glycocalyx contributes to the activation of the endothelial nitric oxide synthase and nitric oxide (NO) synthesis in endothelium. In hypertension, endothelial dysfunction is associated with lower NO concentrations. However, at the end of pregnancy in hypertensive rats (SHR), there is an increase in NO synthesis in vessels. The aim of this study was to analyze whether pregnancy alters glycocalyx synthesis in SHR aortas. We used normotensive (Wistar) and SHR rats, non-pregnant (NP) and pregnant (P). The group of SHR rats had systolic blood pressure (SBP) ≥ 150 mmHg before pregnancy. We evaluated the NOx concentration by the Griess assay, MDA levels by the TBARS technique and protein expression by Western Blotting. Data were presented as mean $\pm$ standard deviation and compared between groups (ANOVA). Animal Use Ethics Committee (UNESP, nº 0097/2021; UEMG, nº 001/2022). SBP was reduced in P rats (Wistar: 86.67 ± 2.56 ; SHR: 134.5 ± 3.22 , n=6; mmHg) compared to NP rats (Wistar: 108 ± 3.47 ; SHR: 161.3 ± 2.82 , n=6, mmHg). Higher [NOx] was observed in aortas of P rats (Wistar: 0.1470 ± 0.004 ; SHR: 0.1034 ± 0.002 , n=8, $\mu\text{M}/\text{mg}$ protein) when compared to NP rats (Wistar: 0.1166 ± 0.003 ; SHR: 0.069 ± 0.002 , n=8, $\mu\text{M}/\text{mg}$ protein). The [MDA] in red blood cells of NP (Wistar: 29.86 ± 0.66 ; SHR: 35.63 ± 0.60 , n=6, nM/mg protein) were decreased by pregnancy (Wistar: 23.50 ± 0.62 ; SHR: 30.83 ± 0.62 , n=6, nM/mg protein). In Wistar P aortas, the expression of Syndecan-1 (1.29 ± 0.06 , n=6, UA) and Glypican-1 (1.03 ± 0.04 , n=6; UA) was lower than in NP aortas (Syndecan-1: 1.83 ± 0.05 ; Glypican-1: 1.57 ± 0.09 , n=6, UA). However, in aortas from SHR P, the expression of Syndecan-1 (1.81 ± 0.06 , n=6, UA) and Glypican-1 (1.06 ± 0.02 , n=6, UA) was higher when compared to NP (Syndecan-1: 0.97 ± 0.02 , n=6; Glypican-1: 0.64 ± 0.06 , n=6, UA). Pregnancy increases the synthesis of specific endothelial glycocalyx components in aortas from SHR, but reduces it in aortas from Wistar rats.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Study of the cardiovascular bulbar nuclei in the face of progressive heart disease in the MDX mouse, an experimental model of Duchenne muscular dystrophy

Authors:

Wagner CORSINI
Monica Akemi SATO
Mateus Borges da SILVA
Luana Aparecida Pinto de ALMEIDA
Luis Felipe Cunha dos REIS
Túlio de Almeida HERMES

Affiliations:

¹Federal University of Alfenas
²ABC Medical School University Center

Keywords: Heart. Cvl. Muscular dystrophy. Mdx. Rvl.

Abstract:

Cardiorespiratory failure is the main cause of early death in patients with Duchenne muscular dystrophy (DMD). Neural control of the cardiovascular system occurs through afferents that are conducted to the CNS and regulated by groups of neurons located in the bulb. The aim of this study was to correlate changes in the cardiac muscle of mdx mice with the density of neuron bodies in the cardiovascular bulbar nuclei in young (Jv) and adult (Ad) mice. Male mdx mice aged 15 and 54 weeks (n=7), kept in the animal house of the Anatomy Department of UNIFAL-MG, were used in accordance with the ethical principles of COBEA and CEUA approval (0031/2022). Healthy animals were used as controls (Ctrl). The heart (COR) and brainstem (TE) were prepared for histological sections. The COR sections were stained with Hematoxylin and Eosin to analyze the thickness and lumen of the left ventricle (LV), and the TE sections were stained with Cresyl Violet to identify the neuron bodies in the RVL and CVL nuclei, observed under a light microscope. It was found that the COR of the mdx Ad mice (2521.83 ± 311.208) had lower LV wall thickness (in μm), compared to the Ctrl Jv and Ad groups (3812.59 ± 634.508 ; 4020.6 ± 355.835 , respectively) and the mdx Jv group (3358.29 ± 211.15), however, the aforementioned group had lower thickness correlated to Ctrl Ad. For the LV lumen (in μm), the mdx Ad group (7188862 ± 1687175) had a larger diameter than all the groups, the mdx Jv (4571087 ± 994045.7) had a larger diameter related to Ctrl Ad (2480654 ± 634206.4), but not for Ctrl Jv (4435142 ± 1005714), the atrioventricular septum did not show statistical significance. The RVL and CVL nuclei showed no significant differences in neuronal density. We conclude that the mdx phenotype presents structural cardiac alterations with advancing age, in thickness and lumen, but without variations in the density of neurons in RVL and CVL.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Action of Ethyl Ferulate on Muscarinic Receptors in Spontaneously Hypertensive Rats Females

Authors:

Brenda Lois Barros dos SANTOS¹;
Francisca Valdirene de Sousa NUNES¹;
Ariell Alves de OLIVEIRA²;
José Guilherme Veras de ASSUNÇÃO³;
Carlos Eduardo Rodrigues DOURADO⁴;
Samuel de Sousa Pereira ARAÚJO²;
Juliana Alves da SILVA²;
Leonardo Guedes RODRIGUES¹;
José Lopes PEREIRA JÚNIOR¹;
João Paulo Jacob SABINO^{1,2,5}

Affiliations:

¹Graduate Program in Pharmaceutical Sciences, Federal University of Piauí, Brazil
²Multicenter Graduate Program in Physiological Sciences, Federal University of Piauí, Brazil
³Graduate Program in biology, Federal University of Piauí, Brazil ⁴Graduate Program in medicine, Federal University of Piauí, Brazil ⁵Department of Biophysics and Physiology, Federal University of Piauí

Keywords: Arterial hypertension. Ethyl ferulate. Females.

Abstract:

The mechanisms of blood pressure regulation in females present some differences, which seem to be partially dependent on the reproductive hormone estradiol, which culminates in the reduced number of cases of women with systemic arterial hypertension (SAH) during the reproductive period. SAH is caused by several factors, among which endothelial dysfunction, cardiorespiratory reflexes and inflammatory processes stand out. Among the compounds with anti-inflammatory action is ethyl ferulate (EF), which is a natural product belonging to the phenylpropanoid class that can also act as an antioxidant and neuroprotector. Although there are reports suggesting cardioprotective and anti-inflammatory action, there is no evidence on the effect of EF on cardiovascular modulation in spontaneously hypertensive rats (SHR). In this sense, the present study evaluated the effect of EF on mean arterial pressure (MAP) and heart rate (HR) in Wistar and SHR females, as well as the possible mechanism of action involved in these responses. The rats (12 weeks old) underwent vaginal cytological evaluation to determine the phase of the estrous cycle. Then, the animals were anesthetized with ketamine (75 mg/kg) and xylazine (10 mg/kg) intraperitoneally and submitted to the femoral artery and vein cannulation procedure. Forty-eight hours after cannulation, the animals were connected to a pressure transducer coupled to an amplifier to record pulsatile blood pressure and simultaneous derivation of MAP and HR. After the adaptation period (60 min) and the basal recording (30 min), the administration of sodium nitroprusside (NPS - 8 µg/kg, i.v.) was started to confirm venous cannulation. After 30 minutes, the animals were treated with saline (0.1 mL/100 g; i.v.) or PE (7.5; 15; 30 mg/kg; i.v.) to evaluate the behavior of the evaluated parameters. Doses of FE 15 and 30 mg/kg were able to promote reductions in MAP and HR of Wistar and SHR. Blockade of muscarinic receptors managed to attenuate the decrease in MAP and HR in Wistar, and partially prevented the decrease in MAP in SHR. Adrenergic blockade was unable to prevent the decrease in MAP in the two strains evaluated. Blockade of nicotinic receptors did not prevent the decrease in MAP and HR after administration of FE. Thus, the results of the present study indicate that FE may be a possible antihypertensive pharmacological tool, through its action on muscarinic receptors. However, further studies are still needed to elucidate other possible mechanisms of action by which this substance promotes its effects on the cardiovascular system. The study was approved by CEUA-UFPI n-804/2023.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Maternal Behavioral and Nutritional Factors Associated with Cardiometabolic Health in Children

Authors:

Jéssica Gonzaga PEREIRA¹²
Maria Brenda Ellen dos Santos Pereira NASCIMENTO¹²
Cheid Manuel CHONGO¹²
Anna Bheatriz Gomes Ferreira MARINHO¹²
Jéssica Oliveira CAMPOS¹²
Reginaldo Correia da Silva FILHO³
Aiany Cibelle Simões Alves¹²
João Henrique da Costa SILVA¹²

Affiliations:

¹Laboratório Multiusuário de Avaliação Física e Processamento de Sinais -LAPS, Centro Acadêmico de Vitória, Universidade Federal de Pernambuco.

²Laboratório Multiusuário em Nutrição, Atividade Física e Plasticidade Fenotípica-CAV/UFPE.

³Laboratório Multiusuário de Bioquímica Geral, Molecular e do Exercício-CAV/UFPE.

Keywords: Child health. maternal factors. cardiometabolic risk

Abstract:

Children's cardiometabolic health is influenced by maternal factors acting through genetic, epigenetic, behavioral, and environmental mechanisms. Maternal nutrition, nutritional status, and lifestyle habits during the perinatal period and throughout childhood play a fundamental role in the metabolic programming of offspring, impacting clinical and biochemical parameters related to cardiometabolic risk. A quantitative cross-sectional study with 26 mothers and 29 children (7–11 years) from public schools (2024). Maternal perception, dietary intake, body composition, blood pressure, cardiac electrical activity, blood glucose, lipid profile, and redox status were evaluated. Ethics Committee (CAAE: 80956824.3.0000.9430). Among mothers, 20.7% were eutrophic, 34.5% overweight, and 44.8% obese. Among children, 75.9% were eutrophic, 10.3% overweight, and 13.8% obese. Mothers demonstrated perception of their children's weight ($r=0.66$; $p<0.001$). Maternal intake of sugar-rich foods was associated with oxidative stress markers in children, such as TBARS ($r=0.47$; $p<0.05$) and protein carbonyls ($r=0.53$; $p<0.05$). Maternal consumption of ultra-processed foods correlated with children's consumption ($r=0.58$; $p<0.001$). Maternal waist-to-hip ratio correlated with children's VAT/SAT ($r=0.44$; $p<0.05$). Children of eutrophic mothers had higher QT interval ($0.34\pm0.00\text{ms}$) compared to children of overweight/obese mothers ($0.32\pm0.00\text{ms}$; $p<0.05$). Children's systolic pressure correlated with maternal weight ($r=0.38$; $p<0.05$). Maternal ASM/height ratio correlated with children's PRR50 ($r=-0.46$; $p<0.05$) and positively with T-wave amplitude ($r=0.37$; $p=0.05$). Maternal body fat correlated with protein carbonyls in children ($r=0.45$; $p<0.05$). In conclusion, despite maternal awareness, maternal nutritional and behavioral factors negatively affect children's cardiometabolic health, highlighting environmental and epigenetic influences. Maternal interventions may benefit the health of children.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Impacts of maternal voluntary physical activity on cardiorespiratory and biochemical interventions of offspring subjected to perinatal protein malnutrition

Authors:

Reginaldo Luís da Rocha Júnior¹;
Bruna K. A. de Melo Silva⁴;
Danilo A. Ferreira Fontes³;
João H. da Costa Silva²,
Viviane de O. Nogueira Souza².

Affiliations:

¹ Master's student in the Multicenter Postgraduate Program in Physiological Sciences;
² Professor at the Federal University of Pernambuco – Vitória de Santo Antão Academic Center;
³ Laboratory Technician at the Federal University of Pernambuco – Vitória de Santo Antão Academic Center;
⁴ Nutritionist at the Federal University of Pernambuco – Vitória de Santo Antão Academic Center.

Keywords: Biochemical analyses. Cardiorespiratory changes. Phenotypic plasticity. Protein malnutrition. Physical activity.

Abstract:

Reduction in protein percentage during critical phases of development can impact cardiorespiratory and metabolic responses. Voluntary physical activity (VPA) is a tool for modulating such changes. We examined the effects of VFA on the biochemical and cardiorespiratory criteria of mothers and offspring, using Wistar rats (n=30) adapted for 30 days in AFV cages, classified according to level of activity: inactive (n=10), active (n=10) and very active (n=10), followed by mating and insertion of the experimental diet (hypoprotein group=HP: 8% protein or normoprotein group=NP: 17% protein) during pregnancy and lactation. We performed biochemical analyses (total proteins, albumin, glucose, total cholesterol, HDL-c, LDL-c and triglycerides) in mothers and offspring, and cardiorespiratory analyses (blood pressure=BP; heart rate=HR; respiratory rate=RR; tidal volume=VT and pulmonary ventilation=VE) in offspring. The study was approved by the animal experimentation ethics committee (CEEa) of UFPE (nº 23076.062778/2014-38). In mothers, a reduction in albumin (NP-inactive=3.34 ± 0.07g/dL vs HP-inactive=3.04 ± 0.02g/dL, p=0.0093) and cholesterol (NP-very active=126.84 ± 1.23mg/dL¹ vs HP-very active=112.52 ± 5.28mg/dL¹ p=0.04 and HP-active=126.72 ± 1.27mg/dL¹ vs HP-very active=112.52 ± 5.28mg/dL¹ p=0.04) was observed. In the offspring, reduction of total protein (HP-inactive=5.80 ± 0.12g/dL⁻¹ vs NP-inactive=6.19 ± 0.03g/dL⁻¹ p=0.0057 and HP-active=5.91 ± 0.07g/dL⁻¹ vs NP-active=6.28 ± 0.03g/dL⁻¹ p=0.01), albumin (HP-inactive=2.95 ± 0.05g/dL⁻¹ vs NP-inactive=3.25 ± 0.07g/dL⁻¹ p=0.01 and 2.95 ± 0.05g/dL⁻¹ vs 3.22 ± 0.03g/dL⁻¹ p=0.03) and cholesterol (HP-inactive=2.95 ± 0.05g/dL⁻¹ vs NP-inactive=124.91 ± 1.35g/dL⁻¹ p=0.03; HP-active=134.22 ± 1.40g/dL⁻¹ vs NP-active=124.86 ± 1.51g/dL⁻¹ p=0.01 and HP-very active=14.91 ± 0.70 vs NP-very active=121.40 ± 2.43g/dL⁻¹ p=0.0002). Finally, AFV attenuated biochemical changes, which was not observed in cardiorespiratory parameters.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Progesterone treatment attenuates the deleterious effects induced by hormonal deficiency in isolated aortic segments from ovariectomized rats

Authors:

Felipe Fraga do Nascimento¹
Tagana Rosa da Cunha¹
Willian Bergamo de Moura¹
Roger Lyrio dos Santos¹

Affiliations:

Universidade Federal do Espírito Santo

Keywords: Aorta; Progesterone; Treatment; Vascular Reactivity.

Abstract:

Progesterone (P4), a hormone essential for reproduction, also acts on the cardiovascular system, but its effects on the aorta have not yet been fully elucidated. The aim of this study was to evaluate the effects of progesterone treatment on vascular reactivity in aortic rings from ovariectomized Wistar rats. All protocols were approved by Institutional Ethics Committee (#18/2022). Eight-week-old adult rats were divided into: SHAM, Ovariectomized (OVX), OVX and treated with P4 (OVX P4). Treatment was performed with silastic tube implants containing 6mg of P4. Reactivity was performed in thoracic aortic rings with endothelium in response to phenylephrine (PE, 10^{-11} - 10^{-4} M) in the absence or presence of L-NAME (100 μ M) or indomethacin (INDO, 10 μ M). Concentration-response curves to acetylcholine (10^{-12} - 10^{-4} M) were also performed on rings precontracted with PE (10^{-7}). Data expressed as mean $\pm$ SEM and analyzed by Two-way ANOVA test, post Tukey test, $*p < 0.05$. As a result, ovariectomy compromised endothelium-dependent relaxation (%PE: SHAM = 101.1 for OVX = 85% *). P4 treatment was not able to prevent this reduction in relaxation induced by ovariectomy (%PE: OVXP4 = 96.8%). Interestingly, P4 showed a significant anticontractile effect reducing the response to PE even in relation to the SHAM group. (Rmax: SHAM = 2.33 ± 0.14 , OVX = 2.61 ± 0.18 , OVXP4 = 1.70 ± 0.07 g). To better understand the anticontractile effect of P4, we evaluated the NO pathway; ovariectomy reduced the participation of this pathway in the contractile response to PE. However, P4 treatment prevented this reduction (AUC: SHAM = 830.1 ± 63 , OVX = $556.4 \pm 51^{*}$, OVX P4 = 869.9 ± 88 AU). Inhibition of the prostanoid pathway revealed that the synthesis of constrictor prostanoids was likely increased in the OVX group (Rmax: PE = 3.46 ± 0.21 for INDO = $2.89 \pm 0.24^{*}$ g), and P4 treatment was able to preserve the homeostasis of this pathway. These findings suggest that P4 exerts a beneficial role in the aorta.



XXXIX REUNIÃO ANUAL DA FeSB
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Nanoparticles of acetylated CGP containing essential oil from orange peel attenuate isoproterenol-induced cardiac hypertrophy in male mice

Authors:

Juliana Vila Verde RIBEIRO¹
Richard Douglas Rodrigues FERREIRA¹
Ariel Penha Carvalho da MOTA¹
Jullia Ingrid Rodrigues MARTINS¹
Hericles Mesquita CAMPOS¹
Paulo Cesar GHEDINI¹
Jane Sousa NAVES¹
Fernanda Cristina Alcântara dos SANTOS¹
Stefanne Madalena MARQUES¹
Gustavo Rodrigues PEDRINO¹
Carlos Henrique Xavier CUSTÓDIO¹
Karla de Aleluia BATISTA²

Affiliations:

¹ Universidade Federal de Goiás-UFG
² Instituto Federal de Goiás- Campus Goiânia Oeste.

Keywords: Biotechnology. Essential oil. Hypertrophy. Nanotechnology

Abstract:

Cardiovascular diseases encompass a set of conditions that affect the heart and blood vessels, such as hypertrophy, heart attacks and hypertension. Recent studies have shown that *Citrus sinensis* essential oil has physiological effects in the treatment of cardiovascular diseases, with hypotensive, cardioprotective and vasodilatory effects. Thus, this study explored the potential of *Citrus sinensis* essential oil (acCGP@OE) encapsulated onto polymeric nanoparticles derived from acetylated cashew gum (acCGP) in preventing the injuries occasioned by isoproterenol-induced cardiac hypertrophy. The acCGP@OE nanoparticles were characterized regarding their encapsulation efficiency and morphological properties. To evaluate the protective effect of PEJUac@OE on isoproterenol-induced cardiac hypertrophy, C57BL/6 mice (n=10 per group) received a single dose of isoproterenol (160 mg/kg, IP injection) followed by treatment during 14 days with acCGP@OE nanoparticles (100 mg/kg, gavage) (CEUA-UFG no. 051/24). On the 13th day, the animals were subjected to the electrocardiogram test. At the end of the treatment, the animals were euthanized by decapitation, and the organs were collected for biochemical histological analysis. Results evidenced that the treatment with acCGP@OE had a significant effect on prevent cardiac hypertrophy, since it reduced lipid peroxidation and restored the antioxidant balance, mainly in the heart. It reversed the damage caused by isoproterenol, reducing the size of cardiac muscle tissue and improving cardiac function, as noted in the electrocardiogram. Based on the results obtained, it can be stated that acCGP@OE nanoparticles represent an innovative and promising approach in the treatment of cardiovascular diseases, extending the beneficial effects of *Citrus sinensis* essential oil.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Effects of Walker-256 Tumour Ascitic Fluid on Mitochondrial Bioenergetics and Metabolic Profile of H9c2 Cardiomyocytes: The Modulatory Role of Leucine

Authors:

Ninon Melany Flores BARRIOS¹,
Maíara Caroline COLOMBERA¹,
Gabriela Matuoka CHIOCCETTI¹,
Antonio Thiago Pereira CAMPOS²,
Bruno Sergio Maia MADEIRA¹,
Rogério Williams SANTOS¹,
Lais Rosa VIANA¹,
Maria Cristina Cintra GOMES-MARCONDES¹

Affiliations:

¹Nutrition and Cancer Laboratory, Department of Structural and Functional Biology, Institute of Biology, University of Campinas (UNICAMP)

²Physics Graduate Program, Universidade Federal do Ceará (UFC)

Keywords: Cachexia. H9c2 Cells. Leucine. Ascitic fluid. Mitochondria

Abstract:

Cardiac cachexia in cancer is characterised by morphological and functional alterations, impairing cardiac contractility and performance, which is linked to increased mortality and reduced quality of life in these patients. Tumour evolution promotes an inflammatory state and proteolytic factors that affect cardiac tissue, inducing mitochondrial dysfunction, oxidative stress, and proteolysis, all of which contribute to cardiac atrophy dysfunction. Our aim was to evaluate in vitro the modulatory effects of leucine in cardiac cells under a mimicking cachexia state, using ascitic fluid (AF) collected from Walker-256 tumour-bearing rats (CEUA/IB/UNICAMP, protocol 5828-1/2021/2024). Cardiac H9c2 cells were exposed to AF for 48 hours, treated or not with 250 μ M leucine supplementation, and subjected to metabolomic analysis, using the MetaboAnalyst platform. Data from the experimental groups ($n = 3-5$) were analysed by ANOVA followed by Tukey's or Student's t-test ($p < 0.05$). Mitochondrial function, assessed by respirometry (Seahorse XF Analyser), revealed a significant reduction in oxygen consumption rates in AF-exposed cells. Analysis of the expression of proteins from the electron transport chain via Western blot showed a decrease in subunits of OXPHOS complex II. CARS microscopy (Coherent Anti-Stokes Raman Scattering) revealed an increase in the intensity and number of intracellular lipid droplets in AF-exposed cells compared to the control group. NMR-based metabolomics identified alterations in pathways related to energy metabolism. Interestingly, leucine supplementation attenuated some of these dysfunctions, partially restoring mitochondrial bioenergetics and reducing lipid accumulation. These findings suggest that ascitic fluid (mimicking cachexia state) contributed to lead to a phenotype consistent with cardiac cachexia, and leucine treatment exerts partial protective effects on cardiomyocytes. Financial Support: FAPESP (2019/14803-4; 2017/02739-4; 2020/13222-5; 2021/08931-0; 2022/13789-0; 2023/00608-0), CNPq (302997/2022-9); CAPES; FAEPEX; SAE-UNICAMP



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Vascular Responses in Diabetic Rats: The Influence of Sex on Mesenteric Artery Reactivity

Authors:

Nikollas Silva MEIRELLES¹
Nathalie Tristão Banhos DELGADO¹
Wendrea Kaylaine Burgarelli DANIELLI²
Roger Lyrio dos SANTOS¹

Affiliations:

¹ Universidade Federal do Espírito Santo
² Faculdade Multivix Vitória

Keywords: Diabetes Mellitus. Mesenteric Arteries. Sex Differences. Vascular Reactivity. Alloxan.

Abstract:

Diabetes Mellitus is a multifactorial condition characterized by chronic hyperglycemia and vascular complications, being a risk factor for cardiovascular diseases. These complications result from endothelial dysfunction, driven by the increase in reactive oxygen species, which reduce the bioavailability of vasodilatory substances. In this context, we hypothesize that diabetes alters the vascular reactivity of the mesenteric resistance arteries in a sex-specific manner. Therefore, the aim of this study was to evaluate the sex differences in the relaxation and contraction of the mesenteric resistance arteries from DM rats. For the present study, Wistar rats (8-9 weeks of age, CEUA nº 12/2024) were divided into four experimental groups: diabetic (DM) and non-diabetic (C) females and males. Diabetes was induced by alloxan (150 mg/kg, i.p.), and after 30 days, the animals were euthanized. Third-order mesenteric arteries were isolated and analyzed in a wire myograph with phenylephrine (PE, 1 nM - 100 µM), acetylcholine (ACh, 0.1 nM - 10 µM), norepinephrine (NOR, 10 nM - 30 µM) and sodium nitroprusside (SNP, 1 nM - 100 µM). Data were expressed as mean ± SEM and analyzed by two-way ANOVA followed by *Sidak's* test ($P < 0.05$). We did not observe changes in maximal contraction responses between groups. However, in the relaxation curves induced by ACh, DM males showed a reduction in the maximum response compared to the other groups (DM Female: $89 \pm 4.5\%$; DM Male: $63 \pm 22\%$; C Female: $83 \pm 6.2\%$; C Male: $89 \pm 9.6\%$). In the NPS curve, we observed an impairment in the vasodilation of DM Male and C Male groups (DM Female: $90 \pm 5.6\%$; DM Male: $59 \pm 8.5\%$; C Male: $56 \pm 4.7\%$). We conclude that DM does not alter contraction, but compromises relaxation only in DM males. These findings provide significant functional evidence on the effects of DM on vascular function in a sex-specific manner.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Cardiovascular effects of hypertonic potassium chloride infusion in normotensive rats

Authors:

Amanda Bernardo da COSTA¹
Lara Marques NAVES¹
Stefanne Madalena MARQUES¹
Gustavo Rodrigues PEDRINO¹

Affiliations:

¹ Universidade Federal de Goiás

Keywords: Hyperkalemia. Cardiovascular regulation. Hemodynamic responses

Abstract: An abnormal increase in plasma potassium (K^+) concentration can trigger changes in cell membrane polarization, affecting cardiovascular homeostasis. Studies have shown that, in cardiac cells, hyperpolarization is associated with adverse cardiovascular events, including arrhythmias and cardiac arrest. Aldosterone secretion is one of the physiological mechanisms involved in the regulation of plasma potassium levels, promoting its renal excretion and contributing to cardiovascular stability. To evaluate the cardiovascular responses induced by the infusion of a hypertonic KCl solution, including heart rate (HR), mean arterial pressure (MAP), mesenteric blood flow (MBF), renal blood flow (RBF), renal vascular resistance (RVR) and mesenteric vascular resistance (MVR), in normotensive wistar rats. Male rats (350–400 g) were anesthetized with urethane (1.2 g/kg) and underwent surgical procedures for the recording of HR, MAP, MBF, RBF, RVC, and MVC. Following baseline recordings, an intravenous infusion of 3M KCl solution ($1.8\% \cdot \text{kg}^{-1}$) was administered. All procedures were approved by the Animal Use Ethics Committee (CEUA) of the Federal University of Goiás (protocol nº 03152). Data analysis indicated that the infusion of 0.3M KCl did not induce significant changes in MAP, HR, RBF, MBF, RVR, or MVR compared to baseline values. Additionally, the evaluation of these parameters during the 0.3M KCl infusion period did not reveal statistically significant changes. The data obtained do not indicate cardiovascular alterations triggered by the infusion of 0.3M KCl. However, we cannot rule out the possibility that the potassium concentration was insufficient to stimulate mechanisms associated with hyperkalemia. Therefore, our next step will be to analyze potassium concentrations in urine and blood before and after the infusion of 0.3M KCl.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Cardiovascular Dysfunctions in a Rat Model of Preterm Birth

Authors:

Izabella F. ACIPRESTE¹
Matheus F. ITABORAHY¹
Carolina FONSECA
Isadora Z. L. F. FENG¹
Maria Eduarda S. FAVALESSA¹
Glauber S. F. DA SILVA¹
Vitoria Silva OLIVEIRA¹
Cleiton Lopes AGUIAR¹
Marlon Lemos DIAS³
Robson A. S. SANTOS¹
M. Fátima LEITE^{1*}

Affiliations:

¹Departamento de Fisiologia e Biofísica, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

²Laboratório de Neurociências Molecular e Comportamental, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

³Instituto de Biofísica Carlos Chagas Filho, Universidade Federal de Minas Gerais, Rio de Janeiro, RJ, Brazil

*INCT Hepatologia360°

Keywords: Cardiovascular dysfunctions, Preterm Birth, Bronchopulmonary Dysplasia, Vasopressin, Animal Behavior

Abstract:

Studies have shown that bronchopulmonary dysplasia (BPD), a common complication of preterm birth, is linked to cardiovascular alterations such as elevated blood pressure and increased arginine-vasopressin (AVP) levels. It has also been shown that individuals born preterm have a higher tendency of developing anxiety behavior and cognitive deficits. AVP, a vasoactive and neuroactive peptide, has been reported to be elevated in both preterm infants and adults with a history of BPD. Its dysregulation may impact vascular tone and stress responses, but its role in cardiovascular and behavioral outcomes remains unclear. This study used 6 female Sprague-Dawley rats to explore hyperoxia-induced BPD (CEUA: 113/2022). After birth, neonates were assigned to two groups: a hyperoxia group (80% O₂ for 12 h from P3 to P14) and a control group (room air from P3 to P14), kept with their dams to prevent behavioral harms. Systolic and diastolic blood pressure was measured through caudal plethysmography at 30 and 60 days of life to assess cardiovascular impact. AVP plasma levels were measured via an ELISA assay. BPD development was validated using hematoxylin and eosin staining of lung tissue. Novel Object Recognition Test and Open Field were used to preliminarily explore possible behavioral alterations. Hyperoxia-exposed rats showed significantly elevated blood pressure at both time points ($p < 0.05$), along with increased AVP levels compared to controls ($p < 0.05$). BPD was confirmed in the rats exposed to hyperoxia. Behavioral tests at 60 days also revealed an initial tendency of anxiety-like behavior and impaired recognition memory ($p > 0.05$). These findings indicate that neonatal hyperoxia leads to persistent cardiovascular changes and AVP upregulation, replicating key features of preterm birth-related complications observed in humans.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Effect of Dapagliflozin on cardiomyocytes of mice fed a high-fat diet and fructose-enriched water

Authors:

Carolina da Silva VALENTIM¹
Paulo Rogerio Silva de PAIVA JÚNIOR²
Péricles Fabrício GOMES FILHO¹
Raíssa Leal de Carvalho dos Santos CUNHA¹
Rebecca Roosevelt da Conceição Souza GUEDES¹
Isaías dos Santos SILVA¹
Cherley Borba Vieira de ANDRADE¹
Kissila RABELO¹,
Luciana Lontro ALVES¹
Aluana Santana CARLOS²
Jorge José de CARVALHO¹
Jemima Fuentes Ribeiro da SILVA¹

Affiliations:

¹Instituto de Biologia Roberto Alcântara Gomes – UERJ

²Universidade Iguazu – UNIG

Keywords: Dapagliflozin. Diet. Heart. Obesity.

Abstract:

High-fat diets (HFD) and fructose-rich beverages induce obesity, insulin resistance, and chronic inflammation, contributing to myocardial dysfunction through lipotoxicity and oxidative stress. Dapagliflozin (DAPA), in addition to its glucosuric effect, exerts cardioprotective action via NHE1 inhibition and reduction of cytosolic $[Ca^{2+}]/[Na^{+}]$, supporting its investigation in obesity-induced cardiac remodeling. Approved by CEUA/UERJ (010/2024), the study used 45 C57BL/6 mice (3 months old), divided into three groups (n=15): control (C), high-fat (HF), and HF+DAPA. Group C received standard diet; HF and HF+DAPA received HFD and fructose-rich water (HFW) for 16 weeks. From weeks 10 to 16, C and HF received placebo; HF+DAPA received dapagliflozin (10 mg/kg/day). Systolic blood pressure (SBP), energy intake (EI), body mass (BM), fat mass (FM), TOTG, ITT, and cardiomyocyte area (CA) were evaluated. SBP: C (n=4): 125.5±2.45 mmHg; HF (n=5): 156.1±19.19 mmHg (+25.6% vs C, p<0.05); DAPA (n=5): 122.2±12.12 mmHg (-21.7% vs HF, p<0.05). EI: C (n=14): 48.6±6.6 kJ; HF (n=14): 59.4±11.1 kJ (+21.5% vs C, p<0.05); DAPA (n=15): 64.5±12.5 kJ (+11.15% vs HF, p<0.05). BM: C (n=6): 27.3±2.6 g; HF (n=6): 33.8±6.1 g (+23.8% vs C, p<0.05); DAPA (n=6): 31.1±3.4 g. FM: C (n=8): 10.01±0.7%; HF (n=5): 20.4±2.9% (+104.2% vs C, p<0.05); DAPA (n=6): 17.1±2.1% (-16.1% vs HF, p<0.05). TOTG_AUC: C (n=14): 49.9±6.4 mmol/dL; HF (n=14): 67.9±12.7 mmol/dL (+36.1% vs C, p<0.05); DAPA (n=15): 57.4±4.6 mmol/dL (-15.6% vs HF, p<0.05). ITT_AUC: C (n=12): 19.1±2.5 mmol/dL; HF (n=14): 29.8±6.04 mmol/dL (+55.4% vs C, p<0.05); DAPA (n=15): 29.3±2.7 mmol/dL. CA: C (n=5): 268.6±51.8 μm^2 ; HF (n=5): 325.3±69.04 μm^2 (+21.1% vs C, p<0.05); DAPA (n=5): 256.7±63.53 μm^2 (-21.1% vs HF, p<0.05). HE staining showed reduced lipid deposition in DAPA vs HF. DAPA attenuates hemodynamic, metabolic, and structural alterations induced by HFD/HFW, suggesting a cardioprotective effect.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Red Quinoa Hydrolysate: A Functional Food with Therapeutic Potential for Hypertension and Vascular Dysfunction in Rats Exposed to Mercury

Authors:

Paola Zambelli MORAES¹
Samia Hassan Husein ANAAN¹
Katy Yasmin de Souza de OLIVEIRA¹
Gabriela Costa TROFINO¹
Franck Maciel PEÇANHA¹
Dalton Valentim VASSALLO²
Marta MIGUEL-CASTRO³
Giulia Alessandra WIGGERS¹

Affiliations:

¹Laboratory of Cardiovascular Physiology / Multicentric Postgraduate Program in Physiological Sciences, Federal University of Pampa (Unipampa), Brazil-RS. ²Laboratory of Electrophysiology and Vascular Reactivity, Federal University of Espírito Santo, Brazil-ES. ³Instituto de Investigación en Ciencias de la Alimentación, CIAL/CSIC, Madrid, Spain

Keywords: Antioxidant. Anti-inflammatory. Bioactive Peptides. Mercury.

Abstract:

The search for natural, accessible therapeutic alternatives without adverse effects to counteract the damage caused by heavy metals such as mercury (Hg) is essential for future treatments. A novel red quinoa hydrolysate (RQH), known for its antioxidant, hypotensive, and anti-inflammatory properties, was evaluated for its potential to protect the cardiovascular system from damage induced by chronic low-level mercury exposure. Male Wistar rats were divided and treated for 60 days in: A) Control (Ct): oral water + intramuscular saline; B) HgCl₂: initial dose of 4.6 µg/kg, followed by 0.07 µg/kg/day i.m. + oral water; C) RQH: 1 g/kg/day orally + intramuscular saline; D) HgRQH: both treatments combined. RQH was characterized by HPLC-MS, identifying bioactive peptides. Systolic blood pressure (SBP) was monitored non-invasively on days 0, 30, and 60. At the end of the treatment, animals were anesthetized for invasive blood pressure measurement via carotid cannulation. After euthanasia, vascular reactivity of the aorta (Ao) and mesenteric resistance arteries (MRA) was evaluated in isolated organ baths using concentration-response curves to acetylcholine, sodium nitroprusside, phenylephrine (Phe), or norepinephrine (NE). Data are presented as mean ± SEM and analyzed by two-way ANOVA followed by Bonferroni post hoc test (p<0.05). Ethical approval: 002/2024 - CEUA Unipampa. Peptides <300 Da identified in RQH showed predominantly antioxidant, anti-inflammatory, and chelating potential. RQH prevented: a) the increase in SBP after Hg exposure (Non-invasive: Ct 119.7±1.2; Hg 133.4±2.9; RQH 120.5±0.9; HgRQH 125.6±2.4 mmHg. Invasive: Ct 123.0±8.8; Hg 138.3±6.1; RQH 123.1±6.5; HgRQH 115.4±7.3 mmHg); and b) the increased vasoconstriction to Phe (Ao) and NE (MRA). RQH neutralizes vascular toxicity caused by chronic Hg exposure, highlighting its potential as a functional food-based therapeutic agent against environmental pollutants.

Funding: CAPES-CNPq, FAPERGS, FAPES, iCOOP/Spanish Government.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Inverse association between circulating hexosylceramides and cardiometabolic risk factors in adult women with overweight

Authors:

Jéssica de Oliveira CAMPOS^{1,2}
Jéssica Gonzaga PEREIRA¹
Maria Brenda Ellen dos Santos Pereira NASCIMENTO¹
Aiany Cibelle SIMÕES-ALVES^{1,3}
Nathalie VEGA²
Carol Gois LEANDRO^{1,3}
Luciano PIROLA²
João Henrique COSTA-SILVA^{1,3}

Affiliations:

¹ Laboratory of Physical Evaluation and Signal Processing, Academic Center of Vitória, Universidade Federal de Pernambuco-UFPE
² INSERM Unit 1060, CarMeN Laboratory, Lyon Civil Hospitals, Université Claude Bernard Lyon1
³ Laboratory of Nutrition, Physical Activity and Phenotypic Plasticity, Academic Center of Vitória, Universidade Federal de Pernambuco – UFPE.

Keywords: Cardiovascular risk. Ceramides. Metabolomics. Obesity. Sphingolipids.

Abstract:

Altered sphingolipid profiles are associated with obesity and increased cardiometabolic risk, with each lipid class having its own specificity. The current study investigated the association between plasmatic sphingolipids, particularly ceramides and hexosylceramides, and cardiometabolic risk factors in adult women with overweight. Anthropometric measures, food consumption, biochemical measurements, and cardiometabolic parameters were obtained from 37 adult women with normal weight (NW, n=12) or overweight (OW, n=25). We used flow injection analysis coupled with mass spectrometry (FIA-MS) targeted metabolomics to determine the plasma concentrations of sphingolipids. Unsupervised clustering analysis of altered metabolites in women segregated NW and OW classes into two distinct groups, revealing an overall association between altered metabolites and OW. Notably, the OW was associated with significantly decreased concentrations of multiple hexosylceramides and certain ceramides molecular species. Total ceramides were positive correlated to total cholesterol (TC: $r=0.728$; $p=0.001$) and LDL ($r=0.511$; $p=0.002$). In line, the concentrations of total hexosylceramides were correlated with TC ($r=0.538$; $p=0.001$); HDL ($r=0.425$; $p=0.012$) and fasting glucose (FG: $r=-0.446$; $p=0.008$). A specific molecular specie Hex2Cerd18.1/24.1 was negative associated with diastolic blood pressure ($r=-0.375$; $p=0.032$). The consumption of ultra-processed food (UPF) was negatively correlated with total hexosylceramides ($r=-0.367$; $p=0.033$). Thus, low plasma hexosylceramide levels were associated with overweight and high FG in women. Our data suggest that decreased hexosylceramide concentrations may be related to the development of cardiometabolic diseases in adult women with OW, supporting the notion that although the bulk of ceramides is positively associated with cardiometabolic diseases, specific sphingolipids may be protective.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Comparative analysis of lipid profile, glycemic status, anxiety and stress levels in transcendental meditation practitioners at Universidade Estadual de Maringá

Authors:

Wanderson Rocha OLIVEIRA¹
Heloisa Bergamasco VETTOR¹
Lucas Schimmack REDONDO¹
Otalita de Melo Santos CORREA¹
Sérgio Captuleio da Silva FILHO¹
Sidney Barnabé PERES¹
Solange Marta Franzói de MORAES¹

Affiliations:

¹Universidade Estadual de Maringá

Keywords: Lipid profile, Transcendental meditation, anxiety, stress

Abstract: Transcendental Meditation (TM) is an evidence-based mental practice with demonstrated benefits for stress reduction and psychological well-being. As a complementary health intervention, TM shows potential to influence both mental and physical health parameters through its effects on autonomic nervous system regulation. This cross-sectional longitudinal study (approved by Ethical Committee in Human Research: CAAE 77175724.7.0000.0104) examined 14 TM practitioners at Universidade Estadual de Maringá (5 males [35.7%], 9 females [64.3%]; mean age 37.9±16 years; metabolic rate 1379 kcal/day [SD 373]). Using colorimetric analysis of fasting blood samples and standardized psychological measures (Beck Anxiety Inventory, PSS-10), we assessed parameters at baseline (C1) and after 6 months (C2) of practice. Health behaviors included smoking (21.4% [n=3]), alcohol use (50% [n=7] to 57.1% [n=8]), and combined use (64.3% [n=9]). Psychological measures showed stress reduction (71.4% low stress [n=10] to 57.1% [n=8]) and normalized glycemia (85.7% [n=12] to 100%). Despite 85.7% [n=12] reporting poor sleep, 66.7% [n=8] had minimal anxiety. Lipid profiles improved: HDL (35→43.2 mg/dL), triglycerides (118→79.6), with stable total cholesterol (185→189) and LDL (127→130). Body composition changed: weight (70.4→65kg), BMI (24.6→22.6 kg/m²), with stable fat percentage (26.3→26.4%). TM practice correlated with metabolic stabilization and psychological benefits, though sleep disturbances persisted, suggesting complex physiological interactions requiring further study.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Indoxyl Sulfate in AKI and UT model: Dual Roles in Purinergic Vascular and Inflammatory Modulation

Authors:

Jeferson STABILE¹
Jackeline Rodrigues RAMOS¹
Marcela Sorelli Carneiro RAMOS²
Cristina Ribas FÜRSTENAU¹

Affiliations:

¹ Universidade Federal do ABC, Centro de Ciências Naturais e Humanas, Laboratório de Bioquímica Vascular

² Universidade Federal do ABC, Centro de Ciências Naturais e Humanas, Laboratório de Imunologia Cardiovascular

Keywords: Acute Kidney Injury. Aorta. Indoxyl Sulfate. Purinergic Signaling. Vascular Inflammation.

Abstract:

Acute kidney injury (AKI) is a public health concern and a risk factor for cardiovascular diseases. Indoxyl sulfate (IS), a uremic toxin (UT) present in renal dysfunction, contributes to these outcomes. Together, AKI and IS can promote cardiac hypertrophy, renal fibrosis, and impair vascular function. Purinergic signaling plays a role in inflammation and regulates vascular tone. This study aimed to investigate the effects of AKI and IS, focusing on acute inflammation and purinergic signaling. Twenty C57BL/6 mice (6–8 weeks old, 20–28 g) were divided into Sham and IR groups. Ten mice underwent 60-minute unilateral renal ischemia (IR), while ten Sham mice underwent laparotomy. During the 15-day reperfusion period, animals received daily injections of IS (100 mg/kg/day) or saline (sal). Morphological assessments included organ weight/body weight ratios. Gene expression in the thoracic aorta was evaluated via qPCR (housekeeping: β -actin, $2^{-\Delta\Delta CT}$). Data were analyzed using two-way ANOVA and Tukey's post hoc test ($n=5$, mean $\pm$ SEM, relative to their controls, ordered from Sham+sal and IR+sal to Sham+IS to IR+IS). Ethics committee (UFABC) approval numbers: 7284191124, 1467010424. Significant cardiac hypertrophy was observed in the IR+sal group (4.6 ± 0.1) vs. Sham+sal (4.15 ± 0.1). Left kidney mass decreased in IR groups (sal: -26% ; IS: -36%), while right kidney mass increased (sal: $+21\%$; IS: $+12\%$). CRP and IL-6 expression increased in the IR+sal group vs. Sham+sal (CRP: 1 ± 0.34 , 1.5 ± 0.55 ; IL-6: 1 ± 0.28 , 4.1 ± 1.1). IL-10 expression decreased in the IR+IS group vs. Sham+IS (1 ± 0.27 , 0.89 ± 0.13). P2X4 expression was reduced in all experimental groups compared to sham+sal. IS appears to reduce cardiac hypertrophy but not the morphometric changes in the kidneys. IS decreased the expression of pro-inflammatory genes and increased anti-inflammatory gene expression. P2X4 reduction may lead to vascular dysfunction and impaired control of vascular tone.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Neonatal history correlated with cardiac electrophysiological parameters and serum markers of metabolism in childhood

Authors:

Anna Bheatriz Gomes Ferreira MARINHO¹²,
Jéssica Gonzaga PEREIRA¹²,
Jéssica Oliveira CAMPOS¹²,
Maria Brenda Ellen dos Santos Pereira NASCIMENTO¹²,
Cheid Manuel CHONGO¹²,
Reginaldo Correia da Silva FILHO³,
Aiany Cibelle Simões ALVES¹²,
João Henrique da Costa SILVA¹²

Affiliations:

¹Laboratory of Physical Assessment and Signal Processing - LAPS, CAV, Federal University of Pernambuco

²Laboratory in Nutrition, Physical Activity and Phenotypic Plasticity - NAFPF, CAV, Federal University of Pernambuco

³Laboratory of General, Molecular, and Exercise Biochemistry - LABMEX, CAV, Federal University of Pernambuco

Keywords: Biological markers. cardiovascular health. child health

Abstract:

The neonatal period is a critical stage for lifelong health development. Parameters such as birth weight, birth length, head circumference, and gestational age have been widely studied for their association with cardiovascular outcomes in childhood. Alterations in these indicators may reflect changes in cardiac development, impacting the risk of health complications during childhood. A quantitative cross-sectional study was conducted in 2024 with 29 children aged 7 to 11 years. Neonatal history, nutritional status, blood pressure, electrocardiogram, as well as glycemic and lipid serum markers were evaluated. Statistical analysis was performed using Spearman correlation. The study was approved by the Ethics Committee from UFPE (CAAE: 80956824.3.0000.9430). The sample consisted of 29 children, all with adequate height for age (100.0%). Regarding BMI classification, 69.0% were eutrophic, 13.8% obese, 10.3% overweight, and 6.9% underweight. A positive correlation was observed between birth weight and PR interval ($r=0.680$; $p=0.002$) and P wave duration ($r=0.685$; $p=0.014$); between birth length and PR interval ($r=0.646$; $p=0.007$) and P wave ($r=0.743$; $p=0.009$); and between head circumference and PR interval ($r=0.667$; $p=0.035$). Birth weight also correlated with QT interval ($r=0.539$; $p=0.021$). A negative correlation was identified between gestational age and LDL-c ($r=-0.474$; $p=0.035$). As for redox status, birth weight ($r=0.687$; $p=0.007$), birth length ($r=0.779$; $p=0.003$), and head circumference ($r=0.825$; $p=0.012$) were positively correlated with Total Thiols. The observed correlations indicate that the neonatal period may exert a lasting influence on cardiovascular and metabolic health. Recognizing these links contributes to the understanding of early risk determinants, highlighting the need for strategies from the earliest years of life.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Cardiovascular risk biomarkers in transcendental meditation practitioners at the Universidade Estadual de Maringá: A preliminary study

Authors:

Wanderson Rocha OLIVEIRA¹
Augusto César Russi CAMPANHONI¹
Heloisa Bergamasco VETTOR¹
Lucas Schimmack REDONDO¹
Nayara Germano de Oliveira¹
Sidney Barnabé PERES¹
Solange Marta Franzói de MORAES¹

Affiliations:

¹ Universidade Estadual de Maringá

Keywords: Meditation, cardiovascular health, public health

Abstract:

Cardiovascular diseases are recognized as the leading cause of death worldwide. Their occurrence can be modulated by lifestyle habits, including modifiable factors: body weight, diet, sedentary behavior, and daily stress levels. Stress may influence individuals to adopt behaviors that increase cardiovascular risk, such as tobacco use, alcohol consumption, and altered sleep patterns. This study aims to report cardiovascular risk biomarker profiles in transcendental meditation volunteers at Universidade Estadual de Maringá (UEM). An observational cross-sectional longitudinal study with convenience sampling was approved by Ethical Committee in Human Research (CAAE 77175724.7.0000.0104). Biochemical analysis and simple descriptive analyses were performed. The sample included 63 volunteers aged 31-50 years (50.79%), 18-30 years (26.98%), over 51 years (22.22%), and males (60.31%). 22.22% (n=14) smoked, 39.68% (n=25) consumed alcohol. Only 22.22% (n=14) exercised regularly. BMI distribution showed 49.20% (n=31) overweight/obese, 46.03% (n=29) normal weight, and 3.17% (n=2) underweight. Lipid profiles revealed: 44.44% (n=28) hypercholesterolemia, 39.68% (n=25) fasting hyperglycemia, 82.53% (n=52) elevated HDL, 22.22% (n=14) hypertriglyceridemia, and 46.03% elevated LDL. The findings demonstrate significant cardiovascular risk markers in the study population. Lipid profile abnormalities were predominant and may be associated with lifestyle habits. Broader studies are needed to evaluate transcendental meditation's influence on these variables.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Effect of estradiol replacement and central injection of losartan on water and 0.3 M NaCl intake induced by water deprivation in ovariectomized renal hypertensive female rats

Authors:

Sarah Antunes da SILVA¹
José Vanderlei MENANI¹
Eduardo COLOMBARI¹
Débora Simões Almeida COLOMBARI¹

Affiliations:

¹UNESP, Departamento de Fisiologia e Patologia, Faculdade de Odontologia de Araraquara/ SP

Keywords: 2K1C. sodium appetite. estradiol. ovariectomy. water deprivation.

Abstract:

Water deprivation (WD) induces sodium appetite in male and females rats. Renovascular hypertensive rats exhibit an even greater increase in sodium intake induced by WD compared to the normotensive rats and losartan injected intracerebroventricularly (icv) reduced this response. In addition, other studies have shown that in females spontaneously hypertensive rats estradiol replacement associate to icv losartan blocked the increase sodium intake induced by WD. Therefore, in the present study we aimed to evaluate the effect of WD on water and sodium intake in renovascular hypertensive and ovariectomised rats, as well as the impact of icv losartan injection and estradiol supplementation on these responses. CEUA: 08/2023; Female Holtzman (Sprague-Dawley) rats (190-210 g) had the left renal artery partially obstructed using a silver clip of 0.15 mm (2K1C) and 3 weeks later part of the rats underwent bilateral ovariectomy was performed (2K1C-OVX). At the end of the 6th week, estradiol (E2) or vehicle was administered subcutaneously for 12 days (duration of the experiments) and the rats underwent icv cannula. Therefore, performing the following groups, 2K1C-OVX/veh (MAP: 177, n = 4) and 2K1C-OVX-E2 (MAP: 164, n = 3). After 24-h water deprivation (WD), rats were allowed to drink only water for 2 h (thirst test), and then they received a 0.3 M NaCl solution for a salt appetite test for an additional 2 h. Losartan or saline icv were injected 15 min before the water bottle was offered. All rats received losartan and saline in 2 different days in a random manner. The water intake induced by WD was reduced in 2K1C-OVX/E2 compared to OVX group with estradiol treatment exhibited a reduction in water intake similar to the effects of central losartan injection. Furthermore, when the effects of losartan and estradiol supplementation were combined, an additive response was observed ($3,2 \pm 5,2$ vs. vehicle: $10,3 \pm 3$ ml/2h). Regarding the response in sodium intake, it was observed that there was no additive effect; however, the response to losartan and E2 supplementation was comparable. ($5,6 \pm 5,7$ vs. vehicle: $4,1 \pm 5,1$ ml/2h). Estrogen exerted an inhibitory effect on water and sodium intake induced by water deprivation in female rats with renovascular hypertension, and central losartan injection further enhanced this thirst inhibition.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Gi Signaling Activation in Cardiomyocytes Preconditions the Heart and Enhances Recovery After Injury

Authors:

Mateus Chaves da COSTA¹
Marcos ELIEZECK¹
Victor Moura VIDAL¹
Rayssa Lima RODRIGUES¹
Sérgio Ricardo Aluotto SCALZO¹
Gustavo de Oliveira ZANETTI²
Dawit Albieiro Pinheiro GONÇALVES²
Sílvia GUATIMOSIM¹

Affiliations:

¹ Department of Physiology and Biophysics, Institute of Biological Sciences, Universidade Federal de Minas Gerais, Belo Horizonte, Brasil.

² School of Physical Education, Physiotherapy, and Occupational Therapy, Universidade Federal de Minas Gerais, Belo Horizonte, Brasil.

Keywords: Cardiac remodeling. Gi pathway. DREADD. Cardioprotection. Cardiac conditioning.

Abstract:

The Gi pathway (Gi) plays a key role in the heart, being activated by acetylcholine via muscarinic receptors to regulate heart rate and contractility. Our group showed that selective Gi activation in cardiomyocytes (CMs), using the Myh6-Cre/hM4Di model, protects the heart from adrenergic-induced cardiomyopathy. This study explored whether Gi activation could precondition the heart and aid recovery after acute myocardial injury induced by isoproterenol (ISO). Myh6-Cre/hM4Di mice (CEUA: 102/2022) were treated with Clozapine-N-Oxide (CNO) 0,5 mg/kg, for 7 days, ensuring sustained Gi activation. For comparison, another group received ISO (25 mg/kg) to induce pathological remodeling. ISO-treated mice showed hypertrophy, cell death, macrophage infiltration, and fibroblast activation. The CNO-conditioned group presented hypertrophy and increased contractility (shortening (μm^2): CTR 264 ± 51 vs CNO 299 ± 58), yet showed no signs of cell death, macrophages infiltration (CD68+ cells a.u.: CNO 12 ± 4 vs ISO 31 ± 15) or fibroblast activation, indicating physiological rather than pathological remodeling. To further investigate this issue, we assessed nitric oxide production and mitochondrial density. Both parameters were elevated in the CNO group compared to control. We next challenged control and Gi-conditioned mice with a single dose of high ISO (300 mg/kg). Strikingly, CNO-preconditioned mice were protected from high ISO damage, reduced weight loss, cell death, and inflammation (CD68+ cells a.u.: CNO+ISO 24 ± 13 vs ISO 39 ± 5). The physical exercise test revealed enhanced performance in CNO-treated animals, both pre- and post-ISO, compared to the CTR and ISO-only groups. In conclusion, selective Gi activation in cardiomyocytes drives adaptive remodeling, preserving cellular structure, enhancing cardiac function, and increasing resilience to acute injury, highlighting its therapeutic potential in cardiac stress conditions. Funding agencies: CNPq, CAPES, FAPEMIG, and Pró-reitoria de Pesquisa-UFMG.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Myocardial Infarction: Humoral Factors Arising from Remote Preconditioning and the Influence of Comorbidities on Cardioprotection

Authors:

Laís Eduardo MARINHO¹
Alberto Barreto GRIMALDI¹
Dahienne Ferreira de OLIVEIRA²
Maria Eduarda Maciel Fernandes PAVARINO¹
Antônio Carlos Campos de CARVALHO¹
Leonardo MACIEL^{1,3}

Affiliations:

¹Federal University of Alfenas
²ABC Medical School University Center

Keywords: Humoral factors. Ischemic preconditioning. Cardioprotection. Ischemia. Reperfusion.

Abstract:

Introduction: Acute myocardial infarction (AMI) represents, approximately, one-third of deaths worldwide. Given the high mortality associated with AMI, strategies to minimize cardiac injury are of critical importance. Remote ischemic preconditioning (RIPC), which involves cycles of ischemia and reperfusion (I/R) in an organ distant from the heart, has emerged as a promising approach, as it is associated with the release of endogenous cardioprotective factors that can reduce myocardial damage during an infarction. However, the effectiveness of RIPC has been unsatisfactory in clinical studies. **Objective:** This study aims to investigate how comorbidities could interfere in the cardioprotection induced by RIPC. **Methodology:** Plasma samples from 55 healthy volunteers and 21 patients with comorbidities (CAAE 28998819.9.0000.5272) were ultrafiltered (<10 kDa) and perfused into isolated rat hearts using the Langendorff system (CEUA 154.21). Plasma was collected before (placebo) and after (conditioned) RIPC. Experimental groups included healthy rats, spontaneously hypertensive rats (SHR), streptozotocin-induced diabetic rats, and rats with high sodium intake. Isolated hearts underwent 30 minutes of ischemia followed by 120 minutes of reperfusion. Plasma ultrafiltrate at 10 kDa cutoff (1:10 in Krebs solution) were perfused prior to I/R. **Results:** Conditioned <10 kDa plasma from healthy donors reduced infarct size and improved hemodynamic parameters during I/R. In contrast, <10 kDa plasma (placebo or conditioned) from patients with comorbidities failed to induce protection. Likewise, in animals with hypertension, diabetes, or high sodium intake, cardioprotection was abolished or reduced. **Results:** Conditioned <10 kDa plasma from healthy donors reduced infarct size and improved hemodynamic parameters during I/R. In contrast, <10 kDa plasma (placebo or conditioned) from patients with comorbidities failed to induce protection. Likewise, in animals with hypertension, diabetes, or high sodium intake, cardioprotection was abolished or reduced. **Conclusion:** Although conditioned plasma from healthy individuals conferred cardioprotection in healthy rats, comorbidities impaired the action of humoral factors, abolishing the cardioprotection potentially induced by RIPC.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Cardiovascular impacts of an obesogenic diet supplemented with flaxseed oil in female offspring compared to males

Authors:

Filipe de Melo BARBOSA¹
Jonnathan Vinnycius Bento da SILVA¹
Douglas Vinícius do Nascimento LOURENÇO¹
Gemerson Clemenson da SILVA
Jivaldo Gonçalves FERREIRA¹
Sidiane Barros da SILVA¹
Maria da Conceição Correia SILVA¹
Thyago Moreira de QUEIROZ¹

Affiliations:

¹Laboratório de Nutrição, Atividade Física e Plasticidade Fenotípica/Centro Acadêmico da Vitória (CAV)/Universidade Federal de Pernambuco (UFPE)

Keywords: Indirect pressure. Transgenerational effects. Vascular reactivity

Abstract:

Obesity during critical periods of development can trigger cardiovascular diseases and affect future generations. Foods rich in omega-3 may attenuate the deleterious effects of high-fat diets. This study aimed to investigate cardiovascular effects in the offspring of rats whose mothers were subjected to an obesogenic diet and supplemented with flaxseed oil during pregnancy and lactation. Wistar rats were divided into groups control (CT), control/flaxseed oil (CTOL), obesogenic (OB) and obesogenic/flaxseed oil (OBOL). Results were expressed as mean and standard error of the mean, with the null hypothesis rejected when $p < 0.05$. All procedures were approved (CEUA-UFPE 0072/2024). On the 7th day of life, OB group female pups showed lower body mass compared to CT ($m = 16.5 \pm 0.95$ vs. 20.5 ± 0.99 g, respectively). A similar trend was observed in OB males. Blood pressure was measured by plethysmography. No changes were observed in the systolic (SBP) and diastolic (DBP) pressures among females in the different groups. At 60 days, OB, males showed increased SBP and DBP compared to CT, which was prevented in OBOL group. At 90 days, animals were euthanized, the aorta was suspended in isolated organ bath chambers. Regarding vascular relaxation, increase insulin-induced relaxation was observed in the CTOL females compared to CT and OBOL ($E_{\max} = 79.1 \pm 7.2$ vs. $51.8 \pm 7.4\%$, respectively). A similar effect was found in males (CTOL vs. OBOL). In female aortas without endothelium, greater contractile efficacy ($E_{\max} = 119.4 \pm 4.7$ vs. $86.6 \pm 7.3\%$) and potency ($pCE_{50} = 7.64 \pm 0.15$ vs. 7.10 ± 0.13) contractile with phenylephrine were found in OBOL and CTOL, respectively. In conclusion, a maternal obesogenic diet may impair offspring cardiovascular health, such as increasing blood pressure, while flaxseed oil supplementation may worsen aortic contractility. These findings highlight the need for further studies to better understand the role of flaxseed oil in cardiovascular health.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Effects of leucine supplementation and Walker 256 tumour evolution on heart function and cardiac tissue metabolomic profile in pregnant rats

Authors:

Miguel Megara Rodrigues SILVA¹,
Beatrice Candido ALVES¹,
Igor Fernando dos SANTOS¹,
Carla de Moraes SALGADO¹,
Beatriz Cassinga TALASSI¹,
Rogério Williams SANTOS¹,
Lais Rosa VIANA¹,
Maria Cristina Cintra GOMES-MARCONDES¹

Affiliations:

¹Nutrition and Cancer Laboratory, Department of Structural and Functional Biology, Institute of Biology, University of Campinas (UNICAMP)

Keywords: Cardiac metabolism, Leucine supplementation, Metabolomics, NMR, Walker 256 tumour.

Abstract:

Metabolomics has proven crucial for understanding pathological biochemical alterations. Considering cardiac cachexia is one of the worst problems during cancer associated with pregnancy, this study aimed to evaluate the effects of 3% leucine supplementation on cardiac tissue metabolome in pregnant Walker 256 tumour-bearing Wistar rats. Animals were distributed into: P (control), PL (control+leucine), PW (5×10⁵ tumour cells implanted in the subcutaneous tissue of the right flank), and PWL (tumour+leucine) groups (n=10/group) (CEUA/UNICAMP 6336-1/2023). After 21 days, cardiac tissue was collected at euthanasia and analysed by ¹H-NMR (600/500 MHz) following phase correction, baseline adjustment, and water signal suppression. Tumour evolution (PW group) caused no change in heart weight, although it led to a dysfunction on the electrocardiogram measurement showing reduced heart rate (20% less) and decreased T amplitude (44% lower), which was less pronounced in the WL group compared to the control group. Despite having a similar ECG compared to the control group, the WL group had a reduction in QRS interval compared to the other groups. MetaboAnalyst 6.0 analysis revealed PW vs P showed 42% butyrate reduction (p=0.003), 31% NAD⁺ decrease (p=0.01), and tyrosine/carnitine downregulation, while PWL vs PW demonstrated 2.3-fold NAD⁺ increase (p<0.001), elevated ATP/NADP⁺, and 3-hydroxybutyrate upregulation. SPLS-DA (R²Y=0.89) confirmed metabolic profile separation, showing PWL data were similar to the control group. Pathway analysis identified significant alterations in butanoate and nicotinate/niacinamide metabolism (FDR<0.05). These findings indicated that leucine supplementation attenuates tumour-induced cardiac metabolic dysregulation by restoring energy metabolism (ATP/NAD⁺), enhancing antioxidant capacity (NADP⁺), and minimising the ECG dysfunction, suggesting potential therapeutic applications in cardiac cachexia in cancer during pregnancy. Financial Support: FAPESP (2023/01743-9; 2023/01811-4; 2022/02114-2; 2021/08188-5; 2021/08931-0; 2017/02739-4; 2023/00608-0), CNPq 302997/2022-9; FAEPEX; SAE- UNICAMP



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Exogenous 10 kDa-Heat Shock Protein Preserves Mitochondrial Function After Hypoxia/Reoxygenation

Authors:

Brenda Emanoele RODRIGUES
Marcella Borges COUTINHO^{1,2}
Dahienne Ferreira de OLIVEIRA^{1,2}
Jose Hamilton Matheus NASCIMENTO^{1,2}
Leonardo MACIEL^{1,2}

Affiliations:

¹Universidade Federal do Rio de Janeiro
²Centro de Pesquisa em Medicina de Precisão

Keywords: Heart. HSP10. Ischemia. Reperfusion.

Abstract:

Ischemic heart disease is the leading cause of mortality in Brazil and worldwide and represents a major public health challenge due to its high socioeconomic cost and therapeutic complexity. During a heart attack, the interruption of blood flow leads to an imbalance between oxygen supply and demand. Reperfusion is essential for myocardial survival, but can aggravate cell damage. A promising strategy to reduce this damage involves the use of cardiomyokines, such as the 10 kDa heat shock protein (HSP10), secreted in response to ischemic preconditioning. Previous studies have shown that HSP10 is capable of reducing cardiac lesions associated with ischemia and reperfusion, but its mechanisms of action are still not fully understood. This study aimed to investigate the effects of HSP10 on mitochondrial function in models of ischemia and reperfusion. Isolated hearts from Wistar rats (300-350 g, CEUA 154/21) were perfused with Krebs solution, subjected to 30 minutes of global ischemia and 10 minutes of reperfusion with or without prior treatment with HSP10 ($1 \mu\text{mol}\cdot\text{L}^{-1}$). Mitochondrial function was assessed after isolation of the organelles. In another protocol, isolated mitochondria were incubated with HSP10 and subjected to hypoxia/reoxygenation in vitro. HSP10 improved mitochondrial respiration, increased ATP production and reduced the formation of reactive oxygen species (ROS) compared to the I/R group. In vitro, HSP10 preserved mitochondrial respiration (91.5 ± 5.1 vs. 51.5 ± 5.0), increased ATP levels (250.1 ± 9.3 vs. 187 ± 21.7) and reduced ROS production (219.7 ± 9.0 vs. 339.0 ± 14.3 ; $p < 0.001$). HSP10 did not reduce the increase in mitochondrial membrane potential ($\Delta\psi_m$) after I/R, but it did reduce mitochondrial swelling. Complex IV activity and maximum O_2 consumption did not differ between the groups. We conclude that HSP10 exerts a direct protective effect on mitochondria, promoting maintenance of bioenergetic function and reducing oxidative stress.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Impact of Ethyl Ferulate on Blood Pressure and Heart Rate: Investigation of Autonomic Mechanisms Potentially Involved in Wistar and SHR Rat Models

Authors:

Samuel de Sousa Pereira ARAUJO^{1,2}
José Guilherme Veras de ASSUNÇÃO^{1,2}
Juliana Alves da SILVA^{1,2}
Francisca Valdirene de Sousa NUNES^{1,2}
João Paulo Jacob SABINO^{1,2}

Affiliations:

¹Federal University of Piauí

²Department of Biophysics and Physiology

Keywords: Ethyl ferulate; Hypertension; Receptors.

Abstract:

Systemic Arterial Hypertension (SAH) is a multifactorial condition characterized by blood pressure levels above 140/90 mmHg and low-grade chronic inflammation. Ethyl Ferulate (EF), a phenylpropanoid compound with anti-inflammatory, antioxidant, neuroprotective, and cardioprotective properties, was studied to evaluate its acute effects on hemodynamic parameters in Wistar and SHR (spontaneously hypertensive rats). The study, approved by the Ethics Committee of the Federal University of Piauí (No. 804/2023), used male rats divided into 8 groups (n=7): Wistar and SHR rats treated with vehicle or EF at doses of 7.5, 15, and 30 mg/kg. Animals were anesthetized and cannulated in the femoral artery and vein for pulsatile arterial pressure (PAP) recording and intravenous administration of EF or vehicle. After 60 minutes of acclimation, hemodynamic parameters were monitored for 60 minutes, with 20-minute intervals between doses. Mean arterial pressure (MAP) and heart rate (HR) were analyzed by One-Way ANOVA and Tukey's post-test ($p < 0.05$). Results showed that EF significantly reduced MAP in Wistar rats ($\Delta = -22 \pm 5$; -31 ± 6 ; -38 ± 6 vs. 2 ± 3 mmHg) and SHR rats ($\Delta = -22 \pm 6$; -32 ± 4 ; -52 ± 5 vs. 0 ± 2 mmHg) at all doses, compared to the vehicle group. HR also decreased in Wistar rats ($\Delta = -166 \pm 48$; -272 ± 43 ; -291 ± 44 vs. -12 ± 9 bpm) and SHR rats ($\Delta = -187 \pm 44$; -243 ± 32 vs. -8 ± 3 bpm) at doses of 15 and 30 mg/kg. It is concluded that EF induced significant blood pressure reduction and bradycardia in both groups, suggesting a potential antihypertensive effect. However, further studies are required to elucidate EF's mechanisms of action in the cardiovascular system.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Exacerbated vascular remodeling in TgPDIA1 overexpressing mice

Authors:

Júlia Martins Felipe De Souza¹
Leonardo Yuji Tanaka¹
Carolina Gonçalves Fernandes²
Daniela Kajihara¹
Tiphany Coralie De Bessa¹
Amanda De Almeida Silva³
Francisco Rafael Martins Laurindo¹

Affiliations:

¹Vascular Biology Laboratory, Heart Institute (InCor), University of Sao Paulo School of Medicine, Sao Paulo, Brazil
²Santa Casa de São Paulo, School of Medical Sciences (FCMSCSP)
³Laboratory of Hypertension, Heart Institute (InCor), University of Sao Paulo School of Medicine, Sao Paulo, Brazil

Keywords: Disturbed flow. Protein isomerase A-1. Vascular injury.

Abstract:

Vascular response to injury is a model process involving several phenomena typical of vascular diseases. Mechanisms such as redox signaling may affect the natural history of injury response. Protein-disulfide isomerase (PDIA1), a dithiol redox chaperone from the endoplasmic reticulum and cell surface, exerts mechanobiological adjustments of the cell-matrix binomial architecture in vessels. We previously reported that PDIA1 sustains vessel caliber during vascular repair after injury. However, the effect of PDIA1 in global vascular response to a remodeling stimulus is yet unclear. We recently validated a model of transgenic PDIA1 global overexpression. Here we investigated effects of such transgenic overexpression in carotid artery remodeling in a model of partial ligation of carotid branches leading to reduced/oscillatory flow. 10 16-weeks-old mice, wt and TgPDIA1 were used to perform partial ligation surgery. Ultrasound was performed in left and right common carotid to document blood flow changes such as low/oscillatory flow after ligation. Arteries were harvested 14 days after injury for histomorphometry and collagen analysis. Histological analysis showed that TgPDIA1 mice display increased medial layer (53,18%; $p=0,047$) and neointima area (212,24%; $p=0,014$) along with loss of high birefringent collagen deposition (80,45%; $p=0,0107$) 14 days after ligation. The overall arterial caliber was enhanced (85,12%; $p=0,028$) in TgPDIA1 mice, indicating exacerbation of an outward remodeling. Similar results were observed in a model of total carotid ligation, with enhanced total caliber and increased wall thickness. Endothelial cells were harvested from arteries 2 days after injury by intimal RNA isolation to perform gene expression plus the left over segment. Gene expression analysis documented the endothelial or smooth muscle cell (VSMC) respective phenotypes. Decrease of alpha actin 2 expression (63,2%; $p=0,033$) in media+adventicia showed early effect of carotid ligation in 2 days in WT mice, but not in TgPDIA1. TgPDIA1 exhibit an exacerbated outward vascular remodeling and vessel wall thickening after low/oscillatory flow-induced remodeling. This may reflect the overdifferentiation VSMC phenotype previously reported by us in these mice. Comitê de Ética: CEUA (Comissão de Ética no Uso de Animais) nº 1661/2021.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Development of an experimental model of PRKAG2 cardiac syndrome: Cardiological evaluation of mice carrying the PRKAG2 P.His398Gln mutation

Authors:

Yzabella Alves Campos Nogueira¹
Mariana Barreto Martins¹
Luciene Paschoal Braga Dias²
Isabella de Moura Folhadella Pires²
Alessandra de Almeida Ramos²
Cristiano Magalhães Ferreira Sobrinho²
Janaina Barcelos Porto Ferreira²
Glauber Monteiro Dias¹

Affiliations:

¹Universidade Estadual do Norte Fluminense Darcy Ribeiro

²Fundação Oswaldo Cruz

Keywords: PRKAG2 Cardiac Syndrome; AMPK; Cardiomyopathy; CRISPR/Cas9

Abstract:

Introduction: PRKAG2 cardiac syndrome (PCS) is an autosomal dominant disorder caused by mutations in the PRKAG2 gene encoding the AMPK γ 2 subunit. It is characterized by glycogen accumulation in cardiomyocytes, cardiac hypertrophy (HV), ventricular pre-excitation (VPE), and an increased risk of sudden cardiac death (SCD). Our group identified a novel missense variant, p.His401Gln, in a Brazilian family with early-onset left ventricular hypertrophy (LVH), VPE, and SCD. Objective: To generate a mouse model carrying the orthologous mutation p.His398Gln via CRISPR/Cas9 to study the pathophysiology of PCS. Methods: Following CEUA-UENF approval (Protocol No. 534), C57BL/6J females were superovulated and mated. Zygotes were microinjected with Cas9, guide RNA, and donor DNA containing the c.1230C>A substitution. One F0 female was homozygous for the mutation [knock-in (KI)] and was mated to a wild-type (WT) male. The descendants have been genotyped and interbred to establish the mice colony. Only heterozygous animals were included in the study. Electrocardiography and echocardiography were performed on 11 WT and 10 KI females and 6 WT and 7 KI males. Results: KI females exhibited atrial fibrillation, supraventricular tachycardia, VPE, and LV overload. KI males had first degree AV block and LV overload. Echocardiography revealed mid and proximal hypertrophy in KI females and apical hypertrophy in KI males. KI females reproduced the human phenotype with sinus tachycardia, shortened RR and PR intervals, and atrial fibrillation. KI males showed prolonged PR intervals, QRS widening and left axis deviation, suggesting fascicular block. PCS-like features such as shortened PR intervals, increased heart rate, and ventricular hypertrophy were confirmed. Conclusions: We report the first animal model of PCS with the p.His398Gln mutation. The model recapitulates important clinical features and supports future investigations into the pathophysiology of PCS.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Parental fructose overload induces cardiorenal dysfunction associated with impaired blood pressure variability: a study of sex-dependent effects

Authors:

Tânia Plens SHECAIRA¹
Camila PAIXÃO¹
Victor Hugo Martins MIRANDA¹
Antônio Viana do Nascimento FILHO¹
Pietra Petrica NEVES²
Danielle da Silva DIAS¹
Maria Cláudia IRIGOYEN³
Kátia DE ANGELIS^{1,2}

Affiliations:

¹ Translational Medicine PhD Program, Federal University of São Paulo (UNIFESP)
² Science Rehabilitation PhD Program, Universidade Nove de Julho (UNINOVE)
³ Hypertension Unit, Heart Institute, School of Medicine, University of São Paulo (InCor-HCFMUSP)

Keywords: Fructose. Autonomic dysfunction. Renal oxidative stress. Sex differences.

Abstract:

Parental fructose exposure may impair offspring cardiorenal function. In this study, the metabolic, hemodynamic, autonomic, and renal oxidative stress profiles of offspring of parents exposed to fructose overload were investigated, stratified by sex. Wistar rats received 10% fructose in their water or in their standard diet for 60 days prior to mating, gestation, and lactation. The offspring were divided into the following groups: control male (CM), control female (CF), fructose male (FM), and fructose female (FF). The rats were evaluated at 51 days of life. CEUA No. 1817160922. Metabolic parameters, blood pressure (BP) by direct recording, autonomic modulation, and renal oxidative stress were analyzed. FM and FF had higher body weights than CM and CF. Blood glucose levels were higher in FF and FM vs. CF (CF: 101.3 ± 1.8 ; FF: 113.9 ± 2.7 ; FM: 111.6 ± 3.0 mg/dL). Insulin sensitivity was lower in FM and FF vs. CM. Mean BP increased in FF vs. CM and CF (CM: 103.9 ± 2.9 ; CF: 102.3 ± 3.1 vs. FF: 117.5 ± 2.3 mmHg). There were no differences observed in heart rate, low-frequency (LF) power, high-frequency (HF) power, or the LF/HF ratio. Systolic BP variance (VAR SBP: CM 12.2 ± 1.5 , FM 20.3 ± 1.3 , CF 10.4 ± 1.6 , and FF 19.7 ± 1.2 mmHg²) and sympathetic vascular modulation (LF-SBP: CM 1.13 ± 0.4 , FM 4.1 ± 0.5 , CF 2.0 ± 0.3 , and FF 5.1 ± 0.5 mmHg²) were higher in FM and FF vs. CM and in FF vs. CF. NADPH oxidase activity increased in FM and FF vs. CM and CF (FM: 0.29 ± 0.1 ; FF: 0.27 ± 0.01 vs. CM: 0.19 ± 0.01 ; CF: 0.19 ± 0.02 nmol/mg protein). Protein oxidation was higher in FF vs. CM and CF (FF: 5.4 ± 0.14 vs. CM: 4.4 ± 0.3 ; CF: 4.4 ± 0.3 nmol/mg protein). In conclusion, offspring from fructose-exposed parents showed metabolic and cardiorenal impairment, associated with increased blood pressure variability. These adverse cardiorenal effects were exacerbated in females, suggesting an increased risk for this sex. Financial Support: FAPESP (2022/04050-1); CAPES; CNPq



XXXIX REUNIÃO ANUAL DA FeSB
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Antithrombotic and antiplatelet effects of polysaccharide extract from *Libidibia ferrea* associated with rivaroxaban in arterial thrombosis model induced by iron chloride in rats

Authors:

Carlos Jorge Maciel Uchoa GADELHA¹
Ana Carollyne Carvalho LIMA¹
Isabely Alencar BARRETO¹
Francisco Matheus dos Santos MACIEL²
Laisa Maria Pinto LEITE²
Ana Maria Sampaio ASSEREUY¹
Nilberto Robson Falcão do NASCIMENTO¹
Maria Gonçalves PEREIRA^{1,2}

Affiliations:

¹ Universidade Estadual do Ceará, ISCB, Brasil
² Universidade Estadual do Ceará, FECLESC, Brasil

Keywords: Jucá. plant polysaccharides. platelets. thrombosis.

Abstract:

Studies from our laboratory demonstrated antiplatelet and antithrombotic effects of polysaccharide extract from *Libidibia ferrea* (PE-Lf) in venous thrombosis models through different routes of administration. Additionally, the association of PE-Lf and low dose of rivaroxaban (RIVA; direct inhibitor of coagulation factor Xa) by oral route potentiates the antithrombotic effect without increasing bleeding. Aimed to evaluate the association PE-Lf plus RIVA in platelet aggregation and arterial model of thrombosis induced by FeCl₃. Rats (n=6; 280-320g; CEUA 11053402/2022) were treated (*p.o.*) with saline, PE-Lf (5 mg/kg), RIVA (0.1 mg/kg) or association, after 90 minutes the animals were anesthetized (5% isoflurane), the right carotid artery exposed and isolated through a cervical incision and ultrasonic probe was used to monitor blood flow. After capturing a stable flow, filter paper (4 mmx4 mm) soaked in 35% FeCl₃ was placed on the ventral surface of the artery for 3 minutes. After removing the paper, the vascular occlusion time was monitored for up to 30 minutes, the time recorded, and blood was collected for platelet aggregation assay. Blood was centrifuged to obtain platelet-rich plasma (1000 rpm; 8 min) and platelets were stimulated by ADP (5 µM). Animals treated with PE-Lf (854±80.5s) and PE-Lf+RIVA (835±113.4s) showed antithrombotic effect, increasing the vascular occlusion time by 82% and 78%, respectively, while RIVA (610±167s) did not show statistical difference in relation to FeCl₃ (468±78s). Platelet aggregation test showed antiplatelet effect in the groups treated with EP-Lf (34.7±2.4%) and EP-Lf+RIVA (34.8±3.2%) with inhibition of 31% and 32%, respectively, in comparison to ADP (52±6%). In conclusion, PE-Lf alone or associated with RIVA presented antithrombotic and antiplatelet effects. Regarding arterial thrombosis is more specific to platelet function, it is reasonable to attribute the inhibited platelet function leading to reduced thrombus formation.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Spectral analysis of blood pressure and heart rate variability in spontaneously hypertensive rats (SHR): Study of sympathetic and hormonal modulation of blood pressure

Authors:

João Ricardo Martinez BALDIN¹
Stephanie Furtado GEROLIN¹
Gustavo dos Reis MARTINS¹
Fernanda Mano Tagliapietra DA SILVA¹
Ruy Ribeiro de Campos JUNIOR¹
Rodrigo Lantyer Marques DANTAS¹
Cassia BERGAMASCHI¹

Affiliations:

¹ Universidade Federal de São Paulo - UNIFESP

Keywords: Heart Rate Variability. Autonomic Nervous system. Spectral Analysis.

Abstract:

Introduction: Hypertension is a highly prevalent chronic condition and a major risk factor for cardiovascular disease and premature mortality. Its pathophysiology is multifactorial, involving interactions among environmental, genetic, mechanical, neural, and hormonal factors. To investigate these mechanisms, the analysis of Blood Pressure Variability (BPV) and Heart Rate Variability (HRV) has proven valuable, providing indirect estimates of autonomic modulation of the cardiovascular system. BPV primarily reflects sympathetic vasomotor control, while HRV provides insights into cardiac autonomic regulation. Both rely on spectral analysis to infer autonomic nervous system oscillations. The power spectrum is divided into three frequency bands—Very Low Frequency (VLF), Low Frequency (LF), and High Frequency (HF)—each associated with distinct physiological mechanisms of cardiovascular regulation. Despite sharing spectral band nomenclature, BPV and HRV differ in physiological interpretation. In HRV, HF is linked to parasympathetic activity, while LF reflects combined autonomic influences. In BPV, LF indicates sympathetic vasomotor modulation and HF is related to respiratory influences. VLF, less well understood, has been associated with neuro-hormonal mechanisms. This study compared BPV and HRV spectral components between spontaneously hypertensive rats (SHR) and normotensive Wistar rats. Materials and Methods: Two groups of adult rats (>8 weeks) were studied: SHR (n = 19) and Wistar (n = 17). Data were obtained from experiments approved by the UNIFESP Ethics Committee. Signals were sampled at 4 kHz and interpolated for processing using Welch's method to estimate power spectral density. Frequency bands were defined as follows: VLF (0.02–0.2 Hz), LF (0.2–0.6 Hz), and HF (0.6–3.0 Hz). Groups were compared using the Mann-Whitney test, with significance set at $p < 0.05$. Analyses were performed using Python. Results: In BPV analysis, VLF spectral power was significantly higher in SHR than in Wistar rats ($p = 0.001$). Similarly, SHR exhibited greater VLF power in HRV compared to Wistar ($p = 0.001$). Conversely, LF power was higher in Wistar rats compared to SHR ($p = 0.001$). Conclusions: The increase in VLF power in both BPV and HRV in SHR suggests that elevated blood pressure may be associated with hormonal alterations. Future studies will include losartan administration protocols to assess the role of the renin-angiotensin system in autonomic modulation.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Intracerebroventricular Administration of Alamandine Reduces the Compulsive-Like Behavior Observed in Spontaneous Hypertensive Rats

Authors:

Natalia Aparecida de Lima Benevides^{1,2}
Lucas Rodrigues-Ribeiro^{1,2}
Nícia Pedreira Soares^{1,2}
Sthéfanie Chaves de Almeida Gonçalves²
Robson Augusto Souza dos Santos²
Thiago Verano-Braga^{1,2}

Affiliations:

¹Núcleo de Proteômica Funcional (NPF)

²INCT-Nanobiofar. Departamento de Fisiologia e Biofísica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brasil.

Keywords: Renin-Angiotensin System, Alamandine, Compulsive-Like Behavior, Marble Burying

Abstract:

INTRODUCTION: Anxiety and depression can be associated with cardiovascular dysfunctions, including arterial hypertension. In addition to hypertension, spontaneously hypertensive rats (SHR), exhibit hyperactivity and compulsive-like behaviors suggesting a relationship between the cardiovascular and central nervous systems. Alamandine (Ala), an endogenous peptide of the renin-angiotensin system (RAS) is a MrgD receptor agonist. An ongoing study from our research group suggests that Ala/MrgD modulates anxiety. Here, we investigated whether Ala can reduce hyperactivity and compulsive-like behaviors in SHR. **METHODS:** Wistar Kyoto rats (WKY) and SHR (8–14 weeks) were divided into four experimental groups: WKY + saline, WKY + Ala, SHR + saline, and SHR + Ala. Animals received Ala or saline via intracerebroventricular (ICV) administration via a cannula previously implanted in the lateral ventricle using stereotactic surgery. After recovery, they were subjected to the open field test (OFT) and marble-burying test (MBT). Experiments were approved by the local ethical authority (CEUA:312/2022). **RESULTS:** Control SHR animals showed a compulsive-like behavior while the SHR treated with Ala showed a significant reduction in the compulsive-like behavior [14.8 ± 3.7 (SHR + saline, $n=5$) vs. 0.8 ± 1.3 (SHR + Ala) buried marbles, $p<0.05$]. Animals showed similar levels of locomotion between groups in the OFT. **CONCLUSION:** Central administration of Ala improves the compulsive-like behavior observed in SHR while not impacting motor activity. Financial support: Capes, Cnpq, and Fapemig.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Effect of obesity associated with water deprivation on blood pressure maintenance in female rats

Authors:

Sara de Moraes LEITE¹
Helena de Oliveira DERÓBIO¹
Gabriela Maria LUCERA¹
José Vanderlei MENANI¹
Eduardo COLOMBARI¹
Débora Simões de Almeida COLOMBARI¹.

Affiliations:

¹UNESP, Departamento de Fisiologia e Patologia, Faculdade de Odontologia de Araraquara/SP

Keywords: Blood pressure. Obesity. Water deprivation.

Abstract: Obesity is a significant health problem and affects men and women. In rodents, it can be induced by a diet rich in fats and carbohydrates. One of the aims of our study was to determine whether a high-fat diet affects blood pressure in female rats. Another situation that increases blood pressure is water deprivation, a common occurrence in both humans and animals. Thus, in the present study, we combined water deprivation with a high-fat diet to verify the effect of both on the blood pressure of female rats. For this purpose, we used Holtzman female rats (280-300 g) that were fed either a standard diet (SD, 11% calories from fat) or a high-fat diet (HFD, 45% calories from fat) for 6 weeks. At the end of the 6th week, the femoral artery was cannulated for recording the baseline mean arterial pressure (MAP). Afterwards, the rats were left in water deprivation (WD) for 24 hours to obtain a new blood pressure measurement under this condition. Rats were euthanized by placing them under deep anesthesia with sodium thiopental (100 mg/kg of b. wt, i.p.) and transcardially perfused with 4% paraformaldehyde. C-Fos was labelled by immunohistochemistry, and the images were captured using a microscope and analyzed in ImageJ software. We observed an increase in blood pressure in hydrated rats fed a HFD (116 ± 3 vs SD: 108 ± 3 ; $p < 0.05$). However, WD did not increase the blood pressure of these rats (124 ± 2 vs HFD-EUH: 116 ± 3 ; $p > 0.05$). Concerning the SD diet, WD increased blood pressure when compared to hydrated rats (116 ± 5 vs SD-EUH: 108 ± 1 ; $p < 0.05$). Qualitative c-FOS data suggest that this increase in blood pressure during water deprivation is due to increased activation of the paraventricular nucleus of the hypothalamus (data under analysis). The data show that WD induces an increase in MAP only in SD fed rats, suggesting a diet-dependent blood pressure regulation in this situation. CEUA 11/2024.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Impact of *Ananas comosus* L. Merrill residue flour on the cardiovascular system of the spontaneously hypertensive rats

Authors:

Lizandra Henrique de Farias ABREU¹
Mirelly Cunha da SILVA¹
Douglas Vinicius do NASCIMENTO¹
Filipe de Melo BARBOSA¹
Thyago de Oliveira RODRIGUES¹
Regina Souza AIRES¹
Maria da Conceição Correia SILVA¹
Christine Lamenha LUNA-FINKLER¹
Thyago Moreira de QUEIROZ¹

Affiliations:

¹ Universidade Federal de Pernambuco

Keywords: Antioxidant. Flour. Hypertension. Pineapple. SHR.

Abstract:

The pineapple of the species *Ananas comosus* L. Merrill is known as a great source of flavonoids, vitamin C and phenolic compounds, which are considered potent antioxidants. This study aimed to evaluate the cardiovascular effects of the pineapple waste flour in spontaneously hypertensive rats. To assess the cardiovascular effects, the treatment with pineapple flour (FLO) was performed in Wistar and SHR rats aged 13 weeks, divided into 4 groups: W CT; W FLO; SHR CT; and SHR FLO. The flour was administered for 21 days via gavage (60 mg/kg), and the following parameters were evaluated: body weight, water and food intake, indirect blood pressure measurement. After euthanasia, the vascular reactivity, biochemical analysis, oxidative stress investigation and organ weights were performed. All protocols were approved by the CEUA/UFPE (# 0053/2023). Treatment with FLO did not reduce body mass or abdominal circumference in the groups. FLO reduced food intake in SHR compared to W FLO (64.81 ± 18.06 g vs 150.89 ± 29.51 g, respectively), and water intake in the SHR CT group was significantly higher than in the W CT group (232.48 ± 39.81 ml vs. 149.14 ± 13.93 ml, respectively). Regarding diastolic blood pressure (DBP), there was a reduction in the SHR FLO group compared to SHR CT (96.69 ± 9.06 mmHg vs. 109.83 ± 5.82 mmHg, respectively). In vascular reactivity, treatment with the flour decreased the maximal contraction of rings with endothelium in the W FLO group compared to the SHR FLO group (55.0 ± 2.8% vs. 85.8 ± 8.6%). No changes were observed in vasorelaxant responses with ACh or SNP. The SHR CT group showed increased NADPH oxidase levels compared to W CT (202543 ± 39636 RLU/mg vs 83944 ± 4668 RLU/mg, respectively). There were also no statistical differences in serum concentrations of glucose, cholesterol, and triglycerides. The FLO treatment, under the assessed conditions, was able to reduce weight gain and DBP but was not able to alter the vascular or biochemical parameters.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Characterization of the hematopoietic system in deep vein thrombosis

Authors:

Kalil Auzier Martins COSTA¹
Lídia Raquel da Silva NUNES²
Vinicius Duarte PIMENTEL²
Nathália Castelo Branco BARROS²
Boris Timah ACHA²
Fernanda Dutra Santiago BASSORA³
Éverton José Ferreira de ARAÚJO^{2,4}
Aldeídia Pereira de OLIVEIRA^{2,5}

Affiliations:

- ¹ University of São Paulo (USP)
² Federal University of Piauí (UFPI)
³ University of Campinas (UNICAMP)
⁴ Department of Pharmacy Program Coordination, Federal University of Piauí (UFPI)
⁵ Department of Physiology and Biophysics, Federal University of Piauí (UFPI)

Keywords: Bronchoalveolar lavage. Deep vein thrombosis. Hematopoietic system

Abstract:

Thrombosis is characterized by a disruption in endothelial hemostatic function, resulting from an impaired ability to regulate normal physiological coagulation processes. To date, no studies have been published that investigate the relationship between deep vein thrombosis (DVT) and hematopoietic system outcomes. This study aims to characterize the hematopoietic system in the DVT. All experiments were approved by the Animal Use Ethics Committee (CEUA/UFPI: 562/2019). *Rattus norvegicus* (Wistar) were used for the 28-day DVT induction protocol. DVT was induced by complete stasis of blood flow in the inferior vena cava for 28 days. Peripheral blood, femoral bone marrow, bronchoalveolar lavage (BAL) and blood plasma samples were collected for total cell counts and differential morphological analysis. Peripheral blood analysis revealed alterations in the erythroid lineage, as well as changes in the leukogram ($10.4 \pm 1.20 \times 10^3$ vs. $6.68 \pm 0.54 \times 10^3$ cells/mm³) and platelet count, consistent with previously reported findings. To assess whether this stimulation originated from a self-limited peripheral pool or from central (bone marrow) signaling, a myelogram was performed, revealing increased total bone marrow cellularity ($4.81 \pm 0.51 \times 10^7$ vs. $2.80 \pm 0.63 \times 10^7$ cells/mL). Although post-thrombotic leukocytosis has been described as transient, we propose that these persistent alterations in both hematopoietic compartments may have been triggered by tissue-level outcomes, as evidenced by leukocyte infiltration in the lungs, corroborated by the increased cellularity in bronchoalveolar lavage (BAL) ($4,84 \pm 1,40 \times 10^3$ vs. $1,73 \pm 0,66 \times 10^3$ cells/mm³). These findings suggest that deep vein thrombosis (DVT), by impairing blood circulation, may stimulate bone marrow hematopoiesis and may not be self-limiting when adjacent tissues are affected. Further studies are required to determine the extent of tissue involvement and to elucidate the pathways underlying cellular migration.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Effect of Angiotensin-(2-7) on Vascular Reactivity in Diet-Induced Obese Mice

Authors:

Carolina Fonseca de Barros¹
Amanda de Sá Martins de Bessa¹
Nícia Pedreira Soares¹
Natalia Aparecida de Lima Benevides¹
Robson Augusto Souza dos Santos¹

Affiliations:

¹Institute of Biological Sciences, Hypertension Laboratory, Federal University of Minas Gerais (UFMG)

Keywords: Cardiovascular. Endocrine. Obesity. Peptides. Vascular.

Abstract:

It is well known that obesity is associated with cardiovascular risk and vascular abnormalities. The renin-angiotensin system (RAS) plays an important role on the vascular function. Ang-(1-7) as favorable vascular actions of human obesity. We have recently identified Ang-(2-7) as a new biological active peptide of the RAS. This hexapeptide display potent antihypertensive effect in SHR. The aim of this study was to evaluate the effects of Ang-(2-7) on blood pressure and vascular function in High-fat diet treatment. We used 8-10 weeks-old C57BL6/J male mice (n=28). The animals were initially divided in two groups: Standard diet (SD) and High fat group (HF). After four weeks, we started the treatment with Ang-(2-7) or saline using osmotic mini pumps, for 28 days. Systolic blood pressure measurements were done by tail plethysmography in the week before starting the diet, in the four weeks after diet treatment and at the end of the treatment (16 weeks). Subsequently, the animals were euthanized, and the vascular function was evaluated using cumulative concentrations of acetylcholine (Ach, in vessels with endothelium) or Sodium Nitroprusside (SNP, in endothelium denude) and Phenylephrine (Phe). Statistical analysis was performed using one-way, two-way ANOVA or t-test ($p < 0.05$), according to the data analyzed: CEUA: 113/2022. There were no statistically significant differences in blood pressure between the groups. As expected, HF-diet induced impairment of the vascular relaxant effect of Ach (HF Sal $Emax: 36 \pm 5,10$; Log $IC_{50}: -5,87$ vs. SD Sal: $Emax: 58,80 \pm 4,50$). The treatment with Ang-(2-7) improved the vasorelaxing effect of Ach in the SD and HF groups (SD Sal: $Emax: 58,80 \pm 4,50$; Log $IC_{50}: -6,00$ vs. SD Ang-(2-7): $Emax: 74,32$; Log $IC_{50}: -6,41$; HF sal: $Emax: 36 \pm 5,10$; Log $IC_{50}: -5,87$ vs HF Ang-(2-7): $Emax: 75,41 \pm 10,79$ Log $IC_{50}: -6,15$). In addition, in endothelium denuded vessels, vasoconstrictor effect of Phe was attenuated in the SD group (SD Sal: $Emax: 50,19 \pm 6,68$; Log $IC_{50}: -6,82$ vs. SD Ang-(2-7): $Emax: 35,69 \pm 8,41$; Log $IC_{50}: -6,40$). These results unmasked a significant effect of Ang-(2-7) on vascular function.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: SGLT2 inhibitor reduces airway morphological changes in OVA-induced potentiated by methylglyoxal

Authors:

Matheus L. Medeiros¹
Akila L. Oliveira²
Edson Antunes³

Affiliations:

Department of Translational Medicine, Pharmacology Area, Faculty of Medical Sciences, University of Campinas (UNICAMP).

Keywords: Methylglyoxal, Empagliflozin, Lung, Asthma and Remodeling

Abstract:

Asthma is a chronic inflammatory symptom in the airways that affects millions of people worldwide. The classic form of the disease, known as type 2 asthma (or high Th2 asthma), is characterized by the predominant presence of eosinophils. Several scientific studies have shown that conditions such as obesity and type 2 diabetes can exacerbate asthma symptoms. Methylglyoxal (MGO) is a byproduct formed mainly during the glycolysis process, whose elevated levels have been associated with increased inflammatory responses. Additionally, studies in animals with insulin resistance have revealed an intensification of allergic pulmonary inflammation, which was partially reversed with the use of antidiabetic drugs. In this study, our aim was to investigate the action of empagliflozin (EMP) in reducing MGO and its impact on allergic pulmonary morphology in male C57BL6/Junib mice aged 4 weeks, all protocols were approved by UNICAMP animal ethics committee (CEUA) under protocol no. 6081-1/2022. The mice were treated with MGO at 0.5% in drinking water and challenged or not with ovalbumin (OVA), and they were treated or not with EMP (10mg/kg by gavage) for 15 days. The statistical analysis was performed using ANOVA, followed by Tukey's post-hoc test. We used the lungs to evaluate H&E, Masson, and PAS techniques, and MGO levels were measured by ELISA and immunohistochemistry. Histological analysis demonstrated a significant increase in inflammatory cells in MGO-treated animals, as well as an increase in collagen deposition (Masson's technique) and mucus production in the lung (PAS technique) ($p < 0.05$). We also observed an increase in MGO levels in the BALF of MGO-treated and OVA-challenged animals ($p < 0.05$). However, EMP treatment, was able to inhibit of MGO-induced lung morphological change ($p < 0.05$), as well as reduction in MGO levels ($p < 0.05$). This suggests that pharmacological intervention with EMP is capable of inhibiting airway alterations in OVA-induced allergic animals by attenuating the potentiating effects of MGO.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Effects of lateral parafacial glutamatergic interneurons activation on the ventilatory parameters of mice during normocapnia and hypercapnia

Authors:

Juliana Reis SOUZA¹
Nathalia SALIM¹
Renato Willian MARTINS SÁ¹
Benedito Honório MACHADO¹
Davi José Almeida MORAES²

Affiliations:

¹Department of Physiology, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto/SP - Brazil.

²Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, São Paulo/SP - Brazil.

Keywords: Active expiration. Glutamatergic interneurons. Lateral parafacial region. Pulmonary ventilation.

Abstract:

The lateral parafacial region (pFL), responsible for generating expiratory motor activity during hypercapnia, contains excitatory neurons and interneurons. The role of pFL excitatory interneurons in regulating ventilation is unclear. We used mice expressing the Cre recombinase in VGLUT2-positive neurons [VGLUT2^(cre/cre)] that were microinjected with cre-dependent virus to express excitatory hM3D-Gq receptors only in pFL glutamatergic interneurons and evaluated the effects of its chemogenetic activation, using JHU37160 (0.1mg/kg; i.p.), on ventilatory parameters of non-anesthetized mice during resting and hypercapnia (7% CO₂) (CEUA #1143/2022). At rest, JHU injection reduced respiratory frequency (f_R : 149.2±53.19 vs 213.9±60.56 cpm; $p=0.002$) and increased tidal volume (V_T : 10.2±3.36 vs 7.91±1.31 mL.Kg⁻¹; $p=0.02$), without affecting ventilation (V_E : 1620±1044 vs 1732±738.3 mL.Kg⁻¹.min⁻¹). The respiratory cycle duration increased (380.1±124.5 vs 287.6±64.37 ms; $p=0.01$), as a result of an increase in expiration (DE: 287.8 ± 138 vs 159.9 ± 40.27 ms; $p=0.01$). After JHU, the area of both early expiration (4.17±1.12 vs 2.87±0.52, $p=0.006$) and late expiration (3.06±1.05 vs 0.69±0.23, $p=0.0001$), as well as the ratio between them, increased (0.74±0.24 vs 0.24±0.06, $p=0.0004$), suggesting the recruitment of active expiration. JHU injection did not change the hypercapnia-induced ventilatory responses of VGLUT2^(cre/cre+hM3D-Gq) when compared to VGLUT2^(cre/cre) [$(\Delta f_R$: 69.2± 59.51 vs 108.4±48.93 cpm; $p=0.10$, ΔV_T : 3.51±4 vs 6.21±4.85 mL.Kg⁻¹; $p=0.18$, ΔV_E : 1729±1591 vs 2794±1986 mL.Kg⁻¹.min⁻¹; $p=0.15$, Δ respiratory cycle duration: -77.6±81.58 vs -133.3±71.91 ms; $p=0.10$, Δ DI: -90.34±55.24 vs -55.72±30.05 ms; $p=0.06$], except the magnitude of reduction in DE, which was abolished in Vglut2^(cre/cre + hM3D-Gq) (Δ DE: 12.74±69.39 vs -77.53±47.95 ms; $p=0.001$). We showed that the pFL glutamatergic interneurons control the ventilatory parameters of mice during resting and hypercapnic conditions.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Airway reflexes: review and proposal of teaching material

Authors:

Ricardo Murilo Pereira Emídio¹

Roseli Soncini¹

Affiliations:

¹Universidade Federal de Alfenas, Alfenas, Brasil

Keywords: Airway reflexes; Respiratory physiology; teaching material; active learning; cordel literature.

Abstract:

Airway reflexes, such as sneezing and coughing, are an important mechanism in regulating respiratory function in humans, highlighting their fundamental role in protecting the airways and maintaining pulmonary health. This topic represents an essential element in the field of respiratory physiology in higher education and clinical practices. Therefore, understanding the airway reflexes is essential for the identification and appropriate management of respiratory conditions, as well as for the practical application of this knowledge in clinical contexts. This study proposes an updated literature review and the development of educational resources that employ active learning approaches, such as "cordel literature" and comic strips. The goal is to reinforce and complement the teaching-learning process of higher education physiology students, providing them with teaching materials that enable a deeper understanding of this topic of respiratory physiology, in order to enable students to understand and apply these concepts effectively. The partial results include the creation of educational "cordel verses" about the reflexes of sneezing, coughing, swallowing, laryngeal adductor reflex and expiration reflex, each containing a standardized structure, characterized by six verses per stanza with rhymes between the 2nd, 4th and 6th verse and seven verses per stanza with rhymes between the 2nd, 4th and 7th verse and between the 5th and 6th verse. Furthermore, the "cordel verses" present the physiological mechanisms and the importance of the respective reflexes in a poetic and accessible manner, aligning scientific content with culturally significant language.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Ovariectomy Exacerbates Neurodegeneration and Respiratory Impairments in a Parkinson's Disease Mouse Model

Authors:

Giovanna Matos RODRIGUES¹

Yasmin Cestari AQUINO¹

Thiago dos Santos MOREIRA²

Ana Carolina TAKAKURA¹

Affiliations:

¹ Department of Pharmacology, Instituto de Ciencias Biomedicas, Universidade de Sao Paulo, SP, 05508-000, São Paulo, SP, Brazil

² Department of Physiology and Biophysics, Instituto de Ciencias Biomedicas, Universidade de São Paulo, SP, 05508-000, Sao Paulo, SP, Brazil

Keywords: Estrogen. Ovariectomy. Parkinson's disease. Respiratory deficits. Sex dimorphism.

Abstract:

Parkinson's Disease (PD) is a neurodegenerative disorder characterized by dopaminergic neuron loss in the substantia nigra pars compacta (SNpc), leading to motor impairments and respiratory impairments. PD is twice as prevalent in men, suggesting a potential role for female sex hormones. This study investigated the influence of estrogen on neuroanatomical and physiological respiratory dysfunction in PD. Adult female C57BL/6 mice (CEUA: 3325170822) underwent bilateral ovariectomy (OVX) or sham surgery. Two weeks later, PD was induced by bilateral 6-hydroxydopamine (6-OHDA) or vehicle injections into the striatum. Respiratory parameters were assessed by whole-body plethysmography, and neuroanatomical changes were analyzed via immunohistochemistry. OVX/6-OHDA mice exhibited increased estrogen receptor alpha expression in the SNpc (87.0 ± 2.5 vs. sham/6-OHDA: 54.5 ± 6.2 cells, $p < 0.0014$), pre-Bötzing Complex (preBötC; 53.0 ± 4.5 vs. sham/6-OHDA: 34.5 ± 3.2 cells, $p < 0.0288$), and Retrotrapezoid Nucleus (RTN; 37.0 ± 2.97 vs. sham/6-OHDA: 19.3 ± 1.8 cells, $p < 0.0004$). They also showed a greater loss of dopaminergic neurons in the SNpc (30.8 ± 1.6 vs. sham+6-OHDA: 63.8 ± 2.4 , $p < 0.0014$), reduced neurokinin 1 receptor density in the preBötC (28.3 ± 0.7 vs. sham+6-OHDA: $33.6 \pm 0.6\%$, $p < 0.0060$), and fewer phox2b+ neurons in the RTN (35.0 ± 1.8 vs. sham+6-OHDA: 44.3 ± 1.2 , $p < 0.0399$). Respiratory frequency declined earlier and more severely in OVX/6-OHDA (day 5: 161.94 ± 1.35 vs. sham/6-OHDA: 179.75 ± 0.41 bpm, $p < 0.0001$; day 10: 151.1 ± 2.4 vs. sham+6-OHDA: 161.0 ± 1.2 bpm, $p < 0.0001$). SNpc lesions at day 5 were similar to those at day 10 (33.0 ± 3.9 vs. sham/6-OHDA: 66.4 ± 3.4 neurons, $p < 0.0174$) but degeneration appeared earlier in the preBötC (44.95 ± 1.2 vs. sham/6-OHDA: $54.1 \pm 1.1\%$, $p < 0.0001$) and RTN (41.3 ± 2.6 vs. sham/6-OHDA: 53.5 ± 2.8 neurons, $p < 0.0162$). OVX accelerates neurodegeneration in the SNpc, preBötC, and RTN, worsening 6-OHDA-induced respiratory decline. These findings support estrogen's neuroprotective role in PD-related respiratory dysfunction. Financial support: FAPESP, CNPq, and CAPES/PROEX



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Number of granular cells in the olfactory bulb of BALB/c mice may be dependent on oxidative stress in an experimental model of hyperoxia

Authors:

Giulia Ferreira DAMASCENO¹
Isabela Azevedo de ALMEIDA¹
Laura Barbosa GROSSI¹
Fernando Monteiro AARESTRUP¹
Beatriz Julião Vieira AARESTRUP¹
Frank Silva BEZERRA²
Akinori Cardozo NAGATO^{1,3}

Affiliations:

- ¹ Laboratory of Immunopathology and Experimental Pathology, Center for Reproductive Biology—CRB, Federal University of Juiz de Fora (UFJF), Juiz de Fora, Minas Gerais, Brazil.
² Laboratory of Experimental Pathophysiology (LAFEx), Department of Biological Sciences (DECBI), Center of Research in Biological Sciences (NUPEB), Federal University of Ouro Preto (UFOP), Ouro Preto, Brazil.
³ Physiology Department, UFJF, Juiz de Fora, Minas Gerais, Brazil.

Keywords: Hyperoxia. Mice, inbred BALB C. Nitric oxide synthase. Olfactory bulb.

Abstract:

Supraphysiological supply of oxygen (O₂) aims to prevent or reverse hypoxemia, but long-term 100% O₂ leads to reactive O₂ species (ROS), stress and oxidative damage, directly affecting the respiratory epithelium. Fat-soluble O₂ diffuses easily throughout the body, including the olfactory bulb (OB). The aim of this study was to determine whether hyperoxia modifies the expression of induced nitric oxide synthase (iNOS) in the OB of mice. The study was approved by the Ethics Committee (UFOP-No.092/2012). Female BALB/c mice (6-8 weeks old; 24.53±0.31g) were maintained in room air (Control group - CG) or exposed to O₂ (1.00 FiO₂ for 24h) and then euthanized. Tissue sections were stained with hematoxylin-eosin and toluidine blue for histopathological and stereological analysis (by light microscopy). The expression of iNOS was verified by immunohistochemistry. The granular cell density (N_v) of the OB was investigated in the experimental groups by counting the number of nuclei per microscopic field ([N_v/μm²] × 10³). The expression of iNOS was determined by the area occupied by diaminobezidine per microscopic field (μm²×10³). The normality of the sample was tested by the D'Agostino & Pearson test. Data were expressed as the mean ± standard error. Differences between the groups was tested by unpaired Student's T-test (normal) or Man-Whitney test (non-normal). The significance level was 5% (p<0.05). Hyperoxia increased the number of granular layer cells (H&E) when compared to room air (O₂: 6.193±0.2788; CG: 3.777±0.2218; p<0.0001). The expression of iNOS was higher in the oxygen group when compared to room air (O₂: 254.3±46.02; CG: 36.94±8.662; p<0.0001). The results suggest a direct relationship between OB granule cell VN and iNOS expression. Further studies may clarify whether hyperoxia has the potential to induce cell division in the OB and whether this is modulated by oxidative stress.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: The Role of GSK3- β and PKM2 in Respiratory Nuclei in Parkinson's Disease

Authors:

Estela Correia Leandro dos SANTOS¹
Luiz Fernando de Araújo Trindade PEDRÃO¹
Pamela Ojuorun Santos MEDEIROS¹
Barbara FALQUETTO¹

Affiliations:

¹University of São Paulo, ²Institute of Biomedical Sciences.

Keywords: GSK3- β , PKM2, Neurodegeneration, Respiratory Nuclei, Parkinson's Disease.

Abstract:

Parkinson's disease (PD) is a neurodegenerative disorder marked by dopaminergic neuron loss in the substantia nigra (SN), leading to motor and non-motor symptoms, including respiratory dysfunction. The 6-OHDA model mimics PD, inducing neuronal degeneration in respiratory nuclei such as rVRG, preBötC, and RTN. Oxidative stress activates glial cells, triggering neurotoxic pro-inflammatory responses. GSK3- β autophosphorylated in residue Tyr216 and p-PKM2 regulate survival and apoptotic pathways involved with neurodegeneration. Were used male mice (n=30, ~22g), received intrastriatal 6-OHDA (10 μ g/ μ l) or vehicle (VH). After six days, brainstem slices were analyzed by Western Blot (WB). TH immunoreactivity confirmed dopaminergic loss. Statistical analysis: Student's t-test, p<0.05. CEUA: 7992180820. 6-OHDA reduced TH+ neurons in SN (6-OHDA:143.5 $\pm$ 27.1 vs. VH:475.8 $\pm$ 81.8, T10=9.443; p<0.0001). WB showed increased GSK3- β in RTN (6-OHDA:1.25 $\pm$ 0.14 vs. VH:0.86 $\pm$ 0.30, T8=2.375; p=0.0449) and decreased levels in rVRG+preBötC (6-OHDA:0.60 $\pm$ 0.28 vs. VH:0.92 $\pm$ 0.30, T10=2.362; p=0.0398). pGSK3- β Tyr216 was reduced in rVRG+preBötC (6-OHDA:0.67 $\pm$ 0.20 vs. VH:1.03 $\pm$ 0.35, T13=2.418; p=0.0310) but unchanged in RTN (6-OHDA:1.53 $\pm$ 0.77 vs. VH:1.03 $\pm$ 0.29, T9=1.576; p=0.1494). PKM2 and p-PKM2 remained stable in rVRG+preBötC (p-PKM2: 6-OHDA: 1,07 $\pm$ 0,26 vs. VH:1,13 $\pm$ 0,19, T10=0,3918; p=0,5127; PKM2: 6-OHDA: 1,10 $\pm$ 0,29 vs. VH: 1,11 $\pm$ 0,22, T10=0,0824; p=0,5520). The p-PKM2/PKM2 ratio showed no changes (6-OHDA:0.98 $\pm$ 0.08 vs. VH:1.02 $\pm$ 0.08, T10=0.7549; p=0.8940). GSK3- β increased in RTN and decreased in rVRG + preBötC, while pGSK3- β Tyr216 decreased in rVRG + preBötC reports that the GSK-3 β pathway may play a role in the neurodegeneration of the hidden nuclei of PD, with its involvement varying depending on the specific nuclei and progression over time. No changes in PKM2 indicate that analyzes preserving its dimeric and tetrameric conformation are permitted to explore its role in the neurodegeneration of respiratory nuclei in PD.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Hyperoxia has the potential to modify the immune phenotype in an experimental asthma model through the influence of Treg cells

Authors:

Giulia Ferreira DAMASCENO¹
Isabela Azevedo de ALMEIDA¹
Maiza da Silva PAULA¹
Fernando Monteiro AARESTRUP¹
Beatriz Julião Vieira AARESTRUP¹
Frank Silva BEZERRA²
Akinori Cardozo NAGATO^{1,3}

Affiliations:

¹ Laboratory of Immunopathology and Experimental Pathology, Center for Reproductive Biology—CRB, Federal University of Juiz de Fora (UFJF), Juiz de Fora, Minas Gerais, Brazil.
² Laboratory of Experimental Pathophysiology (LAFEx), Department of Biological Sciences (DECBI), Center of Research in Biological Sciences (NUPEB), Federal University of Ouro Preto (UFOP), Ouro Preto, Brazil.
³ Physiology Department, UFJF, Juiz de Fora, Minas Gerais, Brazil.

Keywords: Asthma. Cells, Treg. Forkhead transcription factors. Hyperoxia. Mice, inbred BALB C.

Abstract:

Experimental models of allergic asthma (AA) classically exhibit an immune phenotype with a prominent T-helper cell type 2 (Th2) response, and interleukins: IL4, IL5, IL9 and IL13. In acute crises, there is also stress and oxidative damage, a Th17 (IL17) response, neutrophilia, bronchoconstriction, dyspnea and hypoxia. Supplemental oxygen (O₂) serves to reverse hypoxemia, although it generates reactive O₂ species and drives inflammation towards a Th1 phenotype, especially when superimposed on a previous inflammatory background (Th2). Regulatory T cells (Treg) have been reported to suppress the Th2 response, when the transcription factor Forkhead box p3 (Foxp3) induces IL10 synthesis in Treg. The aim of the study was to verify whether hyperoxia, superimposed on airway inflammation induced by ovalbumin (OVA), is capable of altering the expression of Foxp3 in the lungs of BALB/c mice. The study was approved by the Ethics Committee (No.024/2018-CEUA). Female BALB/c mice (6-8 weeks; 24.53±0.31g) were divided into: Control Group (CG, room air), PBS (room air + intraperitoneal injection of phosphate buffer), O₂ (1.00 FiO₂ for 24h), OVA (sensitized and attacked with OVA) and OVOX (OVA+O₂). Lung tissues were stained with hematoxylin-eosin (histopathology and stereology). Foxp3 expression was verified by immunohistochemistry and determined by the area occupied by diaminobenzidine per lung parenchyma (mm²×10⁶). The normality of the samples was tested by the D'Agostino & Pearson normality test. The difference between the groups was tested by the Kruskal-Wallis test, and the inter-group difference by Dunn's post hoc test. A 95% confidence interval was considered significant when p<0.05. Hyperoxia induced greater expression of Foxp3 when compared to the control (O₂: 142.8±17.17; CG: 64.37±28.02; p=0.0240). The data suggest that hyperoxia may induce a Treg response and this may contribute to a change in the phenotype of the immune response in experimental models of AA.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Effects of exposure to sodium hypochlorite on inflammatory and functional mechanisms in an experimental model of chronic allergic lung sensitization

Authors:

Gabrielle Santos MATOS¹
Anna Lima da COSTA²
Suellen Karoline Moreira BEZERRA²
Bianca Turatti MARRETO¹
Clara Carvalho Tavares LAZARIN¹
Leandro Correa SOUZA²
Iolanda de Fátima Lopes Calvo TIBÉRIO²
Renato Fraga RIGHETTI²
Beatriz Manguiera Saraiva ROMANHOLO²

Affiliations:

¹University City of São Paulo (UNICID), São Paulo, Brazil. ²School of Medicine, University of São Paulo (FMUSP), São Paulo, Brazil.

Keywords: Asthma. Anti-IL17. Chlorine. Inflammation. Remodeling.

Abstract:

The Global Asthma Initiative shows that 5 to 20% of new cases are caused by occupational irritant particles. The possibility of an anti-il17 treatment allows us to understand how the control of alarmines can represent not only possible "sign of inflammation", but also new therapeutic strategies. This study aimed to evaluate to hyperresponsiveness, inflammatory profile and pulmonary tissue remodeling in a model of chlorine exposure and chronic allergic lung sensitization. This study was approved by the Committee on Ethics in the Use of Animals of the Faculty of Medicine of University of Sao Paulo (Brazil) (n°7654). The animals will be divided into 08 groups: Control; Asthma; Chlorine; Asthma+Chlorine; Asthma+anti-IL17; Chlorine+anti-IL-17; Asthma +Chlorine+anti-IL-17. On days 1, 14, 28 and 42 the animals will receive intraperitoneal injection with 50µg of Ovoalbumine and from day 21 Ovoalbumine 1% inhalations, 3 times a week until the end of the protocol. Exposure to chlorine will occur through inhalation with sodium hypochlorite solution (NaClO) 1%, 5 times a week. The anti-IL17 will be administered by intraperitoneal injection 30 minutes before each administration of aerosol ovalbumin, in the last 3 inhalations. Data were collected from lung function (Gtis, Htis, Rrs, Ers and Raw) and materials for analysis of BALF and Histology (HE). The Sigma Stat 11.0 software (San Jose, CA, USA) was used for all statistical analyses. A one-way variance analysis was performed, followed by the Holm-Sidak test . All values are expressed as the meann±nstandard error (SE). The significance level (pn<n0.05). Changes in pulmonary resistance and elastance were observed, increased inflammatory cells, edema, mononuclear and polymorphonuclear cells. We concluded that lung function was altered, remodeling was induced, and inflammation was increased in the exposed and co-exposed groups, however when treated with anti-IL17 parameters obtained significant decrease.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Selective chemogenetic manipulation of Guanylyl Cyclase D+ neurons and their projections to the vNDB has no impact on the hypercapnic ventilatory response

Authors:

Phelipe E. SILVA^{1,2}
Emmanuel V. ARAUJO¹
Kara A. FULTON²
Ana C. TAKAKURA³
Sandeep R. DATTA²
Thiago S. MOREIRA¹

Affiliations:

¹ Department of Physiology and Biophysics, University of São Paulo, SP, Brazil
² Department of Neurobiology, Harvard Medical School, Boston, MA, USA
³ Department of Pharmacology, University of Sao Paulo, São Paulo, Brazil

Keywords: Chemosensation, Olfactory system

Abstract:

The regulation of breathing involves a network maintaining basal respiratory rhythms and specialized chemosensors detecting blood gases (CO₂, O₂). These chemosensors activate pathways adjusting respiratory output for homeostasis. Within the olfactory system, Guanylyl Cyclase D+ (GC- D+) neurons detect CO₂ and project to the vertical limb of the nucleus of the diagonal band of Broca (vNDB), involved in social transmission of food preferences (STFP), an important behavioral phenomenon in mice. This study investigated whether GC-D+ neurons and their projections to vNDB contribute to the hypercapnic ventilatory response (HCVR) (CEUA # 8033310719). Genetically modified mice (Tbx21-Cre/VGluT2-Flpo, GCD-KO, GCD-Cre) and chemogenetic tools (DREADDs) were used to selectively manipulate GC-D+ neurons and GCD-PNs in vNDB. Whole-body plethysmography assessed breathing in conscious, unrestrained mice. Chemogenetic inhibition of GCD-PNs (hM4Di, Gi) did not significantly alter ventilatory responses to hypercapnia (7% CO₂) (VE: 354.6 ± 111.5% saline vs. 424.6 ± 155.8% C21, p = 0.526, N = 5). Activation of GCD-PNs (hM3Dq, Gq) did not change VE (311.9 ± 107.5% saline vs. 400.4 ± 137.5% C21, p = 0.090, N = 8). GC-D+ inhibition in GCD-Cre mice had no impact on VE (277.3 ± 156.5% saline vs. 377.7 ± 229.6% C21, p = 0.490, N = 6). GCD-KO genetic knockout did not affect HCVR (VE: 384.3 ± 93.8% WT vs. 480.5 ± 181.1% KO, p = 0.326, N = 4). Findings suggest neither GC-D+ neurons nor their vNDB projections influence HCVR, implying broader circuits in olfactory modulation of breathing. Further studies should explore alternative pathways linking olfactory and respiratory systems.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Recruitment of inflammatory cells in the upper airways of BALB/c mice after an experimental model of acute asthma and hyperoxia opens a reflection on the concept of a single airway

Authors:

Isabela Azevedo de ALMEIDA¹
Giulia Ferreira DAMASCENO¹
Fernando Monteiro AARESTRUP¹
Beatriz Julião Vieira AARESTRUP¹
Frank Silva BEZERRA²
Akinori Cardozo NAGATO^{1,3}

Affiliations:

¹ Laboratory of Immunopathology and Experimental Pathology, Center for Reproductive Biology—CRB, Federal University of Juiz de Fora (UFJF), Juiz de Fora, Minas Gerais, Brazil.
² Laboratory of Experimental Pathophysiology (LAFEx), Department of Biological Sciences (DECBI), Center of Research in Biological Sciences (NUPEB), Federal University of Ouro Preto (UFOP), Ouro Preto, Brazil.
³ Physiology Department, UFJF, Juiz de Fora, Minas Gerais, Brazil.

Keywords: Asthma. Hyperoxia. Inflammation. Ovalbumin. Trachea.

Abstract:

Acute asthma results in recruitment of inflammatory cells, lower airway obstruction, and the need for supraphysiologic oxygen to reverse hypoxemia. According to the single airway concept, recruitment of inflammatory cells should also be observed in the upper airways and a change in histoarchitecture may occur, but it is uncertain whether this occurs in experimental models of acute asthma induced by ovalbumin (OVA), especially when hyperoxia is offered over this pre-existing inflammatory background. The aim of this study was to determine whether OVA and hyperoxia alter the density of inflammatory cell numbers (Nvcells) and the density of collagen volume (Vvcollagen) in the trachea of BALB/c mice. The study was approved by the Ethics Committee (UFOP-No.107/2012). Female BALB/c mice (6-8 weeks, 20-25g) were divided into Control group - "CG", "PBS" (2mg Al(OH)₃ dissolved in 200 µL of PBS), "OVA" (20mg OVA in 2mg Al(OH)₃ dissolved in 200 µL of PBS + 50 µL of 1% OVA), "O₂" (oxygen at 10L/min for 24h) and "OVA+O₂". Histopathologic and stereologic analyses were performed on tracheal sections stained with Gomori's trichrome and analyzed by light microscopy. Nv cells in the tracheal lamina propria and Vv collagen were quantified by area (µm²). After checking the normality of the samples using the D'Agostino & Pearson test, the data were expressed as mean ± standard error. The significance level was set at 5% (p<0.05). The Nv of the OVA+O₂ group (97.29±8.32) was higher (p<0.0001) compared to the CTR groups: 3.174±0.2586; PBS: 7.468±0.5369; OVA: 5.959±0.5257; O₂: 5.532±0.3138. Vv in the OVA+O₂ group (6.454±0.5295) was lower (p<0.0001) than CTR: 324.9±28.98; PBS: 105.3±10.44; OVA: 173.9±22.10. The results suggest that the recruitment of inflammatory cells in the upper airways is similar to that in the lower airways, but this is not observed when the amount of collagen in the airways is considered.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Functional respiratory disorders in the MitoPark model of Parkinson's disease

Authors:

Yasmin Cestari AQUINO¹
Maria Aparecida OLIVEIRA¹
Luiz Marcelo OLIVEIRA³
Thiago dos Santos MOREIRA²
Ana Carolina TAKAKURA¹

Affiliations:

¹Department of Pharmacology and
²Department of Physiology and Biophysics, Instituto de Ciencias Biomedicas, Universidade de Sao Paulo, Sao Paulo 05508, Brazil;
³Center for Integrative Brain Research, Seattle Children's Research Institute, Seattle, Washington 98101

Keywords: Breathing. MitoPark. Parkinson's disease.

Abstract:

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons in the substantia nigra (SN). In addition to the classical motor symptoms, such as tremors and bradykinesia, PD patients may also exhibit respiratory disturbances, which remain underexplored despite being the leading cause of mortality in individuals with PD. The MitoPark genetic model has been widely used to study non-motor symptoms of PD due to its slow disease progression, which closely resembles that of humans. However, respiratory deficits in this model have not yet been investigated. Therefore, this study aimed to evaluate potential respiratory deficits in the MitoPark model throughout its development. For this purpose, the rotarod test was performed to evaluate motor impairments, whole-body plethysmography and FlexiVent were used for functional analysis and respiratory mechanics, and immunohistochemistry was conducted to quantify dopaminergic neurons in the SN and the density of the neurokinin 1 receptor (NK1r) in the preBötzing complex (preBötC) (CEUA 3325170822). The results showed that MitoPark mice presented a 40% reduction in the total number of dopaminergic neurons ($p < 0.0001$) with a 59% reduction in the latency to fall on the rotarod ($p = 0.0141$). Regarding respiratory activity, a 7% reduction in respiratory rate ($p = 0.0062$) and a 23% reduction in estimated tidal volume ($p = 0.0005$) were observed in the 16th week of life of MitoPark animals at baseline. In addition, minute ventilation decreased by 24% in basal conditions ($p = 0.0003$), 20% during the hypercapnic challenge ($p = 0.0175$) and 16% during the hypoxic challenge ($p = 0.0287$). NK1r density in preBötC was 20% lower ($p = 0.0024$), while respiratory mechanics remained unchanged. In conclusion, the MitoPark model shows significant respiratory and motor deficits, linked to dopaminergic neuron loss and reduced NK1r density in the preBötC. These findings highlight the impact of Parkinson's disease on breathing.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Mitochondrial Dysfunction in Anxiety: The Impact of Early Life Stress and Hypercapnia

Authors:

Camila Migliano de OLIVEIRA¹
João Vitor Paris XAVIER¹
Derek Felipe CAMPOS¹
Luciane Helena GARGALIONI¹
Luana TENORIO-LOPES¹

Affiliations:

¹Universidade Estadual Paulista (UNESP)- Faculdade de Ciências Agrárias e Veterinárias (FCAV)

Keywords: Stress, Neonatal Maternal Separation (NMS), Hypercapnia (H), Mitochondria, Reactive Oxygen Species (ROS).

Abstract:

We previously showed that only female mice exposed to neonatal maternal separation (NMS) decrease the *in vivo* hypercapnic ventilatory response (HcVR). Further, NMS caused an increase in hypothalamic orexin, contributing to the altered HcVR. Here, we tested the hypothesis that a hypothalamic mitochondrial dysfunction plays a key role in mediating anxiety-related responses following NMS and/or hypercapnia. C57BL/6 mice were exposed to NMS (pups separated from their mothers 3 h/d, P3-12). At adulthood, control and NMS female mice (n=5-7) were exposed to 1 h normocapnia (N) or 15 min hypercapnia (H; 7% CO₂). After isoflurane euthanasia, the hypothalamus was isolated and immersed in a Biops solution for the analysis of oxygen uptake and reactive oxygen species (ROS) production through the Oroboros Oxygraph (O2k) and DatLab 2 software. A total of 0.3 mg of tissue was homogenized in MiRO5 respiration medium. 5 mM saponin was used to permeabilize cell membranes. To assess mitochondrial respiration, substrates were added to measure Leak respiration, Complex I (CI), Complex I+II (CI+II) and ETS. The integrity of the outer mitochondrial membrane was tested with 10 µM cytochrome c. Finally, 2.5 µM antimycin A was added to inhibit CIII and determine residual oxygen consumption. ROS production was measured by adding SOD, peroxidase and Amplex red. The Respiratory Control Ratio was calculated as the ratio of oxidative phosphorylation (OXPHOS) capacity (after ADP addition) to Leak respiration. Our results revealed that control mice exhibited a decrease in CI+CII activity ($P=0.03$) in response to H, indicating a reduction in OXPHOS and a strong increase in ROS production ($P<0.001$) when compared to N and NMS (during N and H) ($P<0.001$). NMS mice in H did not show increased inner membrane fluidity or ROS production, indicating a possible adaptive response to stress. Mitochondrial function during H may affect ROS production, causing cellular damage only in the control group. CEUA: 2732/2024; 8855191239/2025. Funding agency: FAPESP (Process number: 2024/20925-3).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Impact of Hypoxemia, Normoxia, and Moderate to Severe Hyperoxemia on Brain-lung Crosstalk in Experimental Acute Focal Ischemic Stroke

Authors:

Isadora Antunes Botelho¹
Giselle Carvalho de Sousa¹
Pedro Henrique Lima Conceição¹
Raquel Ferreira de Magalhães Sacramento¹
Cynthia dos Santos Samary^{1,2}
Pedro Leme Silva¹
Patrícia Rieken Macedo Rocco¹

Affiliations:

¹ Laboratory of Pulmonary Investigation, Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.

² Department of Cardiorespiratory and Musculoskeletal Physiotherapy, Faculty of Physiotherapy, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.

Keywords: Acute Focal Ischemic Stroke. Hyperoxemia. Hypoxemia. Mechanical Ventilation.

Abstract:

Introduction: Patients with acute focal ischemic stroke (AIS) often require intensive care management, including mechanical ventilation and supplemental oxygen. Observational studies suggest a "U"-shaped relationship between PaO₂ levels and mortality in AIS, yet the mechanisms driving cerebral and pulmonary damage across different oxygen levels are not well understood. This study aimed to evaluate the extent of brain and lung injury following exposure to varying PaO₂ levels in an experimental model of focal AIS. Methods: Thirty-two male Wistar rats (CEUA 131/23) underwent focal AIS induced by thermocoagulation of pial blood vessels. After 24 hours, rats were randomly assigned to one of four groups (n=8 per group): normoxia (NORMO: PaO₂ = 80-120 mmHg), hypoxia (HYPO: PaO₂ < 80 mmHg), moderate hyperoxia (MOD: PaO₂ = 121-299 mmHg), or severe hyperoxia (SEV: PaO₂ ≥ 300 mmHg). Rats were ventilated for two hours with a positive end-expiratory pressure of 1 cmH₂O and tidal volume (V_T) of 6 ml/kg. Lung function was assessed at INITIAL and FINAL of the experiment. Lungs and brains were gathered for histological analysis. Results: PaO₂ levels at FINAL were 95±15 mmHg in the NORMO group, 51±6 mmHg in the HYPO group, 184±52 mmHg in the MOD group, and 350±47 mmHg in the SEV group (p<0.001). Peak and plateau airway pressures increased over time across all groups (p<0.001), with no significant differences among groups. Diffuse alveolar damage (DAD) score was higher in MOD and SEV than NORMO groups (p=0.042, p=0.048; respectively). DAD score showed a positive and significant correlation with PaO₂ levels (r=0.48, p=0.005). In the brain, neutrophil infiltration in ischemic penumbra indicated blood-brain barrier dysfunction, according to PaO₂ levels. Conclusion: This study showed an association between lung structural changes and PaO₂ levels. In brain, it was observed neutrophil infiltration in ischemic area according to PaO₂ levels.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Incidence of Long Covid syndrome: clinical, spirometric, metabolic and inflammatory profile after recovery from SARS-Cov-2 infection.

Authors:

Camila Silva FERREIRA¹

Maria Thereza de Araújo FRAGOZO¹

Ana Beatriz Faria DE SOUZA¹

Marcelo Carlos RIBEIRO¹

Frank Silva BEZERRA¹

Affiliations:

¹ Universidade Federal de Ouro Preto

Keywords: Covid Longa. Pulmonary function. Obesity. Inflammatory profile. Metabolic profile.

Abstract:

Long Covid syndrome is characterized by the persistence of at least one symptom, such as dyspnea, for months or years after infection with SARS-CoV-2, and it is more common in women. This longitudinal study compared the spirometric, metabolic, inflammatory and clinical profile of obese and non-obese individuals with a previous diagnosis of Covid-19, with or without a history of hospitalization. This study was approved by the Research Ethics Committee of the Federal University of Ouro Preto (CEP/UFOP) under the protocol number 68165523.9.0000.5150. Our study included 40 individuals over the age of 18 with Covid-19 infection confirmed by laboratory tests. After signing the Informed Consent Form, the individuals underwent anamnesis, physical examination, pulmonary function assessment by spirometry and blood sample collection for analysis of inflammatory, oxidative and biochemical parameters. Statistical analysis was carried out using R and STATA software, with results expressed as means, standard deviations, medians and interquartile ranges. Individuals at high cardiovascular risk, identified by neck circumference (>37 cm), had higher triglyceride concentrations (173.2 mg/dL ± 83 vs. 132 mg/dL ± 110 ; $p < 0.05$). In addition, a moderate positive correlation was identified with levels of 25OH Vitamin D (0.33 ; $p = 0.03$) and body mass index (0.5071 ; $p = 0.0008$), and a weak negative correlation with levels of total complement (CH50) (-0.314 ; $p = 0.04$), when considering cardiovascular risk. Obese individuals had higher levels of glycated hemoglobin ($5.7\% \pm 1.5$ vs. $5.2\% \pm 1.04$; $p < 0.05$) and leptin (2146.5 pg/mL vs. 2021.8 pg/mL; $p < 0.05$) compared with non-obese individuals. A moderate positive correlation was observed between the presence of Long Covid symptoms and the degree of improvement in the Tiffeneau Index (FEV1/FVC ratio) (0.3645 ; $p = 0.225$) after bronchodilator use. Our preliminary results suggest that individuals with long COVID have greater pulmonary dysfunction.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: The effects of hypothalamic peptides vasopressin and oxytocin on breathing via interaction with the retrotrapezoid nucleus

Authors:

Emmanuel V. Araújo¹
Phelipe E. Silva¹
Ana C. Takakura²
Daniel K. Mulkey³
Thiago S. Moreira¹

Affiliations:

¹Department of Pharmacology and
²Department of Physiology, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil
³Department of Physiology and Neurobiology, University of Connecticut, Connecticut, USA.

Keywords: Neuropeptides. Oxytocin. Vasopressin. Breathing control. Retrotrapezoid nucleus.

Abstract:

The respiratory parafacial region, which encompasses the retrotrapezoid nucleus, (pFRG/RTN) modulates breathing to maintain arterial CO₂ levels and blood pH, significantly adjusting respiration during sleep and exercise. The paraventricular nucleus of the hypothalamus (PVN) is involved in several homeostatic processes including maintenance of metabolic, autonomic, and respiratory function. More than 30 distinct neuromodulators are synthesized by PVN neurons; most abundantly are vasopressin (VP) and oxytocin (OX), however, despite PVN projections to the pFRG/RTN, the roles of VP and OX in regulation of RTN function are largely unknown. To address this question, we evaluate whether oxytocinergic and vasopressinergic signaling influences the activity of RTN neurons in vitro and whether oxytocin can induce changes in breathing in vivo. Male or female C57B6/J, Phox2b::Cre;Ai14 or Oxt::Cre;Ai32 mice [P7-11 (neonate) or P100 (adult)] were used (CEUA- ICB/USP: nº 9750170720). At the cellular level, cell-attached recordings from RTN neurons show that exposure to the vasopressin agonist (Arg8-Vasopressin or VP: 25 nM) increased firing rate from 1.2 ± 1.2 Hz to 3.5 ± 1.2. This response was decreased by 44% in the presence of fast neurotransmitter receptor blockers. Similarly, exogenous application of the OX agonist (TGOT: 2 nM) increased activity of RTN neurons by 1.8 ± 0.5 Hz under control conditions and 2 ± 0.8 Hz in the presence of the synaptic blocker cocktail. In vivo experiments demonstrate that TGOT (1 µM - 30 nL) injection or optogenetic stimulation of oxytocinergic terminals in the RTN region increased intercostal electromyography (IntEMG) amplitude (16.3 ± 10.9% vs. saline: 2.7 ± 4.2%, and optogenetic stimulation: 18.7 ± 8.3% of baseline), but without changing IntEMG frequency. Our findings indicate that VP and OX signaling exerts an excitatory effect within the RTN neurons, and that oxytocin plays a significant role in modulating respiratory activity.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Effects of chemoreceptors on arousal from obstructive sleep apnea in rats

Authors:

Henrique Alves TEVES¹
Guus H. M. SCHOORLEMMER¹
Sergio Luiz CRAVO¹

Affiliations:

¹São Paulo Medical School, Federal University of São Paulo (EPM-Unifesp).

Keywords: Obstructive Apnea. Hypoxia. Chemoreceptors. Sleep. Hyperoxia.

Abstract:

We investigated the role of chemoreceptor reflexes in arousal from obstructive sleep apnea in rats. To induce apnea, a tracheal prosthesis containing an inflatable balloon was implanted in 5 male Wistar rats (300–400g). EEG and EMG electrodes were implanted for real time sleep staging, and FiO₂ was monitored. Apneas were induced intermittently for up to 6 h/day by inflating the balloon during sleep, interrupting airflow through the trachea. Apneas automatically terminated almost immediately when the rat awoke (judged from increased EMG activity). FiO₂ was periodically increased (35–50%) or reduced (to about 15%) by infusion of O₂ or N₂ into the cage. Apnea duration was defined as the interval between balloon inflation and the latency to arousal, indicated by an increase in the EMG signal. Means $\pm$ SD were compared using ANOVA followed by Tukey's test ($p < 0.05$) applying the Mixed Linear Mode assuming animals as the aleatory component. A total of 3236 apneas were recorded in 5 animals. Under normoxia, mean duration was 8.1 ± 3.3 s; hypoxia significantly reduced it 5.8 ± 2.1 s, and hyperoxia increased it 9.6 ± 4.5 s. Under NREM sleep a total of 2703 apneas were recorded. In normoxia, apneas made during NREM sleep lasted 7.8 ± 3.4 s. Apnea duration fell to 5.8 ± 2.3 s with apneas made in hypoxia and increased to 9.5 ± 4.5 s when apneas were made during hyperoxia, indicating that the latency to arousal from sleep increased when FiO₂ increased ($p < 0.05$). With apneas made in REM sleep ($n = 533$), a similar pattern was observed (hypoxia: 6.0 ± 1.9 s; normoxia: 8.9 ± 3.4 s; hyperoxia: 12.9 ± 5.1 s), but the effect of FiO₂ on apnea duration was larger ($p < 0.05$). Preliminary data in two chemoreceptor-denervated rats showed that apnea duration was longer than in controls, but the modulation by O₂ concentration persisted. These findings suggest that arousal from obstructive apnea is mediated by multiple mechanisms, with chemoreceptors playing an important, though not exclusive, role.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Mechanisms underlying MuRF1 inhibition mediated protection against disuse-induced diaphragm dysfunction and atrophy

Authors:

Fernando RIBEIRO¹
Paulo Roberto JANNIG²
Siegfried LABEIT³
Anselmo Sigari MORISCOT¹

Affiliations:

¹ Department of Anatomy, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil
² Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden
³ DZHK Partner Site Mannheim-Heidelberg, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany

Keywords: Muscle wasting. Respiratory muscle. TRIM63. Ubiquitin-proteasome pathway.

Abstract:

Background: Disuse-induced diaphragmatic dysfunction (DIDD) is characterized by the rapid and progressive loss in diaphragm mass and contractile function. Patients undergoing prolonged mechanical ventilation or phrenic nerve injury commonly develop DIDD. Muscle RING-finger protein-1 (MuRF1) is thought to play a crucial role in DIDD pathophysiology. Aim: To investigate the effect of small-molecule mediated inhibition of MuRF1 upon DIDD in an experimental model. Methods: Wistar rats were allocated into three groups ($n=8$): Sham 12h, Denervated 12h + vehicle, and Denervated 12h + MuRF1 inhibitor (205). Unilateral diaphragm denervation was induced and intravenous delivery of either vehicle or MuRF1 inhibitor (50 mg/kg bw) was executed over 12h. Diaphragm muscle was collected for functional, morphometric, transcriptomic profiling (RNA-seq), and protein level analysis. Statistical significance was accepted as $p < 0.05$ (CEUA ICB#8728030320 and #5143091020). Results: MuRF1 inhibition protected against diaphragm contractile dysfunction ($\sim 50\%$, $p < 0.05$) and fibers atrophy ($\sim 20\%$, $p < 0.05$) compared with vehicle. RNA-seq analysis shows 1606 differentially expressed genes (DEGs) in denervated + vehicle versus Sham. 205-treated animals showed 72 DEGs compared with vehicle. Gene set enrichment analysis (GSEA) revealed that MuRF1 inhibition promoted the upregulation of RNA processing, cell survival and growth, ribosome biogenesis and unfolded protein response pathways. At the protein level, PI3K-AKT-MTOR signaling markers were upregulated in response to MuRF1 inhibition. On the other hand, 205 treatment downregulated some pro-atrophic enzymes (e.g. HDAC4, FOXO1 and MuRF2), genes involved with muscle remodeling (e.g. collagen deposition and adipogenesis), mitigating intramuscular lipid accumulation. Conclusion: MuRF1 inhibition protects against early DIDD by enhancing pro-survival and growth factors while suppressing pathways linked to muscle atrophy, fat deposition and fibrosis.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Repeated Seizures Attenuate CO₂ Chemoreception in Sleep and Wakefulness in Wistar Rats due to partially reduced RTN sensitivity

Authors:

Hanna Lury UMEZU¹
Dafne Monteiro
Carvalho FREIRE¹
Vitória Silva
OLIVEIRA¹
Victor Rodrigues SANTOS²
Glauber dos Santos Ferreira DA SILVA¹

Affiliations:

¹Departamento de Fisiologia e Biofísica, ICB, Universidade Federal de Minas Gerais
²Departamento de Morfologia, ICB, Universidade Federal de Minas Gerais

Keywords: CO₂ chemoreception. Repeated seizures. RTN. PTZ.

Abstract:

In epilepsy, SUDEP (Sudden Unexpected Death in Epilepsy) is the leading cause of death in patients with uncontrolled seizures. Importantly, evidence suggests that SUDEP patients have reduced ventilatory response to CO₂ (HCVR). This study aimed to evaluate HCVR during sleep and wakefulness in Wistar rats after repeated seizures induced by PTZ (pentylene-tetrazol). Adult Wistar rats (CEUA nº279/2022) received PTZ (40 mg/kg, i.p.) or vehicle on alternating days (10 injections). After which, animals underwent surgery to implant EEG and neck EMG electrodes to identify sleep-wake states. In a second experiment, with EEG and EMG, a microdialysis cannula was implanted in the retrotrapezoid nucleus (RTN) for focal acidification. The RTN is a well-documented central CO₂ chemoreceptor. Respiratory frequency (FR), tidal volume (VT), and ventilation (VE) were recorded during hypercapnia (7% CO₂) in the first experiment, and during RTN acidification (aCSF equilibrated with 25% CO₂) in the second, during sleep and wakefulness. Data are expressed as relative from baseline (%), mean ± SEM. First, the repeated seizures elicited an attenuated HCVR during sleep and wakefulness (PTZ n=12; control n=6). During wakefulness, PTZ animals had lower VE responses to CO₂ (243.8% ±14.1 vs 335.1% ±10.5; p<0.0001), with reduced FR (131.4% ±5.7 vs 166.3% ±7.1; p<0.0001) and no changes in VT. During sleep, all variables were attenuated in PTZ animals: FR (144.8% ±6.1 vs 167.4% ±4.4; p=0.006), VT (186.4% ±7.5 vs 222.8% ±9; p=0.001), and VE (273.5% ±19.8 vs 373.8% ±15.2; p=0.0003). To investigate the involvement of a specific central chemoreceptor site, we performed focal acidification in the RTN. The RTN acidification increased VE in control group (n=8) in a state-dependent manner. PTZ animals (n=9) showed reduced VE only in wakefulness (114.2% ±5.8 vs 128.6% ±5.9; p=0.04). These results suggest PTZ-induced seizures impair CO₂ chemoreception, partially due to reduced RTN sensitivity. Financial Support: FAPEMIG, CNPq, CAPES



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Selective optogenetic stimulation of the vagus nerve prevents respiratory dysfunction and astrogliosis in a Parkinson's disease model

Authors:

Laís Maria Cardoso CABRAL¹
Thiago dos Santos MOREIRA²
Lauro Casqueiro VIANNA³
Ana Carolina TAKAKURA¹

Affiliations:

¹ Department of Pharmacology, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil

² Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil

³ Faculty of Physical Education, University of Brasília, Brasília, DF, Brazil

Keywords: 6-OHDA. Breathing. Neuroinflammation. Parkinson's Disease. VNS.

Abstract:

Neuroinflammation plays a pivotal role in neuronal death in Parkinson's Disease (PD). In the 6-hydroxydopamine (6-OHDA) model of PD, glial-mediated neuroinflammation contributes to the degeneration of respiratory nuclei, including the preBötzinger complex (preBötC) and the retrotrapezoid nucleus (RTN), leading to respiratory deficits. Vagus nerve stimulation (VNS) has been shown to reduce the production of pro-inflammatory cytokines through the cholinergic anti-inflammatory reflex and may represent a strategy for PD prevention. To investigate the effects of stimulating the afferent and efferent portions of the vagus nerve (X) in the 6-OHDA model, VGLUT-cre: Ai32 or Chat-cre: Ai32 mice (CEUA nº 4061131021) underwent stereotaxic surgery for striatal injection of 6-OHDA (10 µg/µl) or vehicle. Five days post-surgery, the right X was isolated and stimulated with blue light (5Hz, 10 min). Another five days later, diaphragmatic activity was recorded via electromyography, followed by perfusion and immunohistochemical analysis of the brain. Diaphragmatic activity analysis revealed that the 18.4% (VGLUT-cre: Ai32) and 28.6% (Chat-cre: Ai32) reduction in diaphragm firing frequency observed in the 6-OHDA group was prevented in animals receiving stimulation of the afferent ($p=0.01$) and efferent ($p=0.01$) portions of X, respectively. No significant differences were observed in firing amplitude (VGLUT-cre: Ai32 $p=0.37$, Chat-cre: Ai32 $p=0.16$), inspiratory time (VGLUT-cre: Ai32 $p=0.31$, Chat-cre: Ai32 $p=0.23$), or expiratory time (VGLUT-cre: Ai32 $p=0.56$, Chat-cre: Ai32 $p=0.85$). Although X stimulation did not prevent the 81% loss of tyrosine hydroxylase-positive neurons in the substantia nigra pars compacta ($p<0.0001$), efferent X stimulation prevented astrogliosis in this region ($p=0.02$) and reduced local astrocytic density in the RTN ($p=0.02$). These findings underscore the potential of VNS as a promising therapeutic strategy for alleviating respiratory deficits in this PD model.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Early and severe respiratory impairments in aged model of Parkinson's disease

Authors:

Taina Oliveira MACEDO¹
Maria Aparecida OLIVEIRA¹
Thiago dos Santos MOREIRA²
Ana Carolina TAKAKURA¹

Affiliations:

¹ Department of Pharmacology, Instituto de Ciencias Biomedicas, Universidade de Sao Paulo. São Paulo, SP, Brazil

² Department of Physiology and Biophysics, Instituto de Ciencias Biomedicas, Universidade de Sao Paulo. São Paulo, SP, Brazil

Keywords: Parkinson's Disease. 6-OHDA. Breathing. Aging.

Abstract:

Parkinson's Disease (PD) is primarily characterized by the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and motor impairments, but it also leads to respiratory deficits. Aging is a major PD risk factor, may worsen these deficits. This study assessed the impact of aging on respiratory dysfunction in a PD murine model induced by bilateral 6- hydroxydopamine (6-OHDA; 10 µg/µl) striatal injections. We used adult (3 months) and aged (>18 months) C57BL/6 mice, plus hypomorphic Klotho mice, which exhibit accelerated aging (CEUA: 4476210224). After lesion induction, whole-body plethysmography assessed respiratory function. Histological analysis confirmed more than 70% SNpc dopaminergic neuron loss in 6-OHDA groups, validating the model. At baseline, aged mice exhibited a lower respiratory rate than adults (160.7±2.7 vs. 175.5±1.8 breaths/min; P=0.003). In aged PD mice, this reduction became evident as early as the 4th day post-lesion (148.2±1.6 vs. 158.2±2.4 breaths/min; P=0.0202), whereas in adults, respiratory deficits emerged only on the 8th day (164.3±2.5 vs. 176.4±2.6 breaths/min; P=0.0226). The hypercapnic ventilatory response was also significantly lower in aged mice (p<0.001), with an even greater reduction in aged PD mice by the 6th day (P=0.0367). Additionally, aged mice showed a decrease in NK1r density in the preBötzinger complex and rostral ventral respiratory group, along with reduced Phox2b⁺ neurons in the retrotrapezoid nucleus, with these deficits being more pronounced in aged PD mice. Notably, Klotho mice displayed a respiratory profile similar to naturally aged mice. Peripheral respiratory parameters differed between aged and adult mice, with changes in elastance, tissue, and airway resistances (P<0.05). These findings indicate that aging exacerbates respiratory deficits in this PD animal model, leading to earlier and more severe impairments compared to adults.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Physical Exercise Prevents Cardiorespiratory Deficits and Neurodegeneration in the 6-OHDA Model of Parkinson's Disease

Authors:

Pamela Ojuorun Santos MEDEIROS¹
Luiz Fernando de Araujo Trindade PEDRÃO¹
Bárbara FALQUETTO¹

Affiliations:

¹ Department of Pharmacology, Institute of Biomedical Science, University of São Paulo. São Paulo, Brazil.

Keywords: Cardiorespiratory deficits. Neural control of breathing. Parkinson's Disease. Physical exercise

Abstract:

Parkinson's disease (PD) is a neurodegenerative disease marked by the progressive loss of dopaminergic neurons in the Substantia Nigra (SN). In addition to its hallmark motor symptoms, PD also presents cardiorespiratory deficits. In the 6-hydroxydopamine (6-OHDA) PD animal model, neurodegeneration extends to cardiorespiratory regions, leading to significant impairments in cardiorespiratory function. This study aimed to evaluate whether physical exercise (EX) can prevent neurodegeneration in cardiorespiratory nuclei and mitigate cardiorespiratory deficits in 6-OHDA animals. Male adult rats received intrastriatal injections of either 6-OHDA (24mg/ml) or vehicle (CEUA: 1342290421). EX was initiated 12 days after PD induction and continued for 28 days. On the 40th day, animals underwent whole-body plethysmography, vein and arterial cannulation, baroreflex sensitivity assessments (mean arterial pressure – MAP, and heart rate – HR), perfusion, and brain dissection. 6-OHDA administration significantly reduced the number of TH+ neurons in the SN, and EX failed to reverse this effect, confirming the PD model ($p < 0,0001$). At normoxia, 6-OHDA animals showed a reduced respiratory frequency (fR), which was prevented by EX ($p < 0,0001$). This protective effect of EX was also observed under hypercapnia ($p = 0,0062$). Additionally, EX prevented the reduction of phox2b-expressing neurons in cNTS ($p < 0,0001$), iNTS ($p < 0,0001$), and RTN ($p = 0,0003$), as well as the loss of NK1R-expressing neurons in rVRG and preBotC, and in ChAT+ neurons in NA ($p < 0,0001$). Baroreflex sensitivity was impaired in the sedentary 6-OHDA animals, but EX preserved this function (Phenylephrine – MAP: $p < 0,0001$; HR: $p < 0,0001$; Sodium nitroprusside – MAP: $p < 0,0001$; HR: $p < 0,0001$). EX effectively prevented cardiorespiratory impairments and neurodegeneration in the 6-OHDA PD model, highlighting its potential as a non-pharmacological intervention for preserving autonomic and respiratory function.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Adenosine A_{2A} receptors modulate respiratory responses to hemoreflex activation in mice exposed to 2 hours of sustained hypoxia

Authors:

Luiz Henrique César VASCONCELOS¹
Karla Lima RODRIGUES¹
Juliana Reis SOUZA¹
Benedito Honório MACHADO¹

Affiliations:

¹Department of Physiology, School of Medicine of Ribeirão Preto, University of São Paulo

Keywords: Breathing. Chemoreceptors. Hypoxia. Purinergic.

Abstract:

In previous studies, we documented that adenosine A_{2A} receptors modulate baseline respiratory frequency in awake mice. Herein, we evaluated the impact of two hours of sustained hypoxia (2h-SH) on phrenic nerve discharge (PND) in wild-type balb/C (WT) and A_{2A} knockout (KO) mice in response to central and peripheral chemoreflex activation. Frequency of PND was recorded in baseline and during peripheral or central chemoreceptor activation in mice (6-8 weeks old) previously exposed to 2h-SH (FiO₂:0.10), using the *in situ* working heart-brainstem preparation. Peripheral chemoreflex was activated with KCN (i.v.), and central chemoreceptors by hypercapnia (10%CO₂). Institutional ethical committee approved the experiments (CEUA #070/2021). In normoxia, baseline PND of A_{2A} KO mice was higher than WT (WT=0.27±0.09Hz, n=17; A_{2A} KO=1.76±0.50Hz, n=11), indicating that A_{2A} receptors modulate baseline PND. Peripheral chemoreceptors activation increased PND in WT (ΔPND=0.65±0.22Hz, n=18), but reduced in A_{2A} KO (ΔPND=-0.45±0.73Hz, n=13). In response to hypercapnia, PND was not altered in WT (5%CO₂=0.27±0.09Hz, n=17; 10%CO₂=0.30±0.12Hz, n=18), but it was reduced in A_{2A} KO (5%CO₂=1.76±0.50Hz, n=11; 10%CO₂=0.38±0.24Hz, n=12), indicating that A_{2A} receptors modulate the respiratory responses to both peripheral and central chemoreceptors activation. When exposed to 2h-SH, baseline PND of A_{2A} KO was higher than WT (WT=0.28±0.18Hz, n=13; A_{2A} KO=1.66±0.43Hz, n=11). Peripheral chemoreceptors activation increased PND in WT (ΔPND=0.58±0.31Hz, n=11), but reduced in A_{2A} KO (ΔPND=-0.66±0.50Hz, n=11). In response to hypercapnia, PND was not altered in WT (5%CO₂=0.28±0.18Hz, n=13; 10%CO₂=0.15±0.07Hz, n=11), but it was reduced in A_{2A} KO (10%CO₂=1.66±0.43Hz, n=11; 10%CO₂=0.38±0.25Hz, n=11), indicating SH does not modify respiratory frequency. We conclude that adenosine A_{2A} receptors modulate baseline respiratory frequency and the respiratory responses to chemoreflex activation.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Progesterone and etonogestrel combined with estradiol increase ventilatory responses to hypercapnia in female rats

Authors:

Vitoria Silva OLIVEIRA¹
Beatriz Rodrigues APGAUA¹
Paloma Grazielle BITTENCOURT- SILVA²
Hanna Lury UMEZU¹
Ana Clara CAMPIDELI-SANTANA¹
Roberta ARAÚJO-LOPES¹
Raphael Escorsim SZAWKA¹
Glauber Santos Ferreira DA SILVA¹

Affiliations:

¹ Federal University of Minas Gerais, Department of Physiology and Biophysics, Belo Horizonte, Brazil

² Marquette University, Milwaukee, Wisconsin, EUA

Keywords: Hypercapnia. NTS. Progesterone. Progestin. Female rats.

Abstract:

Evidence points to the effects of progesterone (P4) and progestin (ETO) on the ventilatory responses to CO₂. We tested: i-the effects of hormonal replacement with P4 or ETO alone (P4;2.5mg/0.2 mL/animal, s.c.) and combined with estradiol (E2;10µg/0.1 mL/animal, s.c.) in the hypercapnic ventilatory responses, and ii- the role of the NTS as possible brainstem region involved in the effects. Ovariectomized (OVX) (200-270g) female rats were divided into two groups: 1) received pre-treatment with E2 (n=10) and 2) received pre-treatment with oil (n=9). Both groups received a one-day and three-day treatment with P4. Ventilatory measurements were taken: 1) in diestrus, before treatment; 2) after pre-treatment, 3) 2h after 1-day treatment, and 4) one day after 3-day treatment. To test the effects of ETO we divided the animals into 5 groups: 1) oil (n=8); 2) 0.25µg/0.1 (n=9); 3) 2.5µg/0.1 (n=9); 4) 250µg/0.1 (n=9); and 5) 250µg/0.1 mL/ animal+E2 (n=8). The female rats were treated (21 days) and submitted to the ventilatory measurements. Immunohistochemistry was performed to assess progesterone receptors (nPR) in OVX rats treated for 3 days with E2. Finally, focal acidification of the NTS was performed (microdialysis), in animals treated with P4+E2 (CEUA 21/2018). Our results show that treatment with P4+E2 and ETO+E2 significantly enhanced the ventilatory response to CO₂ (%VE 309.9±19.6 and 294.3±9.02%), while treatment with P4 or ETO alone had no effect. The animals treated with E2 increased the expression of nPR in the NTS and focal NTS acidification promoted an enhanced increase (p<0.05) in ventilation (136.99±11.07%) in animals treated with P4 and E2. In conclusion, the increase in ventilatory response to CO₂ suggests that both hormonal replacements (P4 and ETO+E2) modulate an excitatory response of the respiratory adjustments to hypercapnia. Additionally, we propose that E2 is necessary for the effects of P4 in the ventilatory responses to CO₂, and the NTS plays a role.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Optogenetic activation of somatostatinergic interneurons increases breathing in mice

Authors:

Nathalia Salim¹

Renato Willian Martins Sá¹

Davi José de Almeida Moraes²

Affiliations:

¹ Department of Physiology, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto/SP - Brazil.

² Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, São Paulo/SP - Brazil.

Keywords: Optogenetic. Parafacial region. Somatostatinergic interneurons. Ventilatory

Abstract:

The parafacial region (pFL) contains somatostatinergic (Sst) interneurons, but their role in controlling pulmonary ventilation remains unclear. We hypothesized that the pFL Sst interneurons are inhibitory and control ventilation. To test this, we used genetically modified mice (CEUA 1142/2022) expressing Cre recombinase in Sst neurons, and injected, into the pFL region, a combination of Cre- and FLP-dependent viruses to express channelrhodopsin (ChR2) in pFL Sst interneurons. To evaluate the phenotype, we crossed Sst-Flp, Vgat-Cre, Ai65F and Ai6 mice to express GFP in inhibitory neurons and td-Tomato in Sst neurons. For optogenetic experiments, an optical fiber was implanted to deliver light to the target area, and we then measured respiratory frequency (fR), tidal volume (VT), pulmonary ventilation (VE), and the respiratory cycle duration, inspiration (DI), and expiration (DE) during normocapnia and hypercapnia (7% CO₂) in unanesthetized mice. To activate Sst neurons, a 473 nm laser (10 mW, 20 Hz) was used for 5, 20, or 60 seconds. Data are expressed as mean ± standard deviation. During normocapnia, optogenetic activation of pFL Sst neurons for 5s (n=8), 20s (n=6), and 60s (n=6) increased fR (p=0.008; p=0.01; p=0.02), VT (p=0.0002; p=0.001; p=0.0006), and VE (p=0.001; p=0.005; p=0.005), while decreased the respiratory cycle duration (p=0.001; p=0.009; p=0.006) and DI (p=0.007; p=0.004; p=0.005), without affecting DE. During hypercapnia, similar effects were observed with the 5s protocol (n=6) (fR p=0.01; VT p=0.01; VE p=0.006; respiratory cycle duration p=0.02; DI p=0.008; DE p=0.2). The 20s protocol (n=6) increased VT (p=0.03), VE (p=0.03), and decreased DI (p=0.03), without affecting other parameters, while the 60s protocol (n=6) affected only DI (p=0.03). We also found that all Sst neurons in pFL region are inhibitory (n=4). We conclude that pFL Sst interneurons are inhibitory and control pulmonary ventilation of mice during normocapnia and hypercapnia.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Role of BK channels in the respiratory response to CO₂/pH during development in male and female mice

Authors:

Bianca de Avila MARTINS¹
Luis Gustavo Alexandre PATRONE¹
Mariana Bernardes RIBEIRO¹
Kênia Cardoso BÍCEGO¹
Luciane Helena GARGAGLIONI¹

Affiliations:

¹Dept of Animal Morphology and Physiology, São Paulo State University, FCAVJ, Brazil

Keywords: Channelopathy. Chemoreception. Development. Sex difference. Ventilation.

Abstract:

Large-conductance Ca²⁺-activated K⁺ (BK) channels are widely expressed in mammalian tissues, suppressing action potential excitability and vesicular signaling molecule release. In the central nervous system (CNS), BK channels also provide a negative feedback mechanism to regulate intracellular Ca²⁺ levels, modulating the firing rate (FR). However, the role of these channels in the respiratory response to hypercapnia, including potential sex differences, remains unknown. To investigate this, 39 mice (CEUA – protocol nº9486/22), 15 males and 24 females, were divided into three genotypes: knockout (BKKO; n=13), heterozygous (HET; n=16) and wildtype (WT; n=10). Animals were monitored from postnatal day (P)0 to P80. Ventilation (VE), tidal volume (VT), respiratory rate (fR), O₂ consumption (VO₂) and air convection requirement (VE/VO₂) were recorded under normocapnia and hypercapnia (7% CO₂) at P0, P6, P12, P30 (juvenile) and P80 (adult), using whole-body plethysmography. Data were analyzed by two-way ANOVA comparing genotypes and exposure conditions. In females, BKKO mice showed increased VE under hypercapnia at several stages of development. At P6, VE was 5.09±0.33 vs. WT 4.04±0.55 and HET 3.70±0.49 mL·g⁻¹·min⁻¹. At P12, VE was 6.47±1.02 vs. HET 5.09±0.79. At P30, VE remained higher (11.93±0.57 vs. WT 10.51±1.59). VO₂ was also elevated at P80 in BKKO females (0.204±0.036 vs. WT 0.122±0.029 and HET 0.117±0.048 mL·g⁻¹·min⁻¹). In males, two significant effects were observed under hypercapnia. At P12, BKKO mice had increased VT (0.023±0.008 vs. WT 0.014±0.005 mL·kg⁻¹). At P30, VO₂ was higher in BKKO (0.114±0.004 vs. WT 0.066±0.008 and HET 0.059±0.024 mL·g⁻¹·min⁻¹). These findings demonstrate that BK channels significantly modulate ventilatory and metabolic responses to CO₂, with a clear influence of sex and age, indicating that the role of BK channels in respiratory control is both developmentally and sexually regulated.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Post-natal development of hypoxic ventilatory response in normotensive and spontaneously hypertensive rats

Authors:

Beatriz N Vieira¹
Emilson Donizete PEREIRA-JUNIOR¹
Milede Hanner Saraiva PAES¹
Daniel B. ZOCCAL¹

Affiliations:

¹Department of Physiology and Pathology, School of Dentistry, São Paulo State University (UNESP), Araraquara, SP, Brazil.

Keywords: breathing, hypertension, hypoxia, postnatal.

Abstract:

Introduction: The carotid body chemoreceptors (CB) are immature at birth and undergo a maturational process during the first weeks of life, enhancing their sensitivity to oxygen (O₂). Spontaneously hypertensive rats (SHR), a model for neurogenic hypertension, display increased CB sensory activity, which appears to contribute to the maintenance of elevated blood pressure levels in adulthood. Objective: This study analyzed the hypoxic ventilatory response (HVR) in normotensive and SHR animals during postnatal development. Methods: Pulmonary ventilation (VE), respiratory frequency (fR), and tidal volume (VT) were assessed in normotensive (Holtzman, n=14; and WKY, n=12) and SHR rats (n=18) at different ages (P0-2, P4-6, and P10-12) under normoxic and hypoxic conditions (10% O₂ for 10 minutes). Data analyses: 2-way ANOVA, Bonferroni's post-test. Ethical approval: CEUA 02/2023. Results: At P0-2 age, hypoxia exposure caused similar rapid increases in the VE of NT and SHR (Δ VE: 20-60%, $P < 0.05$). As the hypoxia condition continued, this initial hypoxic-induced response was followed by a depression that fell below baseline values, similarly in both groups (Δ VE: -25 to -10%, $P < 0.05$). This secondary reduction occurred due to a progressive decrease in fR during the exposure period while VT remained higher (Δ fR: -39 to -30 %, $P < 0.05$ and Δ VT: 10-75%, $P < 0.05$). As the animals aged, the NT group exhibited a gradual increase in the magnitude of increase HVR ($P < 0.05$) due to larger increases in VT ($P < 0.05$). In contrast, the magnitude of the HVR in P4-6 and P10-12 SHRs was similar to P0-2 animals ($P > 0.05$). This occurred because, unlike the NT group, VT changes during hypoxia markedly depressed P4-6 and P10-12 SHR animals. In both groups, at older ages (P4-12) fR remains unchanged during exposure to hypoxia. Conclusions: Our findings suggest that the postnatal maturation of CB is impaired in SHRs, reducing their ability to mount an appropriate ventilatory response to hypoxia. The consequences of the depression in response to hypoxia during postnatal life development in the context of arterial hypertension remain to be fully elucidated.

Apoio Financeiro: FAPESP, CNPq. Código do Comitê de Ética: 02/2023.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Effects of chronic caffeine treatment on pulmonary ventilation and arterial blood pressure in spontaneously hypertensive rats

Authors:

Letícia Rodrigues MENDES¹
Andressa Bárbara EVANGELISTA²
Beatriz Nunes VIEIRA³
Emilson Donizete PEREIRA-JUNIOR⁴
Milede Hanner Saraiva PAES⁵
Pedro Lourenço KATAYAMA⁶
Daniel Breseghello ZOCCAL⁷

Affiliations:

¹São Paulo State University (UNESP)

Keywords: Breathing. Hypertension. Postnatal. Caffeine.

Abstract:

Introduction: Breathing irregularities are common during early postnatal life and can lead to hypoxic events in newborns. Spontaneously hypertensive rats (SHR), a widely used model of essential hypertension, exhibit respiratory disturbances and hypoxemia at birth, which are considered risk factors for the development of elevated arterial blood pressure (ABP) in adulthood. Caffeine, a respiratory stimulant, is routinely administered to neonates experiencing apneic episodes to mitigate these risks. Objective: This study aimed to investigate the effects of chronic caffeine administration during the early postnatal period on pulmonary ventilation and ABP in normotensive (NT) and SHR rats. Methods: NT (Sprague-Dawley) and SHR pups received daily subcutaneous injections of either caffeine (15 mg/kg) or vehicle (saline) from postnatal day 0 (P0) to 15 (P15). Resting pulmonary ventilation was measured at P8, while ABP was evaluated in adulthood (P80–P90). Data were analyzed using one-way ANOVA followed by Bonferroni's post hoc test. Ethical approval was obtained from CEUA (protocol no. 02/2023). Results: At P8, caffeine-treated SHR ($n = 15$) demonstrated higher baseline pulmonary ventilation (1901 ± 399 vs. 1528 ± 192 ml/kg/min, $P < 0.05$) compared to vehicle-treated SHRs ($n = 6$) due to increased tidal volume. Caffeine treatment did not affect resting pulmonary ventilation in normotensive rats ($n = 6$) relative to saline-treated NTs ($n = 6$), although it increased respiratory frequency (186 ± 5 vs. 152 ± 20 bpm, $P < 0.05$). In adulthood, SHRs that received postnatal caffeine exhibited lower ABP levels compared to the vehicle-treated SHR group (117 ± 14 vs. 134 ± 17 mmHg, $P < 0.05$). In contrast, caffeine treatment did not alter ABP in normotensive animals (97 ± 11 vs. 102 ± 8 mmHg). Conclusions: Our findings indicate that postnatal caffeine treatment improves pulmonary ventilation in neonate SHRs and attenuates the blood pressure levels in adult life, supporting the idea that postnatal hypoxemia is a significant risk factor for developing essential hypertension. Additional studies are required to deepen the understanding of the mechanisms underlying caffeine's beneficial effects in this experimental model.

Apoio Financeiro: FAPESP, CNPq. Código do Comitê de Ética: 02/2023.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Cardioprotective effects of extracellular vesicles obtained from hypoxia-preconditioned mesenchymal stem cells in experimental pulmonary arterial hypertension

Authors:

Clara Rocha Souza PASTOR¹,
Renata Trabach SANTOS¹,
Cássia Lisboa BRAGA¹,
Maria Eduarda de Sá Freire ONOFRE¹,
Carla Medeiros da SILVA¹,
Nazareth de Novaes ROCHA¹,
Rodrigo Gonzaga VERAS¹,
Douglas Esteves TEIXEIRA²,
Beatriz Toja MIRANDA³,
Miria Gomes PEREIRA⁴,
Christina Maeda TAKIYA⁵,
Monique Ramos de Oliveira GARCIA⁶,
Celso Caruso NEVES²,
Patricia Rieken Macêdo ROCCO¹,
Fernanda Ferreira CRUZ¹,
Pedro Leme SILVA¹

Affiliations:

¹ Laboratory of Pulmonary Investigation, Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro, Rio de Janeiro / RJ, Brazil ² Laboratory of Biochemistry and Cell Signaling, Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro, Rio de Janeiro / RJ, Brazil ³ Laboratory of Cellular and Molecular Cardiology, Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro, Rio de Janeiro / RJ, Brazil ⁴ Laboratory of Cell Ultrastructure Hertha Meyer, Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro, Rio de Janeiro / RJ, Brazil ⁵ Laboratory of Immunopathology, Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro, Rio de Janeiro / RJ, Brazil ⁶ Toxinology Laboratory, Center for Technological Development Health, Oswaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro / RJ, Brazil

Keywords: Cardiac tissue. Extracellular vesicles. Hypoxia. Proteomic. Pulmonary arterial hypertension

Abstract:

Pulmonary arterial hypertension (PAH) increases vascular resistance, leading to right ventricular (RV) overload and heart failure. Current vasodilator therapies have limited effects on vascular remodeling. Bone marrow-derived mesenchymal stem cells (BM-MSCs) may attenuate this process; however, clinical use is limited by low potency and high cell requirements. Hypoxia preconditioning can enhance and modify the extracellular vesicle (EV) content of BM-MSCs. This study evaluated the effects of EVs from normoxia- (EV-N) and hypoxia-preconditioned (EV-H) BM-MSCs on cardiac tissue in experimental PAH. BM-MSCs from male Wistar rats (CEUA 043/22) were cultured under normoxia (21% O₂) or hypoxia (1% O₂) for 48 h. EVs were isolated by ultracentrifugation and analyzed by proteomics. Rats received monocrotaline (60 mg/kg) or saline (CTRL). On day 14, they were treated with saline, EV-N, or EV-H after PAH confirmation. On day 28, echocardiography, RV puncture, and heart collection were performed. A total of 695 proteins were identified: 203 unique to EV-H, 51 to EV-N, and 441 shared. EV-H was associated with MAPK inhibition and apoptosis suppression, while EV-N was linked to MAPK activation and hypertrophic cardiomyopathy. The PAT/PET ratio was lower in PAH (0.25±0.01) vs. CTRL (0.40±0.01) and increased only with EV-H (0.30±0.01). RV outflow diameter decreased only in the EV-H group. RV systolic pressure was



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

higher in PAH (39 ± 2 mmHg) vs. CTRL (22 ± 2 mmHg) and reduced by EV-N (32 ± 5 mmHg) and EV-H (29 ± 4 mmHg). Expression of c-Myc decreased with both therapies, but only EV-H significantly reduced p-GSK3 β -Ser9. These findings reinforce the therapeutic potential of MSC-derived EVs in PAH and suggest that hypoxic preconditioning enhances their effects. Extracellular vesicles from hypoxia-preconditioned MSCs exhibit superior cardioprotective effects in experimental PAH, with relevant functional, molecular, and hemodynamic benefits.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Impacts on function and vascular remodeling of combined sildenafil and mitochondrial transplantation (mitotherapy) in rats with pulmonary arterial hypertension

Authors:

Julia Dalvi BATISTA¹,
Maria Eduarda de Sá Freire ONOFRE²,
Renata Trabach SANTOS³,
Dayene de Assis Fernandes CALDEIRA⁴,
Johnatas Dutra SILVA⁵,
Monique Martins MELO⁶,
Mayck Medeiros Amaral da SILVA⁷,
Jéssica Alexandre de Souza FIGUEIREDO⁸,
Clara Rocha Souza PASTOR⁹,
Rodrigo Gonzaga VERAS¹⁰,
Miria Gomes PEREIRA¹¹,
Fernanda Ferreira CRUZ¹,
Patrícia Rieken Macêdo ROCCO¹,
Pedro Leme SILVA¹

Affiliations:

¹Laboratory of Pulmonary Investigation, Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro / RJ, Brazil

²Hertha Meyer Cell Ultrastructure Laboratory, Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro / RJ, Brazil

Keywords: Mitochondrial transplantation. Pulmonary arterial hypertension. Vascular remodeling

Abstract:

During pulmonary hypertension (PH) development, endothelial and smooth muscle cells may acquire a proliferative phenotype due to metabolism disorder, so-called Warburg effect. To halt this effect, it was hypothesized that the delivery of exogenous mitochondria (mitotherapy) combined with current therapy (PDE-5 inhibitor sildenafil) may lead to normalization of metabolism with relevant outcomes to right ventricular (RV) function in experimental PH. Forty male Wistar rats (CEUA 159/21) were divided into CTRL and PH groups. On day 14, PH rats received saline, sildenafil (SIL), mitochondria (MITO) or the combination (SeM). On day 28, cardiac function and pulmonary vascular histology were analyzed. RV outflow tract diameter decreased in SIL (0.31 ± 0.05 cm, $p=0.002$) and SeM (0.32 ± 0.05 cm, $p=0.01$) compared with PH group. RV systolic pressure decreased in SIL (36 ± 8 mmHg, $p=0.009$), MITO (32 ± 13 mmHg, $p=0.01$), and SeM (30 ± 4 mmHg, $p=0.006$) compared with PH group (52 ± 8 mmHg). Arteriolar α -actin was higher in PH ($58 \pm 5\%$) than CTRL ($19 \pm 4\%$, $p<0.001$), while decreased in MITO ($29 \pm 4\%$, $p<0.001$) and SeM groups ($26 \pm 3\%$, $p<0.001$). The endothelial-mesenchymal transition was inverted in SeM group, decreasing vimentin ($p=0.006$) whereas increasing VE-cadherin expression ($p=0.002$). Bcl-2 expression decreased in SIL and SeM compared with PH group. Caspase-3 expression reduced in those animals treated with MITO, achieving similar levels compared to CTRL, while no changes in caspase-9 was observed. Complex I activity increased in SIL and MITO ($p=0.04$ for both), while complex IV activity increased in MITO ($p<0.001$), suggesting protection by mitotherapy. Mitotherapy, alone or in combination with sildenafil, optimized cardiac function variables, attenuated pulmonary vascular remodeling and restored mitochondrial function. The mechanism may rely on endothelial-mesenchymal transition.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Potential effects of Lippia Grata plant extract complexed in β -cyclodextrin in a mice model of pulmonary emphysema

Authors:

Pablo Galasso MENDONÇA¹,
Ellen Games Jacob PEREIRA¹,
Stephanie CASTRO¹,
Valéria Cunha MOREIRA¹,
Patrícia Santos Carvalinho LOPES¹,
Beatriz Soares SILVA¹,
Adriana Prado ZANELLA¹,
Rodrigo Portes URESHINO^{2,3},
Carolina Prado de França CARVALHO¹,
Fernanda Degobbi Tenorio dos Santos LOPES⁴,
Lucindo José QUINTANS-JÚNIOR⁵,
José Ronaldo dos SANTOS⁶,
Roberta Sessa Stilhano YAMAGUCHI⁷,
Alessandra Mussi RIBEIRO¹,
Carla Máximo PRADO¹

Affiliations:

¹ Departamento de Biociências, Instituto de Saúde e Sociedade, Universidade Federal de São Paulo, Campus Baixada Santista, Santos, SP, Brasil; ² Departamento de Ciências Biológicas, Universidade Federal de São Paulo, Diadema, SP, Brasil; ³ Laboratório de Endocrinologia Molecular e Translacional, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, SP, Brasil; ⁴ Faculdade de Medicina da Universidade de São Paulo, São Paulo, SP, Brasil

⁵ Universidade Federal de Sergipe, Centro de Ciências Biológicas e da Saúde, Laboratório de Neurociências e Ensaios Farmacológicos - LANEF. Aracaju, SE, Brasil; ⁶ Universidade Federal de Sergipe, Centro de Ciências Biológicas e da Saúde, Departamento de Biologia. São Cristóvão, SE, Brasil; ⁷ Departamento de Ciências Fisiológicas, Santa Casa de São Paulo, Escola de Ciências Médicas, São Paulo, SP, Brasil.

Keywords: Animal models. COPD. Inflammation

Abstract:

Introduction: Pulmonary emphysema is marked by lung expansion, alveolar damage, and intense inflammation which may contribute to cognitive decline. *Lippia grata*, a plant from Verbenaceae family has been linked to antioxidant and anti-inflammatory effects. Aim: To investigate whether memory deficits occur in a model of elastase-induced emphysema and whether *Lippia grata* (LIP) extract complexed with β -cyclodextrin can modulate lung and cognitive alterations. Methods: Male C57BL/6 mice received an intranasal instillation of porcine pancreatic elastase (PPE, 0.667 UI, 50uL) to induce emphysema or saline as a control. LIP treatment (5mg/Kg) was administered orally once daily from days 21 to 29 in the SAL+LIP and PPE+LIP groups. Control animals (SAL+Ve and PPE+Ve groups) received the vehicle following the same protocol. On day 29, the novel object recognition task (NORT) was performed to assess short-term memory, and lung alterations were evaluated on day 30. Lung alterations were evaluated on day 30. Results: The PPE+Ve group exhibited an increased alveolar diameter and a higher density of inflammatory cells in the



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

peribronchovascular region and lung parenchyma compared to the SAL+Ve group ($P < 0.05$). Moreover, PPE animals showed memory deficits compared to SAL ($P < 0.05$). In the PPE-LIP group, LIP treatment significantly reduced inflammatory cells density in peribronchovascular area ($P < 0.05$) but did not affect other parameters. Conclusion: These results provide clear evidence that experimental emphysema leads to cognitive impairments in mice. While Lippia grata extract exhibited anti-inflammatory properties, especially in the peribronchovascular region, it did not succeed in preventing memory deficits or reversing the progression of emphysema. Approved by the ethics committee for the use of animals, CEUA number 4034100822.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Influence of Maternal Inflammation and Vitamin D Supplementation on the Respiratory Responses of Mice

Authors:

Maria Emanuelle de França Antunes Reis¹

Alana Tercino Frias¹

Kênia Cardoso Bicego¹

Luciane Helena Gargaglioni¹

Affiliations:

¹ Dept of Animal Morphology and Physiology, São Paulo State University, FCAV, Jaboticabal - SP, Brazil.

Keywords: Development. Gestation. LPS. Neonates.

Abstract:

Introduction: Maternal inflammation during pregnancy can impact fetal development, leading to alterations in respiratory function, which may compromise respiratory control in the first days of life. Vitamin D (VitD) supplementation has the potential to reduce placental inflammation and offers protective factors for the neurocognitive development of the offspring. **Objective:** The aim of this study is to investigate the impact of maternal inflammation on neonatal respiratory function and the benefits of VitD supplementation. **Methodology:** Pregnant C57BL/6 female mice (Protocol CEUA/FCAV-UNESP: n°3321/2024) received daily supplementation with vitamin D (18 pmol/day) via subcutaneous osmotic mini-pumps, starting on the first day of gestation. On gestational day 16, the animals were intraperitoneally injected with either a saline solution or lipopolysaccharide (LPS, 50 µg/kg), resulting in four experimental groups: Vehicle + Saline (VEI+SAL), Vehicle + LPS (VEI+LPS), VitD + Saline (VitD+SAL), and VitD + LPS (VitD+LPS). Ventilation was recorded by whole-body plethysmography in both room air and hypercapnia (7% CO₂). Oxygen consumption (VO₂) was measured by indirect calorimetry using an open-circuit respirometry system. **Results:** Preliminary results show that, under room air and CO₂ conditions, the VEI+LPS group exhibited an increase in oxygen consumption (VO₂), which was normalized by vitamin D supplementation. Additionally, vitamin D supplementation reduced the CO₂-induced hyperventilatory response by decreasing tidal volume. Furthermore, VitD supplementation reduces the respiratory equivalent in pups born to mothers who received LPS. **Conclusion:** The data suggest that VitD supplementation during pregnancy may attenuate the respiratory effects of maternal inflammation on the offspring. **Funding:** FAPESP (2020/01702-2; 2022/05057-0), CNPq, and Capes.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Elimination of noradrenaline synthesis in the Locus coeruleus neurons disrupts CO₂ ventilatory and metabolic responses of mice in the early stages of development

Authors:

Mariana BERNARDES-RIBEIRO¹
Luis Gustavo Alexandre PATRONE¹
Bianca de Avila MARTINS¹
Kênia Cardoso BÍCEGO¹
Nicholas PLUMMER²
Patricia JENSEN²
Luciane Helena GARGAGLIONI¹

Affiliations:

¹Departamento de Morfologia e Fisiologia Animal, FCAV/UNESP, São Paulo/SP, Brazil
²Department of Health and Human Services, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA.

Keywords: Development. Hypercapnia. Locus coeruleus. Noradrenaline.

Abstract:

The Locus coeruleus (LC), is the largest noradrenergic (NA) nucleus in the central nervous system and acts as a CO₂/pH chemosensor. Age-related changes in LC chemosensitivity are reported in rats, but their occurrence and impact on ventilatory responses in mice remain unclear. The present study evaluated ventilation, apnea occurrence and metabolism (VO₂) during room air and 7% CO₂ at postnatal period (P0-1, P6-7, P12-13) of male and female wild type C56BL/6 mice (Dbhwt/wt), mice lacking Dbh expression required for NA synthesis in the LC (DbhLC-null) and mice with reduced Dbh expression in LC and other NA neurons (Dbhhypo). All protocols were approved by ethical committee (CEUA: n°9485/22). Our data show that at P0-1, Dbhhypo males increased VO₂ compared to control mice during normocapnia (Dbhwt/wt:0.025 ± 0.003 vs Dbhhypo:0.040 ± 0.0012 and DbhLC-null:0.037 ± 0.004 mL. g⁻¹.min⁻¹). At P6-7, Dbhhypo and DbhLC-null males had reduced tidal volume (Dbhwt/wt:0.008 ± 0.0008 vs Dbhhypo:0.006 ± 0.0006 and DbhLC-null:0.007 ± 0.0010 mL. g⁻¹) and increased VO₂ (Dbhwt/wt:0.033 ± 0.007 vs Dbhhypo:0.054 ± 0.023 and DbhLC-null :0.050 ± 0.008 mL. g⁻¹.min⁻¹) which caused a hypoventilation under room air (Dbhwt/wt:48.5 ± 6.51 vs Dbhhypo:28.7 ± 10.23 DbhLC-null:27.0 ± 5.23). During hypercapnia, Dbhhypo and DbhLC-null males reduced ventilation (Dbhwt/wt:4.15 ± 1.11 vs Dbhhypo: 2.47 ± 0.59 and DbhLC-null:3.03 ± 0.59 mL.g⁻¹.min⁻¹) due to a lower tidal volume (Dbhwt/wt: 0.015 ± 0.002 vs Dbhhypo: 0.009 ± 0.002 and DbhLC-null:0.011 ± 0.001 mL. g⁻¹) which led to a hyperventilation. Regarding females, both Dbhhypo and DbhLC-null reduced the tidal volume (Dbhwt/wt:0.016 ± 0.0019 vs Dbhhypo:0.012 ± 0.0016 and DbhLC-null:0.011 ± 0.0024 mL. g⁻¹.min⁻¹) during CO₂ exposure. Our findings suggest that LC-NA is important for ventilatory and metabolic responses during development in both sexes, under room air and hypercapnia conditions. Financial support: FAPESP, CNPq and CAPES.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: New insights of aged alveolar cells under cyclic stretch that simulates mechanical ventilation

Authors:

Letícia Almeida da SILVA¹;
Sabrina Sodré de Souza SERRA¹;
Iris Krause CONTRERAS¹;
Marianna Ribeiro CABRAL¹;
Monique Martins de MELO¹;
Mayck MEDEIROS¹;
Maíra Rezende LIMA¹;
Ygor S. F. das CHAGAS²;
Fernando de Azevedo Cruz SEARA²;
Antonio C. C. de CARVALHO²;
Johnatas Dutra SILVA¹;
Christina Maeda Takiya³;
Jorge Miguel Carona FERREIRA⁴;
Fernanda Ferreira CRUZ¹;
Pedro Leme SILVA¹

Affiliations:

¹Laboratório de Investigação Pulmonar; Universidade Federal do Rio de Janeiro, Rio de Janeiro/RJ.

²Laboratório de Eletrofisiologia Cardíaca Antonio Paes de Carvalho; Universidade Federal do Rio de Janeiro/RJ.

³Laboratório de Imunopatologia, Universidade Federal do Rio de Janeiro/RJ. ⁴Universidade Técnica de Dresden/Alemanha

Keywords: Aging. Mechanical Cyclic Stretch. Ventilation Induced Lung Injury.

Abstract:

Although mechanical ventilation (MV) is a life support for patients with respiratory failure, it can also damage the lungs due to excessive alveolar stretch which is often underappreciated. To overcome this limitation, primary alveolar cells can be stretched in vitro in innovative equipment that simulates what happens in vivo. Additionally, elderly patients need more MV and the effects of cyclic stretch in aged alveolar cells are largely unknown. The aims of the present study are to: 1) characterize the senescent alveolar cells; 2) evaluate the biological effects of cyclic stretch on primary alveolar epithelial cells from young and old rats. Alveolar cells were extracted from young (2-3 months) and old (20-22 months) male Wistar rats (CEUA 031/21). Senescence was analyzed by senescence associated beta-galactosidase (SA- β -gal) expression and by gene expression of p16, p21 and p53. Cells were cyclic stretched for 4 hours under 0.25Hz. Static primary alveolar cells were used as control. Cell viability was assessed by LDH and MTT assays. Phalloidin immunofluorescence was used to evaluate cellular actin filaments. p16 and p53 gene expressions and SA- β -gal protein expression were higher in cells obtained from old than young rats ($p=0.005$, $p=0.01$ and $p=0.003$, respectively). LDH was higher in both young (Abs, Absorbance [A490-A680] 0.15 ± 0.8) and old cells (Abs, 0.13 ± 0.06) under cyclic stretch compared to respective static conditions (Abs, 0.08 ± 0.06 and 0.06 ± 0.04). Aged alveolar cells under cyclic stretch showed reduced actin filament and changes in their orientation compared with aged alveolar cells under static condition. In conclusion, markers of senescence were observed in aged cells. Under cyclic stretch, aged alveolar cells showed changes in actin filaments which may reflect their fragility. Thus, we provided evidences of aged alveolar cells under cyclic stretch, which corroborate the worse outcome observed in elderly population under mechanical ventilation.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Aging and Parkinson's Disease aggravation of anatomofunctional respiratory deficits: considerations upon the 6-OHDA rat model

Authors:

Luiz Fernando de Araujo Trindade PEDRÃO¹
Pamela Ojuorun Santos MEDEIROS¹
Silvio de Araujo FERNANDES-JÚNIOR¹
Ana Carolina TAKAKURA¹
Thiago MOREIRA²
Bárbara FALQUETTO¹

Affiliations:

¹ Department of Pharmacology, Institute of Biomedical Science, University of São Paulo. São Paulo, Brazil.

² Department of Physiology and Biophysics, Institute of Biomedical Science, University of São Paulo. São Paulo, Brazil.

Keywords: Aging. Neural control of breathing. Parkinson's Disease. Respiratory

Abstract:

Parkinson's disease (PD) is a neurodegenerative disease with death of dopaminergic neurons in the Substantia Nigra (SN). It presents classic symptoms and respiratory deficits. Ageing is a known aggravating factor. There is neurodegeneration in respiratory regions noted in 6-hydroxydopamine (6-OHDA) animal model, which reflects on the ventilation. Evaluate the effects of aging on respiratory function, the anatomy of respiratory nuclei, and respiratory deficits in 6-OHDA animals. 38 male rats, aged 12 months (CEUA 1342290421), were injected with 6-OHDA (24mg/ml) or vehicle into the striatum, and 13 young male rats were used as control. On day 40, all animals underwent whole-body plethysmography. Half of the animals was euthanized and pulmonary mechanics was performed, and the other half underwent perfusion and immunohistochemistry in the brain. 6-OHDA led to a 65% reduction in TH+ neurons in SN, while aged animals showed no such reduction ($p < 0.05$). Under normoxia, 6-OHDA animals exhibited a 28.1% decrease in respiratory frequency (fR) and a 37.9% reduction in minute ventilation (VE) ($p < 0.05$). During hypercapnia, tidal volume (VT) and VE were reduced by 26% and 23%, respectively ($p < 0.05$). In hypoxia, only VT was reduced (24.2%, $p < 0.05$). Aged animals showed reduction in VT and VE in normoxia (18.9% and 37.9%, respectively), hypercapnia (22.7% and 24.3%) and hypoxia (28.9% and 27.5%) ($p < 0.05$). 6-OHDA and aged rats showed 60.2% and 100% reduction in complacency, respectively ($p < 0.05$). A 32% reduction in phox2b neurons was observed in the cNTS ($p < 0.05$), 53% in the RTN ($p < 0.05$), and a decrease in NK1R-expressing neurons in rVRG (15.2%) and preBotC (14.7%) ($p < 0.05$) in 6-OHDA, but not in aged animals. Both aging and 6-OHDA rats showed reduced ventilation, but central degeneration was observed only in 6-OHDA animals. In aged rats, the absence of structural changes in these regions suggests that respiratory deficits may involve peripheral or functional neuromotor mechanisms.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: NET measurement in the BALF of patients with interstitial lung disease: a possible biomarker in fibrosis?

Authors:

Rebeca Cavalheiro do NASCIMENTO¹
Julia Rodrigues Ferraz SILVA¹
Bianca Peixoto Pinheiro LUCENA²
Monique Martins MELO¹
Mayck Medeiros Amaral da SILVA¹
Marcos de Carvalho BETHLEM²
Nadja Polisseni GRAÇA²
Nina Rocha Godinho dos Reis VISCONTI²
Michelle Cailleaux Cezar FERREIRA²
Carla Conceição dos SANTOS²
Fernanda Carvalho de Queiroz MELLO²
Pedro Leme SILVA¹
Fernanda Ferreira CRUZ¹

Affiliations:

¹Laboratory of Pulmonary Investigation, Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro / RJ, Brazil

²Thoracic Disease Institute, IDT, Federal University of Rio de Janeiro / RJ, Brazil.

Keywords: Bronchoalveolar lavage fluid. Fibrosis. Interstitial lung disease. Neutrophils. NETs.

Abstract:

The presence of neutrophils in bronchoalveolar lavage fluid (BALF) of patients with interstitial lung disease (ILD) denotes a worse prognosis. Neutrophils can form extracellular traps (NET) but how this is associated with ILD and fibrosis is largely unknown. The aim of the present study is to evaluate levels of NET according to the presence of fibrosis on chest tomography (CT) and possible associations with clinical and functional data. Cross-sectional study (CAAE: 66958223.9.0000.5257), with prospective allocation, recruited patients from May 2023 to December 2024 in an ILD Brazilian center. Patients undergoing ILD investigation were referred for bronchoscopy with BALF collection. NET was measured in the supernatant through free DNA quantification. MPO and citrullinated histone were also evaluated. Patients were divided into 2 groups: 1) fibrosis on CT (traction bronchiectasis, honeycombing) and 2) no fibrosis on CT (ground glass opacification). 40 patients were enrolled: 28 had fibrosis on CT. Those with fibrosis showed higher NET ($U = 36.00$, $Z = -3.250$, $p=0.001$) and MPO ($U = 31.00$, $Z = -4.043$, $p<0.001$) than those with no fibrosis on CT. Hypersensitivity pneumonia and unclassifiable ILD were the main diagnoses in high NET group, while sarcoidosis in low NET group. NET levels showed positive correlation with BALF's neutrophils ($R=0.49$, $p<0.005$), MPO ($R=0.71$, $p<0.001$) and pulmonary systolic blood pressure ($R=0.40$, $p=0.031$). In conclusion, there was an association between NET and MPO BALF levels and the presence of fibrosis findings.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Ventilatory, metabolic, and thermal responses to hypercapnia in female mice: effect of progesterone throughout the estrous cycle

Authors:

Beatriz DOMINQUINI-MORAES¹
Mariana BERNARDES-RIBEIRO¹
Alana Tercino FRIAS¹
Kênia Cardoso BÍCEGO¹
Hélio ZANGROSSI JR²
Luciane Helena GARGAGLIONI¹

Affiliations:

¹Faculdade de Ciências Agrárias e Veterinárias, FCAV-UNESP
²Faculdade de Medicina de Ribeirão Preto, FMRP-USP

Keywords: Chemosensitivity, estrous cycle, panic, progesterone

Abstract:

Panic attacks (PA) are triggered by an abnormal sensitivity of brain chemoreceptors to CO₂, that leads to respiratory manifestations such as hyperventilation. Hormonal changes during the menstrual cycle may play a role in the occurrence of PA, as changes in respiratory function appear to be influenced by levels of circulating progesterone. Here, we evaluated the effect of nuclear progesterone receptor blockade on the respiratory response to a panicogenic stimulus (20% CO₂) throughout the estrous cycle (CEUA 1594/2021). Female mice in proestrus, estrus, metestrus or diestrus received an acute administration of RU486 (progesterone antagonist; 10 µg.kg⁻¹) or vehicle (DMSO 20%), and their pulmonary ventilation (V_E), oxygen consumption (VO₂), and body core temperature (T_B) were measured during normocapnia followed by 20% CO₂. The air convection requirement was evaluated by calculating the V_E/VO₂. Data were analyzed by a two-way ANOVA followed by Tukey post hoc testing (significance level of p<0.05). Pharmacological blockade of nuclear progesterone receptors did not affect V_E, VO₂, or T_B of females in any stage of the estrous cycle under normocapnia condition. However, it attenuated CO₂-induced hyperventilation in the proestrus stage by reducing V_E/VO₂ (p = 0.015). In contrast, acute RU486 administration increased ventilatory response to hypercapnia during estrus stage by increasing V_E (p = 0.048) and V_E/VO₂ (p = 0.040). Finally, blockade of nuclear progesterone receptors did not affect the respiratory response to CO₂ in metestrus and diestrus, or T_B in any stage of the estrous cycle. Therefore, our data suggest that progesterone can influence chemosensitivity to CO₂ in a cycle-dependent way.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Effects of Caffeine on Respiratory Disturbances in a Murine Model of Parkinson's Disease

Authors:

Rakhel Dayanne DAMASCENO-SILVA¹
Nicole de Castro MIRANDA¹
Laís Maria Cardoso CABRAL¹
Luara BATISTA¹
Thiago dos Santos MOREIRA²
Ana Carolina TAKAKURA¹

Affiliations:

¹Department of Pharmacology and

²Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil

Keywords: 6-OHDA. Breathing. Caffeine. Parkinson's Disease. Neurodegeneration.

Abstract:

Parkinson's disease (PD) is marked by dopaminergic neurodegeneration in the substantia nigra (SN), leading to motor and respiratory dysfunctions. While most common motor symptoms are widely studied, respiratory alterations remain underexplored, and pharmacological treatments for these symptoms are limited. Caffeine, an adenosine receptor antagonist, shows neuroprotective effects in PD models and may modulate breathing by acting on brainstem regions. This study investigates caffeine's impact on respiratory dysfunction in a 6-OHDA-induced PD model (CEUA: 5513240518, n= 52). To determine the optimal dose, a dose-response curve was performed in adult male C57/Bl6 mice, which received vehicle (control) or 0.3, 3, and 10 mg/kg of caffeine, followed by respiratory and behavioral assessments. Behavior tests included open field, light-dark box, elevated plus maze and whole-body plethysmography, conducted on the first and last days of the experiment. The 0.3 mg/kg dose was selected, as it did not change breathing or most anxiety-related parameters. Five days after bilateral striatal injections of 6-OHDA (10 µg/µl) or vehicle, they were treated daily with intraperitoneal caffeine (0.3mg/kg) or vehicle for five days. Post-treatment, our data revealed a reduction of over 70% in dopaminergic neuron in the SN 10 days after 6-OHDA injections. Caffeine treatment prevented the reduction in respiratory rate ($p=0.0037$) and attenuated the reduction neurokinin 1 receptors density in the pre-Bötzinger complex ($p=0.0369$), but did not change the loss of phox2b-expressing neurons in the retrotrapezoid nucleus ($p=0.3791$) observed in the PD model. No changes in these parameters were noted in control animals treated with caffeine. These findings highlight the therapeutic potential of subchronic caffeine administration in preventing respiratory changes in PD model. The statistical analyses were performed using Student's t-test or one-way/two-way analysis of variance (ANOVA), followed by post hoc comparisons using Tukey's test.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Impairment in short-term memory is associated with increased Iba-1 and decreased nicotinic receptors in brain from mice with acute lung injury

Authors:

Priscila Santos BARBOSA¹
Stephanie Nonato de CASTRO¹
Vitoria Caroline de QUEIROZ²
Patricia Santos Carvalhinho LOPES¹
Valéria Cunha MOREIRA¹
Beatriz Soares SILVA¹
Nathalia Montouro Pinheiro MENEGASSO¹
Ayman K HAMOUDA⁴
Rodrigo Portes URESHINO³⁻⁶
Roberta Sessa Stilhano YAMAGUCHI⁵
Alessandra Mussi RIBEIRO¹
Carla Máximo PRADO¹

Affiliations:

¹ Department of Biosciences, Instituto de Saúde e Sociedade, Universidade Federal de São Paulo, Santos, SP, Brazil;
² Department of Medicine, School of Medicine; Universidade de São Paulo, São Paulo, SP, Brazil;
³ Department of Biological Science, Universidade Federal de São Paulo, Diadema, SP, Brazil;
⁴ College of Pharmacy, University of Texas at Tyler, Tyler, TX 75799, USA;
⁵ Department of Physiological Sciences, Santa Casa of São Paulo School of Medical Sciences, São Paulo, SP, Brazil;
⁶ Laboratory of Molecular and Translational Endocrinology, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, SP, Brazil.

Keywords: ARDS. Microglia. nAChR. Neuroinflammation.

Abstract:

Acute lung injury (ALI) is associated with cognitive impairment in ARDS survivors. Immune cell activity and deficits in cholinergic signaling contribute to cognitive disorder in ARDS. Aims: To evaluate if ALI induces changes in Iba-1, BDNF and nicotinic receptors (nAChR) in the brain. Methods: All protocols used in the present study were approved by the Animal Use Ethics Committee of Federal University of São Paulo (protocol code nº 2933110321). Male BALB/C mice received intratracheal saline or lipopolysaccharide (LPS, 5mg/kg) and were divided into two groups: one evaluated 24 hours (LPS 24h), and the other 48 hours (LPS 48h) post-LPS instillation. Short-term memory was assessed using the novel object recognition task (NORT), performed before and after LPS instillation. Brains were used for immunohistochemistry and RT-PCR for nAChR gene expression and lungs for histology and cytokine analysis. Data was analyzed using ANOVA and a $p < 0.05$ was considered significant. Results: LPS induced weight loss, increased polymorphonuclear cells and higher IL-6 and NF- κ B gene expression in the lungs. In the NORT, LPS induced memory impairment. LPS reduced gene expression of nAChR $\alpha 4$ in the hippocampus and in the cortex and nAChR $\alpha 7$ expression in the hippocampus. Brain immunohistochemistry showed an increased Iba-1 marker, but not in BDNF, in hippocampus and cortex in LPS groups. No differences in BDNF expression were observed in the brain. A strong positive correlation was found between weight of loss and Iba-1 expression ($R = 0.732$). Conclusion: ALI impairs short-term memory, alters microglial marker and reduces nAChR expression in the brain, suggesting nAChR may be an important target in brain damage associated with lung inflammation, potentially linked to immune cells activation.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Effect of peripheral BKCa channels blockade on respiratory and metabolic responses to CO₂/pH during mouse development

Authors:

Isabela Xavier MEDALHA¹
Bianca de Avila MARTINS¹
Luciane Helena GARGAGLIONI¹
Luis Gustavo Alexandre PATRONE¹

Affiliations:

¹Department of Animal Morphology and Physiology, São Paulo State University, UNESP/FCAV, Jaboticabal SP, Brazil.

Keywords: BK channels. Development. Paxilin. Mouse.

Abstract: Calcium-activated potassium (BK_{Ca}) channels regulate the electrical properties of the cell membrane and the activity of neural circuits in the brain and spinal cord, providing a negative feedback mechanism on membrane depolarization. Studies indicate that these channels are fundamental in the activity of neurons involved in cardiorespiratory control. Peripherally, their action is relevant to the sensitivity of blood gas sensors and, consequently, to the control of respiratory function. Thus, the present study seeks to understand the role of peripheral BK_{Ca} channels in the control of ventilation and metabolism during postnatal development in mice, under room air conditions and hypercapnia. One hundred and eighteen wild-type C57BL/6 mice of both sexes were used at postnatal days (P) 0-1, 6-7, and 12-13. Pulmonary ventilation (V_E) was determined by whole-body plethysmography in a closed system, while metabolism was assessed by indirect calorimetry (oxygen consumption, VO_2) using an open-flow respirometry system. The animals received intraperitoneal (i.p.) injections of either vehicle (phosphate buffered saline, PBS) or the BK_{Ca} channel blocker (Paxilline: 2.2 μ g/kg; Sigma, USA), in volumes of 0.04, 0.10, and 0.12 mL, depending on age. V_E and VO_2 measurements were performed before and after i.p. treatment, under normocapnic and hypercapnic (7% CO₂) conditions. All experimental procedures were approved by the local ethics committee (9486/22). The data were analyzed by two-way ANOVA, comparing treatment and exposure conditions. The results indicate that Paxilline, under ambient conditions, did not alter ventilatory or metabolic responses. During CO₂ challenge, Paxilline reduced respiratory frequency in P6-7 mice and attenuated the CO₂ induced hyperventilatory response in P12-13 mice by decreasing V_E and tidal volume. Therefore, our data suggest that BK_{Ca} channels play an important role in hypercapnia induced ventilatory responses in older neonates. Financial support: FAPESP, CNPq and PROPE-UNESP.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Involvement of ASIC1a channels in the locus coeruleus in the response to CO₂ exposure in male mice

Authors:

Letícia Rodrigues PINHEIRO¹
Alana Tercino FRIAS¹
Luis Gustavo Alexandre PATRONE¹
Kênia Cardoso BÍCEGO¹
Hélio ZANGROSSI JR²
Luciane Helena GARGAGLIONI¹

Affiliations:

¹Departament of Animal Morphology and Physiology, São Paulo State University at Jaboticabal, FCAV, Brazil.

²Department of Pharmacology, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil.

Keywords: Behaviour. Chemosensitivity. Hypercapnia. Panic disorder. Respiration

Abstract:

Panic disorder (PD) is characterized by intense anxiety associated with acute respiratory symptoms. Evidence suggests that respiratory alterations, such as hypercapnia, may trigger anxiety-related responses, indicating a possible relationship between chemosensory stimuli and the pathophysiology of this disorder. The locus coeruleus (LC) is a chemosensitive region of the brainstem involved in physiological and emotional responses. The ion channel sensitive to acid type 1a (ASIC1a) has been associated with hypercapnia-induced panicogenic response, but its specific role in LC remains unknown. The objective of this study was to evaluate the participation of LC ASIC1a channels in ventilatory and behavioral responses of male and female mice C57BL/6 (*in vivo*) to a hypercapnic stimulus and the role of these channels in the LC chemosensitivity to CO₂ in male (*in vitro*). We investigated the effects of microinjection of ASIC1a antagonist Psalmotoxin-1 (Pstx-1, 50 ng/0.1 µL) in LC of mice (6-7 animals/group CEUA: 3451/22) in respiratory, metabolic and behavioral parameters during exposure to 20% CO₂. We also evaluated *in vitro* the participation of ASIC1a channels in the basal activity and CO₂ chemosensitivity of LC neurons, using patch-clamp records in brain stem slices of male mice. ANOVA with repeated measures (treatment and gas condition) for *in vivo*, and two-way repeated measures ANOVA (CO₂ and inhibitor) and t-test for *in vitro* studies were used. The microinjection of Pstx-1 did not alter the parameters of ventilation or metabolism in conditions of normo- or hypercapnia, in both sexes. However, there was a reduction in the number of jumps in males during hypercapnia, with no effect on females. *In vitro*, Pstx-1 reduced the activity of LC chemoreceptors in males. These findings indicate that ASIC1a channels in LC modulate CO₂-induced behavioral responses in a sex-specific manner and contribute to chemosensitivity in males, without directly affecting ventilatory control.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Electrophysiological properties of the Nucleus Tractus Solitarius neurons from mice activated by allergen-triggered pulmonary inflammation

Authors:

Luiza Henrique de Souza¹
Renato W. M. Sá²
Isabella V. S. Melhado³
Victoria F. Alves³
Wothan T. de Lima³
Davi J. A. Moraes²

Affiliations:

¹Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, São Paulo/SP - Brazil.

²Department of Physiology, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto/SP - Brazil.

³Department of Pharmacology, Institute of Biomedical Sciences, University of São Paulo, São Paulo/SP - Brazil.

Keywords: Asthma, Nucleus Tractus Solitarius, electrophysiology and pulmonary inflammation.

Abstract:

Asthma involves airways bronchoconstriction and mucus hypersecretion, mediated by parasympathetic activation, due to allergen-triggered pulmonary inflammation. This process activates neurons in the Nucleus Tractus Solitarius (NTS) to excite brainstem parasympathetic motoneurons. Herein, we evaluated the electrophysiological properties of NTS neurons from mice activated by allergen-triggered pulmonary inflammation. We crossed Fos-cre and Ai14 mice to label activated neurons following allergen exposure. Male and female animals (#087/2019) were submitted to an experimental model of asthma, which consisted of subcutaneous sensitizations and intranasal challenges of ovalbumin and aluminum hydroxide. Medullary slices were used for the electrophysiological recordings of the NTS neurons, using whole cell clamp technique before and after the superfusion of fast synaptic transmission blockers. The recorded (activated) neurons were identified by the presence of td-Tomato (7 neurons from 6 animals). Data were expressed as mean $\pm$ sd and compared by paired Student's *t* parametric test. NTS neurons activated by pulmonary inflammation presented a capacitance of 15.44 ± 8.65 pF. We found that the blockade of fast synaptic transmission did not affect their resting membrane potential ($p=0.32$), firing frequency ($p=0.62$) and input resistance ($p=0.31$). However, their excitability was reduced (60 pA: 6.85 ± 11.76 vs 28.71 ± 20.52 spikes; $p=0.01$). These animals showed an increase in eosinophils in bronchoalveolar lavage fluid (44.38 ± 25.99 vs 3.37 ± 2.95 ; $p=0.02$; $n=8$). We conclude that the fast synaptic transmission is controlling the excitability of NTS neurons activated by allergen-triggered pulmonary inflammation. These findings advance our understanding about the electrophysiological mechanisms of the NTS neurons controlling airways bronchoconstriction and mucus hypersecretion induced by allergen-triggered pulmonary inflammation in mice.

Financial Support: FAPESP, CNPQ and PUB/USP.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: CO₂ activates astrocytes in the lateral parafacial region to restrain the tachypneic response in mice

Authors:

Renato Willian Martins
SÁ¹ Benedito Honorio
MACHADO² Davi José de
Almeida MORAES¹

Affiliations:

¹Instituto de Ciências Biomédicas, Universidade de São Paulo
²Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo

Keywords: Active expiration. Astrocytes Calcium waves. Central chemoreception

Abstract:

Studies indicate that astrocytes in the medullary respiratory chemoreceptive areas are responsive to CO₂, pH, and O₂ levels, and can regulate ventilation. Nonetheless, the influence of astrocytes in the parafacial lateral region (pFL) on central respiratory chemoreception remains unexplored. This study tests the hypothesis that astrocytes of the pFL region, which contain active expiratory neurons during respiratory challenges, control the ventilatory response to hypercapnia of mice. Using Aldh1l1^{Cre/+}ERT2 mice aged 15-17 weeks (n=10), the experiment involved bilateral injections of AAV-DIO-hM3D(Gq)-mCherry into the pFL region. The contribution of pFL astrocytes to ventilatory control was assessed *in vivo* through the activation of excitatory DREADDs by i.p. injection of JHU37160 (0.1 mg/Kg) under normocapnic or hypercapnic conditions (7% CO₂) in the same group, using whole-body plethysmography. Additionally, the impact of hypercapnia on intracellular [Ca²⁺] of pFL astrocytes was measured in medullary slices via multiphoton microscopy, using transgenic mice expressing genetically encoded calcium indicator (Aldh^{Cre/+}/Gcamp^{fllox/+}). The study received approval from the Institutional Ethical Committee (CEUA-FMRP 1079/2022). Comparisons were performed using two-way ANOVA followed by Bonferroni post hoc test. The findings revealed that sustained activation of pFL astrocytes *in vivo* with JHU37160 did not change ventilation in Aldh^{Cre/+}hM3D(Gq) mice under normocapnia, but it prevented respiratory frequency to increase in response to hypercapnia. Additionally, hypercapnia induced a significant increase in intracellular [Ca²⁺] of pFL astrocytes *in vitro* (p=0.001; 35 astrocytes from 5 separate experiments). While pFL astrocytes are activated by hypercapnia *in vitro*, these results suggest that their functional role is to restrain the tachypneic response to hypercapnia *in vivo*.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Genetic Profile of Lung Adenocarcinoma: A University Oncologic Center-based Survey in Rio de Janeiro

Authors:

Lucas Pinto de AMORIM¹
Tiago de Abreu Amaral SALGADO¹
Pedro Leme SILVA¹
Patrícia Rieken Macêdo ROCCO¹
Maria de Fátima Dias GAUI¹
Vera Luiza CAPELOZZI²
Fernanda Ferreira CRUZ¹
Camila Machado BALDAVIRA²

Affiliations:

¹Universidade Federal do Rio de Janeiro; ²Universidade de São Paulo.

Keywords: Adenocarcinoma. Clinical study. In silico analysis. Lung cancer. NGS.

Abstract:

Lung cancer (LC) is the leading cause of cancer-related death worldwide. Lung adenocarcinoma (LUAD) presents high rates of somatic mutations, genomic rearrangements, and high risk of metastasis, even after surgical resection. However, public health systems still lack routine large-scale gene mutation screening. Next-generation sequencing (NGS) enables comprehensive mutation profiling and may guide personalized therapies, especially in understudied populations such as Brazilians. This study aimed to determine the mutational profile of LUAD patients by NGS in a university oncologic center in Rio de Janeiro and to associate these mutations with clinicopathological features. The TCGA database (LUAD, PanCancer Atlas; n=585) was used as an exploratory cohort to identify mutation frequencies and related protein expressions in 10 genes. A second cohort of 99 LUAD patients underwent NGS and immunohistochemistry to validate the findings and compare with the TCGA data. In the local cohort, 53.1% self-identified as white, 37.5% as brown, and 8.3% as black. Differences in ethnicity, ancestry, smoking history, histological subtypes, and clinical stage were noted. In silico analysis revealed TP53 as the most frequently mutated gene (52%), followed by KRAS (32%) and KEAP1 (19%). ERBB2 and NRAS had the lowest mutation frequencies (4% and 2.5%, respectively). The validation cohort had a higher number of men (51.5%), a mean age of 63 years, and 37.5% of mixed-race individuals. Aggressive histological subtypes (micropapillary and solid) and advanced stages (IIIB/IV) were prevalent in 70.1%, with disease progression in 71% and poor overall survival. The mutation profile in LUAD varies across populations, reinforcing the need for population-specific genomic studies. These preliminary findings support the importance of personalized molecular testing, considering clinical characteristics that influence prognosis and treatment outcomes.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Melanocortin receptor 4 agonist, setmelanotide, treats opioid-induced respiratory depression

Authors:

Mateus R. AMORIM^{1,2}
Noah WILLIAMS¹
Melanie ALEXIS-RUIZ¹
Junia L. DE DEUS¹
Mi-Kyung SHIN¹
O AUNG³
Olga DERGACHEVA⁴
Cole WINSTON¹
Joan ESCOBAR⁴
David MENDELOWITZ⁴
Vsevolod Y. POLOTSKY^{1,4,5,6}

Affiliations:

¹Department of Anesthesiology and Critical Care Medicine, George Washington University, Washington, DC, USA
²Department of Physiology, Medical School of Ribeirão Preto, SP, Brazil
³Medical College of Wisconsin, Milwaukee, WI, USA
⁴Department of Pharmacology and Physiology, George Washington University, Washington, DC
⁵Department of Medicine, George Washington University, Washington, DC
⁶Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Keywords: Breathing, opioids, neural control, pain

Abstract:

The primary cause of death associated with opioids is opioid-induced respiratory depression (OIRD). Naloxone is used to reverse OIRD, but this drug is a competitive antagonist of μ -opioid receptor (MOR) and reverses analgesia, which limits its therapeutic use. Alternative non-opioid receptor antagonist-based approaches to OIRD treatment and prevention are needed. Previous studies suggest that melanocortin 4 receptor pathway (MC4R), which is implicated in body weight regulation, may impact control of breathing. An MC4R agonist, setmelanotide (SET), is approved to treat genetic obesity caused by abnormal melanocortin and leptin signaling. We hypothesized that SET can treat OIRD in mice. C57BL/6J male mice were treated with IP morphine (10 mg/kg) and then 15 min later with either SET (1 mg/kg IP) or vehicle VEH (IP) in a random order. Breathing was recorded by barometric plethysmography, and pain sensitivity was measured by the tail-flick test. All animal experiments were conducted in accordance with protocols approved by the George Washington University Institutional Animal Care and Use Committee (Protocol #A2023-014) and the Animal Ethics Committee (CEUA) of the Ribeirão Preto Medical School, University of São Paulo (Protocol #1343/2024). In mice with OIRD, SET induced a 3-fold reduction of the apnea index, ($p < 0.001$), and decreased apnea duration as compared to the VEH treatment. SET increased respiratory rate and did not affect opioid analgesia. Photostimulation of MC4R+ ChR2-expressing fibers in the parafacial region elicited short-latency postsynaptic current in pre-motoneurons projecting to the phrenic motoneurons in the C3-C4 ventral horns of the spinal cord. SET effectively treated OIRD by increasing respiratory rate and inducing a significant decrease in the number of apneas without decreasing analgesia. Parafacial MC4R (+) neurons are a likely site of respiratory effects of SET. Support: NIH (R01 HL128970, R01 HL133100, and R01 HL138932), AHA (24CDA1270910)



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Relationship Between Motor and Respiratory Function in Duchenne Muscular Dystrophy Patients

Authors:

Karlla Danielle Ferreira LIMA¹
Sarah Leonardo DIAS²
Leandro Augusto de ALMEIDA¹
Tatiana Ribeiro FERNANDES¹
Alódia BRASIL²
Dora Fix VENTURA²
Marco Antônio Veloso ALBUQUERQUE¹
Edmar ZANOTELI¹

Affiliations:

¹ Department of Neurology, Medical School of the University of São Paulo, São Paulo, Brazil

² Department of Experimental Psychology, Institute of Psychology, University of São Paulo, Brazil

Keywords: DMD. Lung function. MFM-32. Motor scale. Respiratory failure.

Abstract:

Duchenne muscular dystrophy (DMD) is a genetic disorder caused by mutations in the DMD gene, leading to the absence of dystrophin in skeletal muscle. This results in progressive muscle degeneration, loss of ambulation during adolescence, and respiratory failure. Motor function is commonly assessed by the 32-item Motor Function Measure (MFM-32), a validated scale evaluating proximal (D1), axial (D2), and distal (D3) motor capacities. Respiratory function is typically evaluated using forced vital capacity (FVC), which declines with age. This prospective observational study evaluated the correlation between motor and respiratory function in 24 DMD patients (aged 0–18) followed at a single neuromuscular center from January 2022 to July 2023. Spirometry was performed to obtain FVC%, and motor function was assessed using MFM-32. Among the patients, 14 were ambulatory. The average FVC was 95% in ambulatory and 78% in non-ambulatory patients. MFM-32 results showed average scores of 28% (± 33.4) for D1, 84.9% (± 19.7) for D2, 86.1% (± 13.2) for D3, and 60.8% (± 20.4) total. Non-ambulatory patients had lower scores across all domains, with age correlating significantly with D1 ($p = 0.006$). FVC% correlated negatively with age ($p = 0.026$, $r = -0.45$). Statistically significant correlations were found between FVC% and MFM-32 domains D1 ($p = 0.005$), D2 ($p = 0.031$), and total score ($p = 0.011$). This study demonstrates a significant relationship between worsening motor function and reduced pulmonary capacity in DMD patients, emphasizing the need for integrated monitoring.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Prevalence of chronic kidney disease in the indigenous population living in the reserve of Aracruz, Espírito Santo, Brazil

Authors:

Gabriela Callo QUINTE¹
Hully Cantão DE SOUZA¹
Adriana Santos SILVA¹
Wagner ALMEIDA³
José Geraldo MILL^{1,2}

Affiliations:

¹Post-graduate Program in Collective Health

²Post-graduate Program in Physiology from Espírito Santo Federal University, Pharmacy Faculty
Bahia Federal University³

Keywords: Heart Disease. Intestinal Microbiota. Metabolic Glomerular filtration rate, chronic kidney disease, indigenous.

Abstract:

Chronic kidney disease (CKD) is a worldwide problem with prevalence of 6.7% according to the National Health Survey (Brazil, 2015). There are few studies investigating factors affecting the glomerular filtration rate (GFR) and CKD in indigenous populations. Objective: To determine the GFR and the prevalence of CKD in the indigenous living in a reserve in Aracruz, ES, inhabited by indigenous of two ethnic groups (Tupiniquim and Guarani, 80% and 10%, respectively) and non indigenous. Methods: Cross-sectional study (2020/22) carried out in adults (≥ 18 years) of this reserve (N=2,982). The invitation to participate was made by health agents from the 5 basic health units attending the 12 villages on the reserve. 1,084 volunteers attended to undergo clinical and laboratory tests in Vitória. Data were collected through questionnaires and clinical and laboratory tests (blood collected in fasting). GFR was calculated using the CKD-Epi equation, and participants with $GFR < 60 \text{ mL/min} \times 1.73 \text{ m}^2$ were considered with CKD. Data are presented as means $\pm$ standard deviation or as % and 95% CI. The Project was approved in the institutional ethics committee (CAAE 22563019.6.0000.5060) Results: 1,076 participants (age mean= 41.6 ± 14.8 y; 57.8% women) were included in this analysis. Two participants were on continuous hemodialysis. Serum creatinine was higher ($p < 0.01$) in men (0.98 ± 0.24 vs 0.73 mg/dL), the opposite occurring with GFR (higher in women -103 ± 18 vs $95 \pm 19 \text{ mL/min} \times 1.73 \text{ m}^2$, $p < 0.01$). A negative and linear decrease of GFR was observed with age (rate of -8.6 mL/min for each 10 years; $r = -0.68$, $p < 0.01$). The prevalence of CKD was 3.5% (95% CI - 2.4-4.6%), more frequent in men than in women (63% vs 37%) and in participants aged > 60 years (93%). Conclusion: The data show a lower prevalence of CKD than in this population as compared with the general Brazilian population of the same age range.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Role of p38MAPK and EGR1 in adriamycin-induced glomerulosclerosis

Authors:

Eduarda Gomes RODRIGUES¹
Juliana Martins da Costa PESSOA¹
Heitor Macedo BRAZ¹
Maria Oliveira de SOUZA¹

Affiliations:

¹Department of Physiology and Biophysics, ICB - University of São Paulo¹

Keywords: Adriamycin, FSGS, proximal tubule injury, p38MAPK/EGR1.

Abstract:

Introduction. Segmental and focal glomerulosclerosis (FSGS) is a common pathology in glomerular diseases, it is progressive and clinically relevant. The molecular pathways involved in glomerular fibrosis and tubular damage are still poorly understood in models of FSGS. **Objective.** To investigate the role of the p38MAPK/EGR1- pathway (mitogen-activated protein kinase and Early Growth Response 1, respectively) in Adriamycin (ADR)-induced FSGS. **Methodology.** Ethics Committee for the Use of Animals (CEUA- ICB 9397130723). *In vivo* study: 8-week-old BALBc mice organized into control or ADR-treated groups (10 mg/kg, single injection) and euthanized after 28 days. *In vitro* study: Proximal tubule cells (TKPTS) treated with ADR (1 μ M) for 1 or 24 hours (h). Protein expression and distribution are investigated by western blotting and immunofluorescence. Renal morphology is assessed after PAS (periodic acid-Schiff reagent) staining. **Statistics:** t-test with Welch's correction ($p < 0.05$). **Preliminary results.** In mice, ADR treatment induced an increase in the ESG index compared to the control group (ADR: 2.23 ± 0.26 (n=8) vs. CTL: 0.11 ± 0.02 ($p < 0.0001$, n=10) and pronounced tubulointerstitial damage, especially in proximal tubules. In TKPTS cells, treatment with ADR resulted in a significant increase in the expression of phosphorylated p38MAPK at 1h [A.U, CTL: 8850 ± 1976 (n=3) vs. ADR: 16704 ± 2644 (n=4), $p = 0.0065$] and 24h [A.U, CTL: 14445 ± 7916 vs. ADR: 28562 ± 4440 , $p = 0.0287$, n= 4]. The immunofluorescence study, still in progress, suggests that in the animal model of ADR, EGR1 expression in the proximal tubule is reduced compared to the control group. **Partial conclusion.** Preliminary data indicate that in ADR-induced FSGS the p38MAPK/EGR1 signaling pathway appears to be important in proximal tubule injury.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Contribution of the p38MAPK pathway to the modulation of MMP10 in an experimental cisplatin model

Authors:

Thayná Vitoria Ribeiro da SILVA¹
Juliana Martins da Costa PESSOA¹
Neydiana Belize PINA¹
Heitor Macedo BRAZ¹
Gabrielly Hilário da SILVA¹
Guilherme Lopes GONÇALVES¹
Maria Oliveira de SOUZA¹

Affiliations:

Department of Physiology and Biophysics, ICB, University of São Paulo¹

Keywords: Cisplatin. MMP10. P38MAPK

Abstract:

Introduction. Cisplatin, an effective antineoplastic agent with nephrotoxic action, induces acute kidney injury (AKI) with tubular damage. Despite the amount of data on the tubular action of cisplatin, the involvement of p38MAPK and MMP10 remains to be investigated. Objective. To investigate the mechanisms involved in the injury and repair of proximal tubule cells in a model of IRA induced by cisplatin (Cis), with emphasis on the p38MAPK/MMP10 pathway. Methodology. The study was approved by CEUA- ICB 1107271022. In the in vivo model, BALBc mice were treated with cisplatin (20 mg/kg, i.p.) or saline solution and euthanized on the 5th day. In the in vitro model, proximal tubule cells (TKPTS) were treated with cisplatin (25 μ M) for 1 or 24 hours. Renal function was assessed and renal morphology was evaluated by H&E staining. Protein expression and distribution are investigated by western blotting and immunohistochemistry. Statistics: t-test with Welch's correction ($p < 0.05$). Preliminary results. Treatment with cisplatin induced a reduction in urinary flow (FU) and an increase in plasma creatinine (Cr) compared to the respective controls [FU, Cis: 0.15 ± 0.09 μ L/min, $n=8$) vs. CTL: 0.47 ± 0.02 μ L/min, $n=12$) ($p < 0.001$); Cr, Cis: 0.53 ± 0.15 mg/dL, $n=8$) vs. CTL: 0.23 ± 0.07 mg/dL, $n=12$]. In addition, there was tubular damage. In TKPTS cells, Cis induced an increase in the expression of phosphorylated p38MAPK at 1h [A.U, CTL: 15913 ± 2225 ($n=4$) vs. Cis: 25551 ± 2447 ($n=4$), $p=0.027$] and 24h [A.U, CTL: 25333 ± 1391 ($n=4$) vs. Cis: 33786 ± 3057 , $p < 0.0001$ ($n=5$). The ongoing immunohistochemistry study suggests that in the Cis animal model the expression of MMP10 in the proximal tubule is increased compared to the control. Partial conclusion. Preliminary data indicate that in cisplatin-induced ARF the p38MAPK/MMP10 signaling pathway appears to be important in proximal tubule injury.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Non-steroidal mineralocorticoid receptor antagonist improves tubule-interstitial injury in subclinical acute kidney injury animal model

Authors:

João Paulo ZUMACK^{1,4}
Rodrigo Alves da Silva PERES¹
Ana Acácia de Sá PINHEIRO^{1,2}
Carlos Perez GOMES^{4,5}
Celso Caruso NEVES^{1,2,3}

Affiliations:

¹Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil
²Rio de Janeiro Innovation Network in Nanosystems for Health-Nano SAÚDE/FAPERJ, Rio de Janeiro, Brazil
³National Institute of Science and Technology for Regenerative Medicine, Rio de Janeiro, Brazil
⁴Hospital Universitário Clementino Fraga Filho, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil
⁵School of Medicine and Surgery. Federal University of State of Rio de Janeiro, Brazil

Keywords: Non-steroidal mineralocorticoid receptor antagonist. Subclinical acute kidney injury. Tubule-interstitial injury

Abstract:

Introduction. Kidney diseases are associated with excessive activation of the mineralocorticoid receptor (MR). Finerenone, a MR antagonists, has emerged as a new pillar of nephroprotective treatment in kidney diseases. Subclinical acute kidney injury (subAKI) is characterized by tubule-interstitial injury (TII) without changes in glomerular function. TII is linked to reduced albumin reabsorption in the proximal tubule, leading to a pro-inflammatory and pro-fibrotic response. Objective. Uncover the effects of finerenone on the development of subAKI. Methods. A subAKI model was developed in male BALB/c mice (6–8 weeks old) (CEUA-UFRJ 031/24): 1) Control (CTL); 2) subAKI, induced by an I.P. injection of 10g/kg of BSA; 3) subAKI+FIRE, subAKI animals treated daily with finerenone (10 mg/kg) by gavage; 4) FIRE, animals treated with finerenone. On day 7, the animals were euthanized, and urine, plasma, and kidney tissue were collected. Results. Arterial blood pressure, plasma potassium levels, and glomerular function (assessed by plasma creatinine, BUN, and creatinine clearance) remained unchanged. 24-hour proteinuria, albuminuria, UPCr, and UACr were elevated in the subAKI group (24±11 mg; 16±8 mg; 35±18 mg/mg; 25±12 mg/mg, respectively) and reduced in the subAKI+FIRE group (10±6 mg; 6±5 mg; 16±7 mg/mg; 10±8 mg/mg, respectively), reaching levels comparable to the CTL group. TII development, assessed by urinary γ -GT activity, increased in the subAKI group (31±12 UI/L) and was reversed in the subAKI+FIRE group (22±8 UI/L). Tubular sodium handling, measured by renal excretion fraction, was elevated in the subAKI group (0.4±0.1%) and reduced in the subAKI+FIRE group (0.3±0.1%). Cortical (Na⁺+K⁺)-ATPase activity decreased in the subAKI group (0.06±0.04) and was restored in the subAKI+FIRE group (0.2±0.04 nmolPi·mg⁻¹·min⁻¹). Conclusion. Finerenone effectively prevented the development of subAKI, suggesting its potential broader use in kidney disease management.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: The Role of Probiotics in Modulating the Gut Microbiota as an Inhibitor of Diabetic Kidney Disease Progression

Authors:

Vitoria Cecilia Souza COSTA¹
Monique Moreira PINHEIRO¹
Giulia Triolo CABREIRA¹
Isabella Bacci BUSTELLI¹
Julia BELLUCI¹
Sara VENTURA³
Luciana Soares Costa SANTOS^{1,2}
Maria de Fatima Fernandes VATTIMO²
Eloiza de Oliveira SILVA^{1,2}

Affiliations:

¹ School of Nursing of the University of São Paulo

² Faculty of Medical Sciences of Santa Casa de São Paulo

³ Heart Institute of the Faculty of Medicine of USP – Laboratory of vascular biology

Keywords: Diabetic Kidney Disease. Probiotic. Redox Profile

Abstract:

Diabetic kidney disease (DKD) is a major complication of type 2 diabetes (T2DM). Studies suggest that gut dysbiosis, an imbalance in the gut microbiota, is commonly observed in DKD patients and may contribute to its pathogenesis. Among microbial metabolites, butyrate, a short-chain fatty acid, plays a key role in antioxidant protein regulation in diabetic patients. Based on this, we hypothesized that probiotic administration in diabetic rats could modulate the redox profile, thereby attenuating kidney disease progression. To investigate the underlying mechanisms, we conducted an in vivo study using Wistar rats with T2DM induced by nicotinamide (NA) and streptozotocin (STZ). Probiotic treatment (*Bifidobacterium longum*, *Bifidobacterium bifidum*, *Lactobacillus rhamnosus*) significantly increased Nrf2 expression (0.028 ± 0.05 vs 0.09 ± 0.005 , $p < 0.005$) and glutathione levels (0.08 ± 0.03 vs 6.2 ± 1.81 nmol/g protein, $p < 0.005$). Additionally, probiotics reduced oxidative stress markers, including urinary peroxidation (67.1 ± 11.20 vs 15.2 ± 9.20 nmol/g CrU, $p < 0.005$) and lipid peroxidation (2.83 ± 0.90 vs 0.22 ± 0.05 nmol/g CrU, $p < 0.005$). Beyond oxidative profile improvements, probiotics also enhanced renal function, reducing serum creatinine (CrS) (1.02 ± 0.25 vs 0.76 ± 0.10 mg/dL, $p < 0.005$), microalbuminuria (227.7 ± 31.50 vs 137.0 ± 24.40 mg/24h, $p < 0.005$), and improving creatinine clearance (Clin) (0.33 ± 0.08 vs 0.61 ± 0.10 100g/ml/min, $p < 0.005$). Our findings indicate that probiotics prevented DKD progression, likely by modulating oxidative stress through the gut microbiota. These results suggest that probiotics may serve as renoprotective agents, potentially reducing DKD morbidity in T2DM.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Tubule-interstitial injury in subAKI is attenuated by dapagliflozin treatment through modulation of O-linked Glc-NAcylation pathway

Authors:

Rodrigo Alves da Silva PERES¹
Ana Carolina Pinto de FIGUEIREDO¹
Douglas Esteves TEIXEIRA¹
Rodrigo Pacheco da SILVA-AGUIAR¹
Carlos Perez GOMES^{2,3}
Wagner Barbosa DIAS¹
Ana Acácia de Sá PINHEIRO^{1,5}
Celso CARUSO-NEVES^{1,4,5}

Affiliations:

¹Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil
²Clementino Fraga Filho University Hospital, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil
³School of Medicine and Surgery, Federal University of the State of Rio de Janeiro, Rio de Janeiro, Brazil
⁴Rio de Janeiro Innovation Network in Nanosystems for Health-NanoSAÚDE/FAPERJ, Rio de Janeiro, Brazil
⁵National Institute of Science and Technology for Regenerative Medicine, Rio de Janeiro, Brazil

Keywords: Subclinical acute kidney injury, SGLT2 inhibitor, dapagliflozin, tubule-interstitial injury

Abstract:

Subclinical acute kidney injury (subAKI) is characterized by markers of tubular injury without changes in glomerular function. The effects of dapagliflozin on the development of subAKI is unknown. This work investigated the effects of dapagliflozin and its associated mechanisms in subAKI. BALB/c male mice (n group=10), 6-8 weeks, were separated in four groups: 1) CTL, received vehicle; 2) subAKI, received BSA (10 g/kg/day i.p.); 3) subAKI+DAPA, received BSA and dapagliflozin (1 mg/kg/day by gavage); 4) DAPA, received dapagliflozin. All treatment lasted 7 days. In the last 24h of treatment all mice were kept in metabolic cages for urine collection and euthanized for blood and kidney collection (CEUA 083/23). The results are expressed as mean $\pm$ SD. There was no change in plasma creatinine, creatinine clearance, and BUN. However, 24h proteinuria, UPCr, and albuminuria were increased in subAKI (21.18 $\pm$ 5.16 mg/24h; 30.52 $\pm$ 9.8; 50.42 $\pm$ 4.92 fold of control) and attenuated in subAKI+DAPA (14.09 $\pm$ 4.57 mg/24h; 19.36 $\pm$ 6.86; 17.56 $\pm$ 3.37 fold of control) compared to CTL (5.48 $\pm$ 2.29 mg/24; 8.31 $\pm$ 1.56; 1.00 $\pm$ 0.45). Urinary β 2-M, γ -GT, and tissue expression of KIM-1 increased in subAKI (18873 $\pm$ 5157 intensity density, 297 $\pm$ 133 U/g creatinine, and 0.52 $\pm$ 0.12 AU) when compared to CTL (0.100 $\pm$ 0.00, 196.40 $\pm$ 75.48 U/gCr, and 0.06 $\pm$ 0.04 AU). This effect was attenuated in subAKI+DAPA group (5628 $\pm$ 3676; 202.40 $\pm$ 95.70; and 0.31 $\pm$ 0.07, respectively). Cortical interstitial space increased in subAKI (20.72 $\pm$ 2.00) were partially reversed in subAKI+DAPA (9.98 $\pm$ 2.95) compared to CTL (7.75 $\pm$ 0.44 % of total area). Total protein O-GlcNAcylation increased in subAKI (4.25 $\pm$ 2.22 fold of control) was attenuated in subAKI+DAPA (1.39 $\pm$ 0.34 fold of control). The expression of OGT was increased in subAKI (1.30 $\pm$ 0.19 fold of control) and reversed in subAKI+DAPA (0.59 $\pm$ 0.25 fold of control). These results suggest that dapagliflozin prevents tubule-interstitial injury observed in subAKI by attenuating increased protein O-GlcNAcylation.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: The role of SGLT2 inhibitor on the pathogenesis of acute kidney injury

Authors:

Gabriel Sette da COSTA¹

Luiza Gomes da FONSECA¹

Rodrigo Alves da Silva PERES¹

Ana Acacia de Sá PINHEIRO^{1,2}

Celso CARUSO-NEVES^{1,2,3}

Affiliations:

¹Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

²Rio de Janeiro Innovation Network in Nanosystems for Health-Nano SAÚDE/FAPERJ, Rio de Janeiro, Brazil

³National Institute of Science and Technology for Regenerative Medicine, Rio de Janeiro, Brazil

Keywords: Acute kidney injury, SGLT2 inhibitor, dapagliflozin

Abstract:

Acute kidney injury (AKI) is a public health concern associated with high mortality and morbidity rates. The treatment of AKI depends on its underlying cause, highlighting the urgent need for new therapeutic approaches. One hope comes from the observation that dapagliflozin (DAPA), an SGLT2 inhibitor, improves outcomes in chronic kidney disease patients, suggesting that glucose metabolism plays a role in proximal tubule epithelial cell (PTEC) injury. Objective: We aimed to investigate the role of DAPA in the development of AKI. Methodology: A folic acid-induced AKI model was established in adult male C57BL/6 mice (n=12) (CEUA 083/23): (1) Control (CTL); (2) FA, mice received a single intraperitoneal dose of folic acid (250 mg/kg); and (3) FA+D, FA mice treated simultaneously with 1 mg/kg/day of DAPA via oral gavage for two days. The animals were housed in metabolic cages and euthanized for blood and kidney sample collection. Data are presented as mean $\pm$ standard deviation (SD). Results: Renal excretion fraction of glucose was significantly higher in the FA+D group compared to both the FA and CTL groups, confirming DAPA's effect on SGLT2 in PTECs. Plasma creatinine and BUN levels increased in the FA group from 0.4 ± 0.2 and 74.2 ± 12.3 to 0.5 ± 0.3 and 294.0 ± 144.2 mg/dL, respectively. Creatinine clearance decreased in the FA group, confirming the establishment of AKI. DAPA treatment did not alter these glomerular parameters. 24-hour albuminuria (16.28 ± 4.87 mg), albumin-to-creatinine ratio (37.19 ± 2.38), and albumin clearance (473.7 ± 42.39 μ L/min), and renal excretion fraction of albumin were increased in the FA group compared to the CTL group (8.92 ± 0.14 mg/24h; 13.55 ± 1.98 ; 298.30 ± 13.60 μ L/min, respectively). All these parameters were reversed by DAPA treatment. Conclusion: These findings suggest that DAPA treatment prevents the development of albuminuria in AKI, which may be linked to changes in PTEC function.

Funding: FAPERJ, CNPq, CAPES.



XXXIX REUNIÃO ANUAL DA FeSBE
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XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Investigation of PKM2 expression in renal tissue of BTBR wild-type and ob/ob male and female animals

Authors:

Letícia Barreto da SILVA¹
Victoria Mel Dussan ANGULO¹
Mariana Charleaux de PONTE¹
Karina THIEME¹

Affiliations:

¹ Departamento de Fisiologia, Instituto de Ciências Biomédicas – ICBUSP

Keywords: Diabetic kidney disease. Glucose metabolism. PKM2. Sexual dimorphism

Abstract:

Obesity and type 2 diabetes are significant contributors to the increasing incidence of diabetic kidney disease (DKD). The enzyme pyruvate kinase M2 (PKM2) stands out as a key element in understanding the progression of the disease, as it connects metabolic changes, inflammation, and fibrosis. This study was conducted using the BTBR model with the ob/ob mutation, divided into four groups: wild type males, ob/ob males, wild type females, and ob/ob females. The animals were observed up to the 8th or 16th weeks of life, when blood and renal tissue were collected. Before euthanasia, animals were kept in metabolic cages for 6-hour fasting, during which urine was collected, water intake volume was recorded, and weight and blood glucose were measured. Metabolic (lactate, cholesterol, triglycerides, and urea) and urinary (creatinine, mass, and glucose concentration) parameters were evaluated. Part of the renal tissue was used for gene expression analysis by real-time PCR. The following genes were analyzed: *Pkm*, hexokinase (*Hk1*), fructose 1,6-bisphosphatase (*Fbp1*), phosphoenolpyruvate carboxykinase (*Pck1*), GLUT1 (*Slc2a1*), GLUT2 (*Slc2a2*), SGLT1 (*Slc5a1*), and SGLT2 (*Slc5a2*). The protein expression of PKM2 and its phosphorylated form was evaluated by Western blot. Half of the renal tissue was stored for histological analysis (immunohistochemistry and immunofluorescence), and the other half was processed for renal homogenate. Weekly, the weight and blood glucose levels of the animals were recorded until they reached 8 weeks. The weight gain in obese females occurred at the 5th week ($p < 0.0001$), and in ob/ob males at the 6th week ($p < 0.0001$). Hyperglycemia was earlier in ob/ob males, observed at the 7th week ($p < 0.0001$), while in obese females, the glucose shift was smaller and not significant. At 8 weeks, ob/ob males showed significantly higher cholesterol and triglyceride levels than WT controls ($p < 0.0001$ and $p < 0.0001$, respectively) and than ob/ob females ($p < 0.05$ and $p < 0.001$, respectively). Urea was higher in ob/ob females compared to WT females ($p < 0.0001$). Additionally, urinary glucose concentration and mass increased in ob/ob males compared to WT controls and females ($p < 0.0001$ and $p < 0.05$, respectively). However, no significant differences were found in lactate and creatinine analyses. Regarding gene expression, *Hk1* showed a significant difference between male and female WT ($p < 0.05$). While *Pkm* expression was significantly higher in ob/ob females compared to WT controls and ob/ob males ($p < 0.0001$ and $p < 0.05$, respectively). Western Blot analysis demonstrated that PKM2 phosphorylation was higher in both WT and obese females ($p < 0.05$). Regarding SGLT2 expression, there was an increase in transporter expression in ob/ob males compared to their respective WT control group ($p < 0.0001$). However, *Pck1* and *Fbp1* expression did not show significant changes between the studied groups, nor did SGLT1, GLUT1, and GLUT2. The results show that, at 8 weeks, ob/ob animals present a significant increase in body weight. Moreover, while both sexes exhibit changes in lipid metabolism, only obese males show elevated blood glucose, reflecting in greater glucose excretion. The increase in SGLT2 expression, responsible for renal glucose filtration, may



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

intensify hyperglycemia in this group. Obese females, although with lower glucose levels than males, show elevated serum urea and greater urinary albumin excretion, indicating worse renal function. Moreover, the higher gene expression of *Hk1* and *Pkm2* in females indicates a differentiated metabolic response, more associated with glycolysis. Finally, PKM2 phosphorylation plays a key role in metabolic regulation and adaptation of proliferating cells, potentially regulated differently in obesity. The increased phosphorylation of PKM2 in females suggests an alternative mechanism in glycolytic regulation, with implications in obesity. Weight and blood glucose levels were also recorded up to the 16th week. In obese females, weight was significantly higher than in obese males at the 15th week ($p < 0.05$), with a marked difference at the 16th week ($p < 0.0001$). Glucose in ob/ob males remained high from the 7th week, peaking at the 10th week ($p < 0.0001$), and stayed elevated until the 16th week. In ob/ob females, glucose increased at the 10th week ($p < 0.0001$) and remained high until the 16th week, but to a lesser extent compared to obese males ($p < 0.05$). Considering these differences, the evaluation of the expression of both glycolysis and gluconeogenesis genes in 16-week-old animals will be important for deeper exploration of glucose metabolism pathways and renal glucose transporter alterations.

In conclusion, in these preliminary results, differences in metabolic and renal parameters were observed, with sex and genotype being important factors. The findings suggest that, in addition to hyperglycemia, metabolic factors contribute to the development of diabetic kidney disease. Thus, this study is relevant for understanding sexual dimorphism in metabolic changes in a pre-clinical model of DKD study. CEUA: 6515020424



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: The lack of TRPV1 partially restores the effect of high sugar/high fat diet in kidney mice

Authors:

Oluwole Samuel ODEYEMI¹
Laura Barroso Ferreira de OLIVEIRA¹
Pedro Lucas Cailaux LUCIANO¹
Diogo de Barros PERUCHETTI¹
Maristela de Oliveira POLETINI¹

Affiliations:

¹Universidade Federal De Minas Gerais

Keywords: Kidney, Obesity Renal function, TRPV1

Abstract:

Transient receptor potential (TRP) type 1 (TRPV1) is a ligand-gated non-selective ion channel, which can be activated by multiple stimuli including noxious heat, low pH, and chemical irritants such as capsaicin. It is primarily expressed in unmyelinated C-fibers and thinly myelinated Ad-afferent nerve fibers, which innervate internal organs including the kidneys. Available evidence has shown that TRPV1 channel has a role in lifestyle-induced metabolic diseases, such as type 2 diabetes (T2D) and cardiovascular disease, often associated with obesity; however, its role on the kidney function is still poorly understood. TRPV1 knockout (KO) mice (Trpv1^{-/-}) and wild-type (WT) Trpv1^{+/+} littermates were used. The animals were fed high-fat and fructose (HFD+Frut) diet for 60 days and renal function tests were done on the serum and urine samples. This research was done in accordance to CEUA UFMG ethics guideline. HFD+Frut diet significantly increased body weight and fasting blood glucose levels in both KO and wild-type mice. Creatinine clearance (a well-known marker of glomerular function) was 4-fold increased after HFD+Frut diet in WT mice, indicating glomerular hyperfiltration; however, this stimulatory effect of diet was attenuated in KO mice. Assessing the tubular function, we observed a significant decrease in fractional excretion of chloride by 87% in WT mice that received the HFD+Frut diet, indicating hyper tubular reabsorption of chloride. This effect was attenuated in KO mice. Furthermore, the results obtained from kidney cortex histology showed a 1.5-fold increase in glomerular cellularity as well as a 1.65-fold increase in interstitial cell number (indicating kidney inflammation) in WT fed with HFD+Frut diet. Interestingly, these structural changes were attenuated in KO mice. This study shows that lack of TRPV1 channel attenuates the kidney damage and dysfunction caused by high sugar/ high fat diet which could potentially be a target for metabolic disorders pertaining to renal functions.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Angiotensin-(1-7) ameliorates hyperglycemia-induced tubular dysfunction and injury in the early phase of Diabetic Kidney Disease

Authors:

Laura Barroso Ferreira OLIVEIRA¹
Mariana Rodrigues CAMPOS¹
Vitória Luiza LACERDA¹
Arthur Fornazari IOST¹
Kamylle Silva FERRAZ¹
Letícia Cristine CARDOSO²
Paula Peixoto Campos LOPES²
Maria Aparecida Ribeiro VIEIRA¹
Celso CARUSO-NEVES³
Maria José CAMPAGNOLE-SANTOS^{1,4}
Robson Augusto Souza SANTOS^{1,4}
Diogo Barros PERUCHETTI^{1,4}

Affiliations:

¹Department of Physiology and Biophysics, Institute of Biological Sciences, Federal University of Minas Gerais

²Department of Pathology, Institute of Biological Sciences, Federal University of Minas Gerais

³Carlos Chagas Filho Biophysics Institute, Federal University of Rio de Janeiro

⁴INCT-Nanobiofar

Keywords: Angiotensin-(1-7), Diabetic Kidney Disease, hydroxypropyl β -cyclodextrin, Renin Angiotensin System, TGR-(A1-7)-3292

Abstract:

Diabetic Kidney Disease (DKD) is a life-threatening complication of diabetes *mellitus* associated with hyperglycemia-induced tubular albuminuria and modulation of intrarenal Renin-Angiotensin System components. However, the potential role of angiotensin-(1-7) [Ang-(1-7)] is still poorly known. We aimed to study the potential role of Ang-(1-7) in the tubular injury and dysfunction observed in the early DKD. Streptozotocin-induced DKD animal model was used. Eight experimental groups were generated: 1) Sprague Dawley (SD)-ND, normoglycemic rats (control, n=8); 2) SD-D, diabetic rats (n=8); 3) L3292-ND, normoglycemic TGR(A1-7)3292 rats [high systemic Ang-(1-7)] (n=9); 4) L3292-D, diabetic TGR(A1-7)3292 rats (n=9); 5) SD-ND+HPBCD, normoglycemic rats treated with empty hydroxypropyl β -cyclodextrin (HPBCD) used as vehicle via gavage (n= 3); 6) SD-D+HPBCD, diabetic rats treated with vehicle (n= 4); 7) SD-D+HA(1-7), normoglycemic rats daily treated with 30 μ g/kg/day Ang-(1-7) included in HPBCD [HPBCD-Ang-(1-7)] via gavage (n= 3); 8) SD-ND+HA(1-7), diabetic rats treated with HPBCD-Ang-(1-7) (n= 4) (CEUA-UFMG n° 300/2024). All the analyses were carried out after 3 weeks after diabetes induction. When compared with normoglycemic groups (SD-ND and SD-ND+HPBCD groups), the diabetic groups (SD-D and SD-D+HPBCD groups) presented increase in: (1) the fractional excretion (FE) of water, K⁺, Na⁺, Cl⁻ and glucose; (2) both urinary protein:creatinine ratio (UP:Cr) and in FE_{proteins} associated with drop in cortical albumin-FITC uptake; (3) urinary lactate dehydrogenase and urinary $\gamma\gamma$ -glutamyl transferase (kidney injury markers); (4) cortical tubule-interstitial injury as well as collagen deposition. Interestingly, all these modifications were attenuated in L3292-D and SD-D+HA(1-7) groups. No significant changes were observed in the L3292-ND and SD-ND+HA(1-7) groups. Our findings suggest that Ang-(1-7) attenuates the development of tubular injury and dysfunction observed in early DKD.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Development of tubular proteinuria associated with tubular injury induced by accumulation of fine particulate matter from air pollution in Wistar rats

Authors:

Enes Francisco Beraldo de QUEIROZ^{1,3}
Sandro Henrique NOGUEIRA¹
Maria Aparecida Ribeiro VIEIRA¹
Adriana GIODA⁴
Carolina Rosa GIODA⁵
Luciano dos Santos Aggum CAPETTINI^{2,3}
Diogo Barros PERUCHETTI¹

Affiliations:

¹Departament of Physiology and Biophysics, Institute of Biological Science, Federal University of Minas Gerais; ²Departament of Pharmacology, Institute of Biological Science, Federal University of Minas Gerais; ³Departament of Pathology, Institute of Biological Science, Federal University of Minas Gerais; ⁴Departament of Chemistry, Pontifícia Universidade Católica do Rio de Janeiro (PUC-Rio); ⁵Biological Science Institute, Federal University of Rio Grande do Sul

Keywords: kidney disease. Particulate matter. Proteinuria

Abstract:

Introduction: Air pollution is an anthropogenic problem that generates particulate matter (PM), and its body accumulation represents a major threat to global public health. PM_{2.5} (0.1 up to 2.5 µm) remain suspended in the atmosphere and, in addition to causing respiratory problems, can reach blood vessels, leading to systemic complications. In this context, little is known about the potential effects of PM on kidneys. **Methods:** PM_{2.5} samples were collected through borosilicate glass microfiber filters. Then, male Wistar rats (4 weeks old) were exposed daily to 5X diluted PM_{2.5} extracts (initial concentration ± 1 g of filter / 100 mL of saline) for 8 consecutive weeks via the intranasal (N-PM group, n = 9) and intraperitoneal (IP-PM group, n = 9) routes. The rats that received only vehicle were defined as the control group (n=9) (CEUA-UFMG nº 249/2022). In the 8th week, the rats were allocated to metabolic cages for 24-hour urine collection followed by biochemical analysis. **Results:** Body weight, water and food intake, as well as urinary flow, did not change between the experimental groups. Furthermore, renal osmolar clearance as well as markers of glomerular function measured by plasma creatinine, blood urea nitrogen (BUN), creatinine clearance (CCr) were also not altered. Significantly higher proteinuria (1.9 times) and UP-Cr (2.1 times) were observed in both the N-PM and IP-PM groups when compared to the control group. Furthermore, the activity of urinary γ-glutamyl transferase (γ-GT), a specific marker of tubular injury, was increased (3.9-fold and 5.0-fold, respectively) in the N-PM and IP-PM groups, when compared to the control. **Conclusion:** Our results indicate that exposure of rats to PM promotes tubular proteinuria associated with tubular injury, since these changes were not accompanied by any changes in glomerular function. These findings contribute to expanding the current knowledge underlying the pathogenesis of kidney disease caused by PM accumulation.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: RAGE inhibitor TTP488 (Azeliragon) reverses bladder dysfunction in leptin-deficient obese mice

Authors:

Akila Lara de OLIVEIRA¹
Matheus Leite de MEDEIROS¹
Edson ANTUNES¹

Affiliations:

¹State University of Campinas - UNICAMP, Faculty of Medical Sciences, Department of Translational Medicine - Pharmacology area

Keywords: AGEs. Bladder. Collagen. RAGE.

Abstract:

Diabetic bladder dysfunction (DBD) is a highly prevalent complication of diabetes mellitus (DM), manifesting mainly as decreased sensation and increased capacity. In hyperglycemia, the generation of advanced glycation end products (AGEs) increases, resulting from the non-enzymatic binding (glycation) of dicarbonyl compounds, such as methylglyoxal, leading to protein dysfunction. AGEs interact with their receptor RAGE and trigger a cascade of intracellular events. However, little is known about the effects of RAGE blockade in DBD. TTP488 (azeliragon) is an orally bioavailable RAGE inhibitor capable of suppressing the receptor binding cascade. Here, we explored the effect of TTP488 on DBD in *ob/ob* leptin-deficient mice. Animal procedures and experimental protocols were approved by the Ethics Committee on Animal Use, State University of Campinas (CEUA-UNICAMP; Protocol No. 6366-1/2023). *B6.V-Lep^{ob}/JUnib (ob/ob)* females received TTP488 (10 mg/kg) in drinking water for 15 days. C57BL6 females were used as lean controls. Serum levels of MGO and AGEs, as well as RAGE expression in the bladder, were assessed. Bladder function was evaluated using a void spot assay with filter paper and *in vitro* contractility tests. Second harmonic microscopy imaging was performed to assess collagen fibers. TTP488 treatment in *ob/ob* mice significantly reduced serum MGO and AGE levels without altering RAGE expression in the bladder. It also reduced voided volume without affecting spot number and decreased bladder hypercontractility in response to carbachol and electric field stimulation, while reducing the increased bladder collagen observed in the *ob/ob* group. These findings suggest that TTP488 normalizes bladder MGO and AGE levels and attenuates voiding dysfunction in *ob/ob* mice, further supporting the critical role of the AGE-RAGE pathway in diabetes associated bladder dysfunction.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Acute Activation of the MGO-AGE-RAGE Axis Promotes Bladder Hypercontractility

Authors:

Clarissa Paiva HORIOKA¹ Akila Lara de OLIVEIRA¹ Matheus Leite de MEDEIROS¹ Edson ANTUNES¹

Affiliations:

¹ State University of Campinas – UNICAMP, Faculty of Medical Sciences, Department of Translational Medicine – Pharmacology area

Keywords: AGEs. Diabetic Bladder Dysfunction. Hypercontractility. RAGE.

Abstract:

Methylglyoxal (MGO) is a highly reactive dicarbonyl compound, primarily formed under hyperglycemic conditions, such as in diabetes and obesity. Studies from our group have shown that chronic MGO administration induces bladder dysfunction. Here, we investigated whether ex vivo incubation with MGO in healthy mouse bladders would reproduce the hypercontractility observed in vivo. The experimental procedures and protocols were approved by the Animal Use Ethics Committee of the Universidade Estadual de Campinas (CEUA-UNICAMP; Protocol No. 6497-1/2024). Male C57BL6 mice (17 weeks old) had their bladders isolated and divided into two strips: one incubated with MGO (10 μ M) and the other with vehicle for 30 minutes. Bladder contractility was assessed in response to carbachol (1 nM–30 μ M) and electrical field stimulation (EFS, 1–32 Hz). We then tested co-incubation with anti-glycation agents: alagebrium (10 μ M), RAGE antagonist peptide (RAP, 1 μ M), and metformin (10 μ M). Incubation with MGO induced significant ($p < 0.05$) detrusor hypercontractility in response to both carbachol and EFS. Co-incubation with alagebrium, RAP, and metformin significantly ($p < 0.05$) reversed this hypercontractility. These findings indicate that MGO acutely activates the MGO-AGE-RAGE axis, promoting detrusor hypercontractility and reinforcing this pathway as a potential therapeutic target for voiding dysfunctions associated with glucotoxic stress.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Oral treatment with angiotensin-(1-7) ameliorates proteinuria in subclinical Acute Kidney Injury animal model

Authors:

Vitória Luiza LACERDA¹
Mariana Rodrigues CAMPOS¹
Laura Barroso Ferreira de OLIVEIRA¹
Arthur Fornazari IOST¹
Maria Aparecida Ribeiro VIEIRA¹
Robson Augusto Souza SANTOS¹
Celso CARUSO- NEVES²
Diogo Barros PERUCHETTI¹

Affiliations:

¹Departamento de Fisiologia e Biofísica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais

²Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro

Keywords: Angiotensin-(1-7). Proteinúria. Subclinical Acute Kidney Injury.

Abstract:

Subclinical Acute Kidney Injury (SubAKI) is a clinically silent syndrome associated with tubular injury and proteinuria without changes in glomerular function. Previous works point out the overactivation of the intrarenal classical arm of the Renin-Angiotensin System (RAS) in this process. It is known that the balance between the classical arm and the alternative arm of RAS, which involves angiotensin-(1-7) [Ang-(1-7)], determines the outcome of different diseases. However, the potential role of Ang-(1-7) in subAKI still needs to be elucidated. We aimed to study the potential effects of oral treatment with Ang-(1-7) on proteinuria in a subAKI animal model. Eight-week-old female and males C57Bl6 mice were used to generate the following groups: 1) CONT (n=15), control; 2) subAKI (n=18), mice treated with i.p. injection of 10g/kg/day bovine serum albumin (BSA) and oral doses of hydroxypropyl β -cyclodextrin (HP β CD) for 7 consecutive days; 3) subAKI+Ang-(1-7) (n=18), mice treated with BSA and 30 μ g/kg/day Ang-(1-7) included in HP β CD [HP β CD-Ang-(1-7)] via gavage; and 4) Ang-(1-7) (n=15), mice treated with HP β CD- Ang-(1-7) alone (CEUA-UFMG #164/2023). Then, the animals were housed in metabolic cages to collect 24h urine for biochemical analysis. No changes were observed in urinary volume, urinary flow, or the glomerular function parameters assessed. Compared to the CONT group, the subAKI group presented an increase in: 1) proteinuria and urinary protein mass (mg/24h) by 10-fold and 8-fold, respectively; and 2) urinary γ -GT activity (U/L), a specific tubular injury marker, by 8-fold. Interestingly, the treatment with HP β CD-Ang-(1-7) attenuated these alterations observed in subAKI (subAKI+Ang-(1-7) group). No significant changes were observed with treatment with Ang-(1-7) alone (HP β CD-Ang-(1-7) group). Our data suggests that the oral formulation of Ang-(1-7) ameliorated the development of proteinuria and tubular injury observed in subAKI.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Tubular injury precedes modifications in glomerular function at early-stage of focal segmental glomerulosclerosis

Authors:

Giulianne Bastos SERPA ¹
Cecília de Jesus SUDARIO¹
Rodrigo Alves da Silva PERES ¹
Ana Acacia de Sá PINHEIRO ^{1,2}
Celso CARUSO-NEVES ^{1,2,3}

Affiliations:

¹ Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.

² Rio de Janeiro Innovation Network in Nanosystems for Health-NanoSAÚDE/FAPERJ, Rio de Janeiro, Brazil.

³ National Institute of Science and Technology for Regenerative Medicine, Rio de Janeiro, Brazil.

Keywords: Focal segmental glomerulosclerosis. Podocyte injury. Tubulointerstitial injury.

Abstract:

Introduction: Focal segmental glomerulosclerosis (FSGS) is one of the leading causes of end-stage renal disease (ESRD). Although FSGS is primarily a glomerular disease, several studies have highlighted the role of tubular injury in its progression. One of the major challenges in FSGS today is understanding its pathogenesis and achieving early diagnosis. Objective: This study aimed to investigate the potential correlation between tubular and glomerular injury in the pathogenesis of secondary FSGS, identifying possible early-stage biomarkers. Methods: (CEUA083/23) A drug-induced FSGS model was established using a single intravenous dose of doxorubicin (10.5 mg/Kg) in adult male BALB/c mice: control (CTL, n=12) and DOX-treated (DOX, n=18). Two weeks post-injection, the animals were housed in metabolic cages for 24 hours and subsequently euthanized. Urine, plasma, and kidney tissue samples were collected for analysis. Statistical significance was set at $p < 0.05$, determined using Student's t-test. Data are presented as mean $\pm$ standard deviation. Results: Our findings showed that the DOX group did not have changes in estimated glomerular filtration rate, as measured by creatinine clearance. However, there was an increase in 24-hour proteinuria (7.16 ± 2.35 vs 22.88 ± 13.86 mg), UPCr (proteinuria/creatinine ratio; 11.17 ± 2.92 vs 23.96 ± 12.27), and UACr (albumin/creatinine ratio; 1.19 ± 0.75 vs 2.26 ± 1.25 mg/g creatinine) compared to the CTL group. An increase in extracellular lactate dehydrogenase activity (0.04 ± 0.03 vs 0.13 ± 0.10 U in 24h) was observed, along with elevated levels of proximal tubule urinary markers such as γ -glutamyltransferase (4.82 ± 2.14 vs 6.89 ± 2.14 U in 24h) in the DOX group. Conclusion: These findings suggest that tubular injury may occur before detectable alterations in traditional glomerular function markers, such as plasma creatinine. Therefore, tubular injury biomarkers could serve as valuable tools for the early diagnosis of FSGS. Funding: FAPERJ, CNPq, CAPES.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Opioidergic transmission in the shell portion of the nucleus accumbens (sNAcc) is involved in cardiovascular and urinary bladder control

Authors:

Rodrigo Pigozzi de CARVALHO¹,
Nuha Ahmad DSOUKI¹,
Eduardo Mazuco CAFARCHIO¹,
Bárbara do VALE¹,
Laurival Antonio DE LUCA Jr.²,
Patrik ARONSSON³,
Monica Akemi SATO¹

Affiliations:

¹Dept. Morphology and Physiology, Centro Universitario FMABC, Santo Andre, SP, Brazil,
²Dept. of Pathology and Physiology, Dentistry School, Sao Paulo State University (UNESP),
Araraquara, SP, Brazil,
³Dept Pharmacology, Institute of Neuroscience and Physiology, Sahlgrenska Academy,
University of Gothenburg, Gothenburg, Sweden.

Keywords: Cardiovascular. Nucleus Accumbens. Opioids receptors. Urinary bladder.

Abstract:

Introduction: GABAergic and glutamatergic transmissions in the sNAcc are involved in cardiovascular (CV) control and urinary bladder (UB) regulation. We investigated the role of opioidergic transmission in the sNAcc on CV and UB control. Method: Adult male Wistar rats (~300 g, CEUA-FMABC 05/2024) with stainless steel guide cannulas implanted bilaterally in the sNAcc 7 days prior to the experiments were isoflurane anesthetized and subjected to cannulation of the femoral artery and vein for mean arterial pressure (MAP) and heart rate recordings (HR). Left renal blood flow (RBF) was recorded by Doppler flowmeter. The UB was cannulated for intravesical pressure (IP) measurement. After the baseline MAP, HR, RBF and IP recordings for 15 min, endomorphine-2 (EM-2, 0,4 mmol/L, 1 μ L, $\mu\mu$ agonist), naloxone (NAL, 5 nmol/100nL, 1 μ L, $\mu\mu$ antagonist), BRL52537 (0,4mmol/L, 1 μ L, $\kappa\kappa$ agonist), ML190 (4mmol/L, 1 μ L, $\kappa\kappa$ antagonist), or saline (vehicle, 1 μ L) (N=6) injections were made bilaterally into the sNAcc and the variables were measured for additional 30 min. Data are as mean $\pm$ SEM and submitted to Student's t test (P<0.05). Results: Injections of EM-2 and BRL52537 into the sNAcc caused significant hypotension (-17 $\pm$ 4 mmHg EM-2, -31 $\pm$ 3 mmHg BRL52537 vs. 2 $\pm$ 1 mmHg saline), bradycardia (-28 $\pm$ 7 bpm EM-2, -20 $\pm$ 3 bpm BRL52537 vs. 3 $\pm$ 1 bpm, saline), increased renal conductance (20.54 $\pm$ 5.70% EM-2, 46.47 $\pm$ 9.12% BRL52537 vs. 1.95 $\pm$ 0.61% saline), and increased IP (161.79 $\pm$ 22.18% EM-2, 194.29 $\pm$ 21.51% BRL52537 vs. - 2.78 $\pm$ 2.87% saline. NAL and ML190 injections resulted in hypertension (19 $\pm$ 2 mmHg NAL, 25 $\pm$ 2 mmHg ML190 vs. 1 $\pm$ 1 mmHg saline), tachycardia (25 $\pm$ 5 bpm NAL, 14 $\pm$ 2 bpm ML190 vs. 1 $\pm$ 1 bpm saline), decreased renal conductance (-15.59 $\pm$ 2.13% NAL, -23.60 $\pm$ 1.67% ML190 vs. - 1.20 $\pm$ 0.71% saline), nevertheless, NAL increased IP (10.83 $\pm$ 3.69%), whereas ML190 decreased IP (-15.56 $\pm$ 3.18%). Conclusion: Opioidergic transmission in the sNAcc participates in the neural circuitry involved in UB and CV control.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Hyperglycemia-Induced Tubular Proteinuria in Non-Obese Diabetic Mice: A Potential Role in Tubular Injury Development

Authors

Larissa Miguel Atanasio¹;
Laura Barroso Ferreira de Oliveira¹;
Maria Laura Limeres¹;
Thais Lemos Ferreira²;
Maria Aparecida Ribeiro Vieira¹;
Luciano Santos Aggum Capettini²;
Cecilia Horta Ramalho Pinto³;
Ana Maria Caetano Faria³;
Diogo Barros Peruchetti^{1,4}

Affiliations:

¹Department of Physiology and Biophysics, Biological Science Institute, Federal University of Minas Gerais (email: lariatanasio278@gmail.com).

²Department of Pharmacology, Biological Science Institute, Federal University of Minas Gerais.

³Department of Biochemistry and Immunology, Biological Science Institute, Federal University of Minas Gerais. ⁴INCT-NANObiofar.

Keywords: Diabetic Kidney Disease; hyperglycemia; proteinuria; proximal tubule

Abstract

Diabetic Kidney Disease (DKD) is a serious public health problem associated with hyperglycemia-induced specific alterations in proximal tubule epithelial cells (PTECs). In this context, there is a strict correlation between PTEC injury and tubular proteinuria in kidney diseases. We aimed to investigate the potential role of PTECs in the development of tubular proteinuria and tubular injury in the early stages of DKD. We used Non-obese Diabetic (NOD) mice, which are susceptible strains for the development of Type 1 diabetes. This model develops type 1 diabetes after reaching 12 weeks of age. To assess the early stages of DKD, we employed NOD mice as well as non-obese diabetes resistant (NOR) mice (approximately 20-22g body weight, n=4/group) at the 3rd week after type 1 diabetes onset (CEUA-UFMG #155/2023). NOR mice were used as controls. It was observed that food and water intakes were significantly higher in the NOD mice when compared with NOR mice (food intake: NOD 7.75±1.37 g vs NOR 3.33±0.33 g; water intake: NOD 22.00±2.82 mL vs NOR 5.33±1.76 mL), which is consistent with the diabetes. In parallel, the results of urinary volume and urinary flow rate were significantly higher (approx. 15 times) in NOD mice when compared with NOR mice. Urinary osmolality was not changed between the experimental groups, spite of the slightly tendency of drop in NOD mice. In relation to renal protein handling, we observed a significant increase in proteinuria and UPCr, markers of renal disease, in NOD mice when compared with NOR mice (proteinuria: NOD 2.97±0.54 mg/24h vs NOR 0.14±0.005 mg/24h; UPCr: NOD 0.81±0.26 vs NOR 0.24±0.094). In addition, we observed that urinary γ -GT levels, specific marker of tubular injury, were significantly higher in the NOD mice when compared to NOR mice (NOD 0.73±0.14 U/L vs NOR 0.18±0.081 U/L). Our preliminary data showed in NOD mice that there is a development of tubular proteinuria associated with tubular injury in the early stages of DKD.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Dapagliflozin and Oxidative Stress: Exploring the Role of SGLT2 Inhibition in the Proximal Tubule

Authors:

Isabela Borges da Mota SILVEIRA¹
Antonio Viana NASCIMENTO-FILHO¹
Debora Conti Kimura LICHTENECKER²
Katia De Angelis Lobo D'AVILA²
Guiomar Nascimento GOMES²

Affiliations:

¹ Universidade Federal de São Paulo – Programa de Pós-Graduação em Medicina Translacional
² Universidade Federal de São Paulo – Departamento de Fisiologia

Keywords: dapagliflozin; oxidative stress; SGLT2; proximal tubule.

Abstract:

The kidneys are involved in glucose metabolism via tubular reabsorption and gluconeogenesis. The SGLT2 cotransporter located in the proximal tubule reabsorbs almost all filtered glucose. Inhibitors of this cotransporter are used in the treatment of type 2 diabetes, due to the produced intense glycosuria. We observed previously that normoglycemic animals treated with dapagliflozin (3 mg/kg) developed hypoglycemia due to elevated glucose urinary excretion. However, this effect may alter glutamine's metabolism, which is essential for renal gluconeogenesis. Considering that this drug can induce changes in tubular cellular metabolism and interfere with the transport of various solutes, we investigated its repercussions on the generation of reactive oxygen species (ROS) in renal tissue and on the excretion of markers of tubular damage. Three-month-old Wistar rats were divided into groups: (C) Control and (D) Dapagliflozin (treated with 3 mg/kg/day via gavage for 4 weeks). After treatment, the animals were housed in metabolic⁴ cages (24 h) for urine and blood collection. Plasma urea [U]p and creatinine [Cr]p, and urinary excretion of glucose, proteins, ammonium (NH⁺), titratable acids (TA), LDH and γ -GT and EROS in renal tissue were analyzed (n=8). CEUA: 5985180423 - UNIFESP. Dapagliflozin resulted in increased NH₄⁺ excretion (C: 1.1±0.1; D: 8.0±0.1 μ Eq/mL), TA (C: 0.50±0.24; D: 2.07±0.84 μ Eq/mL), LDH (C: 0.0±0.0; D: 0.015±0.001 U/L) and γ -GT (C: 0.0±0.0; D: 387.2±37.6 U/L), and also increased [U]p (C: 36.5±1.0; D: 52.3±2.4 mg/dL). In kidney tissue, there was increase in the levels of hydrogen peroxide (C: 4.86±0.36; D: 6.69±0.47 μ mol H₂O₂) and carbonyls (C: 3.73±0.29; D: 4.83±0.13 nmol/mg). Dapagliflozin induced metabolic changes that resulted in increased acid excretion, accompanied by augmented production of ROS in renal tissue and excretion of renal damage markers, highlighting the important impact of this drug on renal homeostasis.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Activation of the Angiotensin II Type 1 Receptor Mediates the Development of Kidney Injury and Proteinuria Observed in Obesity-Related Diabetic Kidney Disease

Authors:

Laura Araújo Fortes RIBEIRO¹;
Augusto Mitsuo Shibata MEIRA¹;
Laura Barroso Ferreira OLIVEIRA¹;
Luciana Souza Brandão MARTINS¹;
Lídia Silva PARREIRA³;
Kamylle Silva FERRAZ¹;
Maria Aparecida Ribeiro VIEIRA¹;
Maria José Campagnole-SANTOS^{1,2};
Maristela Oliveira POLETINI¹;
Luciene Bruno VIEIRA³;
Diogo Barros PERUCHETTI^{1,2}

Affiliations:

¹Department of Physiology and Biophysics, Institute of Biological Sciences, Universidade Federal de Minas Gerais;

²INCT-Nanobiofar;

³Department of Pharmacology, Institute of Biological Sciences, Universidade Federal de Minas Gerais

Keywords: Angiotensin II; Diabetic Kidney Disease; Losartan; Obesity; Type 2 diabetes mellitus.

Abstract:

The increasing cases of type 2 diabetes *mellitus*, associated with obesity, have made diabetic kidney disease (DKD) the leading cause of chronic kidney disease (CKD). The mechanisms of this process still need to be elucidated; however, evidence suggests a potential role of components of the Renin-Angiotensin System since studies have demonstrated the renoprotective effect of losartan in CKD patients. In this context, we aimed to investigate the role of AT1R in the development of kidney injury and proteinuria associated with obesity-related DKD. We used a hyperpalatable diet-induced obesity experimental model. Male C57BL6 mice (5 weeks old) were divided into groups: CONT group, mice fed the AIN93G diet (n=14); HSB group, mice fed the HSB diet for 12 weeks (n=10); HSB+LOS group, mice fed the HSB diet and treated daily with LOS (losartan 30 mg/kg/day by gavage) for the last 4 weeks (n=10); and LOS group, mice fed the AIN93G diet and treated with LOS (n=12). The following procedures were performed: monitoring of body weight and specific fat deposits; glucose tolerance test; tail plethysmography; urine and plasma collection for biochemical and renal functional analysis (CEUA-UFMG #8/2025). No significant differences were observed in plasma creatinine and plasma urea concentrations as well as blood pressure. Compared to the CONT group, the HSB group showed: a significant increase in body weight; increase in epididymal, retroperitoneal, and mesenteric fat deposits; increase in glycemia during the iGTT; reduced urinary volume; a significant increase of urinary protein excretion; and increased urinary excretion of γ -glutamyl transferase, marker of tubular injury. Daily treatment with losartan for 4 weeks attenuated all parameters, except for the iGTT. No differences were observed between the CONT and LOS groups. Our results demonstrate that AT1R act as a mediator in the development of tubular injury and proteinuria induced by obesity-associated DKD.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Activation of Ang II/AT1R axis mediates the tubular injury and fibrosis associated with tubular proteinuria observed in the early stage of Diabetic Kidney Disease

Authors:

Mariana Rodrigues Campos¹
Pedro Henrique de Sena Reis Seabra¹
Laura Barroso Ferreira de Oliveira¹
Vitória Luiza Lacerda¹
MC Moraes^{1,2};
Letícia Cristine Cardoso dos Santos³
Paula Peixoto Campos Lopes³
Maria Aparecida Ribeiro Vieira¹
Celso Caruso-Neves⁴
Diogo de Barros Peruchetti^{1,5}

Affiliations:

¹Department of Physiology and Biophysics, Institute of Biological Sciences, Federal University of Minas Gerais

²Department of Pharmacology, Institute of Biological Sciences, Federal University of Minas Gerais

³Department of General Pathology, Institute of Biological Sciences, Federal University of Minas Gerais

⁴Carlos Chagas Filho Biophysics Institute, Federal University of Rio de Janeiro; ⁵INCT-Nanobiofar.

Keywords: diabetes, kidney disease, proteinuria, angiotensin II.

Abstract:

Diabetic Kidney Disease (DKD) pathogenesis involves tubular dysfunction associated with tubular proteinuria. The mechanism involved in this process is still unknown. Clues come from reports showing the renoprotective effects of losartan, a specific antagonist of the angiotensin type 1 receptor (AT1R), on different renal diseases. Herein, we investigated the potential role of AT1R activation in tubular proteinuria and injury at the early stage of DKD. Ten-week-old male Wistar rats were used to develop DKD using streptozotocin (55 mg/kg i.v.). When indicated, the animals were daily treated with 30 mg/kg/day of losartan via gavage. Four groups were generated: 1) CONT, normoglycemic rats (n=9); 2) STZ, diabetic rats (n=9); 3) STZ+LOS, diabetic rats treated with losartan (n=9); 4) LOS, normoglycemic rats treated with losartan (n=9). All analyses were performed after 8 weeks (CEUA-UFGM#100/2023). Compared to the CONT group, the STZ group showed: (1) increased renal clearance of Na⁺ (CNa⁺, 4-fold) and Cl⁻ (CCl⁻, 2-fold) as well as in their fractional excretion (FE) parameters (FENa⁺, 4-fold; FECl⁻, 2-fold) associated with impaired cortical (Na⁺+K⁺)ATPase activity; (2) increased proteinuria (mg/24h, 2.5-fold), renal protein clearance (12.4-fold), the FE of proteins (4.3-fold) associated with impaired *in vivo* FITC-albumin endocytosis; (3) increased urinary gamma-glutamyl transferase (3.4-fold, tubular injury marker) and urinary lactate dehydrogenase (3-fold, renal injury marker); (4) increased renal injury score associated with an increased interstitial cell infiltration (2.45-fold) and tubular collagen deposition (2-fold). Interestingly, the daily treatment with losartan attenuated all analyzed functional and structural renal parameters (STZ+LOS group). Altogether, our preliminary data indicate that the activation of AT1R mediates hyperglycemia-induced tubular injury and fibrosis associated with tubular proteinuria observed at the early stages of DKD.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Impact of Resistance Training on Renal Electrolyte Balance in Female Wistar Rats Under Cross-Sex Hormone Therapy

Authors:

Isadora Gonçalves ALMEIDA¹
Isabela Borges da Mota SILVEIRA²
Letícia Maria MONTEIRO²
Rogério ARGENTI²
Débora Conte Kimura LICHTENECKER¹
Magnus Régios DIAS-DA-SILVA¹
Guiomar Nascimento GOMES¹

Affiliations:

¹ Federal University of São Paulo – Department of Physiology

² Federal University of São Paulo – Postgraduate Program in Translational Medicine

Keywords: Cross-sex hormone therapy. Electrolyte balance. Progressive resistance training exercise. Renal function.

Abstract:

Cross-sex hormone therapy (CHT) involves administering testosterone to females to induce secondary male characteristics. Experimental studies on CHT models have reported some kidney alterations. In contrast, physical exercise is recognized for its beneficial effects on renal function. Given these different influences, this study aimed to investigate whether resistance training can modulate the renal alterations induced by CHT. Two-month-old female Wistar rats were divided into four groups: SV (sedentary+vehicle), SH (sedentary+CHT), EV (exercised+vehicle) and EH (exercised+CHT). Vehicle or testosterone cypionate (3 mg/kg) were administered intramuscularly every 10 days for 8 weeks. The exercise groups trained on a vertical climbing ladder five times weekly for six weeks with increasing load. After training weeks, animals were placed in metabolic cages for urine and blood collection and analysis. (CEUA/UNIFESP: 9009241022). Mean±SD, ANOVA, $p \leq 0.05$. Creatinine clearance increased with CHT and decreased with exercise (SV: 5.93 ± 0.69 ; SH: 7.28 ± 1.21 ; EV: 5.26 ± 1.07 ; EH: 6.17 ± 0.51 mL/min/Kg; $p = 0.0028$). CHT increased plasma levels of Na^+ (SV: 148.2 ± 4.02 ; SH: 154.7 ± 2.91 ; EV: 143.9 ± 1.57 ; EH: 145.6 ± 2.72 mmol/L; $p < 0.0001$), PO_4^{3-} (SV: 5.03 ± 0.35 ; SH: 6.18 ± 0.22 ; EV: 4.83 ± 0.39 ; EH: 4.64 ± 0.28 mg/dL; $p < 0.0001$) and Cl^- (SV: 104.8 ± 1.67 ; SH: 107.6 ± 2.07 ; EV: 104.6 ± 2.22 ; EH: 103.8 ± 1.67 mmol/L; $p = 0.0014$). EH groups had plasma values of Na^+ and PO_4^{3-} similar to SV. CHT reduced urinary excretion load of Na^+ (SV: 4.42 ± 0.57 ; SH: 3.63 ± 0.83 ; EV: 3.86 ± 0.59 ; EH: 3.29 ± 0.86 mEq/24h; $p = 0.0216$), Ca^{2+} (SV: 3.38 ± 1.78 ; SH: 1.29 ± 0.88 ; EV: 2.56 ± 0.7 ; EH: 3.43 ± 1.52 ug/min/Kg; $p = 0.0047$) and Mg^{2+} (SV: 5.04 ± 2.4 ; SH: 3.30 ± 1.8 ; EV: 8.12 ± 2.6 ; EH: 5.18 ± 1.2 ug/min/Kg; $p = 0.0036$). Plasma and urinary K^+ remained unchanged. In conclusion, CHT and exercise affected renal electrolyte excretion in opposite ways, thus contributing to the balance of ion excretion.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Impairment of postprandial glucagon-like peptide-1 bioavailability and renal glucagon-like peptide-1 receptor activation in experimental hypertension

Authors:

Natália Carolina PEREIRA¹
Acaris Bennetti dos SANTOS ¹
Andreia BOARO ¹
Adriana Castello Costa GIRARDI ¹

Affiliations:

¹ Laboratório de Genética e Cardiologia Molecular, Instituto do Coração (InCor), Hospital das Clínicas HCFMUSP, Faculdade de Medicina, Universidade de São Paulo, São Paulo, SP, BR

Keywords: Dipeptidyl-peptidase IV. Glucagon-like peptide-1. Hypertension.

Abstract:

Glucagon-like peptide-1 (GLP-1) is an incretin hormone primarily involved in glycemic regulation. Beyond its metabolic effects, GLP-1 receptor (GLP-1R) agonists—used in the treatment of type 2 diabetes—have demonstrated extra glycemic benefits, including increased diuresis and natriuresis and reduced blood pressure (BP) in non-diabetic hypertensive models and patients. Based on these findings, we hypothesized that reduced postprandial GLP-1 levels in hypertension impair renal GLP-1R activation, promoting sodium and water retention and contributing to elevated BP. We studied male spontaneously hypertensive rats (SHR) and normotensive controls to test this. The study was approved by the Ethics Committee on Animal Use of the Faculty of Medicine at the University of São Paulo, approval number 1885/2023. Blood was collected under fasting and postprandial conditions to measure total and intact GLP-1 levels; urine was analyzed for cyclic AMP (cAMP); kidneys were harvested for analysis of GLP-1R, phosphorylated and total catalytic PKA, and dipeptidyl peptidase IV (DPP4) activity. Normotensive rats showed a greater postprandial increase in urinary cAMP compared to SHRs. Although intact GLP-1 levels were similar, SHRs exhibited higher total GLP-1, resulting in a lower intact/total GLP-1 ratio. Additionally, SHRs displayed reduced renal expression of GLP-1R and PKA (both total and phosphorylated) and elevated DPP4 activity in serum and urine, but not in kidney homogenates. These findings suggest that hypertension is associated with increased GLP-1 degradation and reduced renal GLP-1R expression and signaling, contributing to impaired natriuretic responses.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Renal effects of a single intravenous irisin injection prior to bilateral kidney ischemia reperfusion injury

Authors:

Raquel Carvalho CASTIGLIONE¹
Rachel Vicente da SILVA¹
Leannon Fundao Leite THOMY¹
Daniela Lopes de AGUIAR¹
Iasmim Bento Leal¹
Rayana Guagliardi Brito¹
Ana Beatriz Castro Ribeiro¹
Giovanna de Andrade de Barros e Vasconcellos¹
Silvio Rodrigues MARQUES NETO^{1,2}
Eliete BOUSKELA¹

Affiliations:

¹Universidade do Estado do Rio de Janeiro ²Universidade Salgado de Oliveira

Keywords: Acute renal injury. Irisin. Renal ischemia/reperfusion injury. Renoprotection. Tubular injury.

Abstract:

Renal ischemia/reperfusion (RIRI) injury generates cellular damage that can compromise homeostasis due to the increase in reactive oxygen species, generating local inflammatory processes, hyperplasia and renal hypertrophy. Irisin is a protein with diverse physiological actions such as anti-inflammatory, anti-oxidative and anti-apoptotic properties. Our aim was to evaluate the renoprotective role of a single low dose of irisin administration against injuries resulting from bilateral RIRI. Male Wistar rats were divided into 4 experimental groups (n=6): sham operated (SHAM), sham operated + irisin (SHAM+IRI), ischemia and reperfusion (I/R) and ischemia and reperfusion + irisin (I/R+IRI). The animals received saline solution or 10 nM irisin via the jugular vein 30 minutes prior to an one hour bilateral renal ischemia. Twenty-four hours after reperfusion, changes in renal function and histopathology were assessed. All experiments were approved by the State University of Rio de Janeiro (UERJ) Committee on Experimental Animals (CEUA/039/2022). All data are presented as mean $\pm$ SD. One-way analysis of variance (ANOVA) followed by Tukey post hoc test was used for all analysis and the significance level was set at $p < 0.05$. Glomerular filtration rate was 75% reduced in I/R group (0.099 ± 0.065 ml/min, $p < 0.05$) related to SHAM (0.39 ± 0.070 ml/min) and restored with irisin administration (0.28 ± 0.11 ml/min, $p > 0.05$). Plasmatic urea levels were higher in I/R group (167.7 ± 41.57 mg/dl, $p < 0.0001$) and partially restored to SHAM values (40.91 ± 7.010 mg/dl) after irisin administration (112.1 ± 35.16 mg/dl, $p < 0.01$). Renal index was higher in I/R (0.012 ± 0.0015 g/g b.w., $p < 0.01$) compared to SHAM (0.010 ± 0.0008 g/g b.w.) and restored to normal levels in I/R+IRI group (12.77 ± 3.285 g/g b.w., $p > 0.05$). Finally, tubular score injury was higher in I/R (0.012 ± 0.0015 , $p < 0.001$) compared to SHAM (0.1867 ± 0.1498) and partially restored after irisin administration (5.856 ± 1.090 , $p < 0.001$). We can conclude that 10 nM of irisin was able to improve renal function and the renal tubular injury score in animals subjected to bilateral ischemia and reperfusion.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Angiotensin converting enzyme 2 enhances megalin-mediated albumin endocytosis in proximal tubule cells by restraining Angiotensin II/AT1R

Authors:

Helena Ribeiro Peixoto¹
Rodrigo Pacheco Silva-Aguiar¹
Diogo de Barros Peruchetti⁴
Ana Acacia Pinheiro^{1,2}
Celso Caruso-Neves^{1,2,3}

Affiliations:

¹Carlos Chagas Filho Biophysics Institute, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

²Rio de Janeiro Innovation Network in Nanosystems for Health-NanoSAÚDE/FAPERJ, Rio de Janeiro, Brazil

³National Institute of Science and Technology for Regenerative Medicine, Rio de Janeiro, Brazil

⁴Department of Physiology and Biophysics, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Brazil;

Keywords: Albumin endocytosis; Angiotensin-converting enzyme type 2 (ACE2); Megalin.

Abstract:

Introduction: Urinary albumin excretion is a risk factor for kidney disease progression and mortality. In proximal tubule epithelial cells (PTECs), albumin is reabsorbed via megalin-mediated endocytosis. Although the renin-angiotensin system modulates this process, the role of angiotensin-converting enzyme type 2 (ACE2) remains unclear. Objective: To investigate the role of ACE2 in albumin endocytosis in PTECs and its link to kidney disease. Methods: PT cell lines (LLC-PK1, HEK-293, and hACE2-HEK-293) were used to evaluate uptake of FITC-albumin, DQ-albumin, and FITC-dextran via fluorescence. Angiotensin II levels were measured by ELISA. Specific inhibitors targeted ACE2, AT1R and MASR. Megalin and cubilin expression were assessed using immunofluorescence and confocal microscopy. A subclinical acute kidney injury model was induced in BALB/C male mice through intraperitoneal injection of bovine serum albumin, with 12 animals divided into control and subAKI groups (CEUA: A13/20-045-17). Human data correlations were performed via the NephroSeq database. Statistical tests included t-test, ANOVA and linear regression ($p < 0.05$). Results: Albumin endocytosis in hACE2-HEK-293 cells was twice as high as in HEK-293 cells. Albumin binding and megalin expression (mRNA and protein) were similarly increased. Extracellular Ang II levels were lower in hACE2-HEK-293 cells. The ACE2 inhibitor MLN-4760 reduced FITC-albumin uptake in both HEK-293 and hACE2-HEK-293 cells; this effect was reversed by co-treatment with losartan. In LLC-PK1 cells, ACE2 inhibition decreased albumin uptake and increased Ang II levels. In the subAKI model, ACE2 expression was reduced and negatively correlated with proteinuria. NephroSeq analysis showed lower ACE2 and higher ACE expression in PTECs from acute kidney injury patients. Conclusion: Reduced ACE2 expression activates the Ang II/AT1R pathway, downregulating megalin and impairing albumin reabsorption, contributing to albuminuria in subAKI.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Preventive exercise improves oxidative stress and inflammation in diabetic kidney disease through increased glutathione peroxidase activity and suppression of TNF- α regulated by the SDF-1 α /CXCR4 pathway

Authors:

Gabriela Freitas Silva BITENCOURT¹
Fernanda Abreu SILVA¹
Cláudia Silva SOUZA²
Thiago Macêdo Lopes CORREIA³
Liliany Souza de Brito AMARAL¹
Rildo Aparecido VOLPINI⁴
Amélia Cristina Mendes de MAGALHÃES¹
Telma de Jesus SOARES¹

Affiliations:

¹Instituto Multidisciplinar em Saúde - Universidade Federal da Bahia *campus* Anísio Teixeira
²Instituto de Ciências Biomédicas - Universidade de São Paulo ³Instituto de Ciências da Saúde - Universidade Federal da Bahia ⁴Faculdade de Medicina de Ribeirão Preto - Universidade de São Paulo

Keywords: Diabetes. Exercise. Inflammation. Kidney. Oxidative stress.

Abstract:

Diabetic kidney disease involves a complex series of pathways to induce renal injury, including oxidative stress and inflammation. Stromal cell-derived factor 1 α (SDF-1 α) and its receptor CXCR4, associated with elevated levels of TNF- α , have been implicated in renal injury. Thus, this study evaluated the impact of physical exercise on renal changes induced by type 1 diabetes mellitus (T1DM) in female rats. Twenty-four female Wistar rats (200-250g) were divided into 4 groups (n=6): sedentary control (SC), sedentary diabetic (SD), trained diabetic (TD) and previously trained diabetic (PTD). Only the PTD group was submitted to four weeks of exercise before the induction of T1DM induced with streptozotocin (40mg/kg, iv). After induction of T1DM, PTD and TD groups underwent 8 weeks of exercise. This study was approved by CEUA/IMS/UFBA (protocol 052/2017). TBARS levels in renal tissue were increased in the SD group (2.2 ± 0.6) and the exercise prevented this increase in the TD (1.2 ± 0.2) and PTD groups (1.0 ± 0.2), $p < 0.01$. Both exercise protocols increased EC-SOD compared to the SD group (75.8 ± 20.8) ($p < 0.05$), while only previously trained animals showed increased GPx activity in the PTD group (1.0 ± 0.14) compared to the SD (0.66 ± 1.3), $p < 0.01$. Only the PTD group showed a reduction in renal TNF- α levels (8.5 ± 2.7), protein expression of SDF-1 α (26.7 ± 4.3), as well as was able to prevent the protein expression of the CXCR4 receptor (38.0 ± 9.7) in relation to the SD group (15.6 ± 7.6), (55.6 ± 16.8) and (55.6 ± 16.8), $p < 0.05$, respectively. Exercise improved renal IL-10 levels in the trained groups in relation to the SD (0.24 ± 0.1), $p < 0.05$. In conclusion, both exercise protocols improved renal TBARS, EC-SOD and IL-10. However, the exercise performed prior to T1DM promoted additional beneficial effects on the kidney by reducing renal oxidative stress through GPx activity, as well as improving the inflammatory process through suppression of TNF- α probably regulated by the SDF-1 α /CXCR4 pathway.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Intermittent fasting modulates renal function in association with estrous cycle variation and autophagy mechanisms

Authors:

Arthur Fornazari IOST¹
Leonardo Oliveira GUARNIERI¹
Laura Barroso Ferreira de OLIVEIRA¹
Mariana Rodrigues CAMPOS¹
Olívia Ramos Costa Val PEDROSA¹
Maria Aparecida Ribeiro VIEIRA¹
Diogo Barros PERUCHETTI²

Affiliations:

¹Department of Physiology and Biophysics, Institute of Biological Science, Federal University Minas Gerais, Belo Horizonte, Brazil;
²INCT-Nanobiofar, Belo Horizonte, Brazil

Keywords: Intermittent fasting. Kidney. Estrous cycle. Autophagy

Abstract:

Introduction: Intermittent fasting (IF) is a dietary restriction used as non-pharmacological therapy of disorders including type 2 diabetes mellitus. One potential mechanism involved is autophagy. In addition, IF can lead to endocrine changes on the estrous cycle what could affect sex hormone-targeted peripheral organs, like kidneys. Moreover, estrogens modulate kidney function and autophagy. Objective: To study the potential impact of IF on kidneys. Methods: Female Wistar rats (10 weeks old) were randomly divided into two experimental groups: 1) CONT group (control, n=4), with free access to chow; and 2) IF group (n=5), rats subjected to the IF protocol [fasting: 18h (from "zeitgeber time" (ZT)=8 up to ZT=24)/ feeding: 6h (from ZT=1 up to ZT=7)] for 4 consecutive weeks (CEUA-UFMG#13-2023). Results: Compared to the CONT group, the IF group showed: 1) reduction in body weight (BW, 15%) and in visceral adipose tissue depots. Regarding renal function, IF group showed: 1) reduced hydric balance due to increased water intake (3.0-fold) and reduction in urinary volume (64%); 2) reduced glycosuria (44%) and fractional excretion (FE) of glucose (FE_{glucose}); 3) increased proteins excretion (1.8-fold) and increased FE_{proteins}; 4) increased natriuresis (20%) associated with reduced cortical (Na⁺⁺K⁺) ATPase activity. No significant changes were observed in urinary kidney injury biomarkers (γ-GT and LDH) and in renal cortex structures. We found positive correlations between proteinuria or glycosuria and estrous cycle in CONT group. IF group showed a difference frequency of meta estrus and diestrus phases [36.36% (IF) / 22.73% (CONT); 23.64% (IF) / 43.18% (CONT), respectively]. Regarding autophagy at renal cortex, we observed reduction in p62 expression as well as increased LC3B expression in IF group. Conclusions: Our findings suggest that IF modulates cortical tubule transport. These effects could be associated with alterations in the estrous cycle and in autophagy.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Valproic Acid Mitigates Hypertension Associated with Ischemia/reperfusion-induced Acute Kidney Injury

Authors:

Danilo Santos ALVES-BEZERRA^{1,2,3}
Isadora Gonçalves ALMEIDA^{4,5}
João Pedro Moreira VELOSO-SANTOS^{2,3}
Amaury PEREIRA-ACÁCIO^{2,3}
Glória COSTA-SARMENTO²
Humberto MUZI-FILHO^{2,3}
Lucienne Silva LARA^{2,6}
Adalberto VIEYRA^{1,2,3}

Affiliations:

¹Graduate Program of Translational Biomedicine, Grande Rio University, Duque de Caxias, Brazil

²Center for Research in Precision Medicine, Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

³National Center for Structural Biology and Bioimaging/CENABIO, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

⁴Departament of Physiology, Federal University of São Paulo, São Paulo, Brazil

⁵Aristides Pacheco Leão Program to Encourage Scientific Vocations, Brazilian Academy of Sciences, Rio de Janeiro, Brazil

⁶Institute of Biomedical Sciences, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

Keywords: Acute kidney injury. Hypertension. Ischemia/reperfusion. Valproic acid

Abstract:

Acute kidney injury (AKI) is associated with a high risk of mortality and progression to chronic kidney disease. The lack of treatments has driven the search for new therapies, including drug repurposing. We hypothesize that valproic acid (Val) may be involved in the pathophysiology of AKI-associated damage through the histone acetylation/deacetylation balance. CEUA/UFRJ approved the protocols (081/23). At 28 days of age, male Wistar rats were randomly divided into two groups, one receiving Val diluted in water ($100 \text{ mg} \times \text{kg}^{-1} \times \text{day}^{-1}$) and the other receiving the vehicle. At 48 days, the groups were further subdivided, with one subgroup undergoing bilateral renal pedicle clamping for 45 min to induce ischemia and AKI, followed by 48 h of reperfusion (I/R). The other subgroup underwent false surgery (SHAM). At 50 days, the animals were placed in metabolic cages to assess urinary flow, plasma and urinary creatinine (pCr and uCr), Na^+ and K^+ excretion (Na_E and K_E), and systolic blood pressure (SBP) by tail-cuff plethysmography. The I/R rats presented higher SBP than SHAM rats (160 ± 9 vs 129 ± 8 mmHg, $n=7-8$), which was completely reversed in the I/R+Val rats (133 ± 12 mmHg, $n=7$). I/R rats presented elevated pCr levels (0.55 ± 0.06 vs 0.37 ± 0.14 mg/dl in SHAM rats, $n=7-8$), which also returned to SHAM values in the I/R+Val rats (0.35 ± 0.13 mg/dl, $n=6$). Furthermore, I/R rats presented reduced Cr clearance (0.54 ± 0.27 vs 1.68 ± 0.86 ml/min in SHAM rats, $n=5-8$), which was restored to SHAM levels in the I/R+Val rats (1.62 ± 0.74 ml/min, $n=6$). I/R rats presented a reduced Na_E (0.50 ± 0.35 vs 1.06 ± 0.32 mEq/24 h in SHAM rats, $n=7-8$) and K_E (1.91 ± 0.84 vs 3.43 ± 0.87 mEq/24 h in SHAM rats, $n=7-8$) while Val did not produce any changes. The results indicate that Val selectively improves altered renal functional parameters and prevents hypertension in the I/R model. These observations suggest that histone acetylation/deacetylation balance is a key molecular mechanism in AKI pathophysiology.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Hypovolemic states affect the urinary bladder responsiveness to the neurotransmitters of the autonomic nervous system and circulating hormones in female rats

Authors:

Maria Eduarda de ALMEIDA¹,
Vinicius PORTELA¹
Gisele GIANNOCCO^{2,3}
Rui M.B. MACIEL²
Barbara do VALE¹
Patrik ARONSSON⁴
Monica A. SATO¹
Eduardo M. CAFARCHIO¹

Affiliations:

¹Dept. Morphology and Physiology, Faculdade de Medicina do ABC, Centro Universitario FMABC, Santo Andre, SP, Brazil,
²Dept. Medicine, Federal University of Sao Paulo, Sao Paulo, SP, Brazil,
³Dept. Biological Sciences, Federal University of Sao Paulo, Diadema, SP, Brazil,
⁴Dept Pharmacology, Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden.

Keywords: Dehydration. Intravesical pressure. Oxytocin. Urinary bladder. Vasopressin.

Abstract:

Urinary bladder (UB) control involves complex mechanisms that are not fully understood. We aimed to investigate the effects of hemorrhage (HH) and hypovolemic shock (HS) on the UB reactivity in rats. Female Wistar rats (~250 g, CEUA-FMABC#04/2020) isoflurane-anesthetized were submitted to catheterization of the femoral arteries for baseline mean arterial pressure (MAP) and heart rate (HR) recordings and blood withdrawal, respectively, and the UB was cannulated for intravesical pressure (IP) recordings. After baseline MAP, HR and IP recordings, rats underwent a HH [n=6, by withdrawal of ~20% of total blood volume (TBV), equal to ~8% of body weight, until MAP achieved a steady level of ~70 mmHg for 40 min] or were submitted to HS [n=6, by withdrawal of ~30% of TBV until the MAP could achieve values of ~45 mmHg for 20 min] or maintained normovolemic (NV, n=6) for 40 min. Subsequently, topical (*in situ*) administration (0.1 mL) of acetylcholine (Ach, 2.0 µg/mL), or norepinephrine (NOR, 2.0 µg/mL), or vasopressin (VP, 1.0 ng/mL), or oxytocin (OT, 1.0 ng/mL), or saline (SAL, Vehicle) was directly performed onto the UB and the MAP, HR, and IP were recorded. Data are as mean±SEM and submitted to One-way ANOVA (Tukey's post-test, p<0.05). Ach increased IP (488.8±24.9%, NV), nevertheless, a significant reduction in the response was observed in HH (51.5±0.74%) or HS rats (36.4±1.58%). NOR decreased IP (-67.5±1.6%, NV), however the response was abolished in HH (-6.1±2.1%) or HS (5.7±1.1%). VP increased IP (110.2±1.9%, NV), in contrast, HH (19.9±0.3%) or HS (13.9±0.7%) showed significantly reduced responses. OT decreased IP (-47.5±1.2%, NV), however the response was abolished in HH (-0.1±0.5%) or HS (0.7±0.5%). SAL caused no changes in IP in NV, HH or HS. Therefore, hypovolemic states as HH and HS affect the UB, strongly reducing the reactivity to the neurotransmitters of the autonomic nervous system, which innervates the bladder, as well as to circulating hormones (VP and OT).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Study of LEAP2 in fasting and renal ischemia and reperfusion in normotensive and hypertensive rats

Authors:

João Victor Melo da ROCHA¹

Jhulle Horrane Moreira CARVALHO¹

Sixta Isabel Atencio Berrio¹

João Pedro Maciel Machado LEITE¹

Juliana Vila Verde RIBEIRO¹

Hanniely Thais Menezes dos SANTOS¹

Michelle Mendanha MENDONÇA¹

Karla de Aleluia BATISTA²

Gustavo Rodrigues PEDRINO¹

Carlos Henrique XAVIER¹

Affiliations:

¹ Universidade Federal de Goiás

² Instituto Federal de Goiás

Keywords: Cardiometabolic diseases. Ghrelin. LEAP2. GHSR. Nephroprotection.

Abstract:

Cardiometabolic diseases, such as hypertension and diabetes mellitus have high morbimortality rates. The study of peptides modulating metabolism and hemodynamics may pave ways for innovative therapeutic routes. Liver expressed antimicrobial peptide 2 (LEAP2) was recently described as GHSR inverse agonist; the latter is the broadly expressed receptor for ghrelin hormone. We previously showed that GHSR antagonism with the synthetic MK677 produces lower responses in spontaneously hypertensive rats (SHR), which in turn displays renal GHSR underexpression. In this study, we investigated the LEAP2 effects on renal function during hypertension and on the deleterious effects of renal ischemia and reperfusion (IR). After ethics committee approval (CEUA-UFG 28/24), we used normotensive (Wistar – WT) and SHR (n=10) injected (ip) with vehicle (NaCl 0.9%) or LEAP2 (6 or 600 nmol/kg), fed *ad libitum* or under a 24h fasting period. After treatments, rats were set in metabolic cages for 24h. At the end, plasma and urinary samples were taken for biochemical analyses. In another set of experiments, SHR were anesthetized (Ketamine 80mg/kg and Xylazine 10mg/kg) and underwent transient kidney ischemia (15min) by clipping the left renal artery, followed by reperfusion, and were set in metabolic cages for subsequent 24h. Statistics: ANOVA followed by Tukey's test. LEAP2 at dose 6 nmol/kg reduced plasma potassium in SHR ($\Delta -0.46 \pm 0.18$ mmol/L; $p < 0.05$), reduced sodium excretion in WT fed *ad libitum* ($\Delta -17.85 \pm 3.4$ mmol/L; $p < 0.05$) and did not affect plasma or urinary creatinine and creatinine clearance in WT and SHR. Fasting effects on urinary creatinine ($\Delta 11.8 \pm 3.03$ mg/kg/24h; $p < 0.05$) were unaffected by LEAP2. The increases in creatinine clearance ($\Delta 6.629 \pm 2.11$ mL/min/kg; $p < 0.05$) evoked by LEAP2 (600 nmol/kg) in SHR were absent in LEAP2 treated IR group. Our preliminary data suggest that LEAP2 modulates renal function of hypertensive kidneys without affecting the IR-related consequences in SHR.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Cellular dehydration-evoked models induce reduction in the responsiveness of the urinary bladder to neurotransmitters of the autonomic nervous system and vasopressin in female Wistar rats

Authors:

Maria Eduarda de ALMEIDA¹
Vinicius PORTELA¹
Gisele GIANNOCCO^{2,3}
Rui M.B. MACIEL²
Barbara do VALE¹
Patrik ARONSSON⁴
Monica A. SATO¹
Eduardo M. CAFARCHIO¹

Affiliations:

¹Dept. Morphology and Physiology, Faculdade de Medicina do ABC, Centro Universitario FMABC, Santo Andre, SP, Brazil,
²Dept. Medicine, Federal University of Sao Paulo, Sao Paulo, SP, Brazil,
³Dept. Biological Sciences, Federal University of Sao Paulo, Diadema, SP, Brazil,
⁴Dept Pharmacology, Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden.

Keywords: Autonomic nervous system. Dehydration. Intravesical pressure. Urinary bladder. Vasopressin.

Abstract:

The maintenance of internal homeostasis depends on an adequate hydroelectrolytic control. This study aimed to investigate if cellular dehydration affects the urinary bladder (UB) responsiveness, shedding light on the potential integration between hydroelectrolytic regulation and lower urinary tract mechanisms in blood volume control. Female Wistar rats (~250 g, CEUA-FMABC #04/2020) were maintained normovolemic (control group, n=6), or subjected to water deprivation (WD, n=6) for 24 h and maintained with chow-pellets *ad libitum*, or submitted to intragastric administration of hypertonic saline solution (HSS, 2 mL of 2 M NaCl) followed by 2 h without food or water. Afterwards, rats were isoflurane-anesthetized and submitted to catheterization of the femoral artery for mean arterial pressure (MAP) and heart rate (HR) recordings, and the UB was cannulated for intravesical pressure (IP) recordings. After baseline MAP, HR and IP recordings, topical (*in situ*) administration (0.1 mL) of acetylcholine (Ach, 2.0 µg/mL), or norepinephrine (NOR, 2.0 µg/mL), or vasopressin (VP, 1.0 ng/mL), or saline (SAL, Vehicle) was directly performed onto the UB and the MAP, HR, and IP were recorded. Data are as mean±SEM and submitted to One-way ANOVA (Tukey's post-test, p<0.05). Ach increased IP (488.8±24.9%, NV), however, a significant reduction in the response was observed in WD (126.0±12.4%) or HSS rats (75.0±2.7%). NOR decreased IP (-67.5±1.6%, NV), nevertheless the response was significantly attenuated in WD (-17.5±1.0%) or HSS rats (-37.0±1.9%). VP increased IP (110.2±1.9%, NV), whereas WD (38.5±1.0%) or HSS rats (41.8±1.2%) showed significantly reduced responses. SAL caused no changes in IP in NV, WD or HSS rats. Therefore, cellular dehydration caused a lower UB reactivity to the neurotransmitters (Ach and NOR) of the autonomic nervous system, which innervates the bladder, as well as to the circulating hormone (VP), likely to help in the reduction of volume loss from the body.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Resistance training evokes inflammation and activates autophagy in the urinary bladder of female Wistar rats

Authors:

Larissa Araujo de ARRUDA¹
Maria Eduarda de ALMEIDA¹
Bárbara do VALE¹
Nuha A. DSOUKI¹
Roberta Goes da SILVA²
Gisele GIANNOCCO^{2,3}
Rui M.B. MACIEL²
Patrik ARONSSON⁴
Monica A. SATO¹

Affiliations:

¹Dept. Morphology and Physiology, Faculdade de Medicina do ABC, Centro Universitario FMABC, Santo Andre, SP, Brazil,
²Dept. Medicine, Federal University of Sao Paulo, Sao Paulo, SP, Brazil,
³Dept. Biological Sciences, Federal University of Sao Paulo, Diadema, SP, Brazil,
⁴Dept Pharmacology, Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden.

Keywords: Autophagy. Inflammation. Resistance training. Urinary bladder.

Abstract:

Bladder dysfunctions predominantly affect women. High-intensity resistance training can impair the urinary bladder (UB) reactivity to neurotransmitters of the autonomic nervous system after 3 wks of exercise, nevertheless, after 10 wks of training the UB responsiveness can be recovered. We investigated if climbing resistance training (CRT) promotes changes in inflammation and autophagy/mitophagy markers in the UB. Adult female Wistar rats (~250 g, CEUA-FMABC#04/2023) underwent CRT for 3 or 10 wks with 75% of body weight load or were maintained sedentary (SED). At the end of the last exercise bout, the UB was harvested for gene expression of inflammation (NFKb, TNF- α , IL-1 β , IL-6, COX-1 and 2, IL-10), autophagy (AMPK, mTOR), and mitophagy (PINK1) markers by RT-qPCR. The gene expression of the transcription factor NFKb (1.4764 ± 0.1253 vs 1.0161 ± 0.0773 SED, $p=0.0108$), IL-10 (1.3970 ± 0.1232 vs. 1.0081 ± 0.0593 SED, $p=0.0175$) and COX-2 (1.7113 ± 0.1078 vs. 1.0205 ± 0.0866 SED, $p=0.0005$) in the UB were significantly higher in rats subjected to exercise for 3 wks compared to SED rats. In addition, the gene expression of NFKb (1.4224 ± 0.1143 vs. 1.0133 ± 0.0722 SED, $p=0.0128$), IL-1 β (1.5438 ± 0.1186 vs. 1.0080 ± 0.0561 SED, $p=0.0022$), IL-6 (1.6552 ± 0.1204 vs. 1.0085 ± 0.0595 SED, $p=0.0007$), COX-1 (1.5641 ± 0.1507 vs. 1.0078 ± 0.0548 SED, $p=0.0060$), COX-2 (1.3267 ± 0.0815 vs. 1.0023 ± 0.0303 SED, $p=0.0039$), AMPK (1.4646 ± 0.1639 vs. 1.0127 ± 0.0701 SED, $p=0.0297$) and mTOR (2.1601 ± 0.3039 vs. 1.0071 ± 0.0531 SED, $p=0.0390$) in the UB were significantly higher in rats subjected to CRT for 10 wks than in SED rats. However, the gene expression of TNF- α (0.6848 ± 0.0984 vs. 1.0170 ± 0.0854 SED, $p=0.0289$) and IL-10 (0.5230 ± 0.1646 vs. 1.0356 ± 0.1286 SED, $p=0.0341$) in the UB was significantly lower in rats trained for 10 wks than in SED rats. Therefore, the data suggest a chronic inflammatory condition in the UB of rats subjected to CRT, however, with the onset of autophagy mechanisms activation after 10 wks of training.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Nephroprotective effect of a low molecular weight peptide fraction from common bean (*Phaseolus vulgaris*) against renal ischemia and reperfusion

Authors:

Jhulle Horrane Moreira CARVALHO¹

Sixta Isabel Atencio BERRIO¹

Daniel GRAZIANI¹

Patrícia Maria FERREIRA¹

Katia Flávia FERNANDES¹

Karla de Aleluia BATISTA²

Juliana Vila Verde RIBEIRO¹

Carlos Henrique XAVIER¹.

Affiliations:

¹ Federal University of Goiás

² Federal Institute of Goiás

Keywords: Common beans. Kidney injury. Oxidative stress. PV3.

Abstract:

Ischemia reperfusion (IR) injury contributes to acute kidney injury. IR-evoked reactive oxygen species (ROS) production is a mechanism targeted by antioxidant molecules obtained from dietary sources. We previously showed that the low molecular weight (<3KDa) peptide fraction from common hardened bean *Phaseolus vulgaris* (PV3), which has low commercial value but is rich in proteins and bioactive peptides, exerts antioxidant and vasodilator effects. We next evaluated whether PV3 may mitigate the ischemic damage related to oxidative stress on the kidney. In vitro tests using DPPH method evaluated the PV3 antioxidant activity. Human endothelial cells were incubated with the nitric oxide (NO) and ROS fluorescent probes, DAF-FM and DHE, and with H₂O₂, treated with PV3 and analyzed by confocal microscopy. In vivo tests (CEUA 057/22) used rats that underwent SHAM surgery or IR procedure and treated with PV3 (50ug/kg) or the potent antioxidant Tempol. PBS Buffer 1X was also used to control for the possible hypercapnia-related acidosis and Vehicle (VEH) group received NaCl 0.9%. The groups (n=10 each) were as follows: VEH+SHAM, VEH+IR, PV3+SHAM, PV3+IR, PV3+PBS+SHAM, PV3+PBS+IR, Tempol+SHAM, Tempol+IR, Tempol+PBS+SHAM, Tempol+PBS+IR. Metabolism and renal function were evaluated in metabolic cages. PV3 exerted antioxidant effects in vitro (DPPH IC₅₀ 7.74±0.27 mg/mL), in addition to increasing NO production (12% p<0.05) and to reduce ROS (-11,5% p<0.05). In rats, the PV3 effects did not differ from Tempol. PV3 reversed the deleterious effects of IR on renal function on Creatinine clearance (IR+VEH:5.7±2.5 and IR+PV3:8.9±0.03 mL/kg/min; p<0.05). PV3 increased the activity Superoxide Dismutase enzyme (91% vs VEH; p<0.05). In conclusion, PV3 attenuated IR impacts on renal function probably due to its antioxidant and oxidonitrergic effects, supporting beans use as a source of nutraceutical peptides to act on pathophysiological paths relying on oxidative stress.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Differential Effects of 48- and 72-Hour Fasting on Renal Redox Status and Function in Rats with Cisplatin-Induced Acute Kidney Injury

Authors:

Laís Mafrá DE BENEDICTIS¹
Lara Fabiana Luz MALHEIRO¹
Caroline Assunção OLIVEIRA¹
Matheus Cardoso Flores MENDES¹
Pedro Henrique Bento da Silva TEIXEIRA¹
Luiz Gustavo Brito LUCAS¹
Pedro Filipe Xavier GOMES¹
Iago Coutinho SANTOS¹
Patrícia da Silva OLIVEIRA¹
Amélia Cristina Mendes de MAGALHÃES¹
Thiago Macêdo Lopes CORREIA²
Márcio Vasconcelos OLIVEIRA¹
Telma de Jesus SOARES¹
Liliany Souza de Brito AMARAL¹

Affiliations:

¹Instituto Multidisciplinar em Saúde, Universidade Federal da Bahia, Programa de Pós-Graduação Multicêntrico em Ciências Fisiológicas, Vitória da Conquista, Bahia 45029-094, Brazil.
²Instituto de Ciências da Saúde, Departamento de Biorregulação, Universidade Federal da Bahia, Salvador, Bahia 40110-100, Brazil.

Keywords: Cisplatin. Fasting. Nephrotoxicity. Oxidative Stress. Renal function

Abstract:

Cisplatin (CP) is a widely used chemotherapeutic agent whose clinical application is limited by nephrotoxicity, primarily mediated by oxidative stress. This study evaluated the effects of two fasting protocols on metabolic parameters, renal function, and renal redox status in male Wistar rats with CP-induced acute kidney injury (AKI). Rats were randomly assigned to 3 groups (n=7): CP with ad libitum feeding (CP), CP with 48-hour fasting (CP-F48), and CP with 72-hour fasting (CP-F72). Fasting started 24h (CP-F48) or 48 h (CP-F72) before a single CP injection (5 mg/kg) and continued for 24h post-injection. On day 7th, blood, urine, and renal tissue were collected. The study was approved by the CEUA of IMS/CAT-UFBA (No. 134/2024). Data are as mean±SD. Fasting did not exacerbate CP-induced weight loss. Capillary glycemia was significantly lower in CP-F48 (75.29±3.8) and CP-F72 (84.43±7.5) compared to CP (115.4±8.1) (p<0.05). Both fasting protocols increased ketone bodies more markedly in CP-F72 (CP: 0.82±0.2; CP-F48: 2.1±0.2; CP-F72: 2.5±0.4) (p<0.05). Serum creatinine levels were similar across groups. The glomerular filtration rate tended to increase in CP-F72, although not significantly. Urinary flow decreased in both fasting groups (CP: 35.7±3.8; CP-F48: 17.9±12.1; CP-F72: 23.2±6.5) (p<0.05). Only CP-F72 showed reduced renal TBARS (CP: 3.26±0.9; CP-F48: 3.15±0.8; CP-F72: 2.08±0.6) and nitrite (CP: 12.2±1.5; CP-F48: 11.3±0.3; CP-F72: 7.6±2.1) levels (p<0.05). GPx activity was lower in CP-F48 (0.90±0.2) and CP-F72 (0.91±0.2) compared to CP (1.26±0.3) (p<0.05). There were no statistical differences in catalase and SOD activity between the groups. In conclusion, 72-hour fasting may activate renoprotective mechanisms independent of classical antioxidant enzymes, whereas 48-hour fasting appears ineffective and may increase susceptibility to oxidative damage in CP-induced AKI.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Evaluation of the effects of raas inhibitors on renal morphology in rats with renovascular disease induced by the two-kidney, one-clip model

Authors:

Raíssa Leal de Carvalho dos Santos CUNHA¹
Péricles Fabrício GOMES FILHO¹
Paulo Rogerio Silva de PAIVA JÚNIOR²
Carolina da Silva VALENTIM¹
Rebecca Roosevelt da Conceição Souza GUEDES¹
Isaías dos Santos SILVA¹
Cherley Borba Vieira de ANDRADE¹
Kissila RABELO¹,
Luciana Lontro ALVES¹
Aluana Santana CARLOS²
Jorge José de CARVALHO¹
Jemima Fuentes Ribeiro da SILVA¹

Affiliations:

¹Instituto de Biologia Roberto Alcântara Gomes – UERJ ²Universidade Iguazu – UNIG

Keywords: 2K1C. Renovascular disease. RAAS. Inflammation

Abstract:

The renin-angiotensin-aldosterone system (RAAS) is highly activated in renovascular disease (RVD), promoting hypertension, inflammation, and renal fibrosis. This study evaluated the effects of losartan, enalapril, and spironolactone on inflammatory markers and renal morphology in Wistar rats subjected to the two-kidney, one-clip (2K1C) model. Approved by CEUA/UERJ (001/2023), 50 rats were divided into SHAM (S), 2K1C, 2K1C+L, 2K1C+E, and 2K1C+Sp. After RVD induction and four weeks of treatment, systolic blood pressure (SBP), ACE, aldosterone, OPN, IL-1 β , TNF- α , CD163, and renal histology (HE, Picrosirius Red) were analyzed. **SBP:** S: 120.1 $\pm$ 7.6; 2K1C: 158.5 $\pm$ 28.1 (+31.9% vs S, p<0.05); 2K1C+L: 124.5 $\pm$ 8.5 (-21.4% vs 2K1C); 2K1C+E: 134.9 $\pm$ 11.9 (-15% vs 2K1C); 2K1C+Sp: 125.4 $\pm$ 19.4 (<20.9% vs 2K1C). **Aldosterone:** S: 37.5 $\pm$ 14.1 pg/mL; 2K1C: 387 $\pm$ 180 (+932% vs S); 2K1C+L: 225.4 $\pm$ 232.8; 2K1C+E: 79.6 $\pm$ 34.9 (-79.4% vs 2K1C); 2K1C+Sp: 166.3 $\pm$ 108. **ACE:** S: 18048 $\pm$ 2387 KDa; 2K1C: 27109 $\pm$ 5116 (+50.2% vs S); 2K1C+L: 20812 $\pm$ 4232 (-23.2% vs 2K1C); 2K1C+E: 20780 $\pm$ 2842 (-23.5% vs 2K1C); 2K1C+Sp: 21758 $\pm$ 3189. **OPN:** S: 17990 $\pm$ 5567 KDa; 2K1C: 30269 $\pm$ 1375 (+68.3% vs S); 2K1C+L: 22571 $\pm$ 3695; 2K1C+E: 18878 $\pm$ 5733 (-37.6% vs 2K1C); 2K1C+Sp: 22296 $\pm$ 7209. **IL-1 β :** S: 22673 $\pm$ 2065 KDa; 2K1C: 30269 $\pm$ 1375 (+33.5% vs S); 2K1C+L: 25175 $\pm$ 5861; 2K1C+E: 22790 $\pm$ 3670 (-24.7% vs 2K1C); 2K1C+Sp: 23125 $\pm$ 3490 (-23.6% vs 2K1C). **TNF- α :** S: 16541 $\pm$ 1975 KDa; 2K1C: 25879 $\pm$ 1942 (+56.4% vs S); 2K1C+L: 20099 $\pm$ 1580 (-22.3% vs 2K1C); 2K1C+E: 21218 $\pm$ 2896 (-18.1% vs 2K1C); 2K1C+Sp: 19882 $\pm$ 2378 (-23.2% vs 2K1C). **CD163:** S: 4.4 $\pm$ 0.7%; 2K1C: 10.7 $\pm$ 5.2 (+143.2% vs S); 2K1C+L: 4.6 $\pm$ 1.8 (-57% vs 2K1C); 2K1C+E: 5.96 $\pm$ 2.3 (-44.3% vs 2K1C); 2K1C+Sp: 5.34 $\pm$ 3.8% (-50.1% vs 2K1C). **HE** showed glomerular damage in 2K1C, partially preserved in treated groups. Picrosirius Red revealed fibrosis in 2K1C, reduced mainly with enalapril and spironolactone. RAAS inhibitors reduced BP in all groups. Although they target the same pathway, they showed distinct effects on inflammation, renal fibrosis, and markers such as TNF- α , CD163, and IL-1 β .



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: SC1, a novel angiotensin II type 1 receptor antagonist, improves renal hemodynamic parameters in a rat model of chronic kidney disease

Authors:

Regina Souza AIRES^{1,2}

Larissa Fernanda Lima FERREIRA³

Janaína Versiani dos ANJOS³

Alice Valença ARAÚJO¹

Affiliations:

¹Centro Acadêmico de Vitória, Universidade Federal de Pernambuco

²Laboratório de Fisiologia e Farmacologia Cardiorrenal, Centro de Biociências, Universidade Federal de Pernambuco

³Laboratório de Síntese Orgânica Medicinal, Centro de Ciências Exatas e da Natureza, Universidade Federal de Pernambuco

Keywords: 5/6 Nephrectomy. hypertension. oxadiazole derivatives. renal function. renin-angiotensin-aldosterone system.

Abstract:

Renin-angiotensin system inhibitors are essential in chronic kidney disease (CKD) treatment but are often limited by adverse effects. SC1, a novel oxadiazol-5-one derivative and AT1 receptor antagonist, was designed for better stability and solubility. This study evaluated its effects on renal hemodynamics in an experimental CKD model. The experimental protocol was approved by the Animal Ethics Committee (CEUA/UFPE, nº 005/2023). Male Wistar rats were randomly assigned to sham (n=5) or 5/6 nephrectomy (5/6Nx, n=5) groups. Fifteen days post-surgery, animals were anesthetized and treated with SC1 (1 µg/kg, i.v.) to assess hemodynamic parameters, including mean arterial pressure (MAP), glomerular filtration rate (GFR) by inulin clearance, renal vascular resistance (RVR) and filtration fraction (FF). Data were analyzed using two-way ANOVA with Holm-Sidak post hoc test, considering $P < 0.05$ as significant. Results are presented as mean $\pm$ standard error of the mean. Intravenous administration of SC1 reduced MAP 13% in the sham group (111 ± 7 mmHg versus 97 ± 8 mmHg) and 21% in the 5/6Nx group (165 ± 16 mmHg versus 130 ± 4 mmHg), without affecting HR in either the sham (403 ± 35 bpm versus 390 ± 22 bpm) or 5/6Nx group (385 ± 19 bpm versus 392 ± 29 bpm). Additionally, SC1 significantly decreased RVR 33% in the sham group (20 ± 3 versus 13 ± 2 mmHg·mL⁻¹·min·100g⁻¹) and 12% in the 5/6Nx group (61 ± 3 versus 53 ± 3 mmHg·mL⁻¹·min·100g⁻¹), with no changes in RBF in either group (sham: 4.3 ± 0.2 versus 5.5 ± 1.8 mL/min/100g; 5/6Nx: 1.9 ± 0.6 versus 2.0 ± 0.6 mL/min/100g). Notably, in the 5/6Nx group only, SC1 treatment markedly increased GFR 800% (0.3 ± 0.1 versus 2.9 ± 0.1 mL/min/100g) and FF 150% (0.6 ± 0.3 versus 1.5 ± 0.2). However, urinary flow remained unchanged in both the sham and 5/6Nx groups. In conclusion, the results demonstrated that SC1 improved renal hemodynamics in a CKD rat model by lowering MAP and enhancing GFR, suggesting a promising renoprotective effect.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Modulatory Effects of Epithelium-Derived 6-Nitrodopamine on Human Ureter Contractility

Authors:

Denis Lima OLIVEIRA¹
Wilmar AZAL NETO^{1,2}
Adriano FREGONESI^{1,2,3}
Flaviano LORENZO¹
Alan LINHARES¹
José BRITTO-JÚNIOR¹
Felipe Fernandes JACINTHO¹
Gilberto DE NUCCI^{1,4}

Affiliations:

¹ Pharmacology department, Postgraduate Program in Pharmacology, University of Campinas, Campinas/SP.

² Department of Surgery, Faculty of Medical Sciences, State University of Campinas, Campinas, Brazil.

³ Department of Surgery, Faculty of Medicine of Jundiaí, Jundiaí/SP, Brazil.

⁴ Department of Pharmacology, Institute of Biomedical Sciences, University of São Paulo (USP), São Paulo, Brazil

Keywords: Catecholamines, Contractility, 6-Nitrodopamine and Ureter

Abstract:

6-Nitrodopamine (6-ND) is a recently identified endogenous catecholamine found in cardiovascular and genitourinary tissues. Its role in human ureter physiology remains unclear. The ureter transports urine from the kidneys to the bladder through peristalsis, a process regulated by pacemaker cells and modulated by cholinergic, adrenergic, and non-adrenergic/non-cholinergic innervation. The epithelium may contribute to local signaling, as previously shown in seminal vesicles. This study aimed to investigate the basal release of 6-ND by the human ureter and its effect on smooth muscle contractility. Distal ureter segments were obtained from 45 brain-dead donors during kidney transplant preparation. A 2-cm portion of the ureter was isolated, incubated in Krebs-Henseleit solution (KHS), and the basal release of 6-ND was quantified by LC-MS/MS. For functional studies, ureteral rings were mounted in organ baths, and isometric contractions were recorded. Tyrosine hydroxylase expression was evaluated by immunohistochemistry. Human ureter presents basal release of 6-ND (0.6 ± 0.3 ng/mL; $n=10$). 6-ND induced contraction in the human ureter rings with $pEC_{50} = 5.3 \pm 0.2$ and $E_{max} = 1.1 \pm 0.3$ mN. Potency rank: 6-ND (5.3 ± 0.2) = AD (4.8 ± 0.2) = NA (4.9 ± 0.2) > DA (4.1 ± 0.1). Pre-incubation of the tissues with 10 (1.6 ± 0.2 vs 2.2 ± 0.1 ; E_{max}) and 100 nM (1.6 ± 0.2 vs 2.4 ± 0.3 ; E_{max}) of 6-ND significantly increased the contractions induced by noradrenaline. The presence of tyrosine hydroxylase was detected in epithelial cells of all human ureter samples. The human ureter epithelium releases 6-ND, which enhances smooth muscle contractility through non-neuronal pathways. This discovery suggests a novel local mechanism regulating ureteral peristalsis and may contribute to future therapeutic approaches for urinary tract disorders.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Late impacts on sperm quality and quantity in rats exposed to a single dose of gadolinium during prepuberty

Authors:

Catarina Conrado de Britto¹
Amanda Seraphim Oliveira¹
Leticia Mingorance Crepaldi¹
Sandra Maria Miraglia¹
Samara Urban de Oliva¹

Affiliations:

¹ Laboratory of Reproduction and Developmental Biology, Department of Morphology and Genetics, Federal University of Sao Paulo, UNIFESP.

Keywords: Gadolinium. Male reproduction. Rat. Sperm quality.

Abstract:

Gadolinium-based contrast agents (GBCAs) are often used during magnetic resonance. Gadolinium (Gd) retention has been reported in various tissues from patients and experimental animals with normal renal and hepatic function and an intact blood-brain barrier. Gadoteric acid (Dotarem®) is approved for use in neonates and children, for whom the blood-tissue barriers in the male reproductive tract are still being formed, with the possibility of a more pronounced Gd retention. Most studies have investigated Gd toxicity after repeated doses of GBCAs, which does not represent a likely clinical scenario. The study aimed to evaluate the effects of a single dose of Dotarem® administered during prepuberty, at 15th postnatal days (pnd), on qualitative and quantitative sperm parameters in adulthood, at 110pnd. Male Wistar rats were divided into 4 groups (n=7): Gd (TG; 0.06mMol/kg, intravenous via - i.v.); subclinical Gd (TsG; 0.006mMol/kg, i.v.); toxic Gd (TtG; 0.12 mMol/kg, i.v.); control (C; 0.9% saline solution). [CEUA UNIFESP approved nº 3141140222]. TG and TtG showed a significant decrease in progressive motility (C:60±13; TsG:16±12; TG:37±11; TtG:11±29), vitality (eosin staining; C:68±11; TsG:66±29; TG:55±10; TtG:52±6), acrosomal integrity (PNA assay; C:86±3; TsG:76±8; TG:75±4; TtG:57±7), mitochondrial function (DAB method; C:90±8; TsG:83±13; TG:80±13; TtG:81±11), chromatin condensation (toluidine blue staining; C:94±3; TsG: 80±3; TG:75±11; TtG:61±5). Via testis and epididymis homogenization, TG and TtG groups also showed a decrease in the number of step 19 spermatids (x106/testis; C:134±19; TsG:131±20; TG:91±18; TtG:103±16) and daily sperm production (x106/testis/day; C:22±3; TsG:21±3; TG:15±2; TtG:17±2). Data are presented as means and standard deviations. The results suggest that a single exposure to gadoteric acid before the establishment of the testicular and epididymal barriers leads to alterations in quantitative and qualitative sperm parameters in adulthood.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Influence of sleep deprivation on sperm function in mice

Authors:

Samara Urban de Oliva¹
Catarina Conrado de Britto¹
João Vitor Gomes de Souza¹
André Felipe Alves Vieira da Silva¹
Renato Borges Tesser²
Sandra Maria Miraglia¹
Fatima Okada¹
Ronni Rômulo Novaes e Brito²

Affiliations:

¹ Laboratory of Reproduction and Developmental Biology, Department of Morphology and Genetics, Federal University of Sao Paulo, UNIFESP.

² São Camilo University Center.

Keywords: Male fertility. Mice. Sleep restriction. Sperm.

Abstract:

Declining sperm quality has been correlated with environmental, occupational, and lifestyle factors. Reduction in sleep hours and quality has become common in today's society. Male reproduction can be influenced by the quality, quantity, and duration of sleep, resulting in alterations in the neuroendocrine-reproductive axis. Thus, understanding the potential effects of sleep restriction on sperm function may contribute to understanding reduced male fertility. The aim of the study was to evaluate the effects of sleep deprivation on sperm DNA and chromatin integrity, acrosome integrity, and mitochondrial activity in adult mice. Male C57BL/6 mice were submitted to paradoxical sleep restriction (RS group; n=8), for 21 consecutive days (CEUA UNIFESP nº 7572041122). The control group (C; n=8) was not exposed to sleep restriction. Epididymal cauda sperm were evaluated for motility, DNA integrity (Comet and acridine orange assay), chromatin compaction (toluidine blue staining), acrosome integrity (PNA assay), and mitochondrial function (DAB method). Sleep restriction caused a significant decrease in the proportion of sperm with progressive motility (C:73.1%±12.6, RS:44.5%±15.1), an intact acrosome (C:81.6%±3.7%, RS:49.8%±4.6%), active mitochondrial function (Class I - midpiece with 100% of staining - C:68.1±9.2, RS:51.9%±11.2) and compacted chromatin (C:77.2%±6.4%, RS:43.7%±10.2). Increase in the proportion of sperm with DNA fragmentation (Comet assay - Grade II and IV - intense DNA migration - C:16.7%±6.3, RS:33.5±9.2; Acridine orange assay - C:83.7%±5.0%, T:62.7%±6.7%) was also observed after sleep restriction. Data are presented as means and standard deviations. The results suggest that sleep restriction leads to changes in mitochondrial function, impairing sperm motility, besides damage to the acrosome membrane, chromatin compaction, and sperm DNA integrity, which may cause failures in the fertilization process.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Basal release of 6-nitrodopamine and from in NOS knockout mice isolated vas deferens

Authors:

Raquel Rios Campitelli¹
Gustavo Mendes^{2,3}
José-Britto Júnior¹
Silvana Chiavegatto⁴
Edson Antunes¹
Gilberto de Nucci^{1,2,3,4}

Affiliations:

¹ State University of Campinas (UNICAMP).
² Metropolitan University of Santos.
³ Faculty of Medicine, São Leopoldo Mandic (SLMANDIC).
⁴ University of São Paulo (USP).

Keywords: Nitrodopamine. Vas deferens. Catecholamines. Knockout.

Abstract:

The basal release of 6-ND from either rat atria or heart ventricles is not affected by previous incubation with the sodium channel blocker tetrodotoxin, indicating that the source of 6-ND in the heart is not neurogenic [1]. In both human [2] and rat vas deferens [3], 6-ND causes contractions, which are blocked by tricyclic antidepressants. The contractions induced by 6-ND in the rat vas deferens are also blocked by 1-, 1- and 1/2-adrenoceptor antagonists. Here we evaluated in nNOS^{-/-}, iNOS^{-/-} and eNOS^{-/-} knockout mice the basal release of 6-ND. Experiments were conducted in C57BL/6, nNOS^{-/-}, iNOS^{-/-} and eNOS^{-/-} adult mice of either sex (18 to 22 g). Euthanasia was performed by isoflurane overdose (> than 5% until 1 min after the breathing stops). The vas deferens was surgically dissected and suspended separately in a 3 mL organ bath containing KHS and ascorbic acid (3 mM) continuously gassed with a mixture (95% O₂ / 5% CO₂) at 37 °C for 30 min. One aliquot of 2 mL of the supernatant was transferred to black microtubes and 6-ND was quantified by LC-MS/MS [1]. All experimental protocols were approved by Ethics Committee for Animal Use (CEUA; Protocol No. 5959-1/2022, 6087-1/2022 and 6067-1/2022). Isolated vas deferens obtained from control or eNOS^{-/-} mice presented a basal release of 6-ND, as detected in the KHS by LC-MS/MS. The 6-ND release from vas deferens obtained from nNOS^{-/-} mice was significantly reduced when compared to vas deferens obtained from control mice (0.90.3 and 0.20.1 ng/mL from control and nNOS^{-/-} mice, respectively; n= 6). In Isolated vas deferens iNOS^{-/-} mice the basal release of 6-ND was significantly increase when compared to vas deferens obtained from control mice (2.51.2 and 7.12.9 ng/mL from control and iNOS^{-/-} mice, respectively; n= 7). The 6-ND release in eNOS^{-/-} (0.80.5 and 1.00.6 ng/mL from control and eNOS^{-/-} mice, respectively; n= 6) mice was not significantly different compared to control animals and eNOS^{-/-} mice. The results clearly indicate that nNOS is the isoform responsible for the synthesis of 6-ND in the vas deferens.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Maternal obesity alters cannabinoid receptor content in pancreatic islets of rat offspring associated with changes in glycemic homeostasis

Authors:

Marina Nigri Salem¹
Clara Figueiredo Reis
Gomes¹ Lucas Santos¹
Mariana Macedo
Almeida¹ Camila
Calvino¹
Carmen Cabanelas Pazos-
Moura¹ Leonardo de
Souza Mendonça² Isis
Hara Trevenzoli¹

Affiliations:

¹ Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro

² Instituto de Saúde de Nova Friburgo, Universidade Federal Fluminense

Keywords: Metabolic programming. Maternal obesity. Endocannabinoid system. Pancreatic islet.

Abstract:

The endocannabinoid system (ECS) is composed of the endogenous ligands anandamide and 2-arachidoylglycerol that bind to the type 1 (CB1) and type 2 (CB2) cannabinoid receptors to regulate several physiological functions such as appetite, lipogenesis, reproduction, stress and inflammation. The ECS also plays an important role in regulating pancreas development and function, with CB1 decreasing insulin secretion. Obesity is related to the ECS hyperactivation, however, the effect of maternal obesity on ECS of the pancreatic islets of rat offspring is unknown. To investigate this question, we used a well characterized rat model of maternal diet-induced obesity, approved by the CEUA/CCS/UFRJ (protocol 129/21). Female rats, aged 60 days, were fed a control diet (C; 9% kcal as lipids) or obesogenic diet (OD; 40% kcal as lipids and 9.5% sucrose) for 9 weeks before mating and during gestation and lactation. Pancreas and blood samples of male and female offspring were collected at birth and weaning for immunohistochemical analysis (CB1 and CB2) and hormonal profile. Data were analyzed by two-way ANOVA (*p<0.05). Maternal OD increased CB1 content in the islets of neonate rats, with a more prominent effect in males (+26.45%) and in the β cells. This profile was associated with decreased insulin levels (-44%*) and glycemia (-11%*). At weaning, maternal OD increased pancreatic islet diameter in female offspring (+5.6%*), without changes in CB1 content in both sexes. On the other hand, there was an interaction effect on CB2 content, with lower content in OD female compared with OD male offspring (-22%*). This profile was associated with increased glycemia (+6,2%*) and insulinemia (+40,9%*) and decreased glucagon (-37,3%*) and GLP-1 (-33% *) serum levels, with males more affected. Collectively, these results indicate that maternal OD can alter the expression of CB1 and CB2 and the development of pancreatic islets in early life, influencing the sex-specific risk of developing metabolic diseases in the long term.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Shifts in bone marrow and blood leukocytes towards a pro-inflammatory state in Goto-Kakizaki rats, a non-obese model of type 2 diabetes

Authors:

Amara Cassandra dos Anjos Alves¹
Janaína Ribeiro Barbosa Pauferro¹
Gabriela Mandú Gimenes¹
Guilherme Ribeiro Silva Resende²
Tania Cristina Pithon-Curi¹
Sandro Massao Hirabara¹
Renata Gorjão¹
Laureane Nunes Masi³
Ricardo Ambrósio Fock²
Rui Curi^{1,4}

Affiliations:

- ¹ Cruzeiro do Sul University (São Paulo, SP).
² University of São Paulo (São Paulo, SP).
³ Federal University of Santa Catarina (Florianópolis, SC).
⁴ Butantan Institute (São Paulo, SP).

Keywords: Diabetes *mellitus*. Haematopoiesis. Inflammation. Insulin resistance. Myelogram.

Abstract:

Insulin resistance (IR) is associated with a chronic and low-grade inflammation and impaired insulin signalling. We hypothesized that changes in the bone marrow microenvironment may lead to differentiation of leukocytes towards a pro-inflammatory state that once released to blood stream cause systemic inflammation and IR, even in non-obese individuals. To evaluate this hypothesis, differential counting of bone marrow and blood cells of Goto-Kakizaki (GK) rats (a genetic model of non-obese type 2 diabetes) at 21 and 120 days of age was performed, and the results were compared with those of age-matched Wistar (WT) rats. This study was approved by the Cruzeiro do Sul University Animal Ethics Committee (010/2024). GK rats at both ages have blood leukocytosis and elevated bone marrow cellularity; both were intensified with age. The bone marrow analysis revealed an increased population of lymphocytes in GK rats at 21 and 120 days of age (31% and 58%, respectively), in addition to an elevation in myeloid lineage cells (2.6-fold of monocytes in 21-day-old rats and 35% of neutrophils in 120-day-old rats). Bone marrow from 120-day-old GK rats exhibit increased CD8⁺ T cells (51%) and Natural Killer cells (4-fold), and decreased (15%) anti-inflammatory (M2) and increased (40%) pro-inflammatory (M1) macrophage population, compared to WT. Taken as a whole, increased bone marrow cellularity and a switch in cells profile towards an inflammatory state may lead to systemic inflammation and IR in GK rats.

Financial support: FAPESP (2018/09868-7; 2023/18436-1), CNPq, CAPES and Cruzeiro do Sul University.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Reduction in Prostate Cancer Diagnoses During the COVID-19 Pandemic: Evidence from Brazil and the World

Authors:

Nicolle de Souza Rodrigues¹
Daiana Vieira Lopes²

Affiliations:

¹ Universidade Federal do Rio de Janeiro (UFRJ), Campus Macaé, Instituto de Ciências Médicas (ICM). Macaé (RJ), Brasil.

² Laboratório Integrado de Morfologia (LIM), Instituto de Biodiversidade e Sustentabilidade NUPEM - Universidade Federal do Rio de Janeiro (UFRJ), Campus Macaé

Keywords: Impact. Covid-19. Diagnosis. Prostate cancer.

Abstract:

The COVID-19 pandemic, which began in December 2019, was responsible for changes in global health dynamics, mainly due to the overcrowding of healthcare services and confinement measures. In this context, modifications occurred in the approaches to comorbidities such as cancer. Prostate cancer, the second most common cancer subtype in men—excluding non-melanoma skin cancers—has early detection guidelines that positively impact disease prognosis. This investigation begins with the Prostate-Specific Antigen (PSA) test and digital rectal examination, followed by a confirmatory biopsy. Thus, the objective of the present study was to analyze the impact of the COVID-19 pandemic on prostate cancer diagnosis in Brazil and worldwide. Primary incidence data on prostate cancer cases available in the DATASUS - TabNet were used to obtain studies related to the Brazilian context. The study also included articles from the PubMed, SCOPUS and BVS databases to identify the reduction in diagnostic tests and their consequences for patient prognosis in Brazil compared to the global scenario. A database search was conducted using keywords and the Boolean operator AND. Inclusion criteria were free full-text articles published between 2020-2024 in English or Portuguese. Studies that did not contain data about prostate cancer during pandemic or that did not align with the research scope were excluded. A decline in diagnostic procedures was observed. Student's t-test showed that the difference in the monthly averages of new cases from 2019 to 2020 in Brazil was statistically significant ($p = 0.001$). A significant reduction was also observed in 2021 ($p = 0.01$). It was noted that, both in Brazil (except for the Northeast region) and globally, the greatest reduction in diagnoses occurred in April 2020, coinciding with the peak of COVID-19 infections. Finally, further studies on the long-term consequences of underdiagnosis on staging and prognosis are of great importance.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Chlorothalonil Disrupts Steroidogenic Pathway in Adult Male Mice

Authors:

Ludmila Rodrigues Pontes¹
Nicolly Caixêta Gonçalves¹
Paula Rafaela Queiroz da Silva¹
Cristiana Livramento Oliveira Pinto¹
Marcos Vinícius da Silva¹
Hugo Felix Perini¹
Gláucia Eloisa Munhoz de Lion Siervo¹

Affiliations:

¹ Universidade Federal do Triângulo Mineiro.

Keywords: Organochlorine. Reproduction. Toxicity.

Abstract:

Exposure to pesticides can cause both acute and chronic health problems, including effects on the endocrine and reproductive systems. The proper production of steroid hormones is essential, as they play a crucial role in reproductive and endocrine health. Chlorothalonil is a widely used organochlorine fungicide in Brazil; however, its effects on the male reproductive system remain poorly understood. Therefore, this study aimed to evaluate the effects of chlorothalonil on the expression of genes associated with the steroidogenic pathway in adult male mice. The project was approved by CEUA-UFTM (Approval N° 23/2023/CEUA/PROPPG). Fifteen adult male C57BL/6 mice (50 days, 22-26g) were randomly assigned to 3 experimental groups: Control (CTRL), receiving only the vehicle (water); Chlorothalonil 1 mg/kg (CHL1), Chlorothalonil 10 mg/kg (CHL10). The compound was administered via gavage for 50 days, and the animals were weighed throughout the experiment. On the day 51, euthanasia was performed by overdose of ketamine and xylazine. Relative gene expression was quantified from in the testes using RT-qPCR, with GAPDH as the reference gene. Exposure to chlorothalonil at the highest dose (10 mg/kg) resulted in significant downregulation of the *Cyp17a1* ($p = 0.009$) and *Cyp19a1* ($p = 0.009$) genes, whereas the lowest dose had no effect on their expression compared to the control group. No significant changes were observed in *Hsd17b3* and *AR* gene expression under the tested conditions. In conclusion, chlorothalonil exposure in adult male mice affects the expression of genes involved in the steroidogenic pathway, potentially disrupting steroid hormone production and impacting reproductive health. These findings underscore the importance of proper monitoring and disposal of this compound. This work was supported by the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) through grant APQ-00221-22.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Three-dimensional model of ovarian granulosa cells for in vitro studies in female fertility

Authors:

Thatiane Luca Marques de Almeida¹
André Lucas Lagôa de Freitas¹
Isabel Nogueira Santos Moreira Vaz¹
Leandra Santos Baptista²
Marlon Lemos Dias³
Tania Maria Ortega-Carvalho¹
Flavia Fonseca Bloise¹

Affiliations:

¹ Laboratório de Endocrinologia Translacional, Instituto de Biofísica Carlos Chagas Filho (IBCCF), Universidade Federal do Rio de Janeiro (UFRJ).

² Numpex-bio, Campus Duque de Caxias, Universidade Federal do Rio de Janeiro (UFRJ).

³ Grupo de Estudos do Fígado, Centro de Pesquisa em Medicina de Precisão (CPMP - IBCCF), UFRJ.

Keywords: Cell-cell interactions. Granulosa cells. Three-dimensional (3D) culture model.

Abstract:

Ovarian granulosa cells (GC) are essential for oocyte development and nutrient supply during folliculogenesis. GC produce steroid hormones (estrogen and progesterone) essential for female fertility. Although bidimensional (2D) culture models are widely used, these models lack the complexity of *in vivo* cellular signaling and architecture where cells interact dynamically. In contrast, three-dimensional (3D) culture models better mimic the tissue microenvironment, promoting cell-cell interactions. This study aims to characterize a functional 3D culture model of human GC and assess their hormonal production. The human GC line COV434 was cultured in 2D, and 3D spheroid models at different cell densities (1×10^5 ; 2×10^5 and 5×10^5) for 1, 4 or 7 days. Viable cells were detected by the PrestoBlue assay, and results showed increased viability in the 5×10^5 group on day 7 of 3D culture. The CFSE assay was used to monitor cell division and revealed a heterogeneous proliferation profile of cell populations over the culture days. At day 1, the majority of the cells are in a low proliferation stage in both 2D (85.6%) and 3D (89.4%), as expected. At day 4, 3D cells show a significant increase in the high proliferation stage (73.1%), while a substantial portion of 2D cells has already progressed to the intense proliferation stage (42.1%). By day 7, both 2D (98.3%) and 3D (86.2%) exhibit the majority of cells in intense proliferation, with fewer cells in the high stage in 2D (1.67%) compared to 3D (13.5%). When compared to the 2D model, spheroids cells exhibited lower proliferation rates ($p=0.0455$). In conclusion, granulosa cells remained viable and functional in the 3D model, and their proliferation profile offers valuable insights for future follicular physiology studies using this model. Further experiments will explore the 3D model's impact on genes related to follicular development and hormonal production.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Effects of Testosterone Absence on Glucose Tolerance and Energy Metabolism

Authors:

Julia Rodrigues Reis Moreti¹
Felipe Garcia da Silva Sucupira¹
João Paulo Gabriel Camporez¹

Affiliations:

¹ Departamento de Fisiologia da Faculdade De Medicina de Ribeirão Preto.

Keywords: Resistência à insulina. Síndrome Metabólica. Testosterona.

Abstract:

Increased consumption of a high-fat diet and physical inactivity are some factors that can reduce testosterone concentration in men, affecting approximately 20% to 40%, contrasting with the prevalence of 4% to 5% in the general male population, triggering health implications, such as reduced skeletal muscle mass, type 2 diabetes, erectile dysfunction, and decreased quality of life. Therefore, this project aims to evaluate the impact of the absence of testosterone on body composition, glucose tolerance, and ectopic accumulation of triglycerides (TAG) in orchiectomized male animals. **Methods:** Male C57BL/6J mice at 8 weeks of age were divided into four groups: sham-operated animals fed a standard diet (SHAM); SHAM animals fed a high-fat diet (SHAM+HFD); orchiectomized animals fed a standard diet (ORX); ORX animals fed an HFD (ORX+HFD). Eight weeks after surgery and the start of HFD, the following analyses were performed: final body mass of the animals, body composition by magnetic resonance imaging, glucose tolerance test, and measurement of TAG concentration in the tissues (skeletal muscle and liver). 1308/2024R1 FMRP **Results:** Both HFD-fed groups, SHAM and ORX, showed significant increases in body mass and body fat compared to their respective groups fed a standard diet. However, there were no differences between the testosterone-depleted groups compared to the SHAM groups. There were also no differences in glucose tolerance between the ORX groups compared to the SHAM groups, either on the standard diet or on the HFD. However, the ORX+HFD group showed significant increases in hepatic and muscle TAG content compared to the SHAM+HFD group. **Conclusion:** Our data suggest that although the absence of testosterone does not induce glucose intolerance and increased fat mass, ORX animals are more prone to tissue accumulation of TAG, which may eventually lead to insulin resistance.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Assessment of the functional impact of IL-17 exposure on human granulosa cells

Authors:

Marianna Ribeiro Cabral¹
André Lucas Lagôa de Freitas²
Mayck Medeiros Amaral da Silva¹
Sabrina Sodr  de Souza Serra¹
Johnatas Dutra Silva¹
Julia Rodrigues Ferraz Silva¹
Juliana Borges Vieira¹
Monique Martins Melo¹
Pedro Leme Silva¹
Flavia Fonseca Bloise²
Fernanda Ferreira Cruz¹

Affiliations:

¹ Laboratory of Pulmonary Investigation, Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.

² Laboratory of Translational Endocrinology, Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.

Keywords: Asthma. COV434. IL-17. Infertility.

Abstract:

Asthma is a common chronic lung disease among women of reproductive age. Th17-mediated inflammation and its main cytokine IL-17 are usually associated with systemic inflammation and severe asthma. Previous studies have highlighted a possible link between asthma and infertility, with systemic inflammation potentially affecting reproductive organs. Therefore, this study aims to investigate the functional impact of IL-17 exposure on ovarian granulosa cells. To examine this, human granulosa tumor-like cell line COV434 was cultured in DMEM, with 10% FBS and 1% antibiotics, incubated at 37°C with 5% CO₂. First, we confirmed the presence of the IL-17 receptors in granulosa cells by qPCR. COV434 cells express the asthma-related inflammatory mediators receptors IL-17RA, IL17RC, and IL-4R (IL-17RA: 0.123±0.02; IL-17RC: 0.103±0.03; IL-4R: 0.066±0.02; IL-5RA: non-detected; IL-12Rβ2: non-detected). Next, we investigated the effects of IL-17 (10, 100, and 1000 pg/ml) on cell viability, proliferation, and progesterone production at 24 and 48 hours. IL-17 exposure did not affect cell viability (24h, CTRL: 96.7%±2.9; 10 pg/ml: 99%±1.7; 100 pg/ml: 97.7%±2.5; 1000 pg/ml: 96.7%±5.8; 48h, CTRL: 98.3%±1.3; 10 pg/ml: 95.7%±2.1; 100 pg/ml: 95.9%±3.1; 1000 pg/ml: 95.3%±2.6), the number of proliferating cells (24h, CTRL: 32.2%±4.6; 10 pg/ml: 29.7%±4.3; 100 pg/ml: 34.6%±6.2; 1000 pg/ml: 39.1%±9.2; 48h, CTRL: 75.8%±3.9; 10 pg/ml: 73.8%±6.1; 100 pg/ml: 72.6%±2.8; 1000 pg/ml: 74.2%±5.4), or the cyclin mRNA expression (CCNE1, CTRL: 1.0±0.3; 10 pg/ml: 0.9±0.3; 100 pg/ml: 0.9±0.2; 1000 pg/ml: 0.9±0.2; CCNA, CTRL: 1.2±0.8; 10 pg/ml: 1.3±0.8; 100 pg/ml: 1.0±0.6; 1000 pg/ml: 1.5±1.5). However, a significant reduction in progesterone levels was observed in the culture supernatant at the lowest IL-17 concentration (48h, CTRL: 11.45ng/ml±3.5; 10 pg/ml: 5.23ng/ml±2.8; 100 pg/ml: 8.74ng/ml±3.8; 1000 pg/ml: 7.38ng/ml±3.4). Thus, IL-17 seems to have a detrimental functional impact on human granulosa cells.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Impact of perinatal exposure to plastic waste on prostate histology in adult rats

Authors:

Vanessa Aguiar ROCHA¹

Marina Cavalheiro¹

Victória Cristina Pinha¹

Patrick Vieira de Souza¹

Natália Magosso¹

Mirella Franco Moreira¹

Vinícius Luís Rocha da Silva Maria¹

Pedro Henrique da Fonte Portinho¹

Wellerson Rodrigo Scarano¹

Affiliations:

¹ Department of Structural and Functional Biology, Institute of Biosciences, São Paulo State University (UNESP), Botucatu, São Paulo, Brazil.

Keywords: Histology. Nanoplastics. Phthalates. Prostate.

Abstract:

Currently, humanity produces approximately 460 million tons of plastic annually, contributing to environmental pollution through nanoplastics (NPs) and phthalates (plastic additives). The prostate is highly sensitive to various external agents, especially endocrine disruptors. Factors such as plastic waste are associated with alterations in the prostate microenvironment, which can impact prostate morphophysiology. Given the critical importance of the topic, this study aims to analyze the histopathological aspects of the ventral prostate (VP) in adult rats exposed to a mixture of phthalate diesters (MP) and NPs during the perinatal period. Pregnant Sprague-Dawley rats were assigned to six experimental groups: Ctrl (vehicle), T1 (20µg/kg/day MP), T2 (200mg/kg/day MP), T3 (NPs), T4 (20µg MP + NPs), and T5 (200mg MP + NPs). The doses were based on the dose of environmental and occupational exposure. The treatment was administered orally from gestational day 10 to postnatal day (PND) 21. The male pups (n=6) were euthanized at PND120, and the VP was collected for study (CEUA nº 1174020523). The data were analyzed using the one-way ANOVA by the Kruskal-Wallis test. Differences were considered significant when $p \leq 0.05$. The histological slides revealed an increase in inflammatory foci in the prostate of animals exposed. Stereological analysis of the prostate gland indicated an enlargement of the stromal compartment in all groups exposed to MP and NPs (T1-T5), and a decrease of luminal compartment in the T1, T3, and T4 groups compared to Ctrl. Quantification of mast cells demonstrated an increase in both total and degranulated mast cells in all groups exposed (T1-T5), compared to the Ctrl group. All groups (T1-T5) showed an increase in collagen volume when compared to the Ctrl group. The results demonstrate that MP and NPs were capable of inducing significant structural damage in the VP, leading to a microenvironment that is susceptible to pathological alterations.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Effects of perinatal exposure to a phthalate mixture and nanoplastics on the reproductive health of male rat offspring

Authors:

Mirella Franco Moreira¹
Patrick Vieira de Souza¹
Natália Magosso¹
Vanessa Aguiar Rocha¹
Matheus Naia Fioretto¹
Isabela Silva Ferreira¹
Victória Cristina Pinha¹
Marina Cavalheiro¹
Wellerson Rodrigo Scarano¹

Affiliations:

¹Department of Structural and Functional Biology, Institute of Bioscience, São Paulo State University – UNESP, Botucatu, São Paulo, Brazil.

Keywords: DOHaD. Phthalates. Nanoplastics. Testicle.

Abstract:

The environmental and human health risks posed by the ubiquity of nanoplastics (NPs) and the transport of organic pollutants, such as plasticizers, into the body are of great concern due to their action as endocrine disruptors. However, the combined toxicity of NPs and a phthalates mixture (PM) on mammalian reproductive systems is not fully understood. Furthermore, the Developmental Origins of Health and Disease (DOHaD) hypothesis postulates that the exposure to these chemicals during pregnancy and infancy may result in lifelong health impacts. This study investigated the effects of maternal exposure to phthalates and NPs on prepubertal toxicity. Pregnant Sprague Dawley rats (n=6-8) were distributed into 5 groups: CTRL: (control, vehicle); T1: 20µg/kg/day PM; T2: 200mg/kg/day PM; T3: 1mg/kg/day polystyrene NPs - 100 nm; T4: 20µg/kg/day PM+1mg/kg/day NPs; T5: 200mg/kg/day PM+1mg/kg/day NPs. Animals were exposed by gavage from gestational day 10 to postnatal day (PND)21 (CEUA: 1174020523). On PND22, one male by litter was euthanized and subjected to biometric, hormonal, and histological evaluations. The statistical test was ANOVA, followed by Tukey. The results showed that the relative weight of testis significantly decreased in T4 (P=0.0374) vs. CTRL, while body weight remained unchanged. Preputial separation did not differ from CTRL, suggesting puberty was unaffected. Testosterone levels were unchanged, but estradiol levels significantly increased in T5 (P=0.0001) vs. CTRL. Seminiferous tubule diameter was unaltered, but the number of apoptotic spermatocytes in pachytene/tubule increased in T5 (P=0.0012) vs. CTRL. Reduced testicular weight in T4 may indicate alteration in the gonadal development, while the increase in the estradiol levels in T5 may be related to the higher spermatocyte apoptosis. Although further evaluation is needed, findings suggest an impaired testicular development without changes in the body growth or puberty installation.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: From pollution to placenta: How the silent invasion of plastics affects the maternal-fetal axis

Authors:

Natália MAGOSSO¹
Patrick Vieira de SOUZA¹
Mirella Franco MOREIRA¹
Vanessa Aguiar ROCHA¹
Matheus Naia FIORETTO¹
Luisa Annibal BARATA¹
Isabella FÁVARO¹
Felipe Cristiano BARBOSA¹
Victória Cristina PINHA¹
Gabriel Aquino de Souza MAIA¹
Vinícius Luís Rocha da Silva MARIA²
Wellerson Rodrigo SCARANO¹

Affiliations:

¹ Department of Structural and Functional Biology, Institute of Biosciences, São Paulo State University (UNESP), Botucatu, São Paulo, Brazil.

² Department of Biological Sciences, Faculty of Sciences, UNESP, Bauru, SP, Brazil.

Keywords: Placenta. Nanoplastics. Phthalates. Fetal development.

Abstract:

The widespread use of plastic has raised public health concerns. Phthalates, plasticizers that enhance plastic flexibility and cosmetic properties, are semi-volatile and degrade into micro and nanoplastics (MNPs), contaminating water, food and air. As endocrine disrupting (EDs), they interfere with hormonal regulation and have been detected in human blood, urine, placenta and breast milk. Early exposure to phthalates and MNPs may impact offspring in adulthood. This study investigates gestational exposure to phthalates mixture (PM) and nanoplastics (NPs), focusing on their effects on placental energy metabolism and fetal development. Pregnant Sprague Dawley rats (CEUA 1174020523) were distributed into 6 groups (n=7/group): C: (control; vehicle); T1: 20µg/kg/day PM; T2: 200mg/kg/day PM; T3: NPs (1mg/kg); T4: 20µg/kg/day PM + NPs (1mg/kg); T5: 200mg/kg/day PM + NPs (1mg/kg). The mixture was based on a proportion observed in pregnant women. Treatment was administered orally from gestational day 5 (GD5) to GD20. On GD20, 7 pregnant rats from each group were anesthetized, fetuses and placentas were collected and weighed. Gene expression and antioxidant enzyme analyses were performed on placenta. Fetuses were assessed for size for gestational age and collected for skeletal and visceral evaluation. The statistical test was ANOVA, followed by Tukey. RT-qPCR analysis showed an increase in the expression of *Igf-1* and *Vegf* in T3 and T4 vs. C; *Hif-1* in T5 vs. C; *Fgf* in T3 vs. C (p<0.05). In relation to the antioxidant markers, TBARS, SOD and GSTpi levels decreased in T2 vs. C, while CAT increased in T3 vs. C (p<0.05). There was a reduction in placental weight (p=0,04) and increase in placental efficiency in male offspring in T3 (p=0,03). In the same group, a reduction in the number of fetuses appropriate for gestational age was observed (p=0,02). These results suggest that these pollutants modulate placental gene expression, energy metabolism, and fetal development.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Maternal Exposure to Phthalates and Nanoplastics Impairs Blood-Testicular Barrier Integrity in Male Offspring

Authors:

Isabela Silva Ferreira¹
Mirella Franco Moreira¹
Patrick Vieira de Souza¹
Natália Magosso¹
Vanessa Aguiar Rocha¹
Matheus Naia Fioretto¹
Victória Cristina Pinha¹
Marina Cavalheiro¹
Wellerson Rodrigo Scarano¹

Affiliations:

¹ Department of Structural and Functional Biology, Institute of Bioscience, São Paulo State University – UNESP, Botucatu, São Paulo, Brazil.

Keywords: Blood-testicular barrier. DOHaD. Phthalates. Nanoplastics.

Abstract:

The growing use of plastics has raised concerns about their impact on both the environment and human health. Over time, these materials break down into micro- and nanoplastics (NPs), which can enter the body. Additionally, phthalates—chemicals added to plastics to enhance flexibility—are easily released and may disrupt the endocrine system. According to the Developmental Origins of Health and Disease (DOHaD) theory, exposure to these harmful substances during critical developmental stages can lead to long-term health consequences. In this context, this study examined how maternal exposure to phthalates and NPs affects testicular development in offspring, with a particular focus on the integrity of the blood-testis barrier (BTB). Pregnant Sprague Dawley rats were distributed into 4 experimental groups: C: control, vehicle, T1: 20µg/kg/day of phthalate mixture-PM, T2: 100nm polystyrene nanospheres at 1mg/kg/day diluted in distilled water-NPs and T3: 20µg/kg/day of PM+1 mg/kg/day of NPs, being treated from gestational day 10 to postnatal day (PND) 21 by gavage (CEUA: 1174020523). The testicles of male offspring were collected at adulthood (PND 120) and subjected to gene expression analysis by RT-qPCR (N=5) and histological analysis with reticulin staining (N=5). The statistical test was ANOVA, followed by Tukey. The expression of *Tjp1*, an essential protein for the maintenance of BTB tight junctions, was significantly reduced in T3 compared to control (P=0.0236), while *Occludin* showed no significant change. Furthermore, an increase in the reticular fibers quantity in the basement membrane was observed in all experimental groups vs. C (p<0.01; ImageJ measurement), which may compromise the Sertoli cells function and, consequently, the functionality of the BTB. The findings of this study suggest that maternal exposure to phthalates and nanoplastics may indicate structural and functional alterations in testicular tissue, compromising the BTB in adulthood of male offspring.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Association between hair cortisol levels and obesity in the ELSA-Brasil cohort

Authors:

Karine Lino RODRIGUES¹
Patricia de Oliveira da Silva SCARANNI²
Evelyn Nunes Goulart da Silva PEREIRA¹
Vivian Vieira Dias da SILVA¹
Raquel Rangel SILVARES¹
Beatriz Peres de ARAUJO¹
Cristina CASTILHOS³
Maria Inês SCHMIDT⁴
Maria de Jesus Mendes da FONSECA⁵
Rosane Harter GRIEP⁶
Anissa DALIRY¹

Affiliations:

- ¹ Laboratory of Clinical and Experimental Physiopathology, Oswaldo Cruz Institution, Oswaldo Cruz Foundation.
² Gaffrée and Guinle University Hospital, UNIRIO, Rio de Janeiro, Brazil.
³ Department of Public Health, UFRGS, Porto Alegre, Brazil.
⁴ School of Medicine, Federal University of Rio Grande do Sul, Porto Alegre, Brazil.
⁵ National School of Public Health, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil.
⁶ Laboratory of Education in Environment and Health, Oswaldo Cruz Foundation, Rio de Janeiro, RJ, Brazil.

Keywords: Chronic stress. Hair cortisol levels. Obesity. Overweight. ELSA-Brasil.

Abstract:

Hair cortisol levels have recently been identified as a promising marker for determining long-term cortisol levels and as a marker of hypothalamic-pituitary-adrenal (HPA) axis activity. However, research on the relationship between obesity and altered cortisol metabolism remains controversial. The aim of this study was to investigate the association between cortisol levels in hair and overweight and obesity in participants of the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil). This was a cross-sectional study with 2,499 participants from the second follow-up (Visit 3, 2017-2019) who participated in research centers in the states of Rio de Janeiro and Rio Grande do Sul. Hair samples were collected and cortisol levels were analyzed using enzyme-linked immunosorbent assay (ELISA) kits. Cortisol levels were categorized as low (< 40 pg/mg), medium (40-128 pg/mg) or high (> 128 pg/mg). Participants were categorized as eutrophic, overweight or obese based on their weight (kg) and height (m²). Odds ratios (ORs) with 95% confidence intervals (95%CI) were estimated. This study was approved by CONEP and the local ethics committee. Of the 2499 subjects, 30% were eutrophic, 40% overweight and 30% obese. It is noteworthy that cortisol levels gradually increased with increasing body weight. Of the participants with high hair cortisol levels, 41.2% were categorized as overweight and 34.2% as obese. Multinomial logistic regression analysis revealed that participants with high cortisol levels were 43% (OR = 1.43; 95%CI: 1.02- 2.03) more likely to be overweight and 72% (OR = 1.72; 95%CI: 1.20-2.47) more likely to be obese than participants with low hair cortisol levels. After adjustment for all covariates, high cortisol levels remained associated with obesity (OR = 1.54; 95%CI: 1.02-2.31) and overweight (OR = 1.33; 95%CI: 0.91-1.94). We conclude that high hair cortisol levels were associated with a higher risk of obesity in the ELSA-BRASIL population.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Effects of gestational and lactational exposure to plastic waste on biometric parameters of male rat offspring

Authors:

Marina Cavalheiro¹
Vanessa Aguiar Rocha¹
Victória Cristina Pinho¹
Patrick Vieira de Souza¹
Natália Magosso¹
Mirella Franco Moreira¹
Wellerson Rodrigo Scarano¹

Affiliations:

¹ Department of Structural and Functional Biology, Institute of Biosciences, São Paulo State University (UNESP), Botucatu, São Paulo, Brazil.

Keywords: DOHaD. Maternal exposure. Nanoplastics. Phthalates.

Abstract:

The intrauterine microenvironment has been the target of significant changes due to the interference of environmental factors, which may have lasting consequences for the offspring. The plastic problem represents one of the greatest public health challenges worldwide. When plastics enter the environment, they degrade into micro and nanoplastics (NPs). Furthermore, phthalates are plastic additives that are not covalently bound to the polymer, making them easily released into the environment. These plastic residues can cross biological barriers, modulate prostate development, and even induce long-term changes. The aim of this study was to evaluate the consequences of intrauterine exposure to a mixture of phthalates (MP) and NPs in the offspring. For this purpose, pregnant Sprague Dawley rats were assigned to four groups, Ctrl (vehicle); T1: 20µg/kg/day MP; T2: NPs; T3: MP + NPs. The doses were based on environmental exposure, and treatment was administered orally from gestational day 10 to postnatal day (PND) 21. (CEUA no. 1174020523). Males offspring were euthanized at PND120 (n=6). The data were analyzed using the ANOVA test followed by Tukey's test, and differences were considered significant when $p \leq 0.05$. The treatment resulted in a decrease in the body mass of male pups at birth in all groups exposed to MP and NPs compared to the Ctrl group. The absolute and adjusted anogenital distance was reduced in the group exposed to the combination of MP and NPs at PND1. In adulthood (PND120), there were no changes in the animals' body weight or in the serum levels of testosterone and estradiol; however, a decrease in the relative weight of the prostate was observed in all groups exposed to MP and NPs. Additionally, it was observed an increase in the index of androgen receptor positive cells in T3 and T4 groups compared to the Ctrl. Intrauterine exposure to plastic waste impacted biometric parameters in the offspring and caused long-term effects on the prostate.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Early overnutrition does not aggravate depressive-like behavior in a rat model of polycystic ovary syndrome

Authors:

Luma Carolina Santos da Silva^{1,2}
Gabriela Heinzmann Garbinatto¹
Luiz Fernando de Souza Santos¹
Leonardo de Carvalho Araujo¹
Tamires Gregorio^{1,2}
Fernanda Barbosa Lima^{1,2}

Affiliations:

¹ Department of Physiological Sciences, Federal University of Santa Catarina-(UFSC)
² Multicenter Postgraduate Program in Physiological Sciences-UFSC.

Keywords: Depression. Overnutrition. Polycystic Ovary Syndrome. Testosterone.

Abstract:

Metabolic changes observed in polycystic ovary syndrome (PCOS) might be associated with the occurrence of emotional disorders. We investigated the effects of early overnutrition on developmental markers and emotional behavior in a rat PCOS model. PCOS-like rats (P) were treated at postnatal day 1 (PND1) with 1.25 mg of testosterone cypionate, s.c., while controls (C) received only oil. At PND3, litters were maintained as normal, with 10 pups (CNL; PNL) or reduced to 3 pups to establish overnutrition (CRL; PRL). Physical development, neurodevelopment, and depressive-like behavior were evaluated in adulthood. Differences among groups were analyzed using Two Way ANOVA (factors: testosterone and overnutrition); data are presented as mean $\pm$ SEM, for $p < 0.05$ (Ethics Committee/UFSC, nº9266220224). PCOS-like rats showed advanced ear unfolding, which was reversed by overnutrition (CNL:4 $\pm$ 0; CRL:4 $\pm$ 0; PNL:3.5 $\pm$ 0.1; PRL:4 $\pm$ 0; $p=0.00069$). There was a delay in disappearing the palmar grasp reflex in PCOS-like rats (CNL:10.1 $\pm$ 0.1; CRL:10.6 $\pm$ 0.3; PNL:11.1 $\pm$ 0.3; PRL:11.1 $\pm$ 0.5; $p=0.0001$). Overnutrition led to increased body weight during lactation (CNL:26.1 $\pm$ 1.1; CRL:33.6 $\pm$ 1.3; PNL:27.0 $\pm$ 0.8; PRL:32.8 $\pm$ 1.0; $p < 0.0001$), while in adulthood, both testosterone and overnutrition contributed to increasing body weight (CNL:206.3 $\pm$ 7.7; CRL:224.1 $\pm$ 2.8; PNL:261.1 $\pm$ 6.6; PRL:291.1 $\pm$ 8.3; $p < 0.0001$). Overnutrition delayed vaginal opening, which was completely absent in PCOS-like rats (CNL:34.0 $\pm$ 0.3; CRL:35.0 $\pm$ 0.2; PNL:0 $\pm$ 0.0; PRL:0 $\pm$ 0.0; $p=0.0406$; $p < 0.0001$). PCOS-like rats reduced sucrose consumption, which was unrelated to overnutrition (CNL:91.2 $\pm$ 1.1; CRL:90.7 $\pm$ 1.8; PNL:81.0 $\pm$ 4.4; PRL:81.2 $\pm$ 3.8; $p < 0.0001$). Only testosterone led to increased immobility in the forced swimming test (CNL:38.5 $\pm$ 6.7; CRL:52.5 $\pm$ 5.4; PNL:60.4 $\pm$ 8.7; PRL:62.8 $\pm$ 7.7; $p=0.0332$). We concluded that PCOS-like rats show developmental impairments and increased depressive-like behavior in adulthood, which was not influenced by early exposure to overnutrition.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Perinatal exposure to plastic pollutants alters hypothalamic reproductive pathway in post-weaning male rats

Authors:

Victória Cristina Pinha¹
Vanessa Aguiar Rocha¹
Natália Magosso¹
Patrick Vieira de Souza¹
Mirella Franco Moreira¹
Matheus Naia Fioretto¹
Vinícius Luís Rocha da Silva Maria¹
Wellerson Rodrigo Scarano¹

Affiliations:

¹ Department of Structural and Functional Biology, Institute of Biosciences, São Paulo State University (UNESP), Botucatu, São Paulo, Brazil.

Keywords: DOHaD. Hypothalamus. Nanoplastics. Phthalates. Reproduction.

Abstract:

The intrauterine microenvironment is susceptible to alterations driven by environmental factors, which may result in long-term consequences for offspring. Among these factors, nanoplastics (NPs), resulting from plastic degradation, and phthalates, widely used in plastic production, have raised significant concerns due to their capacity to traverse biological barriers and function as endocrine disruptors, respectively. Given the ability of nanoplastics to penetrate the blood-brain and placental barrier, this study was aimed at evaluating the effects of gestational and lactational exposure to an environmentally relevant phthalate mixture (MP) and NPs on hypothalamus of post-weaning rats, with a particular focus on reproductive pathways. Pregnant SD rats were assigned into four groups: Ctrl (vehicle), T1 (MP20µg/kg/day), T2 (NPs1.0 mg/kg/day) and T3 (PM+NPs). The doses were based on environmental exposure. Pregnant rats were exposed orally from gestational day 10 to postnatal day (PND) 21 (CEUA 1174020523). At PND22, male offspring (n=6) were weighed and submitted to necropsy. Blood and hypothalamus were collected for hormonal and molecular analysis. Statistical analysis was performed using one-way ANOVA, followed by post-hoc tests. Results are significant if $P \leq 0.05$. Results showed that all treatments reduced androgen receptor and steroid 5 alpha-reductase expressions compared to Ctrl. In the T1 group estrogen receptor (*Er*) and gonadotropin releasing hormone 1 (*Gnrh1*) expressions decreased compared to Ctrl. Also, *Gnrh1* expression decreased in T3 compared to the Ctrl and *Er* expression was increased in T3 vs T1. No significant differences were observed in body weight, relative and absolute hypothalamus weight, plasm testosterone and estrogen levels, or *Gpr54*, *Cyp19a1* expression at PND22. These findings suggest that the treatment impacts the male hypothalamic reproductive targets, highlighting the potential effect of environmental pollutants on neuroendocrine development.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Exploring the interaction between GLP-1 and androgens: an approach using published transcriptome analysis data

Authors:

Rafaella Sanfelice Normann¹
Bruna Selau¹
Jorge Felipe Argenta Model¹
Anapaula Sommer Vinagre¹

Affiliations:

¹Departamento de Fisiologia, Instituto de Ciências Básicas da Saúde (ICBS),
Universidade Federal do Rio Grande do Sul (UFRGS).

Keywords: Androgens. Orchiectomy. Incretin. Transcript.

Abstract:

Glucagon-like peptide 1 receptor analogs (GLP-1RAs) are widespread drugs used to treat obesity and type 2 diabetes mellitus. Recent studies have identified an interplay between androgens and GLP-1. Publicly available transcriptome data from studies involving GLP-1RA treatment and orchiectomy (ORC) in mice were reexamined to determine GLP-1RAs and androgens effects on gene transcription in the liver. The data used in this work can be found in the NCBI Gene Expression Omnibus database with accession numbers GSE226496 and GSE112947. Data analyzed were from livers of mice on a high-fat diet (HFD) treated with semaglutide (0.12 mg/kg/d) (GLP-1 analog) for 12 weeks and their untreated HFD controls, and livers from HFD ORC and their HFD sham controls, collected 10 weeks after surgery. The downloaded reads were mapped to a reference genome using HISAT2, and the gene expression levels were quantified using StringTie. The genes considered differentially expressed at p-value < 0.05 (fold change threshold of 1), were identified using the R package DESeq2. In mice treated with GLP-1RA, 199 differentially expressed genes (DEGs) were found. In the ORC animals' liver, 635 DEGs were observed, and 26 DEGs are shared between GLP-1RA and ORC mice. Among these, 6 DEGs are related to the immune system, 4 to cytochrome P450, 4 to extracellular matrix, 3 to metabolic pathways, 2 are hormone receptors and 7 are not related to other genes or each other. The results found in this study corroborate some known actions of androgens and GLP-1RAs, such as their influence on lipid and glucose metabolism in the liver. However, the results also indicate the importance of poorly understood processes, such as the GLP-1R action on the hepatic extracellular matrix. Few DEGs were shared between ORC and GLP-1RAs treated animals, indicating that the similar systemic effects observed do not derive from the same changes in gene transcription.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Gestational amplification of metabolic disruptions induced by excessive supplementation of polyunsaturated fatty acids

Authors:

Luiza Alves Amorim¹
Mylena Bordin Silvério¹
Djenifer de Toledo Maciel¹
Mariana Inocência Manzano¹
Mônica Maciel²
Katya Naliwaiko²
Matheus Felipe Zazula^{1,2}

Affiliations:

¹ Grupo de Pesquisa em Gestação e Doenças Metabólicas, Universidade Cesumar – Curitiba, Paraná, Brasil.

² Laboratório de Plasticidade Morfofuncional, Departamento de Biologia Celular, Universidade Federal do Paraná – Curitiba, Paraná, Brasil.

Keywords: Polyunsaturated fatty acids. Metabolic disturbances. Reproductive toxicity. Lipids supplementation.

Abstract:

Polyunsaturated essential fatty acids, such as omega-3 (n-3) and omega-6 (n-6), are obtained exclusively through the diet and are known for their health benefits. However, excessive intake of these fatty acids, particularly in the Western diet with an n-6/n-3 ratio of 16:1, well above the recommended 4:1, is associated with obesity, diabetes, cardiovascular diseases, and negative effects on female reproductive health, potentially leading to infertility and hormonal disruptions. This study, conducted in accordance with the ethical guidelines of CONCEA and CEUA/BIO – UFPR (protocol 1459/2022), used 18 female rats divided into three groups: control (CTL), omega-3 supplemented (SPL3), and omega-6 supplemented (SPL6). The rats received daily supplementation for 4 weeks (4 g/kg, orally), then were mated with control males. After confirmation of mating, they were euthanized for biochemical, histological, and statistical analysis. The results showed that the supplemented rats exhibited signs of obesity, diabetes, and dyslipidemia. Histological analyses revealed endometrial thickening in the SPL6 group and reduced uterine thickness in the SPL3 group. Additionally, the supplemented rats showed significant increases in soluble protein concentration and hydrogen peroxide production. The activities of total cholinesterases and superoxide anion production also increased, along with levels of carbonylated proteins and lipid peroxidation. Only the SPL6 rats showed a significant reduction in non-protein thiol concentrations. No changes were observed in metallothionein concentration. Chronic supplementation with n-3 and n-6 induced metabolic syndrome, creating a pro-inflammatory and pro-thrombotic state, increasing the risk of cardiovascular diseases. These results suggest that excessive consumption of these fatty acids may intensify inflammation and negatively impact both general and reproductive health in females, potentially leading to infertility.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Fetal programming induced by unsaturated fatty acids: Delayed testicular development in offspring

Authors:

Otávio SALES¹
Júlia Cristina Avelino dos Santos¹
Pedro Henrique da Silva Braga¹
Mariana Inocêncio Manzano¹
Mônica Maciel²
Katya Naliwaiko²
Matheus Felipe Zazula^{1,2}

Affiliations:

¹ Grupo de Pesquisa em Gestação e Doenças Metabólicas, Universidade Cesumar – Curitiba, Paraná, Brasil.

² Laboratório de Plasticidade Morfofuncional, Departamento de Biologia Celular, Universidade Federal do Paraná – Curitiba, Paraná, Brasil.

Keywords: Lipid exposure. Gonadal maturation. DOHaD. Maternal Metabolic Syndrome.

Abstract:

Unsaturated fatty acids play an essential role in homeostasis; however, their imbalance can impact various systems, including the reproductive system. This study aimed to evaluate histomorphological alterations in the testes of the offspring of Wistar rats chronically supplemented with omega-3, omega-6, and omega-9. A total of 40 female Wistar rats were used, divided into four experimental groups: control (CTLE), supplemented with omega-3 (SPL3), omega-6 (SPL6), and omega-9 (SPL9). Male offspring were euthanized on postnatal day 21, and their testicular tissues were collected for histological analysis. Samples were processed, embedded in paraffin with 5% beeswax, sectioned at 5–7 μm , deparaffinized, rehydrated, and stained with Hematoxylin and Eosin (CEUA 1452/2022). The results indicated a significant delay in testicular descent in approximately 70% of the offspring from the supplemented groups, whereas this process occurred as expected in the control group. Additionally, a reduction in testicular size and weight was observed in the SPL3 and SPL6 groups, associated with alterations in the seminiferous tubules, suggesting delayed cellular maturation. Histological analysis revealed tubular disorganization and immature spermatogenic cells in the SPL3 and SPL6 groups, indicating impairment in the germ cell differentiation process. In contrast, offspring from the SPL9 group exhibited a more preserved morphology, with testicular organization similar to the control group, suggesting that omega-9 has a less detrimental impact on testicular development. These findings indicate that maternal supplementation with omega-3 and omega-6 may impair male reproductive development, whereas omega-9 exerts less pronounced adverse effects. Further studies are needed to elucidate the long-term reproductive consequences of maternal lipid supplementation and whether these alterations may be transmitted to subsequent generations.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Irisin chronic intracerebroventricular administration improves lipid metabolism in mice with diet-induced obesity

Authors:

Guilherme
Santos de
Freitas¹
Tassiana
Cristina Talpo¹
Kelly Cristina
Pereira Bem¹
Guilherme Augusto da Silva
Nogueira² Helena Cristina de
Lima Barbosa¹

Affiliations:

¹ Laboratory of Endocrine Pancreas and Metabolism, Biology Institute, State University of Campinas, São Paulo.

² Laboratory of Cell Signaling, Department of Medical Pathophysiology, School of Medical Sciences, State University of Campinas, São Paulo.

Keywords: Central nervous system. Irisin. Lipid metabolism. Liver. Obesity.

Abstract:

Obesity is associated with metabolic alterations, including the accumulation of triglycerides and cholesterol in the liver. The hypothalamus regulates hepatic metabolism via the autonomic nervous system. Irisin, an adipomyokine released after exercise, could influence the control of hepatic lipid metabolism by central mechanisms in the nervous system since it can cross the blood-brain barrier. C57BL/6J male mice, 4w, were distributed into 3 groups: CTL fed a chow diet 10% fat, treated ICV with vehicle (saline 0,9%); HFD fed a high-fat diet 45% fat, treated ICV with vehicle; HFD-Iri fed a high-fat diet, treated ICV with irisin 300 ng/day. After 13w in diets, right lateral ventricle cannulation surgery was performed and animals were submitted to ICV treatment for 7d. After euthanasia, tissues were collected. Sample size: 4-11 animals. Data were analyzed by One-way ANOVA ($P < 0.05$), followed by Tukey post-test. CEUA/UNICAMP: 5995-1/2022. HFD group showed an increase in body weight (40.8 ± 2.2 g), increase in plasma cholesterol (120.2 ± 9.2 mg/dL), severe steatosis, with increase in fat percentage ($70.7 \pm 1.5\%$), no difference in the hepatocyte/non-hepatocyte ratio (1.56 ± 0.06), no difference in FAS, but an increase in ACC gene expression (1.0 ± 0.18 and 1.59 ± 0.25 , fold change of CTL), no difference in TH protein expression (1.2 ± 0.2 , fold change of CTL), compared to CTL (27.7 ± 0.7 g; 77.3 ± 3.8 mg/dL; no steatosis; 1.68 ± 0.1). HFD-Iri group showed no difference in body weight (39.5 ± 1.2 g), decrease in plasma cholesterol (99.6 ± 3.8 mg/dL), moderate steatosis, with decrease in fat percentage ($40.1 \pm 8.2\%$), increase in the hepatocyte/non-hepatocyte ratio (2.06 ± 0.09), reduction in FAS gene expression (0.51 ± 0.06 , fold change of CTL), increase in TH protein expression (2.5 ± 0.43 , fold change of CTL), compared to HFD. Chronic ICV treatment with irisin reduced plasma cholesterol and decreased the degree of steatosis in the liver, protecting mice against obesity challenges by regulating lipid metabolism.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Assessment of reproductive parameters in hybrid male mice exposed to ethane dimethanesulfonate (EDS)

Authors:

Vitória Gabrielle Piscelli Soares¹
Jorge Willian Franco de Barros¹
Lethicia Valencise¹
Ana Flavia Quiarato Lozano¹
Wilma de Grava Kempinas¹

Affiliations:

¹ Institute of Biosciences of Botucatu – UNESP.

Keywords: Ethane dimethanesulfonate. Leydig cell. Mouse. Reproductive biology. Reproductive toxicology.

Abstract:

The ethylene dimethanesulfonate (EDS) is a molecule with selective cytotoxicity on the Leydig cells (LC) of rats, leading to androgen deprivation and infertility. However, in mice, the efficacy of this molecule remains controversial. Recent studies from our laboratory have shown that EDS can affect mouse LCs in vitro. Thus, this work aims to investigate the effects of EDS on the reproductive parameters of male hybrid mice from the BALB/C and C57BL lines. In this study, adult mice were divided into three groups (n=7/group): control (standard diet), vehicle (diet with diluent without EDS), and EDS (diet with EDS) treated for 30 days via oral ingestion (CEUA no. 6532220424). After treatment, body weight, food consumption, weight of reproductive and vital organs, sperm motility, and fertility were evaluated. The observed results showed that weight and food consumption did not change when comparing the experimental groups. Moreover, the weight of vital organs (brain, heart, lungs, adrenal glands, and kidneys) also remained unaltered. However, among the reproductive organs (testis, epididymis, and seminal vesicle), there was a reduction in the weight of the testis and epididymis in the EDS group. Regarding fertility tests, the EDS group showed fewer implantations than the other groups. Finally, no changes were observed in the motility test among the experimental groups. The results show that EDS, similar to its effects in rats, also impacts male reproduction in mice. Thus, it can be concluded that studies with EDS in mice may serve as an interesting model for evaluating the dynamics of Leydig cells in these animals. Histological and immunohistochemical analyses are being conducted to draw better conclusions and complement this study.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Lack of STAT3 signaling in ventromedial neurons accentuates the effect of estradiol deficiency on body weight

Authors:

Gabriela Lucas FERREIRA¹
Gabriel Henrique Marques GONÇALVES¹
José ANTUNES-RODRIGUES¹
Lucila Leico Kagohara ELIAS¹

Affiliations:

¹ Ribeirão Preto Medical School, University of São Paulo.

Keywords: Hypothalamus, STAT3, Estradiol. Financial support: FAPESP. CNPq

Abstract:

The ventromedial nucleus of the hypothalamus (VMH) is involved in the regulation of food intake and energy expenditure and neurons in this region are target of leptin and estradiol actions. Estradiol is an important component in the regulation of energy homeostasis, as observed by the increase in food intake and body weight gain after ovariectomy in female rats. To better understand the mechanisms underlying the effects of 17 β -estradiol on the central nervous system, we used cre-lox technology to generate female mice with specific deletion of the STAT3 signaling pathway in the SF1 neurons of the hypothalamus to assess the relevance of this pathway to the effects of estradiol on the regulation of food intake, body composition and energy expenditure (CEUA-FMRP Protocol No. 1171/2023). Results showed that ovariectomized females with a deletion of the STAT3 pathway without estradiol replacement exhibited a greater increase in body weight compared to the other groups and particularly in comparison to the control STAT3 flox/flox (n=6/group, p<0,05). This effect was not due to differences in food intake. In addition, ovariectomized SF1-cre (cre/+); STAT3 flox/flox mice showed changes in body composition, with a gain in fat mass and a loss of lean mass. However, the effect of estradiol treatment on body weight reduction in ovariectomized female mice is unaffected by STAT3 deletion in the VMH. These data suggest that the effects of estradiol on body weight do not require the STAT3 pathway in VMH neurons. Nevertheless, in the context of estradiol deficiency, this pathway is recruited and necessary to prevent increased body weight gain.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Inverse correlation of umbilical blood flow with umbilical cord diameter in gestational diabetes mellitus with insulin therapy

Authors:

Eloisa Amanda Trevisol¹
Laís Teles de Sousa Micheli Cristiane Hintz²
Karine Souza da Correggio²
Fernanda Barbosa Lima¹
Laureane Nunes Masi¹

Affiliations:

¹ Federal University of Santa Catarina (Florianópolis, SC).

² University Hospital of Federal University of Santa Catarina (HU-UFSC).

Keywords: Fetal circulation. Gestational diabetes mellitus. Insulin therapy. Maternal hyperglycemia. Umbilical artery flow.

Abstract:

Gestational diabetes mellitus (GDM) is a common pregnancy complication with unclear effects on fetoplacental hemodynamics, particularly in insulin-treated cases. The aim of the study was to examine the impact of insulin therapy on gross morphology and fetal blood flow in GDM. This cross-sectional study included 68 parturients (19 non-GDM, 30 GDM without insulin use and 19 GDM with insulin use (GDMins)) admitted at the University Hospital of the Federal University of Santa Catarina (HU-UFSC/Ebserh). GDM was confirmed at 24-28 weeks of gestation, using one- step strategy. We analyzed Doppler indices (pulsatility index, PI) of the umbilical and middle cerebral arteries, fetal morphology (umbilical cord diameter (UC) and intraventricular septum (IS)), and demographic data from medical records, with Ethics Committee approval and informed consent. GDMins was older with a mean (SD) of 33.8 (3.7) years and had a higher body mass index at the beginning 33.1 (7.2) kg/cm² and end 36.8 (6.7) kg/cm² of pregnancy compared to both groups, with a higher number of cesarean deliveries (63%) and the size of the baby for gestational age (47%) compared to non-GDM. The UC diameter differed significantly between GDMins and non-GDM, 1.9 (0.2) vs. 1.7 (0.2) centimeters; p-value = 0.003 with a negative correlation ($r = -0.6257$, $p = 0.0042$) with the umbilical artery PI in GDMins. The middle cerebral artery PI also differed significantly between GDMins 1.6 (0.3) and the others groups 1.3 (0.3); p-value = 0.005. GDM caused an increase in the IS 6.3 (0.9) and even higher with insulin use 6.9 (0.8) compared to non-GDM 5.4 (0.7); p-value = 0.003 and p-value <0.001, respectively. In insulin-treated GDM mothers, increased UC diameter correlated with lower umbilical artery impedance, possibly linked to higher IS and middle cerebral artery PI. The prognostic role of umbilical artery PI remains uncertain, requiring further study.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Evaluation of nuclear RAR receptors (Retinoic Acid Receptors- α and β) in the ventral prostate of vasectomized rats

Authors:

Anne Beatriz de Souza¹
Leticia Gebra Tardivo¹
Ana Clara Pacheco de Santana²
Vinícius Luis Rocha da Silva Maria²
Cristiane Figueiredo Pinho¹
Sergio Pereira¹

Affiliations:

¹ São Paulo State University (UNESP), School of Sciences (FC), Bauru, São Paulo, Brazil.

² São Paulo State University (UNESP), Institute of Biosciences (IB), Botucatu, São Paulo, Brazil.

Keywords: Prostate. RAR- α . RAR- β . Vasectomy.

Abstract:

Vasectomy is a simple surgical procedure in which the vas deferens is cut to prevent sperm from being expelled during ejaculation. Additionally, it is the fourth most common contraceptive method in the world and the most effective for men. Nonetheless, recent studies have indicated a significant association between vasectomy and the risk of prostate cancer. Retinoids are substances that play a central role in controlling cellular physiological processes, such as proliferation, differentiation and cell death, and maintaining tissue homeostasis, both in normal and pre-malignant and malignant cells. Then, retinoids reduce the risk of carcinogenic initiation and progression, since they control the proliferation of damaged cells. In the intracellular environment, retinoic acid, the active metabolite of retinol, modulates the gene expression of these cellular processes through cellular receptors, including retinoic acid receptors (RAR). Thus, considering the role of retinoids and the possible relationship between vasectomy and the risk of prostate cancer, this study aimed to evaluate the expression of RAR- α and RAR- β in the ventral prostate of vasectomized rats. For this, 30 adult male Wistar rats, approximately 90 days old, were randomly divided into two experimental groups: a Control group and a Vasectomized group. The animals were euthanized 30, 60, or 90 days after surgery, and the ventral prostates were collected and processed histologically for immunohistochemical analysis of the RAR- α and RAR- β receptors. The results showed that animals from the Vasectomized Group at 90 days after surgery exhibited lower intensity and smaller average area of RAR- α immunostaining compared to the Control Group collected at the same time. Regarding RAR- β , no statistically significant differences were found. Therefore, vasectomy altered the expression of retinoic acid receptors in the ventral prostate of rats, potentially impacting the maintenance of homeostasis. The experimental procedure, protocol n°200-CEEA, was approved by the Ethics Committee for Animal Experimentation (CEEA) of the Institute of Biosciences – UNESP/Botucatu.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Effects of pregnancy hormones in Achilles tendon biomechanics and biochemistry in female obese mice

Authors:

Sara Luiza Custódio de Souza¹
Camila Alves Lage Santos¹
Matheus Henrique Alves de Moura¹
Petrus Pires Marques²
Bruno Zavan¹
Flávia da Ré Guerra¹

Affiliations:

¹ Universidade Federal de Alfenas.
² Universidade José do Rosário Vellano.

Keywords: Achilles tendon. Obesity. Pregnancy. Sex hormones. Tendon lesions.

Abstract:

Tendon injuries are common and compromise quality of life, requiring long recovery periods and generating high costs for health systems. Factors such as sex, age, and chronic diseases, especially obesity, increase susceptibility to these injuries, especially in women. The effects of obesity associated with sex hormones and gestational changes in tendon physiology are not yet fully understood. This study assessed the impact of obesity and hormonal changes during pregnancy on the Achilles tendon of obese female mice. Ten male and 60 female Swiss mice (*Mus musculus*) were divided into two groups: control and obese (CEUA/UNIFAL: 54/2017). Each group was subdivided into gestational days (GD) 6, 10, and 16. Body weight was monitored every two days and nasoanal length was measured to estimate adiposity by calculating the Lee Index. The Achilles tendons were subjected to biomechanical analysis to determine their mechanical strengths. These data suggest that sex hormones reduced tensile strength in the control groups from DG 10 and on all gestational days in the obese groups. In addition, obese mice on DG 16 showed a significant reduction in tendon elasticity compared to the control on DG 6, suggesting that the combination of obesity and gestational progression compromises tendon integrity. Therefore, the analyses indicate that female sex hormones influence the biomechanics of the tendon, reducing its resistance to tension during pregnancy, while obesity contributes to a decrease in elasticity, depending on hormonal action. These findings reinforce the need for further studies to understand the underlying mechanisms and develop strategies to minimize the risk of tendon injuries in pregnant women.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Effect of estrogen hormone therapy on memory and hippocampal redox state in periostropause rats

Authors:

Thainá Daguane Esperança^{1,2}
Beatriz Procópio Stringheta-Villar²
Débora Prazias Cavalcante^{1,2}
Luana Galante Douradinho^{1,2}
Júlia Zanuto Douradinho^{1,2}
Isabella Ribeiro Pradela²
Gabriela Alice Fiais²
Antonio Hernandez Chaves-Neto^{1,2}
Fernanda Barbosa Lima³
Rita Cássia Menegati Dornelles^{1,2}

Affiliations:

¹ Programa de Pós-Graduação Multicêntrico em Ciências Fisiológicas, UNESP-SBFis.
² Department of Basic Sciences, School of Dentistry, São Paulo State University, São Paulo/SP.
³ Department of Physiological Sciences, Federal University of Santa Catarina, Santa Catarina/SC.

Keywords: Estrogen. Neuroprotection. Perimenopause. Redox state.

Abstract:

Cellular aging is a natural process that affects all living beings, with the female reproductive system being the first to age. Perimenopause, characterized by cyclical irregularity, is influenced by hormonal changes, impacting the antioxidant and neuroprotective system. Estrogen hormone therapy (EHT) is used to alleviate menopausal symptoms, but its effects on perimenopause are still under study. Therefore, the aim of this study was to evaluate the effect of EHT in periostropause, on potential modulation of hippocampal antioxidant system and on short (STM) and long-term (LTM) memory retention. Thirty animals (18 months-old) with irregular estrous cycles, in persistent diestrus, participated in the study; 15 rats received corn oil (21Mo/Veh group; 0.2mL/sc; 2x/week) and 15 were subjected to EHT (21Mo/E₂ group; 17 β -estradiol/15 μ g/Kg/sc; 2x/week) for 120 days (CEUA process 0850-2021). The object recognition (OR) test was applied to evaluate STM and LTM. The animals were euthanized by decapitation, the hippocampi (HP) were isolated, homogenized and destined for biochemical analyses. The null hypothesis rejection level was set at 5% ($p < 0.05$). After 4 months, the 21Mo/E₂ group showed cognitive improvement, with significant interactions in intervention of test stages (total exploration time: $p = 0.0001$; LTM: $p = 0.0037$) compared to 21Mo/Veh group, the latter being smaller in relation to its 17 months ($p = 0.0008$). The total non-enzymatic antioxidant capacity and catalase enzyme activity were significantly lower in 21Mo/E₂ group compared to 21Mo/Veh group ($p = 0.0064$ and $p = 0.0280$, respectively). In contrast, GSH concentration was significantly higher in 21Mo/E₂ group compared to 21Mo/Veh group ($p = 0.0379$). There was no significant effect on other redox markers in HP, not even in STM. In fact, EHT has been shown to be beneficial for cognitive performance and brain biochemistry, being a good aid in redox balance, both benefits are significant for neurohomeostasis in periostropause.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Effect of maternal apical periodontitis on the activation of inflammatory pathways in the placenta of rats

Authors:

Kelly Fernanda da Silva Soares¹
Ana Carla Thalez Ywabuchi Nobumoto¹
Anna Clara Cachoni²
Angela Vermelho Sarraceni¹
Anielly Gonsalves¹
Mariana Campanha Hilário Pereira¹
Lorena Umbelino Rodrigues¹
Camila Conrado França¹
Louise Elias de Carvalho¹
Doris Hissako Matsushita^{1,2}

Affiliations:

¹ Department of Basic Sciences, São Paulo State University (UNESP), School of Dentistry, Araçatuba, Brazil. Multicenter Postgraduate Program in Physiological Sciences.

² Department of Preventive and Restorative Dentistry, São Paulo State University (UNESP), School of Dentistry, Araçatuba, Brazil. Graduate Program in Dental Science.

Keywords: Apical periodontitis. Inflammation. Fetal programming. Periapical periodontitis. Placenta.

Abstract:

Studies have shown that maternal periodontal disease (PED) is associated with adverse gestational outcomes, such as low birth weight and preterm birth. In addition, periodontal pathogens have been shown to induce changes in placental structure. Apical periodontitis (AP) is an oral inflammation in the region of the apex of the dental root associated with an increase in inflammatory cytokines that can contribute to systemic alterations. The aim of this study was to investigate the inflammatory pathways and placental morphology in pregnant rats with AP. To this, the following assessments were carried out on AP rats and control rats (CN): (1) body mass and food intake during gestation and lactation; (2) absolute placental weight; (3) placental TNF- α content. 18 Wistar rats were assigned to 3 groups (n=6/group): (1) CN rats; (2) rats with 1 AP induced in the 1st upper right molar; (3) rats with 4 APs induced in the 1st and 2nd upper and lower right molars. The AP was induced using a carbon steel drill with a 0.1 mm ball at the end. After 30 days of pulp exposure, the rats in all groups were mated. The rats were euthanized on the twenty-first day of pregnancy and the experiments were carried out. The analyses showed that: (1) there was an unchanged food intake and body weight assessed from the induction of oral inflammations until the last day of pregnancy of the mother rats; a reduction in placental weight and the area of the placental labyrinth only in the AP4 group when compared to the CN and AP1 groups; (3) an increase in TNF- α content in the placenta of AP4 rats compared to the AP1 and CN groups. Data were analyzed by ANOVA with Tukey's post hoc test- $p < 0.05$. Results showed that maternal AP is associated with activation of inflammatory pathways and morphological changes in the placenta, worsened by multiple foci, highlighting the importance of maternal oral health in preventing systemic alterations that affect the intrauterine environment and fetal development. CEUA: 0878-2022. This study was financed, in part, by the São Paulo Research Foundation (FAPESP), Brasil.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Maternal true endo-periodontal lesion activates inflammatory pathways in the adult life of offspring

Authors:

Anielly
Gonsalves¹
Bianca
Elvira
Belardi¹
Maria Sara de Lima
Coutinho Mattera¹ Ana
Carla Thalez Ywabuchi
Nobumoto¹ Bruna De
Oliveira Alves¹
Mariana Campanha
Hilário Pereira¹ Kelly
Fernanda da Silva
Soares¹ Angela
Vermelho
Sarraceni¹ Lorena
Umbelino
Rodrigues¹
Dóris Hissako Matsushita^{1,2}

Affiliations:

¹ Department of Basic Sciences, São Paulo State University (UNESP), School of Dentistry, Araçatuba, Brazil. Multicenter Postgraduate Program in Physiological Sciences.

² Department of Preventive and Restorative Dentistry, São Paulo State University (UNESP), School of Dentistry, Araçatuba, Brazil. Graduate Program in Dental Science.

Keywords: Fetal development, Inflammation, Insulin resistance, Periapical periodontitis, Periodontal diseases.

Abstract:

Maternal periodontal disease-PED and apical periodontitis-AP in rats promote insulin resistance- IR in their adult offspring. True endo-periodontal lesion-EPL is an infectious process that extends to the pulp and periodontal tissue simultaneously, each with its own origin. It became essential to conduct further studies to verify whether maternal EPL can also promote systemic changes in the adult life of the offspring. Aim: verify in adult rats, offspring of rats with AP, PED and EPL: phosphorylation status of the transcription factor NF- κ B(p50/p65) and total content in gastrocnemius muscle-GM by the western blotting technique. 28 Wistar rats(4 groups): control rats-CN; rats with induced AP in the upper right first molar; rats with induced PED in the upper right 2^o molar; rats with EPL, in which PED was induced in the upper right second molar and AP in the upper right first molar. AP was induced by exposing the pulp tissue to the oral environment, using a carbon steel burr with a 0.1 mm ball at the end. PED was induced by ligation in the second molar with sterile silk thread. 30 days after induction of oral inflammation, the rats from all groups were placed for mating. When the male offspring of these rats completed 75 days, the experiments were performed. There was an increase in the phosphorylation status of NF κ Bp65/p50 in GM in the groups of PED-o and EPL-o offspring in relation to the CN-o and AP-o groups. EPL-o group showed an increase in this parameter when compared to the CN-o, AP-o and PED-o groups. Statistical analysis was performed by ANOVA followed by the



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Tukey test ($p < 0.05$). This study shows that maternal EPL was able to promote more exacerbated changes in the health of adult offspring in relation to the results obtained when AP or PED were evaluated separately. These data reinforce the importance of maintaining good maternal oral health to prevent the development of systemic diseases in the adult life of the offspring. CEUA:0446-2022

This study was financed, in part, by the São Paulo Research Foundation (FAPESP), Brasil. Process numbers 19/27662-0; 24/05519-9; 2023/01400-4; 2022/04868-4.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Evaluation of the oxidative stress in adult rats, offspring of rats with true endo-periodontal lesion

Authors:

Mariana Campanha Hilário Pereira¹
Bianca Elvira Belardi¹
Maria Sara Lima Coutinho Mattera¹
Anielly Gonsalves¹
Kelly Fernanda da Silva Soares¹
Ana Carla Thalez Ywabuchi Nobumoto¹
Bruna de Oliveira Alves¹
Estêvão Lopes Pereira²
Marco de Luca Monteiro Sturaro¹
Dóris Hissako Matsushita^{1,2}

Affiliations:

¹ Department of Basic Sciences, São Paulo State University (UNESP), School of Dentistry, Araçatuba, Brazil. Multicenter Postgraduate Program in Physiological Sciences.

² Department of Preventive and Restorative Dentistry, São Paulo State University (UNESP), School of Dentistry, Araçatuba, Brazil. Graduate Program in Dental Science.

Keywords: Periodontal disease. Oxidative stress. Inflammation. Periapical periodontitis. Insulin resistance.

Abstract:

Studies have demonstrated that maternal periodontal disease-PED and apical periodontitis-AP and True endo-periodontal lesions-EPL in rats promote insulin resistance (IR) in their adult offspring. Oxidative stress is a contributing factor in both the onset and progression of diabetes. The aims of this study were: evaluation of glycemia and insulinemia followed by the HOMA-IR index; oxidative stress analysis in GM (Catalase-CAT and Thiobarbituric Acid Reactive Species-TBARS). 28 Wistar rats were assigned to 4 groups: control; with induced AP in the right maxillary first molar; with induced PED in the right maxillary second molar; EPL, in which PED was induced in the right maxillary second molar, and AP in the right maxillary first molar. AP was induced by exposing the pulp tissue to the oral environment using a carbon steel bur with a 0.1 mm spherical tip. PED was induced by placing a sterile silk ligature in the second molar. After 30 days, the rats from all groups were mated with healthy males. When the male offspring rats reached 75 days of age, the experiments were conducted. Offspring of rats with AP(AP-o), PED(PED-o), and EPL(EPL-o) exhibited increased insulin levels and HOMA-IR index compared to the offspring of control rats (CN-o). This parameter was higher in the EPL-o group compared to the AP-o and PED-o groups; 2) catalase enzymatic activity was higher in the EPL-o group compared to the CN-o, AP-o, and PED-o groups; 3) TBARS concentration in GM was significantly reduced in the EPL-o group compared to the CN-o, AP-o, and PED-o groups. Data were analyzed by ANOVA with Tukey's post hoc test-p<0.05. Results demonstrate that maternal AP, PED and EPL promote IR in the adult life of their offspring, and this alteration is worse in EPL-o. Only EPL-o showed alteration in oxidative stress. These findings highlight the importance of maintaining maternal oral health to prevent diseases and ensure the general health of offspring. C EUA: 0446-2022. This study was financed, in part, by the São Paulo Research Foundation (FAPESP), Brasil. Process numbers 2024/22879-9; 2019/27662-0; 2022/04868-4; 2023/01400-4.



XXXIX REUNIÃO ANUAL DA FeSB
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Different Estrogen Treatments in a BPA-Dysregulated Mammary Gland Microenvironment: Receptor Analysis

Authors:

Rafael Henrique Borges Coelho¹;
Leonardo Araújo Caires de Aguiar¹;
Vinícius Savazo Assis¹;
Stella Bicalho Silva¹;
Vitor Grigio¹;
Patrícia Simone Leite Vilamaior¹;
Thalles Fernando Rocha Ruiz^{1,2};
Sebastião Roberto Taboga^{1,2}

Affiliations:

¹ Laboratório de Microscopia e Microanálises / Instituto de Biociências, Letras e Ciências Exatas – UNESP Campus de São José do Rio Preto, Brasil.

² Instituto de Biologia Universidade Estadual de Campinas – UNICAMP, Campinas, Brasil.

Keywords: DOHaD. Endocrine disruption. Histopathology.

Abstract:

The Mammary gland (MG) is highly susceptible to hormonal interactions, mainly estrogens and progesterone. An imbalance caused by endocrine-disrupting chemicals such as bisphenol A (BPA), a xenoestrogen, during developmental windows, can lead to a tumorigenesis. Thus, this study aimed to analyze the hormonal receptors in MG after estrogenic supplementation with BPA perinatal disruption and pro-carcinogenic induction. To assess carcinogenic development under an EDC microenvironment, female gerbil (6mo) was exposed perinatally to BPA (50 µg/kg/daily) and divided into 4 experimental groups: a control group (only BPA exposure) and 3 induced to carcinogenesis with ethyl-N-nitrosourea (ENU) receiving different treatments: 17β-estradiol (E2); 17α-ethinylestradiol (EE2); and only pro-carcinogenic treated (ENU), CEUA/IBILCE nº 235/2022. The incidence of estrogen receptor (ER) α-positive cells increased in the ENU group (25.60%±8.22) compared to the others, indicating the development of ERα-positive tumors. In contrast, the E2 exhibited a low incidence of ERα+ cells (9.94%±5.32), but no significant differences in receptor expression by Western Blotting assay. Regarding ERβ, no significant differences were observed among groups, whereas E2-treated females (1.33±0.38) showed higher protein expression compared to other groups. The progesterone receptor (PR) had a higher incidence in the EE2 (81.53%±12.25), indicating the development of PR+ neoplasia. Prolactin receptor (PRLR) expression in E2 (1.31±0.24) was lower than in only ENU treated group (1.87±0.09), and E2 (24.72%±6.74) had a reduced incidence compared to the BPA (54.11%±8.15) and EE2 (47.46%±19.7). Also, androgen receptor (AR) protein expression was higher in the BPA (1.01±0.01) compared to the E2 (0.73±0.03). The results suggest that endogenous E2 regulates mammary hormones more effectively than synthetic EE2, with reduced PRLR sensitivity and lower ERα responsiveness, while EE2 is linked to more aggressive PR+ cancer.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Vitamin D levels in the indigenous population living in the reserve of Aracruz, Espírito Santo

Authors:

Hully Cantão dos Santos¹
Nathalie Gabriel Ferreire Felberg¹
Gabriela Callo Quinte¹
José Geraldo Mill^{1,2}

Affiliations:

¹ Programa de Pós-Graduação em Saúde Coletiva.

² Programa de Pós-Graduação em Ciências Fisiológicas, Universidade Federal do Espírito Santo.

Keywords: Vitamin D, obesity, indigenous, diabetes

Abstract:

Introduction: Vitamin D (VitD) is critical to maintain calcium balance, as it plays an essential role in intestinal absorption. Insufficient VitD levels contribute to increased bone demineralization due to secondary hyperparathyroidism. Surprisingly, many studies have documented a high prevalence of insufficient VitD in several Brazilian populations, given the abundant sunlight in our country. Most of these studies have primarily focused on urban populations, where sunlight exposure is often limited. Objective: Our aim was to determine VitD levels in adults residing in the indigenous reserve of Aracruz, ES. Methods: A cross-sectional study (2020/22) was performed to assess chronic non-communicable diseases. All adults were invited to participate by indigenous health agents operating in the reserve. Data collection included questionnaires, clinical and laboratory exams conducted on a scheduled day at the Cardiovascular Research Clinic in Vitoria, ES. A fasting blood sample was obtained, with all analyses carried out in a central laboratory. Data are presented as means $\pm$ standard deviation or percentages. Results: The eligible population comprised 2,982 adults (aged ≥ 18 years) and 1,084 (36.4%) attended to the study. The analysis focused on 1,066 participants (64.7% women) with complete data (mean age 41.5 ± 14.8 years). The mean VitD concentration (measured as 25(OH)D) was 32.7 ± 10.0 ng/dL, with men exhibiting higher levels compared to women (34.0 ± 10.8 vs. 31.7 ± 9.2 ; $p < 0.01$). Adequate 25(OH)D levels (≥ 30 ng/dL) were recorded in 56.2% of participants (95% CI: 53.3-59.1%), while 7.8% (95% CI: 6.2-9.4%) displayed deficient levels (< 20 ng/dL). Increased body fat, assessed through body mass index (BMI) or body fat mass, was identified as the primary factor associated with reduced levels of VitD. Conclusion: The concentration of VitD in this population was higher than in urban populations suggesting that a higher sun exposure may contribute to this difference.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Effects of gestrinone treatment on body mass and renal morphofunction in female rats fed on a normo or a high-fat diet

Authors:

Elisângela Pinotti Conci Costa Rosa¹
Kayky Diniz Daniel¹
Maria Lúcia Bonfleur²
Dionizia Xavier Scomparin¹
Rosane Aparecida Ribeiro¹

Affiliations:

¹ Universidade Federal de Ponta Grossa.
² Universidade Estadual do Oeste do Paraná

Keywords: Gestrinone. Kidneys. Obesity.

Abstract:

Obesity is a disease that may impair kidney health. Gestrinone (GES) has gained attention for its off-label use in weight loss. As sex hormones regulate metabolism and kidney function, here we aimed to evaluate the effects of GES on body mass composition and renal morphofunction in female rats. Adult female *Wistar* rats were fed on a normo-(ND) or a high-fat diet (HFD), and received 3 times a week a gavage of soybean oil containing 0.2% DMSO [control (CTL) and HFD] without or with 0.26 (C0.26 and H0.26) or 0.52 mg GES/kg body weight (BW; C0.52 and H0.52 groups) for 12 weeks. At euthanasia, BW, total blood, abdominal fat stores, kidneys and carcasses were collected (CEUA protocol: 23.000077157-5). HFD females displayed increases of 18% in BW, 114% in abdominal adiposity and 71% in carcass fat mass, but reductions of 10% in kidneys weight and of 7% in carcass lean mass. H0.26 and H0.52 females displayed lower BW (13% and 17%), abdominal adiposity (37% and 55%) and carcass fat masses (28% and 43%), but higher carcass lean masses (5% and 8%), than HFD group. Plasma creatinine, urea and uric acid were similar between HFD (0.9 ± 0.2 , 18.5 ± 2.1 and 2.0 ± 0.2 mg/dL) and CTL females (0.9 ± 0.2 , 26.5 ± 3.4 and 2.2 ± 0.2 mg/dL). Such parameters did not differ in GES-treated HFD (H0.26: 0.9 ± 0.2 , 25.2 ± 4.0 and 2.0 ± 0.2 ; H0.52: 0.7 ± 0.1 , 22.6 ± 3.2 and 1.7 ± 0.2 mg/dL) or ND groups (C0.26: 0.7 ± 0.1 , 27.2 ± 4.0 and 1.8 ± 0.2 ; C0.52: 1.1 ± 0.4 , 22.7 ± 2.1 and 2.3 ± 0.3 mg/dL). The size of the renal corpuscle, glomerulus and Bowman's space were similar among HFD (4560 ± 108 , 3248 ± 244 and 1252 ± 235 μm^2) and CTL females (4718 ± 179 , 3487 ± 50 and 1119 ± 180 μm^2). These renal structures were not altered by GES in HFD (H0.26: 4587 ± 239 , 3212 ± 152 and 1017 ± 269 ; H0.52: 4770 ± 273 , 3182 ± 259 and 1558 ± 68 μm^2) or in ND groups (C0.26: 4944 ± 266 , 3494 ± 288 and 1324 ± 109 ; C0.52: 4516 ± 129 , 3208 ± 58 and 1284 ± 170 μm^2). Therefore, GES treatment attenuated some obesogenic effects of a HFD without changes in renal morphofunction.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Androgenic modulation affects neoplastic development in the gerbil male prostate following perinatal exposure to the endocrine disruptor bisphenol A

Authors:

Leonardo Araújo Caires de Aguiar¹
Rafael Henrique Borges Coelho¹
Vinicius Savazo Assis¹
Stella Bicalho Silva¹
Vitor Grigio¹
Patrícia Simone Leite Vilamaior¹
Thalles Fernando Rocha Ruiz^{1,2}
Sebastião Roberto Taboga^{1,2}

Affiliations:

¹ Instituto de Biociências, Letras e Ciências Exatas – UNESP, São José do Rio Preto, Brasil.

² Instituto de Biologia da Universidade Estadual de Campinas – UNICAMP, Campinas, Brasil.

Keywords: Prostate Cancer. Histopathology. EDCs.

Abstract:

Steroid hormone signaling is crucial in prostate cancer development, with disruptions in hormone receptor expression linked to neoplastic potential. In this context, studying endocrine disruptors like bisphenol A (BPA) is essential. We evaluated the histopathological effects of androgen modulation in the ventral prostate of gerbils perinatally exposed to BPA. The animals (Ethics Committee Protocol: CEUA/IBILCE 240/2022) were randomly subdivided into five groups (n=10): control and four BPA-exposed (50 µg/kg/daily), which received androgen supplementation, surgical castration (deprivation), or androgen receptor blockade (enzalutamide). At 12 months, animals were euthanized, and ventral prostates were analyzed by immunohistochemistry and Western Blotting assay. BPA exposure led to a reduction in androgen receptor (AR) in stromal cells ($39.34\% \pm 6.99$) and estrogen receptor beta (ER β) ($55.24\% \pm 22.76$), compared to control. In the deprivation group, AR expression in epithelial cells decreased ($38.05\% \pm 19.28$), and the AR110 isoform was absent. Conversely, under receptor blockade, AR110 (3.21 ± 0.14) and ER β 28 (1.97 ± 1.21) isoforms expression were increased. The AR86 isoform was expressed under androgen supplementation scenario, suggesting an alternative AR signaling pathway. This group also showed higher epithelial proliferation (PHH $_3$: $0.43\% \pm 0.38$) and decreasing in apoptosis (cleaved Caspase-3: 0.26 ± 0.06) than other BPA-exposed groups and control. These findings suggest that androgen supplementation after BPA exposure may enhance neoplastic potential, potentially via alternative AR isoforms, while deprivation and blockade triggered mechanisms that may mitigate this risk.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Evaluation of the Androgen Receptor and Estrogen Receptor Alpha in the ventral prostate of vasectomized rats

Authors:

Leticia Gebra Tardivo¹
Anne Beatriz de Souza¹
Ana Clara Pacheco de Santana²
Vinícius Luis Rocha da Silva Maria²
Cristiane Figueiredo Pinho¹
Sergio Pereira¹

Affiliations:

¹ São Paulo State University (UNESP) School of Sciences, Bauru, São Paulo, Brazil.
² São Paulo State University (UNESP) Institute of Biosciences (IB), Botucatu, São Paulo, Brazil.

Keywords: AR. ER- α . Prostate. Vasectomy.

Abstract:

Vasectomy, a surgery that interrupts the vas deferens, prevents sperm release into the seminal fluid. Although effective, it may cause postoperative complications and raises concerns about its association with severe diseases such as prostate cancer. The prostate, a hormone-dependent organ, is sensitive to androgens and estrogens, making the action of hormonal receptors essential, such as Androgen Receptors (AR) and Estrogen Receptor Alpha (ER α). Previous studies have linked ER expression to prostate cancer, detecting it in lesions ranging from neoplasms to invasive carcinomas. It is, evidence suggests that vasectomy may alter prostate morphology. This study used 30 adult male Wistar rats, approximately 90 days old, divided into two groups: Control Group (CG) and Vasectomized Group (VG). The VG underwent vas deferens disruption, while the CG underwent a sham surgery. After 30, 60, or 90 days, the prostate of the rats was collected, fixed, and analyzed immunohistochemically. Twenty histological field images were captured and quantified using CellProfiler software. Statistical analysis was performed using the Kruskal-Wallis test, followed by Dunn's multiple comparisons test with 5% significance, using Prism© 10.3.0 (GraphPad). Results showed that the average ER α immunostaining intensity did not differ significantly between experimental groups. Regarding AR expression, no statistically significant differences were found between groups at any time point. However, in the VG, significant differences were observed between 30 and 60 days and between 30 and 90 days. The comparison of ER α immunostaining areas between CG and VG revealed significant alterations at 30 and 90 days post-surgery, indicating that vasectomy reduces receptor expression at these points. Prostate homeostasis relies on a finely tuned balance between androgens and estrogens. Future studies should investigate whether the post-vasectomy reduction in ER α expression impacts prostate homeostasis. The experimental procedure, protocol nº200-CEEA, was approved by the Ethics Committee for Animal Experimentation (CEEA) of the Institute of Biosciences – UNESP/Botucatu.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Perinatal exposure to phthalates and susceptibility to prostatic inflammation in adulthood

Authors:

Maria Clara Betete da Silva Fonseca¹
Livia Centofanti Cózaro¹
Vinícius Luís Rocha da Silva Maria²
Ana Clara Pacheco de Santana²
Liara Botão da Silva¹
Júlia Landeira Gomez Chamelete¹
Wellerson Rodrigo Scarano²
Sergio Pereira¹
Cristiane Figueiredo Pinho¹

Affiliations:

¹ Faculty of Sciences, São Paulo State University, Bauru, Brazil.

² Institute of Biosciences, São Paulo State University, Botucatu, Brazil.

Keywords: DOHaD. Dorsolateral prostate. Endocrine disruptors. Mast cells. Phthalates.

Abstract:

Phthalates are the main class of plasticizers found in plastic packaging and everyday cosmetics. Classified as environmental pollutants, these compounds act as endocrine disruptors, causing several morphological, metabolic and reproductive alterations in exposed organisms, especially in hormone-dependent organs such as the prostate. In this context, the present study aimed to evaluate the effects of perinatal exposure to an environmentally relevant mixture of phthalates on the histological structure of the prostate in adulthood, especially regarding the emergence of inflammatory foci. To this end, pregnant Sprague-Dawley rats were randomly divided into 4 experimental groups: **C** (Control, corn oil), **T1** (20 µg/kg/day mixture of phthalates), **T2** (200 µg/kg/day mixture of phthalates), and **T3** (200 mg/kg/day mixture of phthalates). Exposure was administered via gavage from gestational day 10 to postnatal day 21. On postnatal day 120, the offspring were euthanized and the dorsolateral prostate of each male was removed and processed for routine histology. Hematoxylin-eosin staining showed a typical prostate architecture in group C, with predominance of acini with wide lumens and lined by simple columnar epithelial secretory cells. In contrast, perinatal exposure to the mixture of phthalates (T1, T2, T3 groups) had a significant impact on prostate morphology, causing epithelial disorganization and inducing a greater degree of folding towards the lumen. Toluidine Blue staining allowed us to identify a significant increase in the number of periacinar mast cells in T1, T2 and T3 groups, compared to C. In addition, the incidence of inflammatory foci increased in T3, compared to C. These results showed that the exposure to different doses of the mixture of phthalates during prostate organogenesis could lead to morphological alterations in adulthood, increasing the development of inflammatory lesions. Approved by Ethical Committee: 1040-CEUA/ Institute of Biosciences - UNESP. Funding Support: FAPESP 2024/20520-3.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Offspring birth weight classification in an experimental model of mild and severe hyperglycemia induced during pregnancy in rats

Authors:

Caroline Martins SIERRO^{1 2}

Karla Martinucho GODEGUEZI^{1 2}

Milena Silva AGOSTINI^{1 2}

Carla de Moraes MACHADO¹

Ana Carolina Inhaz KISS^{1 2}

Affiliations:

¹ Department of Structural and Functional Biology, Institute of Biosciences of Botucatu, São Paulo State University 'Júlio de Mesquita Filho' (UNESP).

² Graduate Program in General Physiology, Institute of Biosciences, University of São Paulo (USP).

Keywords: Diabetes. Glucose tolerance. Pregnancy. Rodents.

Abstract:

Maternal diabetes, a common pregnancy complication, has long-term implications for both mother and offspring, which can be related to the degree of hyperglycemia. The aim of the present study was to establish an experimental model of mild and severe hyperglycemia induced during pregnancy and to evaluate offspring birth weight classification. Pregnant rats were assigned either to Normoglycemia (N, (n= 12), glycemia up to 120 mg/dL), Mild Hyperglycemia (MH, (n= 14), streptozotocin (STZ) 35 mg/kg i.p. on day 7 of pregnancy, glycemia between 120 and 300 mg/dL) or Severe Hyperglycemia (SH, (n= 11), STZ 50 mg/kg i.p., glycemia above 300 mg/dL) groups (CEUA 3041270422). The oral glucose tolerance test (OGTT) was performed and the area under the OGTT curve (AUC) was calculated (mean $\pm$ SEM, mg/dL/120 min, ANOVA followed by Tukey). Offspring were classified according to their birth weight in small (SPA), appropriate (APA), or large for pregnancy age (LPA) (Fisher's exact test, %). The analysis of the OGTT AUC showed that the expected glycemic range for each experimental group was achieved (N: 12689.4 ± 307.3 , MH: 17462.7 ± 499.3 , SH: 53573.9 ± 2103.84 , group (F (2,34) = 360.391, p < 0.001), NxMH (t = -2.964, p = 0.015), NxSH (t = -24.496, p < 0.001), MHxSH (t = -22.484, p < 0.001). Birth weight classification varied between N (9.83% SPA, 83.60% APA, 6.55% LPA), MH (2% SPA, 89.5% APA, 8.5% LPA), and SH dams (10.32% SPA, 83.87% APA, 5.80% LPA). MH dams had a lower proportion of SPA pups compared to N (p = 0.0015) and SH dams (p = 0.0009). In conclusion, the experimental model of mild and severe hyperglycemia induced during pregnancy was successfully established and the degree of maternal hyperglycemia changed offspring birth weight classification, with mild hyperglycemic dams showing a smaller proportion of small pups. Future studies will further explore the effects of the current experimental model for the mothers and their offspring, such as on maternal behavior.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Gestrinone Administration Decreases Adiposity and Plasma Cholesterol but Increases Hepatic Cholesterol in Obese Female Mice

Authors:

João Pedro Giannotti Alves¹
Ana Caroline Barbosa Retameiro¹
Amanda Götz Lopes¹
Heloisa De Matos Maillard¹
Camila Scolari¹
Rosane Aparecida Ribeiro²
Maria Lúcia Bonfleur¹

Affiliations:

¹ Universidade Estadual do Oeste do Paraná.
² Universidade Estadual de Ponta Grossa.

Keywords: Cholesterol metabolism. Obesity. Gestrinone.

Abstract:

Gestrinone is a 19-nortestosterone steroid originally developed for the treatment of endometriosis. More recently, it has been repurposed as an off-label therapy for fat loss and muscle gain, despite limited data on its safety and efficacy. This study aimed to evaluate the effects of oral gestrinone administration on obesity and lipid homeostasis in female mice fed a high-fat, high-fructose diet (HFFrD). Sixty-day-old female C57BL/6 mice were submitted to obesity induction through HFFrD intake for 8 weeks. Then, 3 times a week the animals received a gavage of soybean oil containing 0.2% DMSO without (OB-CTL group) or with 514 µg gestrinone/kg body weight (BW) (OB-GES group), for an additional 8 weeks. After euthanasia, the fat pad stores, the liver and blood were collected. Liver fragments were subjected to lipid extraction. All experimental procedures were approved by CEUA/Unioeste (07-24). BW was similar between the groups. On the other hand, gestrinone caused significant reductions in the weights of inguinal (3.96 ± 0.43 %/BW; $p=0.0407$), parametrial (2.09 ± 0.27 %/BW; $p=0.0397$), retroperitoneal (0.93 ± 0.11 %/BW; $p=0.0331$), and mesenteric fat pads (1.14 ± 0.09 5 %BW; $p=0.0093$), when compared to OB-CTL (5.49 ± 0.80 , 2.92 ± 0.28 , 1.36 ± 0.15 , and 1.64 ± 0.15 %/BW). Plasma cholesterol (CHOL) levels were 21% lower in OB-GES than in OB-CTL ($p=0.0007$), despite no modification in triglycerides (TG) level. In addition, hepatic CHOL content was higher in OB-GES (0.80 ± 0.04 µg/g liver; $p=0.0032$) than OB-CTL (0.50 ± 0.09 µg/g liver), whereas TG content in the liver was similar between the groups. These findings indicate that gestrinone treatment reduces fat accumulation and alters CHOL metabolism, decreasing circulating cholesterol levels while increasing hepatic cholesterol content. Further investigations are necessary to elucidate the underlying mechanisms and potential clinical implications of such anti-adiposity and modification on CHOL metabolism in female HFFrD mice.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Prolactin attenuates stress-induced activation of the paraventricular nucleus but not corticosterone secretion in male rats

Authors:

Evelyn Cristine dos Santos Alvares¹
Patrícia Costa Henriques¹
Kaoma Stephani da Costa Silva¹
Ana Clara Campidelli-Santana¹
Matheus Viana¹
Roberta Araújo-Lopes¹
Raphael Escorsim Szawka¹

Affiliations:

¹ Departamento de Fisiologia, Instituto de Ciências Biológicas UFMG.

Keywords: Corticosterone. Male rats. Paraventricular nucleus of the hypothalamus. Prolactin. Stress.

Abstract:

Prolactin (PRL) modulates stress responses in females, but whether PRL plays a similar role in males is unclear. We investigated the effects of ovine PRL (oPRL) on neuronal activation in the paraventricular nucleus (PVN) and corticosterone (CORT) and PRL secretion in response to stress in male rats (CEUA 338/2019). Rats received i.c.v. injections of vehicle (Veh), oPRL (PRL), or PRL-R antagonist and oPRL (Ant+oPRL) 10 min before restraint stress (Stress) or no stress (Control). Brains were processed for double-label immunohistochemistry to c-Fos and oxytocin (OT) or corticotropin-releasing hormone (CRH). Stress increased the global expression of c-Fos and the percentage of OT and CRH-neurons expressing c-Fos in the PVN of Veh rats, whereas these responses were reduced by oPRL (% of c-Fos/OT-immunoreactive, ir, neurons: Veh, 3.6 ± 0.7 ; Veh-Stress, 13.6 ± 3.0 ; PRL-Stress, 8.0 ± 1.4 ; $p < 0.05$; % of c-Fos/CRH-ir neurons: Veh 2.3 ± 1.3 ; Veh-Stress 36.9 ± 3.5 ; PRL-Stress 18.8 ± 4.3 ; $p < 0.01$). In jugular-vein cannulated rats, stress increased PRL secretion with peak levels within 5 min of stimulus (Control: 3.2 ± 0.8 ; Stress: 27.4 ± 7.0 ng/mL; $p < 0.001$). CORT secretion, in turn, was strongly elevated but with maximum levels attained 15 min after Stress (Control: 135.6 ± 77.8 ; Stress: 411.5 ± 41.3 ng/mL; $p < 0.001$). Next, rats received the same i.c.v. treatments and were decapitated 15 min after Stress for PRL and CORT measurements. oPRL attenuated PRL response to Stress, which was restored by the PRL-R antagonist, while did not alter PRL secretion in Control rats (Veh-Control, 2.5 ± 0.6 ; Veh-Stress, 23.7 ± 2.4 ; PRL-Stress, 15.0 ± 3.3 ; Ant+oPRL-Stress, 21.5 ± 2.9 ng/mL; $p < 0.05$). Conversely, oPRL did not affect basal or stress-induced CORT secretion (Veh-Stress, 423.3 ± 34.9 ; PRL-Stress, 362.7 ± 39.3 ; $p = 0.5$). Thus, PRL blunts stress-induced neuronal activation in the PVN without altering CORT secretion. We hypothesize that PRL acts on the PVN of males to modulate behavioral but not adrenal-axis response to stress.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Does the past influence the future? Metabolic adaptations following repeated glucocorticoid treatments throughout life

Authors:

Carolina de Moraes Silveira Peixe¹
Alexandre Giusti-Paiva²
Alex Rafacho²

Affiliations:

¹ Universidade Federal de Santa Catarina, PPG em Farmacologia.

² Universidade Federal de Santa Catarina, Departamento de Ciências Fisiológicas.

Keywords: Diabetes. glucocorticoids. metabolism.

Abstract:

Dexamethasone (DEX) is a potent glucocorticoid used across life stages. However, has adverse metabolic effects and is considered diabetogenic. We aimed to investigate whether prior DEX treatments could exacerbate the metabolic disturbances induced by a subsequent DEX treatment in adulthood. Male rats underwent up to three DEX treatment cycles at 30, 60, and 90 days. Each cycle consisted of five daily ip injections of DEX (1.0 mg/kg) or saline. Groups: Control, 90 (DEX only at 90 days), 30+90 (DEX at 30 and 90 days), 60+90 (DEX at 60 and 90 days), and 30+60+90 (DEX in all three cycles) Data and values will follow this sequence and expressed as mean $\pm$ SD. In the first cycle, DEX-treated groups showed reduced weight gain ($35,9 \pm 4,7$; $36,9 \pm 8,0$; $0,3 \pm 4,8$; $39,2 \pm 5,5$; $-2,5 \pm 5,0$). In the second, groups 60+90 and 30+60+90 showed weight loss (13 ± 4 ; 15 ± 2 ; 14 ± 3 ; -46 ± 5 ; -35 ± 5) and reduced food intake (146 ± 6 ; 145 ± 12 ; 142 ± 6 ; 107 ± 6 ; 104 ± 12). All DEX-treated groups had weight loss (4 ± 6 ; -58 ± 6 ; -56 ± 10 ; -56 ± 9 ; -47 ± 8) and hypophagia (2272 ± 140 ; 2100 ± 161 ; 1999 ± 114 ; 2029 ± 102 ; 1871 ± 123) during the third cycle. In the ipGTT, DEX treatment led to increased the area under the curve (AUC; 13116 ± 3766 ; 24748 ± 5834 ; 20559 ± 5388 ; 26646 ± 4652 ; 20477 ± 5873). In the ipITT, groups 90, 30+90, and 60+90 had elevated AUC, whereas 30+60+90 displayed lower AUC values (2343 ± 802 ; 6318 ± 662 ; 4956 ± 499 ; 4875 ± 1829 ; 2430 ± 898). The 90 group exhibited reduced corticosterone levels compared to control and 30+60+90 (144 ± 34 ; 88 ± 35 ; 95 ± 53 ; 98 ± 51 ; 199 ± 93). Groups 90, 30+90, and 60+90 had increased plasma insulin ($2,2 \pm 0,7$; $8,3 \pm 4,0$; $6,4 \pm 2,6$; $5,1 \pm 2,4$; $4,5 \pm 1,5$). Pancreatic beta-cell mass increased in 90 and 60+90 ($0,5 \pm 0,08$; $1,5 \pm 0,62$; $1,2 \pm 0,13$; $1,6 \pm 0,37$; $1,3 \pm 0,34$). Interestingly, repeated DEX exposure attenuates some of the metabolic effects induced by DEX treatment in adulthood. CEUA 8396201023.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Oral gestrinone treatment is refractory to obesity and its comorbidities in male mice

Authors:

Ana Caroline Barbosa Retameiro¹
João Pedro Giannotti Alves¹
Amanda Götz Lopes¹
Heloísa De Matos Maillard¹
Camila Scolari¹
Rosane Aparecida Ribeiro²
Maria Lúcia Bonfleur¹

Affiliations:

¹ Universidade Estadual do Oeste do Paraná.
² Universidade Estadual de Ponta Grossa.

Keywords: Abdominal obesity metabolic syndrome. Anti-obesity drugs. Gestrinone.

Abstract:

Gestrinone is a synthetic progestin derived from 19-nortestosterone that displays antiestrogenic and androgenic actions. Empirical information has been indicated the potential off-label use against adiposity. Since sex steroids may regulate various aspects of intermediary metabolism, here we aimed to evaluate the effects of gestrinone on obesity and glycemic homeostasis in male mice fed with high-fat high-fructose diet (HFFrD). For this, 60-day-old *C57Bl/6* male mice were submitted to obesity induction through ingestion of HFFrD for 8 weeks. After, the obese mice continue to consume HFFrD and received 3 times a week a gavage of soybean oil containing 0.2% DMSO (OB-CTL group) without or with 0.51 (OB-0.51 group) or 1.03 mg gestrinone/kg body weight (BW; OB-1.03 group) for additional 8 weeks. BW was measured weekly. In the last week of treatment, oGTT and ipITT were performed. At euthanasia, the blood and the abdominal adipose tissue were collected (CEUA/Unioeste- Protocol nº 07-24). Total BW gain throughout of gestrinone treatment did not differ between OB-0.51 (43.66 ± 1.8 g), OB-1.03 (42.46 ± 1.6 g) and OB- CTL (43.02 ± 1.7 g). Gestrinone treatment did not alter glucose tolerance or insulin sensitivity in OB-0.51 (282.4 ± 76.52 and 185.5 ± 56.42 mg/dL) or in OB-1.03 group (275 ± 79.19 and 176.3 ± 53.73 mg/dL), when compared to OB-CTL (279.7 ± 81.24 and 185.7 ± 63.48 mg/dL). Total abdominal adiposity, and plasma concentrations of triglycerides and cholesterol were also not modified by gestrinone in both OB- 0.51 (5.28 ± 0.31 g, 70.83 ± 17.38 and 150.5 ± 31 mg/dL) and OB-1.03 mice (5.38 ± 0.39 g, 61.32 ± 17.31 and 147.3 ± 19.72 mg/dL), when compared to OB-CTL (5.68 ± 0.39 g, 67.05 ± 15.04 and 170.5 ± 37.66 mg/dL). In conclusion, the gestrinone treatment in male HFFrD mice didn't regulate body mass or plasma biochemical parameters related to glucose and lipid homeostasis.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Chronic exposure to atrazine-based herbicides exacerbates liver steatosis in adult mice fed a high-fat diet

Authors:

Amanda Gotz Lopes¹
Izadora Redivo¹
Heloisa de Matos Maillard¹
João Pedro Giannotti Alves¹
Ana Caroline Barbosa Retameiro¹
Maria Lúcia Bonfleur¹

Affiliations:

¹ Universidade Estadual do Oeste do Paraná.

Keywords: Pesticide. MASLD. Obesity.

Abstract:

The increasing prevalence of obesity has been accompanied by a rise in metabolic dysfunction-associated steatotic liver disease (MASLD). Among the contributing factors, exposure to environmental pollutants, such as pesticides, plays a significant role. Atrazine-based herbicides (ABHs) are among the most widely used in Brazil, with the potential to contaminate water, soil, air, and food. Several lines of evidence indicate that ABH exposure may influence the development of obesity and associated comorbidities. This study aimed to evaluate the effects of chronic ABH exposure on the development of obesity and hepatic steatosis in adult mice. Eighty-day-old male C57BL/6 mice were divided into two groups: OB-CTL and OB-ABH. Both groups received a high-fat diet (HFD) for 20 weeks, while the OB-ABH group also received 5 mg/kg of ABH in drinking water. Body weight and food intake were recorded weekly. After 150 days, the animals were euthanized, and blood, liver, perigonadal, and retroperitoneal fat were collected. The experimental procedures were approved by CEUA/Unioeste (protocol no. 22/24). No significant differences were observed in total BW gain (OB-CTL: 26.21 ± 0.43 g; OB-ABH: 29.54 ± 1.6 g; $p=0.08$) or food intake (OB-CTL: 61.14 ± 1.03 g; OB-ABH: 72.8 ± 3.9 g; $p=0.1$). Plasma cholesterol (OB-CTL: 508.9 ± 13.73 mg/dL; OB-ABH: 422.8 ± 32.89 mg/dL) and triglyceride levels (OB-CTL: 93.55 ± 8.31 mg/dL; OB-ABH: 110.3 ± 10.88 mg/dL) were similar between groups. However, fasting glycemia was lower in OB-ABH (122.8 ± 4.50 mg/dL) than OB-CTL (135.3 ± 2.69 mg/dL, $p=0.0483$). Adipocyte morphology showed no significant changes. Liver histology revealed more severe steatosis in OB-ABH (60% score 3, 40% score 2) than in OB-CTL (40% score 3, 20% score 2, 20% score 1). In conclusion, chronic ABH exposure worsened liver steatosis in obese mice, suggesting that ABHs may aggravate liver damage in obesity.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Chronic exposure to glyphosate-based herbicide aggravates hepatic steatosis in obese adult mice

Authors:

Amanda Gotz Lopes¹
Izadora Redivo¹
Heloisa de Matos Maillard¹
João Pedro Giannotti Alves¹
Maria Lúcia Bonfleur¹

Affiliations:

¹ Western Paraná State University.

Keywords: Pesticide. MASLD. Obesity.

Abstract:

Obesity is a major risk factor for conditions such as metabolic dysfunction-associated steatotic liver disease (MASLD). Its increasing prevalence is linked to various factors, including environmental pollutants. Evidence indicates that chronic exposure to glyphosate-based herbicides (GBHs) may disrupt lipid metabolism and aggravate liver dysfunction. Thus, we aimed to evaluate whether chronic exposure to GBH exacerbates obesity and contributes to the development of hepatic steatosis in adult mice fed a high-fat diet (HFD). Male C57BL/6 mice (80 days old) were divided into two groups: OB-CTL and OB-GBH. Both were fed a HFD for 20 weeks, with OB-GBH receiving 50 mg/kg GBH in their drinking water. Body weight (BW) and food intake were measured weekly. After 150 days, animals were euthanized; blood, liver, perigonadal and retroperitoneal fat were collected and stored for analysis. All the procedures were approved by the CEUA/Unioeste (protocol no. 22-24). There was no significant difference in BW gain (OB-CTL: 26.21 ± 0.42 g; OB-GBH: 26.26 ± 1.09 g; $p=0.96$), but OB-GBH showed a higher food intake (OB-GBH: 73.8 ± 2.1 g and OB-CTL: 61.1 ± 1.03 g; $p=0.0009$). In addition, adipocyte size and number in adipose tissue were similar between groups. Fasting glycemia was significantly lower in the OB-GBH group (120.1 ± 4.7 mg/dL) compared to OB-CTL (135.3 ± 2.7 mg/dL; $p = 0.0254$), without alterations in insulinemia. Plasma cholesterol (OB-CTL: 508.9 ± 13.73 mg/dl; OB-GBH: 453.7 ± 36.71 mg/dl) and triglyceride levels (OB-CTL: 86.9 ± 5.89 mg/dL; OB-GBH: 74.23 ± 7.52 mg/dL) were not significantly different ($p>0.05$). Histological analysis of the liver revealed increased steatosis severity in the OB-GBH group (80% scored 3 and 20% scored 1) compared to OB-CTL (40% scored 3, 20% scored 2 and 20% scored 1). In conclusion, chronic exposure to GBH worsens hepatic steatosis and may aggravate metabolic disturbances associated with obesity.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Regulation of Foxo3 by Thyroid Hormones in Sepsis: Implications for Muscle Atrophy and Survival

Authors:

Maria Isadora Amaral¹
Matheus da Silva Ferreira¹
Thamires Siqueira de Oliveira¹
Johnatas Dutra Silva²
Pedro Leme Silva²
Tânia Maria Ortiga-Carvalho¹
Flavia Fonseca Bloise¹

Affiliations:

¹ Translational Endocrinology Laboratory, Carlos Chagas Filho Institute of Biophysics, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

² Pulmonary Investigation Laboratory, Carlos Chagas Filho Institute of Biophysics, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

Keywords: Atrophic pathway. Sepsis. Thyroid hormone.

Abstract:

Sepsis is responsible for 11 million deaths per year, thus it is a major public health concern. Septic patients frequently develop the nonthyroidal illness syndrome (NTIS). This condition causes a reduction in serum thyroid hormones (TH) levels with no increase in TSH. The NTIS development and the decrease in muscle mass are predictors of septic mortality. Since TH are crucial for muscle function, we investigated the atrophic pathway in peripheral skeletal muscle from septic mice. Sepsis was induced through a cecal ligation and puncture (CLP) in adult male C57BL6 mice, the control group (Sham) was sham operated. The CLP group was treated with T4 (7.5 ng/g BW dose) plus T3 (250 ng/g BW dose) (CLP+TH) or saline (CLP) 8h and every 24h after surgery for 5 days. At day 5 the animals were euthanized and the soleus muscle collected (CEUA: A10/20-A18-088-15). The gene expression was analyzed by qPCR, and one-way ANOVA was used for statistical analysis. Although *Thra1* expression did not change between groups, the TH positively regulated gene *Hr* expression increased in the CLP+TH group (SHAM: $1.0 \pm 0.2a$ / CLP: $0.5 \pm 0.1a$ / CLP+HT: $12.1 \pm 5.4b$; $p < 0.05$) as expected. Next, we investigated the atrophic-regulated genes *Foxo1* and 3. We only observed a change in *Foxo3* expression, with an increase in septic soleus, and TH treatment reduced it (SHAM: $1.0 \pm 0.3a$ /CLP: $3.4 \pm 0.9b$ /CLP+HT: $2.1 \pm 0.5a$; $p < 0.05$). Since *Foxo3* can induce atrogenic gene expression, we investigated *Fbox32* (Murf-1) and *Trim63* (Atrogin) expression, but did not change between groups. Our findings suggest that sepsis does not regulate the mean atrophic gene expression in the soleus. However, the increased expression of *Foxo3* could indicate that the autophagy pathway is unregulated and controlled by TH. To further elucidate *Foxo3*'s influence, we will investigate its regulation of autophagy and apoptosis.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: The impact of growth hormone secretion on kisspeptin neurons activity

Authors:

Guilherme Andrade ALVES¹
Renata Frazão²

Affiliations:

¹ Department of Anatomy, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, SP, Brazil.

Keywords: Puberty. Kisspeptin. Reproduction.

Abstract:

Growth hormone (GH) influences the expression of hypothalamic genes related to reproduction, like *Kiss1* and *Gnrh1*, during puberty. Kisspeptin neurons, essential for reproduction, reside in GH-responsive hypothalamic regions. This study investigated whether the effects of GH hypersecretion or deficiency on reproduction are influenced by changes in kisspeptin neuron activity. CEUA: N°7681180521. Female mice exhibiting GH hypersecretion (bGH), spontaneous GH deficiency (*Ghrhr^{lit/lit}*), and control littermates were evaluated. Fertility, hypothalamic mRNA expression, and frequency and amplitude of GABAergic postsynaptic potentials (sPSC) recorded from kisspeptin neurons were analyzed. Statistical analyses employed Fisher's exact test, unpaired Student's t-tests. We observed that bGH females were subfertile; 2 out of 10 bGH mice vs. 10 out of 10 controls got pregnant ($p < 0.0001$). Hypothalamic *AgRP* (bGH, 1.5 ± 0.1 ; control, 1 ± 0.1 ; $p < 0.01$) and *Pomc* mRNA expression (bGH, 0.7 ± 0.1 ; control, 1 ± 0.1 ; $p < 0.01$) were, respectively, up and downregulated in bGH mice. Conversely, hypothalamic *AgRP* (bGH, 0.5 ± 0.1 ; control, 1 ± 0.1 ; $p < 0.01$) and *Pomc* mRNA (bGH, 0.6 ± 0.1 ; control, 1 ± 0.1 ; $p < 0.01$) were downregulated in *Ghrhr^{lit/lit}*. Meanwhile, *Kiss1* and *Gnrh1* mRNA were unaltered in both mouse models ($p > 0.05$). GABAergic sPSC frequency and amplitude recorded from kisspeptin neurons were not altered in bGH mice, whereas *Ghrhr^{lit/lit}* mice showed decreased GABAergic sPSC amplitude on AVPV/PeN nuclei (55.8 ± 5.6 pA) compared to control animals (84 ± 4.5 pA; $p < 0.001$). Our findings suggest that GH hypersecretion-induced impairment of fertility depends on neurons upstream of kisspeptin cells or on peripheral action.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Effects of binge eating disorder on the metabolism of mice

Authors:

Yasmim Silveira Goncalves¹
Felipe Alves Alencar¹
Cristian Ferreira Corona¹
Jessica Sabrina Canedo da SILVA Sumensse¹
Kevin Donansan Carvalho¹
Julia Burocco dos Santos Pinto¹
Pedro Henrique Grignet¹
Maria Vitória Mardgan¹
Maria Claudia Gross¹
Heloisa Deola Confortim²
Jean Franciesco Vettorazzi¹

Affiliations:

¹ Federal University for Latin American Integration (UNILA). Latin American Institute of Life and Natural Sciences.

² Federal University of Paraná (UFPR), Medicine Course.

Keywords: Binge eating disorder. Binge-like sucrose. Metabolism.

Abstract:

Approximately 70 million people worldwide suffer from eating disorders, which can affect physical health and psychosocial functioning. Binge eating disorder, an important eating disorder, is characterized by a high consumption of food within a short period of time, accompanied by a feeling of lack of control. In addition to the psychological effects, little is known about the metabolic aspects of this disorder. In this sense, the aim of this study was to investigate the metabolic effects of binge eating disorder in an animal model. To this end, male C57BL6 mice with 30 days were submitted to a control diet or a binge-like sucrose overconsumption protocol receiving 4-h limited access to both 0.5 M sucrose solution and chow for 10 days. All experiments were approved by the animal research ethics committee (23422.013429/2023-4). Throughout the period, animal weight and sucrose consumption were assessed, and at the end of the experimental period, blood was collected to analyze fasting and feed glycemia, triglycerides, and cholesterol levels. The protocol was efficient to induce binge eating in mice, with an increase in sucrose intake (1.35 ± 0.11 g/day in the first day versus 7.60 ± 0.60 g/day in the 10th day of protocol). The binge-like sucrose group presented reduced body weight when compared to control (21.54 ± 0.67 mg/dL versus 25.48 ± 0.55 mg/dL). However, when compared to the control group, there was no significant change in metabolism in terms of fasted glycemia (121.3 ± 13.11 mg/dL in control versus 131.3 ± 4.30 mg/dL in binge group), fed glycemia (159 ± 33 mg/dL in control versus 145.8 ± 4.99 mg/dL in binge group), triglycerides (102.6 ± 11.57 mg/dL in control versus 99.71 ± 6.88 mg/dL in binge group) and cholesterol (150.7 ± 10.72 mg/dL in control versus 153.1 ± 8.08 mg/dL). These data shows that the binge-like sucrose protocol induces overconsumption associated with a reduced body weight, although no significant effects on the animal's metabolism were observed.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Effects of GH Hypersecretion on mice Fertility

Authors:

Paola Pamela Almeida¹
Guilherme Andrade Alves¹
Renata Frazão¹

Affiliations:

¹ University of São Paulo, Institute of Biomedical Sciences III, Department of Anatomy, Laboratory of Neurophysiology of Reproduction.

Keywords: Fertility, Growth hormone, Kisspeptin, Neuroendocrinology, Reproduction.

Abstract:

The regulation of fertility in mammals is a highly sophisticated process, mediated by complex interactions between sex hormones and neurotransmitters. We have previously demonstrated that disruption of growth hormone (GH) and insulin-like growth factor (IGF-1) secretion impairs reproduction. We investigated Albumin-Cre/GHR-Flox (Δ GHR) mice, which have a specific deletion of the GH receptor in hepatocytes, resulting in increased GH secretion despite low IGF-1 levels. We evaluate whether GH hypersecretion alone affects the onset of puberty or fertility of female and male mice. The animals were maintained in a controlled environment. Experimental strategies included assessment of body weight gain during development, sexual maturation, and fertility rate. The study was approved by the Ethics Committee on Animal Use (CEUA), protocol No. 7681180521. Results indicate that male and female Δ GHR mice exhibit lower body weight gain during development compared to control animals ($P < 0.05$). Despite lower body weight gain, the onset of puberty was not affected in Δ GHR female mice (age of vaginal opening, control, 32.0 ± 2.6 (n=6), Δ GHR, 33.6 ± 1.3 days (n=17), $P = 0.6$). Whereas Δ GHR male mice exhibited later puberty onset (age of testicular descent, control, 30.0 ± 1.7 (n=11), Δ GHR, 35.1 ± 1.1 days (n=22), $P = 0.02$). Both male and female animals were fertile and produced a similar number of pups per litter compared to the control mice (n= 5/8 female or male mice per group, $P > 0.05$). Our results demonstrate that reproduction is maintained in the face of GH hypersecretion when IGF-1 levels are suppressed. This finding contrasts with the existing literature, as the effects of GH secretion disruption on reproduction cannot be dissociated from possible IGF-1 effects. This understanding can inform further strategies to prevent reproductive disorders.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Sperm-Protective Action of Chrysin in Mitigating the Effects of Microplastics in *Zebrafish*

Authors:

Davi Araújo Fernandes¹
Guilherme Álvaro Ferreira da Silva²
Talita Sarah Mazzoni²

Affiliations:

¹ Universidade Federal de Alfenas (UNIFAL-MG), Programa de Pós-Graduação em Biociências Aplicadas à Saúde.

² Universidade Federal de Alfenas (UNIFAL-MG), Departamento de Biologia Celular e do Desenvolvimento – Instituto de Ciências Biomédicas.

Keywords: Cellular damage. Environmental pollutants. Infertility. Sperm protection.

Abstract:

Microplastics (MPs) have emerged as a global threat, negatively impacting the health of various species, including humans. Current research primarily focuses on their effects on different tissues, with limited studies on substances that mitigate the damage caused by these pollutants. To investigate chrysin's potential as a "sperm-protective" agent against MP exposure, we established four experimental groups, each with 15 adult male *zebrafish*: control (GC), animals exposed to 0.025 mg/day of chrysin (GCr), animals exposed to 0.5 g/L of MPs (GMP), and animals exposed to 0.5 g/L of MPs + chrysin (0.025 mg/day) (GMPCr). After 30 days, semen samples were collected for computerized sperm motility (CASA) and DNA integrity (Comet Assay) analysis. Additionally, histological analysis of testes was performed 24 hours post-reproduction to assess potential morphological changes in the germinal epithelium. Sperm analysis revealed a significant reduction in motility in the GMP group, while the GCr and GMPCr groups showed motility levels similar to the GC group. The Comet Assay showed that DNA damage was significantly lower in GMPCr compared to GMP, which exhibited the highest damage levels. Histological analysis indicated increased spermatozoa in the seminiferous tubules of GMP animals, suggesting reduced sperm extrusion during reproduction. Vacuolization in interstitial tissue and ruptures in spermatocysts were also observed. However, no morphological changes were found in GCr and GMPCr testes, which were comparable to the GC group. Exposure to MPs impaired sperm release from seminiferous tubules, resulting in poor sperm quality. Chrysin administration appeared to reverse this effect, improving sperm characteristics. Our results suggest that MPs compromise male gamete quality, but chrysin proves effective as a "sperm-protective" agent, ensuring adequate sperm production and efficient gamete emission.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Calcitriol Suppresses Brown Adipose Tissue Thermogenesis in Lactating Rats

Authors:

Matheus Leonardo Moro¹
Aline Zanatta Schavinski¹
João Batista Camargo Neto²
Leticia Cirelli Ruiz¹
Andressa Pereira²
Gabriel Hunzinker Skiba¹
Neusa Maria Zanon¹
Luciane Carla Albericci³
Isis Do Carmo Kettelhut²
Luiz Carlos Carvalho Navegantes¹

Affiliations:

¹ Department of Physiology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, SP, Brazil.

² Department of Biochemistry & Immunology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, SP, Brazil.

³ Department of BioMolecular Sciences, Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil.

Keywords: Brown adipose tissue. Lactation. Vitamin D.

Abstract:

During lactation, the active form of vit. D (calcitriol) rises in parallel with the suppression of brown adipose tissue (BAT) thermogenesis. Although calcitriol inhibits brown adipocytes function *in vitro*, its role in BAT suppression in lactating rats remains unknown. To address this question, female Wistar rats were subjected to dietary vit.D deficiency (VDD) or sufficiency (CT). The core body temperature, BAT temperature and tail heat dissipation were simultaneously monitored in virgin (V) and lactating (L) rats throughout lactation. At weaning (PN21), BAT, blood and milk samples were collected from dams, while offspring were used for body weight and body composition evaluation. The data are expressed as mean±SEM (n=3-12) and statistics were performed using Two-way ANOVA or Student *t*-test ($p<0.05$) (CEUA 1241/2023). Our results showed that the core body temperature (38.8 ± 0.2 versus $37.4\pm0.2^\circ\text{C}$ in V-CT) and serum calcitriol (77 ± 12 versus 8 ± 1 pg/mL in V-CT) increased during a physiological lactation (L-CT), which was associated with a higher heat dissipation (33 ± 1 versus $29\pm1^\circ\text{C}$ in V-CT) and a drop in BAT temperature (36.5 ± 0.3 versus $38\pm0.4^\circ\text{C}$ in V-CT). Concurrently, BAT mass (-50%) as well as UCP-1 (-64%), CINDUFB8 (-72%) and CIVMTCO1 (-36%) protein content decreased in L-CT. VDD diet successfully reduced serum calcitriol before conception and during lactation to values below 20 ng/mL. Coherently, VDD attenuated the rise of calcitriol induced by the lactation (47 ± 7 versus 77 ± 12 pg/mL in L-CT) and reduced both serum (9 ± 0.2 versus 10.6 ± 0.2 mg/dL in L-CT) and milk calcium concentration (217 ± 14 versus 280 ± 13 mg/dL in L-CT). BAT thermogenesis suppression, BAT atrophy, CINDUFB8 and CIVMTCO1 reduction did not occur in L-VDD. Interestingly, VDD reduced the offspring weight, fat and lean mass in male and female pups (not shown). Altogether, these data suggests that calcitriol contributes to BAT thermogenesis suppression during lactation and plays a role in offspring growth.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Influence of gestational and lactational exposure to phthalate and nanoplastics on the reproductive parameters and distribution of mast cells in the lactating mammary gland of Sprague-Dawley rats

Authors:

João Vitor Cronemberger Silva¹
Isadora Penedo de Souza¹
Luis Manuel SARMIENTO-Machado¹
Vanessa Aguiar Rocha¹
Wellerson Rodrigo Scarano¹
Luis Fernando Barbisan¹

Affiliations:

¹ São Paulo State University (UNESP), Institute of Biosciences, Department of Structural and Functional Biology, Botucatu, Brazil.

Keywords: Phthalates, Polystyrene nanoplastics, Mast cells, Lactating mammary gland, Reproductive parameters.

Abstract:

Exposure to endocrine disruptors (EDs) during pregnancy and lactation can compromise maternal reproductive parameters and mammary gland development, impacting the offspring. Among the EDs, plastic waste — such as phthalates, bisphenols, and micro- or nanoplastics — can impact animal and human health. Thus, this study aimed to analyze reproductive and mammary gland changes in dams at the end of lactation, after gestational and lactational exposure to a phthalates mixture (PM) and polystyrene nanoplastics (PNs). Pregnant Sprague-Dawley rats were allocated into 6 groups: C: control; T1: 20µg PM/kg/day; T2: 200mg PM/kg/day; T3: 1.0 mg PNs/kg/day; T4: 20µg PM/kg/day+PNs; T5: 200mg PM/day+PNs. MF and PNs treatments were administered by gavage from gestational day (GD) 10 to postnatal day (PND)21, using environmentally relevant doses for humans. Dams were monitored for body weight, birth, and litter development. At weaning, dams were euthanized, and samples from the 4th abdominal mammary gland were collected and fixed in 10% (v/v) buffered formalin. Histological sections (5 µm) were obtained. All the experimental procedures were approved by the Local Ethics Committee on the Use of Animals (CEUA Nº 1174020523). Sections were stained with hematoxylin-eosin for morphological analysis, toluidine blue pH 4.5 for mast cell count, and Picrosirius red to assess collagen deposition and measure epithelium and alveolar lumen. Data were analyzed using ANOVA test ($p < 0.05$) followed by Tukey's post hoc test. Treatments with MF and NPs did not significantly affect maternal reproductive parameters (number of pups, male/female ratio, and offspring body weight at PND1). Similarly, the mean number of mast cells and the proportion of epithelial components, alveolar lumen, and collagen fibers in the lactating mammary gland remained unchanged among groups. Thus, these findings indicate that maternal MF and PNs exposure did not alter the reproductive and morphological parameters analyzed.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Effects of estrogen therapies in a scenario of endocrine disruption on the female gerbil prostate

Authors:

Vinicius Savazo Assis¹
Leonardo Araújo Caires de Aguiar¹
Rafael Henrique Borges Coelho¹
Lorena Gabriela de Souza^{1,2}
Luara Jesus Ferrato^{1,2}
Caroline Marques Bedolo¹
Stella Bicalho Silva¹
Vitor Grigio¹
Patrícia Simone Villamaior¹
Thalles Fernando Rocha Ruiz^{1,2}
Sebastião Roberto Taboga^{1,2}

Affiliations:

¹ UNIVERSIDADE ESTADUAL PAULISTA – UNESP, São José do Rio Preto.

² INSTITUTO DE BIOLOGIA – UNICAMP, Campinas.

Keywords: Carcinogenesis. Epithelium. Receptors. Lesions.

Abstract:

Estrogens and androgens play critical roles in the female reproductive cycle, maintaining endocrine regulation throughout most of life. However, exposure to endocrine-disrupting chemicals (EDCs) such as Bisphenol A (50 µg/kg/day), combined with pro-carcinogens like N-ethylnitrosourea (ENU), can lead to hormonal dysregulation and promote the development of lesions such as prostatic intraepithelial neoplasia (PIN) and adenocarcinomas. This study investigated the effects of 17-β-estradiol (E2) and 17-α-ethinylestradiol (EE2) supplementation on the female prostate of Mongolian gerbils (*Meriones unguiculatus*), exposed perinatally (39 days). Animals were euthanized at 7 months, in accordance with ethical guidelines (CEUA nº239/2022). Statistical analysis was based on mean ± SD. In the epithelium, androgen receptor (AR) expression decreased in the ENU group (28.34% ± 6.26; 33.68% ± 6.10; 25.88% ± 4.70), while estrogen receptor alpha (ERα) was elevated in the E2 group (39.01% ± 3.87). Estrogen receptor beta (ERβ) showed decreased expression in the EE2 group (32.31% ± 13.0) versus BPA and E2 groups (56.48% ± 10.79; 54.04% ± 6.40). Stromal ERα was reduced in the E2 group (32.67% ± 10.31) versus ENU (56.48% ± 15.38), and all RA groups differed statistically from ENU. No significant differences were observed for ERβ in the stroma. In epithelial PHH3, the BPA group showed increased expression (10.94% ± 3.25) versus E2 (3.34% ± 0.54). In the stroma, EE2 increased PHH3 expression (15.63% ± 3.01) compared to E2 (5.78% ± 2.75). Cleaved caspase-3 was highly expressed in the E2 group epithelium (54.33% ± 29.11) versus ENU (13.57% ± 7.11), and also in the stroma (30.99% ± 15.47 vs. 4.77% ± 2.15). These results suggest that E2 increases ERα and ERβ expression and may enhance apoptosis, while reduced AR suggests androgen-independent lesions. EE2 appears to promote epithelial proliferation.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Ovarian steroids reverse depressive-like behavior induced by chronic unpredictable stress in female rats

Authors:

Tamires
Gregorio¹
Flaviano
Lorenzon¹
Luma Carolina
Santos da Silva¹
Gabriela Baptista¹
Vinicius
Alexandre
Zanotti¹ Ellen
Dal Moro
Piana¹
Fernanda
Barbosa Lima¹

Affiliations:

¹ Departamento de Ciências Fisiológicas, Programa de Pós-Graduação Multicêntrico em Ciências Fisiológicas, Universidade Federal de Santa Catarina, Florianópolis/SC.

Keywords: Depression. Estradiol. Progesterone. Stress.

Abstract:

Hormonal fluctuations and chronic unpredictable stress (CUS) are key risk factors for mood disorders in women. This study investigates how reduced levels of ovarian hormones and CUS affect depressive-like behavior in female rats. Adult Wistar rats were ovariectomized and received 6 injections (twice a week, i.m.) of estradiol E (15µg/kg; OVX+E) or E + progesterone (P) (0.5 mg/rat; OVX+E+P). A CUS protocol was applied daily, 7 days before treatment completion. Animals were tested for sucrose preference and in the forced swim test (CEUA 4926201021). No significant differences were found regarding sucrose preference. Regardless of stress, rats replaced with E or E+P showed reduced immobility time (OVX: 99.6±31.9; OVX+CUS: 124.9±14.1; OVX+E: 6.2±2.1; OVX+CUS+E: 41.5±8.8; p=<0,007p=<0,0001) and (OVX+E+P: 16.1±10.2; OVX+CUS+E+P: 48.8±10.3; p<0,0004). CUS reduced swimming in OVX rats (Effects of CUS: E2: p=<0.009; E2+P4: p=<0.01), which was reversed by E or E+P (OVX: 145.6±55.6 OVX + CUS: 25.6±5.2; OVX+E: 284.7±5.7; OVX+CUS+E: 245.1±15.5; p=<0,0001) and (OVX+E+P: 255.5 ±26.5; OVX+CUS+E+P: 210.3±10.8; p=0,0001). In contrast, rats with hormone replacement decreased climbing (Effects of hormone: E2: p=<0,0001; E2+P4: p=<0,0001), but exposure to CUS reversed this effect (OVX: 56.4±19.5 OVX+CUS: 145.9±10.7; OVX+E: 8.4±3.9; OVX+CUS+E: 26±8.3; p=<0,0001) and (OVX+E+P: 3.2±1.6; OVX+CUS+E+P: 41.4±8.4; p=0,0001). Stress exposure did not affect uterine weight (OVX: 0.03±0.003; OVX+CUS: 0.03±0.007; OVX+E: 0.05±0.004; OVX+CUS+E: 0.05±0.008; OVX+E+P: 0.05±0.008; OVX+CUS+E+P: 0.05±0.004). Our findings confirmed that CUS induces depressive-like behaviors in OVX rats and provided evidence that E and P replacement effectively reverses this effect. These results highlight the detrimental interaction between ovarian steroid deprivation and chronic stress on mood regulation, underscoring a potential neuroprotective role of these hormones in mental health.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: T+E2 treatment induces proliferative lesions in the ventral prostate of adult male gerbils

Authors:

Luara Jesus Ferrato^{1,2}
Lorena Gabriela de Souza^{1,2}
Gervásio Evangelista Brito Filho²
Thalles Fernando Rocha Ruiz^{1,2}
Sebastião Roberto Taboga^{1,2}

Affiliations:

¹ Universidade Estadual de Campinas.
² Universidade Estadual Paulista Júlio de Mesquita Filho.

Keywords: Prostate. Endocrine dysregulation. BPH.

Abstract:

The prostate is regulated by the action of steroid hormones and imbalances in hormone levels can be responsible for the development of lesions, such as benign prostatic hyperplasia (BPH). Thus, this project aims to investigate histopathological repercussion, with the induction of BPH by endocrine dysregulation. Adult male gerbils (*Meriones unguiculatus*) were divided into 4 experimental groups (n=10/group) for evaluation at 7-, 10-, and 30-days post-induction. BPH was induced by subcutaneous administration of testosterone cypionate and 17 β -estradiol (100:1). Histopathological analyses to quantify the lesions and inflammatory foci in the tissue were performed. Statistical analyses: ANOVA or Kruskal-Wallis ($p>0.05$). Preliminary results showed an increase in the incidence of high-grade prostatic intraepithelial neoplasia (HGPIN) in the T+E2 group after 7 days (2.50 ± 0.5), compared to the control group (0.90 ± 0.3). A marked increase in subepithelial inflammation was also observed in the T+E2 group (5.82 ± 0.71) when compared to the control group (0.97 ± 0.27), as well as to the long-term T+E2(30d) (2.31 ± 0.73) and T+E2(10d) (2.09 ± 0.52) treatment groups. Although, there was no difference between the treatments for the other proliferative lesions (low-grade PIN, hyperplasia, microinvasive carcinoma) and other inflammatory foci (intraluminal and periductal). Exposure to doses of testosterone plus 17 β -estradiol has already been demonstrated in literature as a possible inducer of proliferative lesions in the tissue, as demonstrated in this study. At least, current studies show that alterations linked to BPH have inflammatory mechanisms. Therefore, future studies aim to analyze these mechanisms through the induction of chemical prostatitis to better understand the molecular ways for this to happen, concomitant with the induction of BPH via endocrine dysregulation by steroid hormones.

Ethic Committee Protocol number 264/2024.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Biometric data and sperm morphology in obese C57BL/6J mice treated with emulsifiers carboxymethylcellulose and polysorbate 80

Authors:

Victor Hugo CARVALHO¹
Gabriel Prata BACIL¹
Leticia Cardoso VALENTE²
Guilherme Ribeiro ROMUALDO
Luis Fernando BARBISAN⁴

Affiliations:

¹ São Paulo State University (UNESP), Department of Structural and Functional Biology - IBB/UNESP.

² São Paulo State University (UNESP), Experimental Research Unity (UNIPEX) - FMB/UNESP.

Keywords: Emulsifiers. Obesity, Western-type diet, Reproductive parameters, C57BL/6 mice.

Abstract:

Recent studies indicate that obesity and ultra processed foods intake may contribute to sperm dysfunction (concentration and motility), as well as may impact male sex hormones. Thus, this study aimed to evaluate the detrimental effects of the emulsifiers carboxymethyl cellulose (CMC) and polysorbate 80 (P80) in mice fed a western diet (WD) on biometric, biochemical and sperm morphological parameters. Six-week-old C57BL/6J mice (n = 8/group) received a control diet (G1) or DO (G2-G8) [chow containing 30% fat, 0.2% cholesterol, and 20% sucrose and a sugar-rich solution (23.1 and 18.9 g/L d-fructose and d-glucose) in drinking water] for 24 weeks. After 4 weeks of DO, G1 and G2 received the emulsifier dilution vehicle by gavage. G3-G6 received doses of CMC or P80 alone (G3 and G4: 370 and 740 mg/kg/day of CMC, respectively; G5 and G6: 100 and 200 mg/kg/day of P80, respectively) and G7 and G8 received the combination of CMC and P80 in lower and higher doses (G7: 370 and 100 mg/kg/day; G8: 740 and 200 mg/kg/day), 5 times a week for 20 weeks. The animals were subjected to the glucose tolerance test (GTT). After euthanasia, blood samples were collected and the testes, epididymis and adipose tissue (epididymal, retroperitoneal, intestinal and subcutaneous) were collected and weighed. As expected, DO increased body mass, adiposity index, serum total cholesterol levels, and induced glucose intolerance ($p < 0.0001$). DO intake reduced the relative weights of the testes ($p = 0.0017$) and epididymis ($p = 0.007$), but without altering serum testosterone levels or structural damage in sperm. Thus, treatment with emulsifiers for 20 weeks did not significantly interfere with the changes induced by DO intake analyzed to date. (CEUA No. 1410/2022; FAPESP No. 2022/16633-1).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Influence of women's lifestyle on the success of assisted reproduction protocols

Authors:

Murillo Lippi Correa¹

Tábata Martins de Lima²

Affiliations:

¹ Universidade do Estado de Minas Gerais.

Keywords: Assisted Reproductive Technologies (ART). Female Infertility. Lifestyle Factors. Hypothalamic-Pituitary-Gonadal Axis.

Abstract:

Human reproduction is essential for the continuity of the global population and also reinforces the emotional bonds between couples. However, many women face difficulties conceiving due to dysfunctions in the female reproductive system, resulting in infertility. In this context, infertility is treated through protocols established by specialized clinics using assisted reproductive technologies (ART). This narrative literature review aims to explore how women's lifestyle factors may contribute to infertility and influence the outcomes of ART treatments. Understanding the physiology of the female endocrine and reproductive systems is crucial to assess how lifestyle habits impact key infertility-related hormones, particularly those of the hypothalamic-pituitary-gonadal (HPG) axis, which regulates ovulation and embryonic development. In addition to these primary factors, the review also highlights the role of secondary metabolic regulators, such as insulin and leptin. Several ART techniques were examined, including in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI), frozen embryo transfer (FET), and intrauterine insemination (IUI), each chosen based on specific reproductive complications. Lifestyle factors such as obesity, diet, and physical activity can either support or hinder reproductive function and the success of ART protocols. The review concludes that, although further research is needed, current evidence suggests that lifestyle choices significantly influence the efficacy of assisted reproduction treatments.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Maternal low-dose caffeine intake during gestation or lactation disrupts the insulin signaling pathway in metabolic tissues of adult female offspring

Authors:

Mayara da Silva ALMEIDA¹
Iala Milene BERTASSO¹
Beatriz Souza da SILVA¹
Nelyana Oliveira SERPA^{1,2}
Nathalia Medeiros NEHME¹
Egberto Gaspar de MOURA¹
Rosiane Aparecida MIRANDA¹
Patrícia Cristina LISBOA¹
Luana Lopes de SOUZA¹

Affiliations:

¹ Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil.

² Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

Keywords: DOHaD. Gestation. Insulin resistance. Lactation. Maternal caffeine.

Abstract:

Caffeine excess during pregnancy can lead to adaptive responses in offspring, affecting glucose homeostasis. However, the impact of a safe low dose of caffeine during gestation or lactation period is unknown. Previously, in a model of low perinatal caffeine exposure, we showed lower glucose tolerance in female offspring exposed to caffeine only in gestation or only in lactation. Here, we investigated key markers of the insulin pathway in the liver and skeletal muscle of adult offspring. For this, pregnant Wistar rats received intragastric caffeine (CAF) (25mg/kg/day) or vehicle during gestation (GEST) or lactation (LACT) or both periods (G+L). At birth, litters were adjusted to 8 pups (4 males and 4 females) by dam. The oral glucose tolerance test (OGTT) and the insulin signaling in liver and muscle were performed at 120-days-old. Data were analyzed by one way ANOVA followed by Dunnett's post-test. Protocol approved: 26/2019 e 011/2024. Although the glucose intolerance in CAF GEST and CAF LACT females, animals did not show differences in the adiposity, plasma triglycerides and insulin. Regarding the hepatic insulin pathway, we observed unchanged pIRS and pAKT in offspring from both sexes, but lower pAKT/ AKT ratio only in female offspring (CAF GEST: -42% and CAF LACT: -38%, compared to control). In the soleus muscle, we observed lower pAKT (-63%) and GLUT4 expression (-73%) only in the CAF LACT female, with no change in pIRS1. CAF GEST female showed a nonsignificant lower GLUT4 content (-54%). We did not observe changes in OGTT nor in the insulin signaling proteins in the CAF male offspring. CAF LACT female also showed higher plasma testosterone (+152%; $p < 0,05$). Therefore, even in a low dose, maternal caffeine intake only in gestation or only in lactation promote oral glucose intolerance and insulin resistance in female offspring. Interestingly, the maternal caffeine intake during continuous period (CAF GL) did not change the glucose homeostasis of offspring.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Combined training improves physical performance and muscle irisin of senescent female rats

Authors:

Débora Prazias CAVALCANTE^{1,2}
Rafael Augusto Santos SILVA^{1,2}
Luana Galante DOURADINHO^{1,2}
Rodrigo Martins dos SANTOS¹
Thainá Daguane ESPERANÇA^{1,2}
Beatriz Procópio STRINGHETTA-VILLAR^{1,2}
Rafael PEREIRA³
Edilson ERVOLINO⁴
Antonio Hernandez CHAVES-NETO^{1,4}
Fabrisia AMBROSIO⁵
Rita Cássia Menegati DORNELLES^{1,2,4*}

Affiliations:

¹ Multicentric Graduate Program in Physiological Sciences– SBFis/UNESP, São Paulo State University, Araçatuba, São Paulo, Brazil.

² Aging Biology Research Group, São Paulo State University (UNESP), School of Dentistry, Araçatuba, SP, Brazil.

³ Department of Biological Sciences, Universidade Estadual do Sudoeste da Bahia (UESB), Jequié, BA.

⁴ Department of Basic Sciences, São Paulo State University (UNESP), School of Dentistry, Araçatuba, SP, Brazil.

⁵ Department of Physical Medicine & Rehabilitation, Harvard Medical School, Boston, MA, USA.

Keywords: Combined training. Irisin. Oxidative stress. Sarcopenia. Senescent.

Abstract:

Physical exercise is an important non-pharmacological approach to improve muscle function and strength, helping to mitigate the functional decline associated with aging. This study evaluated the impact of regular combined training (CT) performed during periostropause on muscle morphological and functional characteristics, as well as on plasma redox balance. Periostropause rats (17–21 months) were distributed into the untrained (NT; n = 10) and combined training (CT; n = 10) groups. Animals in the CT group performed stair climbing (20 minutes) followed by treadmill running (20 minutes) (3x/week/120 days) on alternate days, totaling 40 minutes per session. Maximum voluntary load capacity and maximal incremental speed test parameters were used to adjust training loads and speed during the training period (CEUA Proc. No. 00826-2018). Analyses two-way ANOVA showed significant improvement in the CT group gait parameters and aerobic capacity ($p=0.05$ between times). The results of Student t test demonstrated that regular CT improved muscle strength ($p<0.0001$) and muscle mass ($p<0.0001$). Muscle irisin immunostaining increased after CT ($p=0.0256$), as did enzymatic antioxidant defenses (SOD $p<0.0001$; CAT $p<0.0055$), while plasma oxidative damage reduced ($p<0.005$) and plasma enzymatic (SOD $p<0.0498$; GPx $p<0.0174$) and non-enzymatic (TAC $p<0.0147$; GSH $p<0.001$) antioxidant defense increased. The results show an important action of CT in preventing sarcopenia in senescent rats, promoting muscle hypertrophy, with possible action of irisin and its autocrine effect. It also improves cardiorespiratory capacity and the ability to neutralize harmful free radicals, maintaining the balance between oxidative stress and antioxidant protection. This set of results suggests that the performance of CT in the periostropause phase has the potential to promote functional improvements and mitigate the damage of aging.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Effects of maternal apical periodontitis on fetal development and placental health

Authors:

Ana Carla Thalez Ywabuchi Nobumoto¹
Thais Verônica Saori Tsosura¹
Maria Sara de Lima Coutinho Mattera¹
Mariana Campanha Hilário Pereira¹
Bianca Elvira Belardi¹
Kelly Fernanda da Silva Soares¹
Bruna De Oliveira ALVES¹
Ângela Vermelho SARRACENI¹
Marco De Luca Monteiro STURARO¹
Luciano Tavares Angelo Cintra²
Dóris Hissako MATSUSHITA^{1,2}.

Affiliations:

¹ Department of Basic Sciences, São Paulo State University (UNESP), School of Dentistry, Araçatuba, Brazil. (Multicenter Postgraduate Program in Physiological Sciences).

² Department of Preventive and Restorative Dentistry, São Paulo State University (UNESP), School of Dentistry, Araçatuba, Brazil. (Graduate Program in Dental Science).

Keywords: Placenta; Inflammation; apical periodontitis; insulin resistance; fetal development.

Abstract:

In recent years, the relationship between oral inflammation and systemic disorders has become an area of great interest in the scientific community. Furthermore, it has been observed that periodontal pathogens cause changes in the placental structure. Apical periodontitis (AP) is an oral inflammatory process in the region of the apex of the tooth root, associated with an increase in inflammatory cytokines that may contribute to systemic changes. Objective: investigate changes in the placenta of rats with AP and examine the effects of maternal AP on neonatal health. For this purpose, 24 two-month-old Wistar rats (200g) were assigned to three groups: 1) control rats; 2) rats with one induced AP in the upper right first molar; 3) rats with four induced APs in the upper and lower right first and second molars. The AP was induced using a carbon steel drill with a 0.1 mm sphere at the tip. After 30 days of pulpal exposure, the rats from all groups were placed for mating. The rats were euthanized on the twenty-first day of pregnancy to carry out the following experiments: (1) IL-6 in the placenta; (2) histological analysis of the placenta and labyrinth area; (2) number of fetuses per rat. Regarding the neonates, the following analyses were performed: TNF- α in the liver. The values were expressed as mean $\pm$ standard error of the mean (SEM). Results: (1) increased IL-6 content in the placenta in the AP1 and AP4 groups compared to the CN group; (2) reduction in the placental labyrinth area; (3) there was no change in the number of fetuses per rat. Regarding the fetus, there was an increase in TNF- α content in the liver from rats with AP1 and AP4 when compared to the CN group. These data demonstrate that maternal AP can lead to alterations in the placenta and fetal development. The presented data emphasize that maintaining good maternal oral health is crucial to prevent systemic changes that can affect the intrauterine environment and fetal development. (CEUA: 0878-2022).

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Evaluation of the nonenzymatic antioxidants in adult rats, offspring of rats with true endo-periodontal lesion

Authors:

Bianca Elvira Belardi¹
Rayara Nogueira de Freitas²
Maria Sara Lima Coutinho Mattera¹
Mariana Campanha Hilário Pereira¹
Anielly Gonçalves¹
Ana Carla Thalez Ywabuchi Nobumoto¹
Anna Clara Cachoni²
Bruna De Oliveira Alves¹
Antônio Hernandes Chaves Neto^{1,2}
Doris Hissako Matsushita^{1,2}

Affiliations:

¹ Department of Basic Sciences, São Paulo State University (UNESP), School of Dentistry, Araçatuba, Brazil.

² Department of Preventive and Restorative Dentistry, São Paulo State University (UNESP), School of Dentistry, Araçatuba, Brazil.

Keywords: Periodontal disease. Oxidative stress. Inflammation. Periapical periodontitis. Insulin resistance.

Abstract:

Research has shown that maternal periodontal disease (PED), apical periodontitis (AP), and true endo-periodontal lesions (EPL) in rats contribute to the development of insulin resistance (IR) in their adult offspring. Additionally, oxidative stress is recognized as a key factor in both the onset and progression of diabetes. The aims of this study were: evaluation of glycemia and insulinemia followed by the HOMA-IR index; oxidative stress analysis (FRAP- ferric reducing antioxidant potential, glutathione-GSH, Uric acid-UA) in gastrocnemio muscle (GM). 28 Wistar rats were assigned to 4 groups: control; with induced AP in the right maxillary first molar; with induced PED in the right maxillary second molar; EPL, in which PED was induced in the right maxillary second molar, and AP in the right maxillary first molar. AP was induced by exposing the pulp tissue to the oral environment using a carbon steel bur with a 0.1 mm spherical tip. PED was induced by placing a sterile silk ligature in the second molar. After 30 days, the rats from all groups were mated with healthy males. When the male offspring rats reached 75 days of age, the experiments were conducted. The offspring of rats with AP (AP-o), PED (PED-o), and EPL (EPL-o) exhibited increased insulin levels and HOMA-IR index compared to the offspring of control rats (CN-o). This parameter was higher in the EPL-o group compared to the AP-o and PED-o groups. There was an increase in GSH activity in the PED-o and EPL-o groups compared to the CN-o and AP-o groups. Moreover, the EPL-o showed an increase in FRAP and UA concentration compared to the CN-o group. These results demonstrate that EPL can cause more changes in the IR and FRAP and UA when compared to other groups. This emphasize the importance of maintaining maternal oral health to prevent diseases and promote the overall health of offspring, and care should be intensified when the mother shows an association between AP and PED. CEUA: 0446-2022.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Impacts of Maternal Exposure to Bisphenol F (BPF) and AF (BPAF) on Thyroid Function in Wistar Rats: Effects During Lactation and Adulthood

Authors:

Nelyana Oliveira Serpa^{1,2}
Nathalia Medeiros Nehme¹
Iala Milene Bertasso¹
Mayara da Silva Almeida¹
Beatriz Souza da Silva¹
Marcelle Novaes Andrade³
Mariana Alencar Cavalcante⁴
Dayane Texeira Ognibene⁴
Rosiane Aparecida Miranda¹
Luana Lopes de Souza¹
Egberto Gaspar de Moura¹
Leandro Miranda Alves³
Andrea Claudia Freitas Ferreira²
Patrícia Cristina Lisboa¹

Affiliations:

¹ Laboratory of Endocrine Physiology, State University of Rio de Janeiro, Rio de Janeiro, Brazil.

² Laboratory of Endocrine Physiology Doris Rosenthal, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.

³ Laboratory of Experimental Endocrinology, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.

⁴ Laboratory of Cardiovascular Pharmacology and Medicinal Plants, State University of Rio de Janeiro, Rio de Janeiro, Brazil.

Keywords: Bisphenol. EDCs. Thyroid.

Abstract:

Endocrine-disrupting chemicals (EDCs), particularly bisphenol A (BPA) and its analogs BPF and BPAF, interfere with thyroid function and may lead to metabolic programming during critical developmental periods. This study evaluated the effects of maternal exposure to BPF and BPAF (10 or 50 µg/kg) during lactation on thyroid mass and biochemical parameters, such as enzymes related to thyroid hormones production and oxidative stress (PCR and in vitro assay), and plasma hormones (ELISA) of male and female offspring at weaning (21 days) and adulthood (120 days). Protocol approved: CEUA/UERJ/031/2022. Dams exposed to BPF10 showed higher DUOX activity (+87%, $p < 0.05$), while BPAF10-exposed dams exhibited higher T4 levels (+181%, $p < 0.05$), despite thyroid mass and gene expression (DUOX, NIS, TSH- R, TPO, NOX2) remained unchanged. In offspring at weaning, BPF10-exposed males had higher DUOX activity (+57%, $p < 0.05$), whereas females showed a significant reduction of this enzyme activity (BPF10: -55%; BPF50: -49%). In adulthood, male offspring from BPAF groups exhibited lower DUOX and TPO expression (~50%, $p < 0.05$), as well as higher oxidative stress (TBARS for BPF50: +185%; TBARS for BPAF50: +132%; $p < 0.05$). Male offspring had higher catalase and hormonal disturbances in BPF50 and BPAF groups (+62% TSH in BPF50; +109% and +114% of T4 in BPF50; +112% and +114% of T4 BPAF groups, $p < 0.05$). Female offspring presented lower DUOX expression (59-66% for BPAF groups, $p < 0.05$) and lower catalase activity, despite unchanged plasma thyroid hormones. Thyroid mass remained unaltered in all groups. Early life BPF and BPAF exposure induces sex- and age-specific thyroid dysfunction, with low-dose effects persisting into adulthood. The sustained oxidative stress and hormonal imbalances suggest metabolic programming of thyroid dysfunction. These results challenge the presumed safety of BPA alternatives and emphasize the need for rigorous assessment of their developmental impacts.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Exposure to microplastics (MP) and nanoplastics (NP) during the perinatal life alters hormone levels in Wistar rat dams and offspring of both sexes and different ages

Authors:

Nathália Medeiros NEHME¹
Nelyana De Oliveira SERPA^{1,2}
Mayara Da Silva ALMEIDA¹
Beatriz Souza da Silva¹
Rosiane Aparecida Miranda¹
Luana Lopes de Souza¹
Egberto Gaspar de Moura¹
Iala Milene Bertasso¹
Patrícia Cristina Lisboa¹

Affiliations:

¹ Laboratório de Fisiologia Endócrina, Universidade do Estado do Rio de Janeiro, Brazil.
² Laboratory of Endocrine Physiology Doris Rosenthal, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.

Keywords: Hormones. Microplastics. Nanoplastics.

Abstract:

Currently, plastic production in the world is around 400 million tons/year. Due to improper disposal, waste is generated that accumulates in the environment, such as MP (particles smaller than 5 mm) and NP (particles smaller than 100 nm), which have already been found in air, soil, water and food. Additionally, MP and NP have been detected in various human tissues, such as the placenta and breast milk, and may affect the endocrine system of offspring. Here, we studied if exposure to MP and NP during pregnancy and lactation can cause hormonal dysfunction in dams and offspring of both sexes during weaning, puberty and adulthood. Experimental design was approved by ethics committee (032/2022). From gestational day 7 until weaning, Wistar rat dams received vehicle (filtered water; control group; n=9), 10µm polystyrene microparticles (25µg/kg body weight, MP group; n=8) or 100nm polystyrene nanoparticles (25µg/kg body weight, NP group; n=8). Hormone levels of dams, male and female pups were evaluated by multiplex assay at weaning (21 days-old), puberty (45 days-old) and adulthood (120 days-old). Corticosterone, T4, TSH, leptin and insulin were analyzed at all ages, while gonadotropins and sex steroids were evaluated at adulthood. Data were analyzed by one-way ANOVA with Dunnett's post-test; p<0.05 as significant. NP dams had lower plasma T4 (-9.63%, p=0.0329), while MP dams did not show hormonal changes. At weaning, male offspring had lower plasma corticosterone (MP -64.85%, p<0.0001; NP - 55.22%, p=0.0001), while females did not show hormone alterations. At puberty, no changes were observed in either sex. In adulthood, male offspring presented higher plasma FSH (MP +53.23%, p=0.0280; NP +50.67%, p=0.0437), while females presented higher plasma TSH (MP +53.62%, p=0.0328; NP +49.75%, p=0.0483). Thus, perinatal exposure to both MP and NP alters the hormonal homeostasis of dams and offspring at weaning and in adulthood, while at puberty, no changes were found.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Melatonin and clock genes in interscapular brown adipose tissue: gene expression in adult rats

Authors:

Caroline Aparecida Pereira de Souza¹
Fernanda Akane Nishino¹
Rafael Américo Pitanga dos Santos¹
Maria Luiza Conti de Freitas Gomes Custódio¹
Fernanda Gaspar do Amaral¹

Affiliations:

¹ Pineal Neurobiology Lab, Department of Physiology, Federal University of São Paulo, São Paulo, Brazil

Keywords: Brown adipose tissue. Clock genes. Melatonin.

Abstract:

The clock genes *Bmal1*, *Clock*, *Cry1* and *2*, *Per1* and *2* are crucial for homeostasis and seem to have their transcription controlled by pineal melatonin. These genes are widespread and also found in the brown adipose tissue (BAT), responsible for non-shivering thermogenesis. The absence of melatonin impairs the interscapular BAT (iBAT) thermogenic activation pathway, and altered clock genes expression in iBAT is responsible for decreased thermogenic potential. The present project aimed to understand the role of pineal melatonin absence on clock genes expression in iBAT. Adult male Wistar Control (C), Pinealectomized (PINX) and Pinealectomized with oral melatonin replacement (1 mg/kg, PINX/Mel) rats were housed in a 12h/12h light/dark cycle with food and water *ad libitum* for 13 weeks. iBAT was collected, every 3 hours over 24 hours, weighed and the expression of clock genes was assessed by real-time PCR. Statistical analyses were performed using Prism and Cosinor software ($p < 0.05$) (CEUA #8074220415). PINX animals' iBAT collected during the light phase was lighter than the same group's iBAT collected during the dark phase and than PINX/Mel rats' iBAT collected during the light phase. No statistical differences were found in clock genes expression among groups. On the other hand, rhythmic analyses by Cosinor showed that PINX/Mel iBAT *Bmal1* mesor, and PINX and PINX/Mel *Bmal1* amplitude were higher in comparison to the C group; PINX iBAT *Cry1* expression did not fit the cosine model and PINX/Mel iBAT *Cry1* acrophase was delayed compared to C iBAT; PINX and PINX/Mel iBAT *Cry2* expression did not fit the cosine model; PINX iBAT *Per1* expression did not fit the cosine model and PINX/Mel iBAT *Cry1* amplitude was decreased compared to C iBAT. The results suggest that the absence of systemic melatonin appears to affect the clock genes expression in iBAT, being one of the possible mechanisms involved in impaired iBAT thermogenic function when this hormone is absent.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Toxic Effects of Mercury and Cadmium Co-Exposure on Reproductive Function in Wistar Rats

Authors:

Julia Karina de Oliveira Maioli¹
Jones Bernardes Graceli^{1,2}

Affiliations:

¹ Federal University of Espírito Santo, Brazil. ²
Southern Illinois University, USA.

Keywords: Cadmium. Endocrine disruptor. Estrous cycle. Female reproduction. Mercury.

Abstract:

Heavy metals contaminate air, water, and soil due to human activities, affecting the environmental balance. According to the World Health Organization, chronic exposure to mercury (Hg) and cadmium (Cd) can impair female reproduction. The aim of this study was to investigate the toxic effects of co-exposure to Hg and Cd on reproductive function in Wistar rats (8–10 weeks). Animals divided into a) control (CON): exposed to saline (mg/kg/day, IM) and b) MIX: Hg (1st dose of 4.6 mg/kg, 2nd subsequent doses of 0.07 mg/kg/day, IM) + Cd (100 mg/L) for 15 days. The estrous cycle was analyzed daily, ovarian and uterine morphology evaluated with H&E and Picrosirius Red, and hormone levels were assessed using ELISA kits. Data are presented as mean $\pm$ SEM. Sample number 5. Statistical analysis performed by Student's t-test, with significance at $p < 0.05$. All protocols approved by CEUA-UFES (04/2023). Exposure to the mixture increased cycle duration in the MIX group (CON: 1.66 ± 0.17 ; MIX: 3.16 ± 0.59 ; $p < 0.05$). Serum E2 and LH levels increased in MIX rats (CON: 28.68 ± 3.01 ; MIX: 12.98 ± 3.20 ; $p < 0.05$) and (CON: 227.10 ± 7.55 ; MIX: 165.40 ± 7.52 ; $p < 0.05$). MIX ovaries showed a reduction in the number of primary follicles and corpus luteum (CON: 1.61 ± 0.19 ; MIX: 0.76 ± 0.27 ; $p < 0.05$) and (CON: 1.39 ± 0.16 ; MIX: 0.84 ± 0.17 ; $p < 0.05$). However, the antral follicle count increased in MIX rats (CON: 0.92 ± 0.10 ; MIX: 1.02 ± 0.20 ; $p < 0.05$). Uterine endometrial and myometrial areas were reduced in the MIX group (CON: 3.44 ± 0.15 ; MIX: 2.31 ± 0.11 ; $p < 0.05$) and (CON: 3.02 ± 2.49 ; MIX: 2.49 ± 2.49 ; $p < 0.05$). The number of glands increased in the MIX group (CON: 0.01 ± 0.0008 ; MIX: 0.02 ± 0.001 ; $p < 0.05$). Collagen deposition was higher in the ovaries (CON: 5.90 ± 0.56 ; MIX: 15.06 ± 1.48 ; $p < 0.05$) but lower in the uterus of the MIX group (CON: 0.201 ± 0.007 ; MIX: 0.134 ± 0.012 ; $p < 0.05$). These results suggest that co-exposure to Hg and Cd negatively affects reproductive function.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Melatonin action in the initial and later steps of insulin signaling in rats with Apical Periodontitis, Periodontal Diseases and Endo-periodontal Lesion

Authors:

Marco de Luca Monteiro Sturaro¹
Thais Paiva Moraes²
Maria Sara Coutinho Mattera¹
Bianca Elvira Belardi¹
Ana Carla Thalez Ywabuchi Nobumoto¹
Ângela Vermelho Sarraceni¹
Estevão Lopes Pereira²
Lorena Umbelino Rodrigues¹
Luciano Tavares Ângelo Cintra²
Doris Hissako Matsushita^{1,2}

Affiliations:

¹ Department of Basic Sciences, São Paulo State University (UNESP), School of Dentistry, Araçatuba, Brazil. Multicenter Postgraduate Program in Physiological Sciences.

² Department of Preventive and Restorative Dentistry, São Paulo State University (UNESP), School of Dentistry, Araçatuba, Brazil. Graduate Program in Dental Science.

Keywords: Inflammation. Periodontal Diseases. Melatonin.

Abstract:

Apical periodontitis (AP), periodontal disease (PD), and true endo-periodontal lesion (EPL) are associated with systemic disorders, such as impaired insulin signaling (IS). Melatonin (MEL) has anti-inflammatory properties and may improve this signaling. This study evaluated whether rats with EPL, AP, or PD develop IS alterations, whether combining these diseases worsens these changes, and MEL's influence on early and late IS pathways. This study was approved by the local ethics committee (protocol number CEUA: 668/2023). For this purpose, fifty-six Wistar rats were where assigned to 8 groups: control rats (C), rats with AP (AP), rats with PD (PD), rats with EPL (EPL), control rats supplemented with MEL (C+M) (0.5 mg/kg by gavage for 60 days), rats with AP supplemented with MEL (AP+M), rats with PD supplemented with MEL (PD+M), and rats with EPL supplemented with MEL (EPL+M). For the AP groups, lesions were induced in the right upper first molar by exposing the pulp tissue using a carbon steel bur with a 0.1 mm spherical tip. The PD were induced in the right upper second molar using a sterile silk ligature. For the EPL groups, AP and PD were induced in the 1st and 2st molars, respectively. After 30 days, supplementation with MEL started. The results were evaluated by two-way ANOVA followed by Tukey's test ($p < 0.05$). The phosphorylation status of pp185, after insulin stimulation, in the AP, PD, and EPL groups was reduced compared to the C group, and it was more pronounced in EPL in comparison with AP and PD, and it reversed by MEL supplementation. Akt phosphorylation status did not differ significantly between groups with or without MEL. However, MEL increased this parameter in the C group supplemented with MEL (C+M) compared to C, and in the EPL+MEL group compared to EPL. These results suggest that oral diseases cause systemic alterations and the EPL exacerbates these alterations. Furthermore, MEL shows therapeutic potential in modulating these changes.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Hypothyroidism differentially affects the bone microstructure of the maxilla in male and female mice

Authors:

Maíza Caroline Alves FERREIRA¹
Ana Bárbara de Paula SILVA¹
Bruno Henrique GOMES¹
Mariana de Souza SANTOS¹
Soraia MACARI²
Raphael Escorsim SZAWKA¹
Paula BARGI-SOUZA¹

Affiliations:

¹ Department of Physiology and Biophysics, ICB, UFMG, Belo Horizonte, MG, Brazil.

² Department of Restorative Dentistry, School of Dentistry, UFMG, Belo Horizonte, MG, Brazil.

Keywords: Bone remodeling. Hypothyroidism. Maxilla. Sexual dimorphism. Thyroid hormones

Abstract:

Bone is a metabolically active tissue and undergoes constant remodeling, involving resorption and deposition of new mineralized matrix. Thyroid hormones (THs) play a key role in regulating this process, although their effects on the maxilla remain poorly explored. This study evaluated the effects of hypothyroidism on the bone microstructure of the maxilla in male and female mice, focusing on potential sex differences. Thirty-six adult C57BL/6J mice (15 females and 21 males) were used, divided into Control and Hypothyroid groups. Hypothyroidism was induced for 45 days using methimazole (0.1%) and sodium perchlorate (1%) in the drinking water. After euthanasia, the maxillae were collected and processed for micro-computed tomography to analyze the following parameters: Bone Mineral Density (BMD), Bone Volume (BV), Bone Volume Fraction (BV/TV%), Trabecular Thickness (Tb.Th), Trabecular Number (Tb.N), Trabecular Separation (Tb.Sp), Structure Model Index (SMI), and Intersection Surface (IS). Histological analyses were also performed using hematoxylin-eosin, Masson's trichrome, and Tartrate-Resistant Acid Phosphatase (TRAP) staining for cell counting. All in vivo experimental protocols were previously approved by the Animal Ethics Committee (CEUA: 170/2021 and 349/2023) of UFMG. Results showed that hypothyroid females exhibited a reduction in BV and BV/TV%, while males showed a decrease in Tb.Th. Regarding the cellular pattern, euthyroid females presented more osteocytes than males, further increasing in the hypothyroid group. The findings indicate that hypothyroidism affects the bone tissue of the maxilla differently between sexes, leading to loss of mineralized matrix and increased cell count in females, suggesting a possible sexual dimorphism in response to TH deficiency.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Absence of the pineal gland alters the daily expression of clock genes and thermogenic molecular machinery genes in cervical brown adipose tissue

Authors:

Rafael Américo Pitanga dos Santos¹
Caroline Aparecida Pereira de Souza²
Fernanda Akane Nishino²
Maria Luíza Conti de Freitas Gomes Custódio²
Fernanda Gaspar do Amaral²

Affiliations:

Pineal Neurobiology Lab, Department of Physiology, Federal University of São Paulo, São Paulo, Brazil

Keywords: Cervical brown adipose tissue. Pineal. Melatonin. Clock genes. Rhythmicity.

Abstract:

Pineal melatonin has been shown to regulate the thermogenesis of interscapular brown adipose (iBAT) tissue daily and seasonally. Cervical BAT (cBAT) is crucial for blood thermoregulation in the central nervous system due to its anatomical location. Although melatonin has been shown to act as a photoperiod signal controlling the molecular machinery of thermogenesis and the expression of clock genes in iBAT, there is a lack of information regarding cBAT. Male Wistar rats control (C), pinealectomized (Pinx), and pinealectomized with melatonin replacement (Pinx/Mel, 0.5 mg/kg daily in drinking water) (200-250 g) were housed in a 12h/12h light/dark cycle with food and water ad libitum. cBAT samples were collected every 3 hours over 24 hours (n=4/time point/group), 13 weeks after surgery (CEUA-UNIFESP #8074220415). Real-time polymerase chain reaction was used to evaluate Clock, Bmal1, Cry1, Cry2, Per1, Per2, Rev-Erba, Dbp, Rora, Adrb3, Dio2, Pgc1a, Cideia, Ucp1, G-actin and RPL37a gene expression. Statistical analyses were performed using Prism and Cosinor software (p<0.05). Two-way ANOVA pointed out that Pinx cBAT showed altered daily variation in Clock, Per1, Per2, Cry1, Cry2, Dbp, Revb, Adrb3, Dio2, Pgc1a, Cideia, and Ucp1 gene expression. Cosinor rhythmic analysis showed that Pinx cBAT presented a phase delay in Bmal1 and Revb gene expression compared to C cBAT, unlike Per1, Per2, and Dbp expressions, which showed a phase advance, and Cry1 and Adrb3, which had their daily rhythm abolished. The rhythmic analysis also showed decreased Per2 and Revb expression amplitude in Pinx cBAT compared to C cBAT. These changes described in PINX cBAT were only partially prevented by melatonin replacement, as demonstrated by the Pinx/Mel cBAT results.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: The impact of estradiol replacement on the browning of white adipose tissue and the maintenance of body temperature in high-fat-fed female mice

Authors:

Bruna Maria Fantini Trinca¹
Alessandra Gonçalves Da Cruz¹
Jessica Denielle Matos dos Santos¹
João Paulo Gabriel Camporez¹

Affiliations:

¹ Department of Physiology, Ribeirão Preto Medical School, University of São Paulo – FMRP/USP. Ribeirão Preto, São Paulo, Brazil.

Keywords: Body temperature. Brown adipose tissue. Browning. Estradiol. White adipose tissue.

Abstract:

The objective of the present study was to analyze the effects of E2 on browning markers in tissues and the morphology of white and brown adipose tissue, as well as its impact on body temperature in female mice fed a high-fat diet. For this, female mice with a C57BL/6J background were used, subjected to ovariectomy (OVX) at 8 weeks of age. In one of the groups, subcutaneous E2 pellets (OVX+E2) were implanted (n=8), while the other group received a vehicle (OVX) (n=8). All animals were fed a high-fat diet (HFD) for 4 weeks. After this period, a glucose tolerance test (GTT) was performed. Subsequently, the animals were euthanized, and the periuterine white adipose tissue (PUT), inguinal white adipose tissue (IGN), and interscapular brown adipose tissue (ICL) were collected. A protein expression analysis of UCP1 in the PUT was performed using Western Blot. A Cold Tolerance Test was also carried out, associated with baseline thermography and post-challenge cold thermography in the animals. Additionally, histological data were collected through H&E staining in the PUT, IGN, and ICL tissues (CEUA-FMRP protocol 1031/2021R1). It was found through the GTT that animals in the OVX+E2 group had a lower area under the curve (AUC). Furthermore, the protein expression analysis of UCP1 in the PUT showed a significant increase in the OVX+E2 group. Moreover, the Cold Tolerance Test demonstrated that the OVX+E2 group had a greater ability to maintain body temperature when challenged with cold, a result that was supported by thermography. Additionally, histological data through H&E staining showed a smaller lipid droplet area and a greater number of cells in the white adipose tissue of the PUT and IGN tissues in the OVX+E2 group. Thus, the significant relationship between estradiol replacement and increased UCP1 in the PUT, along with the greater ability to maintain body temperature in OVX+E2 females, supported by the Cold Tolerance Test and thermography, and the contribution of histological data with browning parameters, suggest a relationship between estradiol and white adipose tissue thermogenesis.

Financial Support: FAPESP.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Effect of melatonin on the expression of TNF- α content in rats with apical periodontitis subjected to passive tobacco inhalation

Authors:

Lorena Umbelino RODRIGUES¹
Maria Sara de Lima Coutinho Mattera¹
Bianca Elvira Belardi¹
Ana Carla Thalez Ywabuchi Nobumoto¹
Anna Clara Cachoni²
Bruna de Oliveira Alves¹
Luciano Tavares Angelo Cintra²
Estêvão Lopes Pereira²
Angela Vermelho Sarraceni¹
Anielly Gonsalves¹
Camila Conrado França¹
Kelly Fernanda da Silva Soares¹
Mariana Campanha Hilário Pereira¹
Marco De Luca Monteiro Sturaro¹
Doris Hissako Matsushita¹

Affiliations:

¹ Department of Basic Sciences, São Paulo State University (UNESP), School of Dentistry, Araçatuba, Brazil. (Multicenter Postgraduate Program in Physiological Sciences).
² Department of Preventive and Restorative Dentistry, São Paulo State University (UNESP), School of Dentistry, Araçatuba, Brazil. (Graduate Program in Dental Science).

Keywords: Apical periodontitis; insulin resistance; melatonin; tobacco.

Abstract:

Apical periodontitis (AP) and smoking may be associated with metabolic syndrome, diabetes mellitus, and insulin resistance (IR). Melatonin (MEL) is a hormone with antioxidant and anti-inflammatory properties. Previous studies have shown that MEL improves insulin sensitivity and signaling in the skeletal muscle of rats with AP. Aim: To evaluate the inflammatory signaling pathway in the skeletal muscle of adult rats with AP subjected to passive tobacco inhalation. A total of 128 Wistar rats, 60 days old, were assigned to 8 groups: control (CN); smoking rats (T); rats with AP (AP); smoking rats with AP (T+AP); control with MEL supplementation (CN+MEL); smoking rats with MEL supplementation (T+MEL); rats with AP and MEL supplementation (AP+MEL); smoking rats with AP and MEL supplementation (T+AP+MEL). The smoking groups were exposed to passive cigarette smoke for 50 days. On the 20th day, AP was induced in the designated groups using a carbon steel drill on the first and second upper and lower molars on the right side. Additionally, animals in the MEL groups received melatonin supplementation (5 mg/kg, orally via gavage) from the 20th to the 50th experimental day. At the end of the treatment, the TNF- α content in skeletal muscle tissue was analyzed using the Western Blotting (WB) technique in gastrocnemius muscle tissue (GM). Results: TNF- α content significantly increased in the AP, T, and T+AP groups, and this increase was reversed by MEL in all respective groups. Conclusion: AP and tobacco exposure lead to an increase in TNF- α levels. MEL was effective in reducing TNF- α levels, highlighting its therapeutic potential in modulating insulin resistance and inflammation associated with AP and tobacco exposure (CEUA: 699-2023).

This study was financed, in part, by the São Paulo Research Foundation (FAPESP) [grant #2019/04183-9; #2022/08872-6; #2023/03786-7; #2023/12488-0; #2023/01400-4; #2022/04868-4] and CNPq [grant 134692/2024-1; 151151/2023-7] São Paulo, SP, Brazil.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Protective Effects of Female Sex Hormones Against CCl₄/HFD-Induced MASH

Authors:

Felipe Nunes de Camargo¹
Anne Raissa Melo Santos¹
Leticia Franco Amaro¹
Jessica Denielle Matos dos Santos¹
Alessandra Gonçalves da Cruz¹
Sandro Leão Matos¹
Ana Livia Dias Maciel¹
João Paulo Camporez¹

Affiliations:

¹ Department of Physiology - FMRP – USP.

Keywords: MASH. Ovariectomy. Sexual hormones.

Abstract:

Introduction: Metabolic Dysfunction-Associated Steatohepatitis (MASH) is defined by steatosis, inflammation, hepatocellular ballooning, fibrosis, and Mallory bodies. Declining female sex hormones during menopause is linked to increased visceral fat and higher risk of metabolic disorders (insulin resistance, type 2 diabetes, and cardiovascular disease). This study evaluates the protective effects of female sex hormones against CCl₄/HFD-induced MASH. **Methods:** Female C57BL/6J mice (n=24) (CEUA-039/2021) were randomly assigned at 8 weeks of age to three groups (n=8): SHAM-RC (sham surgery, regular diet, vehicle), SHAM-CCl₄ (sham surgery, HFD, CCl₄), and OVX-CCl₄ (ovariectomy, HFD, CCl₄). SHAM-CCl₄ and OVX-CCl₄ received 10% fructose in water. CCl₄ (0.5 µl/g) was given i.p. 3 times/week for 4 weeks, diluted in olive oil. Glucose tolerance, body composition, hepatic steatosis, and liver damage were assessed. Statistical analysis used one-way ANOVA (p<0.05). **Results:** OVX-CCl₄ showed ~15% higher body weight vs. SHAM-RC. Whole-body fat increased ~29% in OVX-CCl₄ vs. SHAM-RC and ~24% vs. SHAM-CCl₄. Uterine mass decreased ~69% vs. SHAM-RC and ~61% vs. SHAM-CCl₄. Glucose intolerance increased ~22% in OVX-CCl₄ vs. SHAM-RC and ~34% vs. SHAM-CCl₄. Plasma cholesterol rose ~66% in OVX-CCl₄ vs. SHAM-RC and ~64% in SHAM-CCl₄ vs. SHAM-RC. Hepatic cholesterol was ~255% higher in OVX-CCl₄ vs. SHAM-RC and ~37% vs. SHAM-CCl₄, while SHAM-CCl₄ had a ~158% increase vs. SHAM-RC. Hepatic triglycerides increased ~622% in OVX-CCl₄ vs. SHAM-RC and ~36% vs. SHAM-CCl₄; SHAM-CCl₄ increased ~429% vs. SHAM-RC. Plasma AST increased ~219% in OVX-CCl₄ vs. SHAM-RC and ~160% in SHAM-CCl₄ vs. SHAM-RC. **Conclusion:** The lack of sexual hormones in OVX mice combined with CCl₄/HFD exacerbated metabolic dysfunction by increasing body fat, glucose intolerance, cholesterol, triglycerides, and liver damage. These findings suggest female sex hormones protect against MASH by mitigating metabolic and hepatic disturbances.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Analysis of bone microarchitecture and chemical structure of the femur neck in Wistar rats at different periods of reproductive aging

Authors:

Luana Galante Douradinho^{1,2}
Débora Prazias Cavalcante^{1,2}
Rafael Jesus Gonçalves Rubira³
Rita Cássia Menegati Dornelles^{1,2}

Affiliations:

¹ Programa de Pós-Graduação Multicêntrico em Ciências Fisiológicas, PPGMCF/UNESP/SBFis.
² Departamento de Ciências Básicas, Faculdade de Odontologia de Araçatuba (FOA), Universidade Estadual Paulista (UNESP), Araçatuba, São Paulo, Brazil.
³ Instituto de Geociências e Ciências Exatas, Unesp de Rio Claro.

Keywords: Aging. Bone health. Menopause. Osteoporosis.

Abstract:

This study evaluated trabecular and cortical bone microarchitecture, and chemical information on the mineral components of bone tissue in female rats at different periods of reproductive senescence. Wistar rats aged 9, 16 and 24 months, after confirmation of the estrous cycle, were euthanized (CEUA no. 760/2022). Bone microarchitecture (micro-CT) and chemical structure analyses using the Raman scattering technique were performed on the right femoral neck. Micro-CT analysis of trabecular bone showed that percentage bone volume (9 vs 16: $p < 0.0001$; 9 vs 24: $p = 0.0005$) and trabecular thickness (9 vs 16: $p < 0.0001$; 9 vs 24: $p < 0.0001$) were greater at 9 months. Adult rats showed lower trabecular separation (9 vs 16: $p = 0.0005$; 16 vs 24: $p = 0.0262$). The cortical bone of 9-month-old animals showed greater area (9 vs 16: $p = 0.0012$; 9 vs 24: $p < 0.0001$) and cortical bone thickness (9 vs 16: $p < 0.0001$; 9 vs 24: $p < 0.0001$). Regarding the number of pores, 9 and 16-month-old rats had fewer pores than 24-month-old rats (9 vs 24: $p = 0.0004$; 16 vs 24: $p = 0.0139$). It was observed that the bands (collagen and phosphate) are widely distributed in the bone region of 9-month-old animals. In contrast, mapping of these bands in the bone tissues of 16-month-old animals reveals a lower presence of high-intensity points, with a predominance of dark regions. In 24-month-old animals, there is an even more significant reduction in the intense regions associated with collagen and phosphate bands compared to 9- and 16-month-old animals. The results show a decrease in the volume, area and thickness of cortical and trabecular bone, while cortical porosity and trabecular separation increase. In addition, there is a reduction in amount of phosphate and collagen in the tissues analyzed. Therefore, the structure and chemical composition are determining factors in the modification of bone composition during the reproductive transition periods of females and impact the aging of bone tissue.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Effects of early exposure to polystyrene microplastics on metabolic parameters in zebrafish: impacts on the development of obesity and type II diabetes mellitus in adulthood

Authors:

Bernardo Lannes Monteiro Fontes^{1,2}
Ana Luiza Chagas da COSTA^{1,2}
Gabriel Do Almo GUIMARÃES^{1,2}
Cintia Rodrigues PINHEIRO^{1,2}
Marinaldo Pacífico Cavalcante NETO^{1,2}

Affiliations:

¹ Universidade Federal do Rio de Janeiro.
² Núcleo de Pesquisas Ecológicas de Macaé.

Keywords: glucose; insulin; microplastic; polystyrene; zebrafish.

Abstract:

Introduction: Plastic is widely used and its improper disposal contributes to environmental pollution. Polystyrene (PS) is one of the most common plastics, whose degradation generates microplastics (MPs), particles smaller than 5 mm. These MPs can accumulate in the food chain, acting as endocrine disruptors (EDs) and affecting metabolic health. Studies suggest that early exposure to EDs can alter metabolic programming, increasing the predisposition to diseases such as obesity and type II diabetes mellitus (T2DM). Insulin signaling, mainly via IR receptors, is affected in T2DM conditions, and MPs can interfere with this process. Thus, understanding the impacts of this exposure in the early stages of development is essential to assess health risks in adulthood. Objectives: To investigate the effects of exposure to PS-MPs, from fertilization to the 27th larval day, on endocrine and metabolic parameters related to obesity and T2DM in zebrafish, both at the end of exposure and in adulthood (152 days of life). Material and Methods: Zebrafish embryos will be divided into three groups: control, PS-MPs 100 µg/L and PS-MPs 1000 µg/L, and will be exposed to fluorescent MPs from 2 hpf to 27 dpf. The accumulation of MPs will be evaluated at different stages of development. Blood glucose, insulin, body fat and gene expression of IR, IRS-1 and IRS-2 receptors in the liver will be analyzed by RT-PCR in adults, while in larvae protein detection will be done by immunohistochemistry. Statistical tests such as Dunnett, Kruskal-Wallis and Dunns will be used for data analysis. Results: As a result, we have the accumulation of PS-MPs, changes in weight and length and we assume the alteration of metabolic biomarkers. Conclusion: This study will contribute to the understanding of the effects of environmental contaminants on metabolic programming and health, providing relevant data on the relationship between microplastics and the development of metabolic diseases. CEUA: 006/22.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Sodium Saccharin Exposure During Gestation and Lactation Induces Prostate Histological Changes Across Generations in Rats

Authors:

Sarah Cordeiro Yamashita¹
Alana Rezende Godoi Rebeschini¹
Ana Clara Pacheco de Santana¹
Vanessa Caroline Fiorante¹
Francisco Eduardo Martinez¹
Patricia Fernanda Felipe Pinheiro¹.

Affiliations:

¹ Structural and Functional Biology Department, Institute of Biosciences of Botucatu – UNESP.

Keywords: Artificial sweeteners. DOHaD. Male reproductive tract.

Abstract:

Sodium saccharin (SS) is an artificial sweetener present in foods and beverages; however, its use is still the subject of many controversies. The study investigated the potential intergenerational inheritance in the developmental programming of male offspring (F1 and F2) resulting from the exposure of female rats (F0) to SS during gestational and lactational periods, and its implications on ventral prostate. Sprague-Dawley rats, males (n=10, 90 DOA, 350g) and females (n=20, 90 DOA, 250g) were distributed in: Control Group (CG): dams fed a rodent standard diet and filtered water ad libitum during gestation and lactation (n=10); Saccharin Group (SG): dams fed a rodent standard diet and filtered water ad libitum sweetened with sodium saccharin at 0.3% during gestation and lactation (n=10) (Nº 8109101219 CEUA). All animals were weighed until the day of their deaths. Samples from the ventral lobe of the prostate were weighted and stained with HE, Picrosirius Red and Masson's Trichome for histological analysis. Dams from both CGs and SGs showed similar body weight evolution in both generations. The offspring's body weight and ventral lobe of prostate weight showed no statistical difference. Individuals from the F1 and F2 generations of the control and sodium saccharin groups didn't show differences in the testicular descent timing. However, the evaluation of complete preputial separation indicated a delay in the SG-F1 (CG-F1:41,06±0,57; SG-F1:42,75±0,58*; CG-F2:41,33±0,78; SG-F2:42,25±1,36; *p<0.05). Comparative histological analysis revealed an intense inflammatory infiltrate, an increase in the density of connective fibers in some areas of stroma, smaller distal and intermediate acini and a greater presence of secretion and cell debris in the lumen of the SGs of the F1 and F2 generations. Therefore, this study highlighted the intergenerational impact of SS consumption in rats, especially in the ventral lobe of the prostate.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Fetal and placental morphometric alterations induced by prenatal stress

Authors:

Letícia Rodrigues Pinheiro¹
Luana Tenorio-Lopes¹
Rodrigo Da Silva Nunes Barreto¹
Kênia Cardoso Bicego¹
Raí André Querino Candelária¹
Luciane Helena Gargaglioni¹

Affiliations:

¹Departament of Animal Morphology and Physiology, São Paulo State University at Jaboticabal, FCAV, Brazil.

Keywords: Development. Gestation. Placenta. Stress.

Abstract:

Prenatal stress (PNS), resulting from the exposure of pregnant women to stressful factors, can impact maternal health and affect fetal development. Rodent models exposed to restraint stress in the last week of gestation alter the offspring's neurological development, leading to cognitive and behavioral consequences in adulthood. Experimental models indicate that PNS can cause changes in the placenta and predispose offspring to metabolic, neurobehavioral, respiratory and cardiovascular disorders throughout life, but it is not known what mechanisms are involved in these changes. Given the impact of PNS on placental and fetal parameters, it is essential to further investigate how these alterations manifest. Therefore, this study aimed to evaluate the effect of PNS on placenta-fetal morphometry of pregnant females. Pregnant C57BL/6 female mice (CEUA- protocol nº 4978/2024) were submitted to the PNS by restraint stress exposure for 3 h/ day, during 5 consecutive days between gestational days (GD) 14 to 18. On day GD18, the females were euthanized, and a sterile cesarean section was performed to remove the uterus. The placentas and deciduous were excised, and the diameter, thickness and placental weight were evaluated. The fetuses were also excised, and the fetal length, thickness and weight were evaluated. The results showed significant differences between the PNS (n=34 animals, from 4 different litters) and control groups (n=33, from 4 different litters). The PNS showed a significant reduction in placenta diameter (p=0.008), placenta thickness (p<0.0001), placenta weight (p=0.006), fetal length (p<0.0001), fetus thickness (p<0.0001) and fetal weight (p=0.01). PNS exposure impairs the development of the placenta and fetus, resulting in smaller placentas and impaired fetal growth. These data highlight the importance of the intrauterine environment in fetal development and help to understand the negative effects of maternal stress on pregnancy.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Effect of Melatonin on the Modulation of Skeletal Muscle Inflammation Induced by Apical Periodontitis and Smoking in Rats

Authors:

Maria Sara de Lima Coutinho Mattera¹
Bianca Elvira Belardi¹
Bruna De Oliveira Alves¹
Lorena Umbelino Rodrigues¹
Ana Carla Thalez Ywabuchi Nobumoto¹
Anna Clara Cachoni²
Anielly Gonsalves¹
Camila Conrado França¹
Kelly Fernanda da Silva Soares¹
Mariana Campanha Hilário Pereira¹
Angela Vermelho Sarraceni¹
Marco De Luca Monteiro Sturaro¹
Estevão Lopes Pereira²
Luciano Tavares Angelo Cintra²
Doris Hissako Matsushita¹

Affiliations:

¹Department of Basic Sciences, São Paulo State University (UNESP), School of Dentistry, Araçatuba, Brazil. (Multicenter Postgraduate Program in Physiological Sciences).

²Department of Preventive and Restorative Dentistry, São Paulo State University (UNESP), School of Dentistry, Araçatuba, Brazil. (Graduate Program in Dental Science).

Keywords: Apical periodontitis; Melatonin; Tobacco; Insulin resistance.

Abstract:

Apical periodontitis (AP) and smoking may be associated with metabolic syndrome, diabetes mellitus, and insulin resistance (IR). Melatonin (MEL) is a hormone with antioxidant and anti-inflammatory properties. Aim: To evaluate the inflammatory signaling pathway in the skeletal muscle of adult rats with AP subjected to passive tobacco inhalation. A total of 128 Wistar rats, 60 days old, were assigned to 8 groups: control (CN); smoking rats (T); rats with AP (AP); smoking rats with AP (T+AP); control with MEL supplementation (CN+MEL); smoking rats with MEL supplementation (T+MEL); rats with AP and MEL supplementation (AP+MEL); smoking rats with AP and MEL supplementation (T+AP+MEL). The smoking groups were exposed to passive cigarette smoke for 50 days. On the 20th day, AP was induced in the relevant groups using a carbon steel drill on the first and second upper and lower molars on the right side. Additionally, animals in the MEL groups received melatonin supplementation (5 mg/kg, orally via gavage) from the 20th to the 50th experimental day. At the end of the treatment, the phosphorylation levels of NFκB p50 and p65 proteins and their total contents in the gastrocnemius skeletal muscle (GM) were analyzed using the Western blotting (WB) technique. Results: An increase in the phosphorylation level of p-NFκB p65 was observed in the AP group compared to the CN, CN+MEL, and T+MEL groups. Moreover, MEL reduced the phosphorylation of this protein in the T+AP+MEL group. Regarding the phosphorylation level of p-NFκB p50, the AP group showed an increase compared to the CN, CN+MEL, T+MEL, and T+AP+MEL groups. MEL was able to reverse this change when AP comparing to



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

AP+MEL groups. Conclusion: AP and tobacco exposure activate the inflammatory NF-kB p50/p65 pathway. Furthermore, MEL proved effective in reducing NF-kB p50/p65 phosphorylation, highlighting its therapeutic potential in modulating insulin resistance and inflammation associated with AP and tobacco. (CEUA: 699-2023). This study was financed, in part, by the São Paulo Research Foundation (FAPESP) [grant #2019/04183-9; #2022/08872-6; #2023/03786-7; #2023/12488-0; #2023/01400-4; #2022/04868-4] and CNPq [grant 134692/2024-1; 151151/2023-7] São Paulo, SP, Brazil.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Metabolic effects of chronic exposure to glyphosate at the acceptable daily intake dose in Wistar rats

Authors:

Samara Cristine Knebel^{1,5}
Laura Schleder Corrêa^{1,5}
Anna Karolina Kretschmann Florencio de Souza Bagetti^{1,3}
Isadora Sulzbacher Ourique¹
Laura Braun Eickhoff^{1,3}
Luis Felipe Pedrolo Hickmann^{1,3}
Maria Eduarda Todendi de Bragas^{1,2}
Eduarda Bordignon^{1,2}
Diovana Gelati de Batista^{1,4}
Mirna Stela Ludwig^{1,5}
Thiago Gomes Heck^{1,4,5}

Affiliations:

¹ Grupo de Pesquisa em Fisiologia (GPef), Universidade Regional do Noroeste do Estado do Rio Grande do Sul (UNIJUÍ).

² Curso de Biomedicina, Universidade Regional do Noroeste do Estado do Rio Grande do Sul (UNIJUÍ).

³ Curso de Medicina, Universidade Regional do Noroeste do Estado do Rio Grande do Sul (UNIJUÍ).

⁴ Programa de Pós-Graduação em Modelagem Matemática e Computacional, Universidade Regional do Noroeste do Estado do Rio Grande do Sul (UNIJUÍ).

⁵ Programa de Pós-Graduação em Atenção Integral à Saúde, Universidade Regional do Noroeste do Estado do Rio Grande do Sul (UNIJUÍ).

Keywords: Glyphosate. Chronic exposure. Acceptable daily intake. Metabolism. Glycemia. Wistar rats.

Abstract:

I: The use of glyphosate-based herbicides (GLY) has increased in recent decades, and this scenario may represent a risk for metabolic effects under chronic exposure, even at doses considered safe. **O:** We aim to investigate whether chronic exposure to GLY induces metabolic alterations. **M:** Male Wistar rats (n=12), aged 4–5 months, were divided into Control (CTRL) or Glyphosate exposure for 12 weeks (GLY, 0.5 mg/kg/day in drinking water, representing an acceptable daily intake dose, ANVISA; Roundup Original Mais®). Body weight and food and water intake were assessed weekly. A glucose tolerance test (GTT) was performed after a 12-hour fast (glucose 80%, 1 g/kg, i.p.), before and after 12 weeks. The animals were euthanized, and adiposity was assessed. Serum lipids and liver and kidney enzymes were analyzed. Data were compared by T-test or two-way ANOVA (P<0.05, GraphPad), CEUA 282024. **R:** The GLY group intake 0.49±0.02 mg/kg/day of GLY per animal, with no differences in water intake (P=0.385). Both groups started with similar body weight (g) (P=0.200), and there was an increase in food intake (g/day) in the GLY group (CTRL=23.7±1.0 g; GLY=25.4±1.1 g; P=0.0008), resulting in a weight gain over the 12 weeks (P<0.0001). Thus, higher body weight was observed in the GLY group (CTRL=487±23; GLY=522±34; P=0.036). No differences were found between groups in adiposity (P=0.615), fasting glycemia (P=0.580), alanine transaminase (P=0.571), alkaline phosphatase (P=0.153), urea (P=0.826), creatinine (P=0.07), cholesterol (P=0.608) and triglycerides (P=0.792). However, while the CTRL group showed no alterations in GTT after 12 weeks (AUC mg/min/dL, initial=14,262±2,380 vs. final=12.720±2.693, P=0.064), the GLY group showed an increase in glycemic values at the end of the study (initial=12.04±1.202



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

vs. final=15.010±1.786, P=0.0025). **C:** Chronic exposure to glyphosate, even at a dose considered acceptable, may promote weight gain and altered glycemic response, suggesting metabolic effects.

Funding/Financiamento: CNPq, #407329/2016-1, #444286/2024-1, #403136/2024-5, #405546/2023-8, #307926/2022-2 to TGH, CAPES, FAPERGS e UNIJUÍ.



XXXIX REUNIÃO ANUAL DA FeSB
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Morphometric-stereological study of the dorsolateral prostate from perinatal exposure to a relevant environmental mixture of phthalates

Authors:

Lívia Centofanti Cózaro¹
Maria Clara Betete da Silva Fonseca¹
Vinicius Luis Rocha da Silva Maria²
Ana Clara Pacheco de Santana²
Liara Botão da Silva¹
Júlia Landeira Gomez Chamelete¹
Ariana Musa de Aquino³
Sergio Pereira¹
Cristiane Figueiredo Pinho¹

Affiliations:

- ¹ Faculty of Sciences, São Paulo State University, Bauru, Brazil.
² Institute of Biosciences, São Paulo State University, Botucatu, Brazil.
³ Department of Pancreatic Cancer Research, Henry Ford Hospital, Detroit, USA.

Keywords: DOHaD, morphology, phthalates, prostate.

Abstract:

Exposure to environmental endocrine disruptors, such as phthalates, has been associated with the development of several neoplasms, including prostate cancer, by altering the concentration and metabolism of steroid hormones. Phthalates represent approximately 85% of plasticizers currently used and exhibit antiandrogenic activity in the prostate, increasing susceptibility to preneoplastic and malignant lesions. In this context, the present study aimed to evaluate whether gestational and lactational exposure to different concentrations of an environmentally relevant mixture of phthalates is capable of altering the histological architecture of the prostate of rats in adulthood. Thereunto, pregnant Sprague-Dawley rats were randomly divided into 4 experimental groups: **C** (Corn oil), **T1** (20 µg/kg/day mixture of phthalates), **T2** (200 µg/kg/day mixture), and **T3** (200 mg/kg/day mixture). Exposure was administered via gavage from gestational day 10 to postnatal day 21. On postnatal day 120, the offspring were euthanized and the dorsolateral prostate of each male was collected and subjected to histopathological and morphometric-stereological analyses, using hematoxylin-eosin staining. The results showed that group C presented a typical glandular structure, with predominance of acini with wide lumens, lined by simple columnar epithelial cells. In contrast, exposure to the mixture of phthalates (T1, T2, T3) caused epithelial disorganization and induced a greater degree of folding towards the lumen, culminating in a significant increase in the volume of the epithelial compartment and a decrease of the luminal compartment, as confirmed by stereological analysis. Notably, the most significant alterations were observed in the T2 group. These results suggest that perinatal exposure to phthalates can modify prostate morphogenesis, altering tissue morphology in the adult gland, which may be associated with the carcinogenic process. Approved by Ethical Committee: 1040-CEUA/Institute of Biosciences

- UNESP Funding Support: FAPESP:2024/20520-3.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Changes in hypothalamic norepinephrine activity associated with hot-flash symptoms in estrogen-deficient female rats

Authors:

Tunde Femi Abraham¹
Kaoma Stephani da Costa Silva¹
Andre Fernandez Gomes¹
Raphael Escorsim Szawka¹

Affiliations:

¹Universidade Federal de Minas Gerais, Belo Horizonte, Brazil.

Keywords: Arcuate nucleus. Estradiol. Hot flash. Norepinephrine. Preoptic area.

Abstract:

Hot flashes (HF) are caused by estradiol (E2) leading to disruption of normal thermoregulatory responses in the preoptic area (POA). Norepinephrine (NE) neurons project to the POA and arcuate nucleus of hypothalamus (ARC) and is associated with HF. We investigated changes in NE activity in subregions of the POA and ARC associated with HF in the ovariectomized (OVX) rat model. Rats (CEUA:246/2024) were sham operated or OVX and randomly assigned to 3 groups: Sham on diestrus (n = 8), OVX (n = 8), and OVX treated with E2 (OVX+E2, n = 8). Tail temperature sensors were implanted subcutaneously to measure tail skin temperature (Tskin). Fifteen days after ovariectomy, Tskin were recorded every 5 min for 1 h at 24 ± 0.5 °C. Brains were collected for microdissections of the median preoptic area (MnPO), medial preoptic area (MPO), middle ARC (mARC) and posterior ARC (pARC) to quantify the concentration of NE and its metabolite 3-methoxy-4-hydroxy-phenylglycol (MHPG) by HPLC. Tskin significantly increased in OVX rats compared with diestrus ($P < 0.001$), which was prevented in the OVX+E2 group. Concomitantly, MHPG levels were higher in the MPO of OVX rats compared with the OVX+E2 group ($P < 0.05$) and there was a trend towards increase in the MHPG/NE ratio in OVX rats ($P = 0.06$). NE concentrations in the pARC were higher in OVX rats than in the other groups ($P < 0.05$), while MHPG levels were increased OVX rats compared with diestrus ($P < 0.05$). No change in NE parameters were found in the MnPO or mARC. OVX rats display increase in Tskin, which is associated with higher NE release in the MPO and pARC. The MPO is a key hypothalamic nucleus involved in thermoregulation and neurokinin-B neurons in the ARC have been linked to increased heat dissipation. Thus, our findings suggest that NE projections to the MPO and pARC play a role in the generation of HF in females under E2 deficiency.

Financial support: FAPEMIG, CNPQ, CAPES.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Raloxifene therapy attenuates the development of MNU-induced prostatic tissue lesions in rat

Authors:

Liara Botão da Silva¹
Ana Clara Pacheco de Santana²
Vinicius Luis Rocha da Silva Maria²
Lívia Centofanti Cózaro¹
Maria Clara Betete da Silva Fonseca¹
Júlia Landeira Gomez Chamelete¹
Sergio Pereira¹
Cristiane Figueiredo Pinho¹

Affiliations:

¹ Faculty of Sciences, São Paulo State University, Bauru, Brazil.

² Institute of Biosciences, São Paulo State University, Botucatu, Brazil.

Keywords: Histopathology; morphology; Prostate Cancer; Raloxifene.

Abstract:

Prostate Cancer (PCa) is the second most diagnosed malignant neoplasm in Brazil and the fifth leading cause of death among men in the world. PCa ability to progress to a non-responsive phase to androgen deprivation therapy emphasizes the importance of study on adjuvant treatments. Recent research suggests estrogen receptors as possible therapeutic targets in neoplasias and they can be modulated by drugs such as Raloxifene. In this context, the present study evaluated the histoprotective effects of Raloxifene therapy on the prostate of rats induced to prostatic carcinogenesis through exposure to the carcinogen N-metil-N-nitrosourea (MNU). Thereunto, 20 male Fisher 344 rats were inoculated with MNU (15 mg/kg) in the prostate and received subcutaneous doses of testosterone cypionate twice a week for 220 days. Then, they were divided into two experimental groups: Raloxifene (10 mg/kg of body weight/day of Raloxifene for 30 consecutive days); Induced (corn oil for 30 consecutive days). Another 10 animals belonging to the Control group received only corn oil for the same period. After euthanasia, prostate was collected and subjected to histopathological and morphometric-stereological analyses. The results showed that Induced group presented epithelial disorganization and a greater degree of folding towards the lumen, culminating in a significant increase in the volume of the epithelial compartment and a decrease of the luminal compartment, as confirmed by stereological analysis. Additionally, this group presented the highest indices of inflammatory, premalignant and malignant lesions, including microinvasive adenocarcinomas. On the other hand, the Raloxifene treatment was responsible for reducing the appearance of epithelial atypia, dysplasia and NIP, with no animal presenting inflammatory foci and adenocarcinoma. These data suggest that Raloxifene had a histoprotective effect, attenuating histopathological alterations caused by the process of tumor induction by MNU. Approved by Ethical Committee: 718-CEUA/ Institute of Biosciences – UNESP.

Funding Support: FAPESP:2023/12378-0.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Toxic effects of tributyltin and cadmium co-exposure on the reproductive axis in Wistar rats

Authors:

Amanda dos Santos Nunes¹
Jones Bernardes Graceli²

Affiliations:

¹Instituto Federal do Espírito Santo.
²Southern Illinois University, USA.

Keywords: Cadmium, endocrine disruptor, heavy metals, female reproduction, tributyltin.

Abstract:

Daily exposure to chemicals in water and food poses risks to human health. Cadmium (Cd) and tributyltin (TBT) are known for their harmful effects on the endocrine system, as well as on female reproductive health, acting as endocrine-disrupting chemicals. However, the effects of co-exposure (Cd+TBT) to these substances remain poorly understood. This study aimed to investigate the impact of co-exposure on the reproductive axis of Wistar rats (8–10 weeks). Rats were divided into two groups: control (CON, n=9) at doses of saline and treated with TBT+Cd (MIX, n=10), at doses of 100 ng/kg/day and 100 mg/L. The estrous cycle was monitored for 15 days. After treatment, tissues were histologically analyzed using H&E and Alcian Blue, and hormone levels were measured by ELISA kits. The study was approved by CEUA-UFES (04/2023). Data were expressed as mean $\pm$ SEM. Exposure to MIX resulted in increased uterine weight (CON: 0.39 ± 0.029 ; MIX: 0.48 ± 0.020 , $p < 0.05$) and reduced ovarian weight (CON: 0.13 ± 0.006 ; MIX: 0.11 ± 0.007 , $p < 0.05$). Estrous cycle duration was shorter (CON: 7.16 ± 0.59 ; MIX: 5.65 ± 0.28 , $p < 0.05$), with reduced proestrus phase (CON: 2.23 ± 0.36 ; MIX: 0.93 ± 0.06 , $p < 0.05$). Estrogen and testosterone levels decreased (E2: CON: 29 ± 3.16 ; MIX: 19 ± 2.86 ; T: CON: 0.60 ± 0.04 ; MIX: 0.33 ± 0.02 , $p < 0.05$). The ovarian reserve was reduced (CON: 0.31 ± 0.04 ; MIX: 0.11 ± 0.02 , $p < 0.05$), along with fewer corpora lutea (CON: 0.29 ± 0.07 ; MIX: 0.11 ± 0.03 , $p < 0.05$). Uterine analysis showed increased myometrium and luminal epithelium thickness (CON: 1.36 ± 0.07 ; MIX: 1.74 ± 0.06 ; CON: 15.6 ± 1.1 ; MIX: 20.8 ± 1.4 , $p < 0.05$), and reduced endometrial area (CON: 0.57 ± 0.04 ; MIX: 0.49 ± 0.007 , $p < 0.05$). Mast cell increase indicated ovarian inflammation (CON: 0.40 ± 0.19 ; MIX: 1.29 ± 0.23 , $p < 0.05$) and uterus (CON: 0.0009 ± 0.0002 ; MIX: 2.32 ± 0.40 , $p < 0.05$). These findings demonstrate that co-exposure to MIX has detrimental effects on reproductive structure and function in female rats.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Impact of maternal periodontal disease on offspring: miRNA expression modulation and long-term health consequences

Authors:

Camila Conrado França¹
Maria Sara De Lima Coutinho Mattera¹
Bianca Elvira Belardi¹
Ana Carla Thalez Ywabuchi Nobumoto¹
Anna Clara Cachoni²
Bruna de Oliveira Alves¹
Edilson Ervolino¹
Estêvão Lopes Pereira²
Angela Vermelho Sarraceni¹
Anielly Gonsalves¹
Lorena Umbelino Rodrigues¹
Kelly Fernanda da Silva Soares¹
Mariana Campanha Hilário Pereira¹
Marco De Luca Monteiro Sturaro¹
Doris Hissako Matsushita¹

Affiliations:

¹ Department of Basic Sciences, São Paulo State University (UNESP), School of Dentistry, Araçatuba, Brazil. (Multicenter Postgraduate Program in Physiological Sciences). ² Department of Preventive and Restorative Dentistry, São Paulo State University (UNESP), School of Dentistry, Araçatuba, Brazil. (Graduate Program in Dental Science).

Keywords: Periodontal disease. miRNA.

Abstract:

The fetal programming hypothesis suggests that stimuli or insults during intrauterine life can result in permanent changes in the offspring's physiology and metabolism, increasing the risk of diseases in adulthood. Previous studies have shown that maternal periodontal disease-PD promotes insulin resistance-IR, increases plasma cytokine concentrations, and reduces GLUT4 content. Maternal PD was able to activate inflammatory pathways in the muscle tissue of adult offspring, as evidenced by increased expression of TNF- α , NF- κ Bp65/p50, IKK α / β , and ERK1/2. More studies are needed to investigate other mechanisms involved in these alterations, such as miRNA expression patterns in other adipose tissues of adult offspring. Aim: To evaluate, in adult rats born to mothers with periodontal disease, the global expression of miRNAs. The rats were divided into two groups: PD group, in which the disease was induced by silk thread ligature around the first lower molar, and control rats-CN. After seven days of ligature placement, rats from both groups were placed for mating, and vaginal smears were performed daily to confirm the day of copulation. When the male offspring reached 75 days of age, global miRNA expression (microarray technique) was analyzed in adipose tissue. Results: Maternal PD led to low birth weight in offspring and, in adulthood, modulation of 12 miRNAs [5 upregulated (miR-25-5p; miR-3120; miR-3562; miR-702-5p; miR-187-5p) and 7 downregulated (miR-128-3p; miR-28-3p; miR-29c-3p; miR-18a-5p; miR-10b-3p; miR-138-5p; miR-3557-3p)]. miR-128a has been shown to be negatively controlled by TNF- α , a cytokine that contributes to inflammation in diabetes, demonstrating that maternal PD can modify microRNAs expression important for the development of IR. These results demonstrate the short-term impact of periodontal disease on intrauterine life and its long-term effects on the predisposition to certain diseases in adulthood (CEUA 00407-2019).

This study was financed, in part, by the São Paulo Research Foundation (FAPESP) [grant #2019/04183-9; #2022/08872-6; #2023/03786-7; #2023/12488-0; #2023/01400-4; #2022/04868-4] São Paulo, SP, Brazil.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Effects of Peruvian Maca (*Lepidium meyenii*) on ovariectomized rats: Behavioral, Biometric and Brain Oxidative Stress Markers

Authors:

Anna Karolina Kretschmann Florencio de Souza Bagetti¹
Elisângela de Fátima Balzan Valentini¹
Lucas Machado Sulzbacher^{1,2}
Samara Nicole Friske¹
Paula Taís Friske^{1,2}
Giuseppe Potrick Stefani^{1,3,4}
Thiago Gomes Heck^{1,2,5}
Mirna Stela Ludwig^{1,2}

Affiliations:

¹ Physiology Research Group (GPeF), Regional University of the Northwest of the State of Rio Grande do Sul (UNIJUÍ), Ijuí, RS, Brazil.

² Postgraduate Program in Comprehensive Health Care (PPGAIS). Regional University of the Northwest of the State of Rio Grande do Sul (UNIJUÍ), Ijuí, RS, Brazil.

³ Olympic Studies Research Group (GPEO). School of Health and Life Sciences. Pontifical Catholic University of Rio Grande do Sul (PUCRS). Porto Alegre, RS, Brazil.

⁴ Postgraduate Program in Nutrition Sciences (PPGNUT). Federal University of Health Sciences of Porto Alegre (UFCSA). Porto Alegre, RS, Brazil, Ijuí, RS, Brazil.

⁵ Postgraduate Program in Mathematical and Computational Modeling (PPGMMC), Regional University of the Northwest of the State of Rio Grande do Sul (UNIJUÍ), Ijuí, RS, Brazil.

Keywords: Anxiety. Brain. Maca. Menopause. Oxidative Stress.

Abstract:

Menopause is associated with metabolic and behavioral alterations. Peruvian maca (MACA, *Lepidium meyenii*) has been demonstrated as a strategy to attenuate postmenopause effects by its antioxidant properties. We aim to evaluate whether MACA supplementation may produce alterations in the biometric, biochemical, and behavioral variables in ovariectomized rats. Wistar rats (n=27, 5 months of age) were divided into 4 groups: Control (C, n=5); Ovariectomy (OVX, n=6); Maca (M, n=8); Maca+Ovariectomy (M+OVX, n=8). The rats were ovariectomized or sham operated (Phase I) and, after 4 weeks (Phase II), they received MACA (1 g/kg/day o.v.) for 28 days. Body mass, feed intake, and behavior (Elevated Cross test) were assessed. After euthanasia, brain oxidative stress was analyzed. Data were evaluated by 3-way-ANOVA (Time x MACA x OVX) or 2-way-ANOVA (MACA x OVX). CEUA 023/21. Ovariectomized rats presented higher feed consumption compared to the other groups (OVX factor $P<0.001$), with effect of time ($P<0.001$) and MACA consumption ($P=0.007$), with interaction (OVX x MACA, $P=0.016$). However, body weight gain was influenced by time ($P=0.002$) and OVX ($P=0.002$), with no effect of MACA consumption ($P=0.074$) or interaction ($P=0.077$). In the Elevated Cross test, there was a decrease in the time spent in the open arm (Phase I vs. Phase II, $P<0.001$), without effects of MACA ($P=0.088$) or OVX ($P=0.094$), but with interaction ($P=0.021$). The time spent in the center of the elevated cross was not affected by the time ($P=0.308$), MACA ($P=0.634$), OVX ($P=0.088$), or interaction ($P=0.404$). Also, there were no effects on brain lipid peroxidation levels or SOD and CAT enzymes activities by MACA, OVX or their interaction ($P>0.05$). The consumption of MACA may affect feed intake and behavior in the postmenopausal period, but does not modify body mass or redox state in the brain. CNPq, 407329/2016-1, 444286/2024-1, 403136/2024-5, 405546/2023-8, 307926/2022-2 to TGH, CAPES, FAPERGS, UNIJUÍ.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Effect of perinatal exposure to BPA and glyphosate on prostate morphology in gerbil males

Authors:

Lorena Gabriela de Souza^{1,2}
Gervásio Evangelhista Brito-Filho²
Luara Jesus Ferrato^{1,2}
Vinícius Savazo Assis²
Leonardo Araújo Caires de Aguiar²
Thalles Fernando Rocha Ruiz^{1,2}
Sebastião Roberto Taboga^{1,2}

Affiliations:

¹ Universidade Estadual de Campinas (UNICAMP).

² Universidade Estadual Paulista (UNESP).

Keywords: Prostate. Gerbil. Bisphenol-A. glyphosate. Morphology.

Abstract:

Exposure to endocrine disruptors (EDCs) affects the morpho-physiology of biological systems, with a greater impact on endocrine pathways, such as hormone-responsive organs. Bisphenol A (BPA), a xenoestrogen, acts through estrogenic pathways and alters the morphophysiological properties of the prostate. Among herbicides, one of the most used is glyphosate, whose harmful effects on other organs have been described, and is also a xenoestrogen. In this study the effects of the two composts on the prostate of gerbil males exposed during the perinatal period are comparatively evaluated. Three experimental groups (N=18) were used: control, BPA (50 µg/kg/day), and glyphosate (70 mg/kg/day). The prostate of the males was analyzed during adulthood (6 months of age). Histopathological analyses to assess morphological changes in the prostatic epithelium were performed. Statistical analysis: ANOVA or the Kruskal-Wallis test ($p < 0.05$). Histopathological analyses showed an increase in the number of microinvasive carcinoma (5.6 ± 1.37) and intraluminal inflammation (8.48 ± 3.57) in the glyphosate- treated group compared to the control (0.48 ± 0.07 and 2.08 ± 0.62 , respectively) and the BPA groups (0.43 ± 0.16 and 1.63 ± 0.18 , respectively). There is also an increase in prostatic epithelial neoplasia (PIN) in the glyphosate group (22.55 ± 6.01) compared to the control (10.06 ± 1.93). The BPA-treated males showed a reduction in the number of atrophies (5.85 ± 1.08) compared to the glyphosate (12.13 ± 0.84). Preliminary results point to glyphosate favouring a pro-proliferative imbalance, observed in the presence of proliferative alterations, such as PIN and microinvasive carcinoma. In addition, the endocrine dysregulation caused by exposure to glyphosate, as already described in other organs, seems to influence the inflammatory process in the prostate. However, further analysis is needed to understand the real effects of BPA and glyphosate on the prostate.

CEUA/IBILCE 263/2024.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Follicle-stimulating hormone pulsatile secretion in female rats shows higher synchronicity with luteinizing hormone in the absence of estradiol

Authors:

Ana Clara Campideli-Santana¹
Kaoma Stephani da Costa Silva¹
Roberta Araujo- Lopes¹
José Donato Jr²
Raphael Escorsim Szawka¹.

Affiliations:

¹ Departamento de Fisiologia e Biofísica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brasil.

² Departamento de Fisiologia e Biofísica, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, São Paulo, Brasil.

Keywords: FSH. LH. Estrous cycle. Rat. Pulses.

Abstract:

Estradiol (E2) inhibits luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion through the negative-feedback effect. LH pulsatile secretion is driven by the equally pulsatile release of gonadotropin-releasing hormone (GnRH) in the pituitary portal blood. In turn, a similar pulsatile secretion of FSH has been reported only in ovariectomized (OVX) rat models and little is known about its features and control. Here, we investigated the pulsatile secretion of FSH in female rats under different E2 conditions and determined the synchronicity between LH and FSH pulses. Tail-tip blood samples were collected at 6-minute intervals for 180 minutes on diestrus (n = 6), OVX (n = 6), and OVX treated with E2 (OVX+E2; n = 5) rats (Ethics committee protocol # 272/2020). FSH and LH levels were measured in the same blood samples by ultrasensitive ELISA. FSH pulsatile secretion was detected in diestrus, OVX, and OVX+E2 rats. On diestrus, LH and FSH pulse frequencies were 1.78 ± 0.69 and 1.67 ± 0.52 pulses/hour, respectively. OVX rats displayed increased pulse frequency, baseline, and mean levels of LH and FSH compared with diestrus ($P < 0.01$). E2 replacement in OVX+E2 rats decreased the pulse frequency ($P < 0.01$) and mean levels ($P < 0.05$) of LH and FSH compared with OVX rats. However, the amplitude of FSH pulse, but not of LH, decreased in OVX+E2 compared with OVX and diestrus rats ($P < 0.01$). The percentage of FSH pulses happening simultaneously with LH pulses, considering total number of LH pulses as 100%, was altered by the E2 milieu, being $54 \pm 15\%$, $75 \pm 11\%$, and $46 \pm 22\%$ in diestrus, OVX, and OVX+E2 rats, respectively ($P < 0.05$). Our data demonstrate that FSH exhibits a pulsatile pattern of secretion negatively modulated by E2 in female rats. Furthermore, the synchrony of LH and FSH pulses is higher in the absence of E2, suggesting that E2 interferes more with the response of FSH than of LH to the GnRH stimulation.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Selective celiac vagal transection avoids hyperinsulinemia without alter adiposity on hypothalamic obese rats

Authors:

Helen de França Kailer¹
Bruna SchumAker Siqueira¹
Vanessa Marieli Ceglarek²
Sandra Lucinei Balbo¹
Sabrina Grassioli¹

Affiliations:

¹ Universidade Estadual Do Oeste do Paraná – UNIOESTE.

² Universidade Federal do Rio Grande do Sul – UFRGS.

Keywords: Obesity. Pancreas. Vagotomy.

Abstract:

The Vagus Nerve (VN) hyperactivity is associated with the development of hyperinsulinemia and obesity. Total subdiaphragmatic transection of VN induces reduction in adiposity and improvement in insulin secretion and action. The celiac vagal branch is primarily responsible for pancreatic innervation and could avoid hyperinsulinemia presenting lesser collateral effects. Here we compare the effects of Subdiaphragmatic Vagotomy (SV) and Celiac Vagotomy (CV) on adiposity, metabolism and histomorphometry of pancreatic islets in rodents with hypothalamic obesity. Ethics Committee Approval nº 38-19. Obesity (OB) was induced by neonatal treatment with monosodium glutamate (4g/kg). Control animals (CT) received equimolar saline. At 60 days, OB animals were randomly subdivided into subgroups according to surgical procedures: OB-SHAM; OB-SV and OB-CV. At 150 days, the rats were euthanized, and adiposity parameters [White Adipose Tissue (WAT) and Lee Index (LI)]; plasma variables (glycemia and insulin) and islets histology (size and number) were assessed. Despite lower body weight ($p=0.002$) OB-SHAM rats had higher LI ($p=0.0371$), WAT content ($p=0.0031$), and insulin ($p=0.014$) compared to CT rats; without changes in the glycemia, number and size of pancreatic islets. OB-SV and CV groups reduced LI ($p<0.05$) and plasma insulin concentration ($p<0.05$), as well as increased pancreatic weight ($p<0.05$) in relation to OB-SHAM rats. However, only OB-CV reduced islets number compared to OB-SHAM ($p=0.0315$). OB-SV showed significantly reduced inguinal WAT ($p=0.0152$) and increased stomach weight ($p=0.007$) compared to the OB-SHAM group; an effect not found in the OB-CV rats. None of the surgeries had an effect on the retroperitoneal WAT ($p>0.05$). Conclusion: CV was effective in reducing the number of pancreatic islets, reducing plasma insulin, without altering adiposity, an effect more evident in SV, suggesting that vagal control of WAT maybe mediated by different vagal branches.



XXXIX REUNIÃO ANUAL DA FeSB
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Selective activation of tuberoinfundibular dopaminergic neurons suppresses prolactin and stimulates luteinizing hormone secretion in male mice

Authors:

Matheus Viana¹
Ana Clara Campidelli-Santana¹
Isabele Beatriz Pereira Alves¹
Sílvia Carolina Guatimosim¹
José Donato Jr²
Raphael Escorsim Szawka¹

Affiliations:

¹ Departamento de Fisiologia e Biofísica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brasil.

² Departamento de Fisiologia e Biofísica, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, São Paulo, Brasil.

Keywords: TIDA. Dopamine. Luteinizing hormone. Pulses. DREADD.

Abstract:

Tuberoinfundibular dopaminergic (TIDA) neurons, located in the arcuate nucleus of the hypothalamus (ARC), inhibit prolactin (PRL) secretion. PRL autoregulates its secretion activating dopamine release from TIDA neurons and suppresses luteinizing hormone (LH) secretion inhibiting gonadotrophin-releasing hormone (GnRH) release. Because GnRH neurons are inhibited by dopamine, we hypothesized that TIDA neurons may also control LH secretion. Male C57BL6 dopamine transporter (DAT)- and tyrosine hydroxylase (TH)-Cre mice, expressing Cre-recombinase under the control of DAT or TH promoter, respectively, were bilaterally injected intra-ARC with an adeno-associated-virus (rAAV-hSyn-DIO-hM3D(Gq)-mCherry-WPRE-hGH; 2×10^{12} vg/mL) encoding a Cre-dependent DREADD excitatory receptor. Wild-type (WT) mice intra-ARC injected with vehicle were used as control (CEUA 240/2022). After 1 week, between 8 and 11 h, mice were administrated with clozapine N-oxide (CNO; 1.5 mg/kg, i.p.) or saline, randomly on two different occasions 1-week apart, 15 min before collection of 4uL tail-tip blood samples at 6 min intervals over 120 min for PRL and LH pulse analysis. Hormone levels were determined by ELISA. CNO treatment reduced PRL release by half during the 2h period evaluated in both DAT-Cre and TH-Cre mice ($P < 0.01$), confirming activation of TIDA neurons. Pulse analysis showed that CNO increased LH pulse amplitude and mean LH in both DAT-Cre and TH-Cre mice ($P < 0.05$), with no effect on pulse frequency or baseline levels. CNO altered neither PRL nor LH in WT mice. The selective activation of TIDA neurons suppresses PRL secretion in both DAT-Cre and TH-Cre male mice, likely due to the expected increase in dopamine release. Surprisingly, TIDA neuron activation enhanced the LH pulse amplitude and overall secretion, probably caused by the corelease of a yet-to-be-determined excitatory neurotransmitter to GnRH.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Estrogen Replacement Therapy and Its Effects on BPA-Induced Ovarian Disruption in Mongolian gerbils

Authors:

Clara Bazi de Sousa¹
Thalles Fernando Rocha Ruiz^{1,2}
Lorena Gabriela De Souza^{1,2}
Luara Jesus Ferrato^{1,2}
Stella Bicalho Silva¹
Vitor Grigio¹
Patrícia Simone Villamaior¹
Sebastião Roberto Taboga^{1,2}

Affiliations:

¹ UNIVERSIDADE ESTADUAL PAULISTA – UNESP, São José do Rio Preto.
² INSTITUTO DE BIOLOGIA – UNICAMP, Campinas.

Keywords: Endocrine disruptor. hormone replacement. ovaries.

Abstract:

Exposure to the endocrine disruptor (ED) bisphenol A (BPA), a polycarbonate plastic monomer with estrogenic activity, during critical developmental windows can significantly compromise female reproductive functions. This study aimed to investigate the ovarian effects of estrogenic replacement therapies (ERT) following endocrine disruption caused by BPA during the pregnancy-lactation windows of susceptibility. In accordance with the Ethics Committee Protocol: CEUA/IBILCE 240/2022, female Mongolian gerbils were exposed to 50 µg/kg/day of BPA for 39 days during pregnancy and lactation, followed by six months of ERT: 17β-estradiol (E2) at 0.5 mg/kg/day, ethinylestradiol (EE2) at 35 µg/kg/day, and the phytoestrogen genistein (GEN) at 240 mg/kg/day. At 18 months of age, the animals were euthanized, and their ovaries were analyzed by histopathology and Western blotting. Serum hormone levels were evaluated using ELISA. Statistical analyses were performed using one-way ANOVA with Tukey's post-hoc test or Kruskal-Wallis with Dunn's test ($p < 0.05$). Control ovaries exhibited low densities of primordial, primary, and secondary follicles. BPA impaired follicular development by reducing the number of secondary follicles while increasing primordial and primary follicles. ERT treatments stimulated all types of growing follicles, indicating a protective effect on ovarian function. Slight reductions in atresia were observed following E2 and EE2 treatments. BPA decreased estrogen receptor alpha (ERα) and androgen receptor (AR), while increasing ERβ. ERT restored ERα, without altering AR. BPA downregulated 17β-HSD and upregulated 5α-reductase. ERT increased aromatase expression in follicles and interstitial cells. Therefore, it was demonstrated that ERT partially counteracts BPA's adverse effects on ovarian function, preserving follicular reserve and modulating receptor and enzyme expression, indicating a protective hormonal influence.



XXXIX REUNIÃO ANUAL DA FeSB
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Vagus nerve modulates oral glucose patterns in hypothalamic-obese rats through a splenic-dependent mechanism

Authors:

Bruna Schumaker Siqueira¹
Vanessa Marieli Ceglareck²
Marianela Andrea Díaz Urrutia¹
Caroline de Maman Oldra¹
Sabrina Grassioli¹

Affiliations:

¹ Programa de Pós-graduação em Biociências e Saúde, Universidade Estadual Do Oeste do Paraná – Campus Cascavel, Cascavel, Paraná, Brazil.

² Programa de Pós-graduação em Ciências Biológicas – Fisiologia; Departamento de Fisiologia, Instituto de Ciências Básicas da Saúde da Universidade Federal do Rio Grande do Sul – UFRGS.

Keywords: Obesity; Glucose; Vagus Nerve; Spleen.

Abstract:

Introduction: The vagus nerve (VN) innervates endocrine pancreas, liver and intestine exerting strong influences on glucose homeostasis. Anti-inflammatory vagal reflex, a mechanism spleen dependent, would have repercussion on glycemia. Objective: Here we evaluated the impact of VN and spleen absence on oral glucose pattern in the hypothalamic obese rats. Methodology: Male Wistar rats were induced to hypothalamic obesity through neonatal administration of monosodium glutamate (MSG, 4 g/kg). Control (CT) animals received equimolar saline (1.25 g/kg). At 60 days of age, MSG rats underwent either Sham surgery, splenectomy (SPL), vagotomy (VAG), or a combined SPL+VAG procedure. An oral glucose tolerance test (OGTT; 2g/Kg) was performed in the 150-old days. The body weight (BW), naso-anal length (NAL), visceral white adipose tissue (WAT-v) and fasting plasmatic values of insulin, glucose, and triglyceride were done. Results: Compared to CT animals, the MSG rats had lower BW (-23%), NAL (-13%), increased WAT-v content (+25%), hyperinsulinemia (+86%), and hypertriglyceridemia (+42%), besides delayed time to glycemic peak during OGTT (+235%). MSG-SPL rats showed lower insulinemia (-378%); higher glucose (+28%) and triglycerides (+47%) in relation to MSG-sham, without altering OGTT pattern. MSG-VAG rats had smaller BW (-16%), WAT-v (-77%), insulinemia (-272%), and triglycerides (-45%) with higher glycemic peak (+13%) and reduced time to peak (-196%) compared to MSG-Sham. In the SPL+VAG rats were noted lower insulinemia (-415%) and increased glucose levels (+37%) without modifying pattern of OGTT. Conclusion: Hypothalamic obese rats showed perturbed oral tolerance characterized by prolonged time to glycemic peak, a response that elevated risk for diabetes. The VN transection restores time to glucose peak on MSG-obese, a response spleen-dependent, suggesting that vagus-spleen interaction plays a central role in modulating glucose tolerance during hypothalamic obesity.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Role of GABAergic Transmission from Kisspeptin Neurons in Sexual Maturation and Fertility: Insights from Vgat Ablation Study

Authors:

Henrique Rodrigues VIEIRA¹

Estela Guidini FERRUFINO¹

José DONATO JR.²

Renata FRAZÃO¹

Affiliations:

¹ Universidade de São Paulo, Instituto de Ciências Biomédicas, Departamento de Anatomia, São Paulo, Brazil.

² Universidade de São Paulo, Instituto de Ciências Biomédicas, Departamento de Fisiologia e Biofísica, São Paulo, Brazil.

Keywords: Hypothalamic-pituitary-gonadal axis. Luteinizing hormone. Puberty. Estrous cycle cyclicity. Hypothalamic gene expression.

Abstract:

GABAergic transmission plays a vital role in reproductive functions by controlling the secretion of the gonadotropin-releasing hormone. Most kisspeptin neurons are GABAergic cells, but it is not clear whether GABAergic transmission from kisspeptin cells is required for puberty onset and fertility of female and male mice. To determine the role of GABAergic transmission from kisspeptin cells, we used brain slices obtained from female *Vgat*-Cre/GFP mice to determine the percentage of kisspeptin-ir cells that coexpress *Vgat* (CEUA: 2903071119). In female mice, most of the kisspeptin neurons located at the anteroventral periventricular and rostral periventricular nuclei are GABAergic, and the percentage of GABAergic kisspeptin neurons did not vary along the estrous cycle ($P > 0.05$). Next, we use Cre-loxP technology to induce the selective ablation of *Vgat* from kisspeptin cells (*Kiss1*-Cre/*Vgat*^{flox/flox}) or not (control). We evaluate the reproductive and metabolic profiles of *Kiss1*-Cre/*Vgat*^{flox/flox} and control female and male mice. *Kiss1*-Cre/*Vgat*^{flox/flox} animals exhibited increased body weight ($P < 0.05$), while total fat and lean mass was similar ($P > 0.05$) compared to control mice. The lack of *Vgat* expression in kisspeptin cells was sufficient to downregulate *Vgat*, *Gnrh1*, and *Kiss1r* expression in the anteroventral periventricular nucleus and *Gnrh1* mRNA in the arcuate nucleus of the hypothalamus compared to control mice ($P < 0.05$). *Vgat* gene ablation from kisspeptin cells did not disrupt puberty onset or fertility of female or male mice ($P > 0.05$), even though female *Kiss1*-Cre/*Vgat*^{flox/flox} tended towards lower luteinizing and follicle-stimulant hormones serum levels at the proestrus ($P = 0.06$). Our results reinforce that GABAergic transmission from kisspeptin neurons contributes to the estrogen-positive feedback mechanism but is not required for sexual maturation or fertility, suggesting that these phenomena are not dependent on GABAergic transmission from kisspeptin cells.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: LH and FSH secretion in male and female rats determined by highly sensitive ELISA

Authors:

André Fernandes Gomes¹
Roberta Araújo Lopes¹
Mariana de Souza Santos¹
Ana Clara Campideli Santana¹
Soraia Macari²
Raphael Escorsim Szawka¹

Affiliations:

¹ Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas
² Universidade Federal de Minas Gerais, Faculdade de Odontologia

Keywords: Gonadotropins. Elisa. Rats.

Abstract:

Serum levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are key in diagnosing reproductive disorders. Accurate hormonal measurement in both animals and humans is essential. We developed a novel, highly sensitive ELISA for FSH and used tail-tip blood sampling to assess longitudinal LH and FSH secretion in male and female rats under different hormonal conditions. Blood was collected hourly. All procedures were approved by the Ethics Committee for the Use of Animals (CEUA Nº 216/2022). Castration surgeries (orchietomy – ORX and ovariectomy – OVX) simulated high LH/FSH levels; SHAM surgeries served as controls. In Experiment 1, the ELISA was validated via a parallelism test, confirming no interference by rat plasma. In Experiments 2–4 (cross-over model), each rat served as its own control. The ELISAs detected increased LH/FSH secretion in OVX rats versus SHAM (diestrus). In ORX males (n=6), LH and FSH significantly rose vs. intact ($p<0.01$ and $p<0.001$). After testosterone treatment (ORX+T), LH returned to baseline ($p<0.01$), and FSH was partially restored ($p<0.05$). Across the estrous cycle, LH/FSH were basal during diestrus (n=7) and peaked in proestrus (n=6); on estrus (n=6), LH normalized while FSH declined. OVX increased both hormones vs. diestrus ($p<0.01$ and $p<0.001$), but estradiol treatment (OVX+E2) decreased them in the morning, especially FSH. In the afternoon, E2 induced an LH surge (17–18h) and a gradual FSH rise (17–19h). These data provide a recharacterization of LH and FSH secretion in rats using ELISA from tail-tip samples in a longitudinal design.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Sexual dimorphic effect of the ablation B2R from kisspeptin cells in metabolic and reproductive profile thought development and adulthood in mice

Authors:

Henrique Rodrigues Vieira¹
José Dotano Jr.²
Frederick Wasinski³
Renata Frazão¹

Affiliations:

¹ Department of Anatomy, Institute of Biomedical Sciences - University of São Paulo.
² Department of Physiology and Biophysics Institute of Biomedical Sciences - University of São Paulo, São Paulo, Brazil.
³ Neurology and Neurosurgery of Paulista Medical School - Federal University of São Paulo, São Paulo, Brazil.

Keywords: neuroendocrinology, hypothalamic-hypophyses-gonads axis, metabolism, kallikrein-kinin system, sexual dimorphism.

Abstract:

The sexual steroids feedback under gonadotropin-releasing hormone (GnRH) neuron activity depends on kisspeptin neurons, which are sited in the anteroventral periventricular nucleus and rostral periventricular nucleus (AVPV/PeN) and arcuate nucleus of the hypothalamus (ARH). The bradykinin (Bk) receptors, type I (B1R) and type II (B2R), are distributed in those areas of various species. Bk stimulates GnRH release in male and female rats in hypothalamic explants via B2R but not B1R. Whether B2R plays a role in the kisspeptin neurons is unclear. To verify the role of B2R signalization from kisspeptin cells, we crossed female and male C57BL6 mice carrying Cre-LoxP to induce a selective ablation of the gene *Bdkrb2* from kisspeptin cells (B2RKO) or not (CEUA: 4138240719). Male and female mice's reproductive and metabolic profiles were analyzed until 90 days old. B2R ablation from kisspeptin cells downregulates *Bdkrb2* gene expression in the AVPV/PeN ($P < 0.05$). Also, B2RKO female showed a downregulation of *Kiss1* and *Kiss1r* in the POA ($P < 0.05$). No difference was found in the *Kiss1*, *Bdkrb1*, and *Bdkrb1* gene expression of ARC, AVPV/PeN (males), brown adipose tissue, liver, testicle, and ovary ($P > 0.05$). The lack of *Bdkrb2* on kisspeptin cells induced a later puberty onset in male mice, despite no effect on female B2RKO ($P > 0.05$). Female and male B2RKO mice exhibited lower body weight than the control group. In adulthood, male B2RKO mice presented lower sperm viability and normal morphology compared to the control group ($P < 0.05$), whereas females exhibited no changes in the reproductive profile ($P > 0.05$). Female B2RKO mice showed an increased respiratory exchange ratio at night ($P < 0.05$), while a similar effect was observed during the day for male mice compared to the control animals ($P < 0.05$). In conclusion, lack of B2R in kisspeptin neurons leads to impaired male sperm quality and disbalances the energy homeostasis in both sexes along the lifespan of mice.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Modulation of genes related to mitochondrial dynamics and mitophagy in the testis of hypothyroid rats

Authors:

Lorena Cristina de Souza e Souza¹
Roberta da Fonseca Coutinho Pontes¹
Juliana Santos Romão¹
Karen De Jesus Oliveira¹

Affiliations:

¹ University Federal Fluminense.

Keywords: Autophagy. Fusion. Fission. Thyroid hormones.

Abstract:

Thyroid hormones (TH) regulate testicular functions and hypothyroidism can impair male gonadal function. Mitochondria are crucial for maintaining testicular homeostasis. They undergo dynamic processes such as fission and fusion and damaged mitochondria can be degraded through mitophagy. However, the influence of hypothyroidism on mitochondrial dynamics in the testis remains incompletely understood. Male Wistar rats (n=16) were divided into euthyroid (n=8) and hypothyroid (n=8) groups. Hypothyroidism was induced via administration of 0.03% methimazole in drinking water for 3 weeks. Testis were collected and stored at -80°C for analyses. Gene and protein expression were evaluated by qPCR and Western blotting, respectively. Serum levels of free T4 were measured by ELISA. All experimental procedures were approved by CEUA/UFF #248811022. Hypothyroidism was confirmed with reduced free T4 levels. Regarding mitochondrial fission, mRNA expression of *Fis1* was reduced (p=0.0086), while *Mff* and *Dnm1l* showed no differences, and total DRP1 protein levels remained unchanged. Among fusion-related genes, *Mfn1* (p=0.0009) and *Mfn2* (p=0.0055) were downregulated, whereas *Opa1* expression was unaltered. In the mitophagy, *Bnip3l* (p<0.0001) and *Bnip3* (p=0.0018) expression levels were decreased, while *Park2* and *Fundc1* showed no significant changes. In the autophagy pathway, all analyzed genes were downregulated, including *Map1lc3b* (p=0.0014), *Becn1* (p=0.0224), *Atg7* (p=0.0106), and *Lamp2* (p=0.0398). Protein analysis revealed an increase in p62 (p=0.0431). Regarding mitochondrial biogenesis, *Ppargc1a* expression was reduced (p=0.0005), while *Tfam* expression remained unaffected (p=0.3100). These findings suggest a reduction in both mitochondrial fission and fusion processes. The downregulation of all assessed autophagy-related genes and protein alterations, combined with decreased expression of *Bnip3* and *Bnip3l*, may indicate impaired mitophagy in the testis under hypothyroid conditions.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Estradiol modulates thermogenic activity and metabolic parameters in high-fat-fed female mice

Authors:

Alessandra Gonçalves da Cruz¹
Guilherme Fancio Bovolin¹
Bruna Maria Fantini Trinca¹
Anne Raissa Melo Santos¹
Jessica Denielle Matos dos Santos¹
Felipe Nunes de Camargo¹
João Paulo Gabriel Camporez¹

Affiliations:

¹ Department of Physiology, Ribeirão Preto Medical School, University of São Paulo – FMRP/USP. Ribeirão Preto, São Paulo, Brazil.

Keywords: Browning, Estradiol, Thermogenic Activity, UCP1.

Abstract:

Estrogen deficiency is associated with metabolic disturbances and impaired thermoregulation. Our objective was to evaluate the role of estradiol in modulating metabolic function and thermogenic activity in high-fat-fed female mice. Eight-week-old female C57BL/6J mice underwent bilateral ovariectomy (OVX) or sham surgery (SHAM) and were fed HFD 45% of calories from fat. After 4 weeks, body composition, glucose tolerance test (GTT) and cold tolerance test (CTT) were performed. The inguinal (ING), periuterine (PUT) and brown (BAT) adipose tissues were collected to analyze protein and gene expression of browning and thermogenic activity markers (*Ucp1*, *Prdm16*, *Cidea*, *Dio*, *Pgc1a*, *Tbx1*) and quantify noradrenaline concentration. In addition, primary beige adipocyte cell cultures were treated with palmitate (500µM), estradiol (0.1µM) or both, and UCP1 gene expression was analyzed (CEUA 1111/2022R1). OVX group exhibited greater weight gain and increased body fat percentage compared to SHAM. GTT results indicated that OVX group had a reduced glucose tolerance and a higher area under the curve. CTT demonstrated that OVX group had an impaired ability to maintain body temperature under cold exposure. *Ucp1* gene expression demonstrated lower levels in ING and PUT from OVX group. In ING, *Prdm16* expression was increased, while *Dio* expression was decreased in the OVX group. Protein analysis confirmed a reduction in UCP1 expression in OVX animals. Noradrenaline concentration was decreased in ING and BAT tissues in the OVX group. In beige adipocyte cultures, treatments with palmitate resulted in decreased *Ucp1* expression, whereas estradiol treatment led to higher *Ucp1* expression. The results confirm the positive influence of estradiol on glucose tolerance, body composition, and cold tolerance. Moreover, estradiol emerged as a key modulator of thermogenesis-related genes. Noradrenaline quantification further suggested a potential modulation mediated by the sympathetic nervous system.



XXXIX REUNIÃO ANUAL DA FeSB
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Potential of acCGP nanoparticles containing *Citrus sinensis* essential oil in the treatment of Type 1 Diabetes

Authors:

Juliana Vila Verde Ribeiro¹
Richard Douglas Rodrigues Ferreira¹
Ariel Penha Carvalho da Mota¹
Jullia Ingrid Rodrigues Martins¹
Rodrigo de Melo Gomes¹
Jane Sousa Naves¹
Fernanda Cristina Alcântara dos Santos¹
Carlos Henrique Xavier Custódio¹
Karla De Aleluia Batista²

Affiliations:

¹ Universidade Federal de Goiás-UFG
² Instituto Federal de Goiás- Campus Goiânia Oeste.

Keywords: Biotechnology. Essential oil. Nanoparticle. Metabolism. Type 1 diabetes.

Abstract:

Type 1 diabetes (T1D) is a multifactorial disorder resulting from a complex interplay between host genetics, the immune system and the environment, that culminates in the destruction of insulin-producing beta cells, resulting in hyperglycemia. In this study, a nanoparticle system based on acetylated cashew gum polysaccharide (acCGP) and *Citrus sinensis* essential oil (acCGP@OE) was evaluated in streptozotocin-induced type 1 diabetes in male mice (CEUA-UFG no. 051/24). T1D in Swiss mice was induced with streptozotocin (STZ - 40 mg/kg, IP injection) for 5 days and in the 17th the animals started to be treated with the nanoparticles of acCGP@OE (75 mg/kg, gavage) for 28 days. The animal weight, food and water intake were determined weekly. On the 27th day, the glucose tolerance test was performed, and on the 28th day, the animals were euthanized, and the organs were removed for histological and biochemical analyses. The results evidenced that despite the nanoparticles of acCGP@OE were not able to reduce the glycemic levels, they reduced the glucose resistance and attenuated the oxidative stress on pancreas, liver and kidney, reducing the lipid peroxidation levels and increasing the activity of antioxidant enzymes. These findings demonstrate that acCGP@OE nanoparticles could be used to ameliorate the metabolic injury and tissue damages associated to the development of type 1 diabetes



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Impacts of herbicide exposure on the adrenal cortex of Mongolian gerbils

Authors:

Gabriel Ribeiro BERNUSI¹

Vitor Grigio¹

Stella Bicalho Silva¹

Thalles Fernando Rocha Ruiz ^{1,2}

Sebastião Roberto Taboga ^{1,2}

Patricia Simone Leite Vilamaior¹

Affiliations:

¹ Instituto de Biociências, Letras e Ciências Exatas – Universidade Estadual Paulista, São José do Rio Preto, Brasil.

² Instituto de Biologia, Universidade Estadual de Campinas – UNICAMP, Campinas, Brasil.

Keywords: Adrenal gland; EDCs; Pesticides

Abstract:

Herbicide usage has increased exponentially in modern agriculture. Water contaminated with residues of these herbicides from plantations has exposed an increasing number of people. Among these, glyphosate, atrazine and 2,4-dichlorophenoxyacetic acid (2,4-D) based herbicides are the most commonly used. Environmental protection organs established limits to the amount that can be traced in tap water, being 700µg/L, 3µg/L and 700µg/L to glyphosate, atrazine and 2,4-D respectively. The adrenal gland is an endocrine organ that, due to its high vascularity, is highly susceptible to contaminants. The objective of this study was to evaluate the impacts of herbicide exposure in the cortex of the adrenal gland of Mongolian gerbils (*Meriones unguiculatus*). 6 months old male gerbils were divided into 5 groups (n=6): Intact control (IC) that received no treatment, Glyphosate (GLY), Atrazine (ATZ) and 2,4-D groups were all treated with the dosage established by the USA EPA, and mixture group (MIX) that were treated with a mix of the three herbicide dosages (CEUA Prot. No. 244/2023). Biometric data showed a decrease in right adrenal weight on MIX group (0.020 ± 0.001 g) when compared to IC (0.029 ± 0.005 g) ($p=0.0039$). Capsule thickness was reduced in the MIX group ($5.09 \pm 1.58\mu\text{m}$) in comparison to IC ($6.79 \pm 2.28\mu\text{m}$) ($p<0.0001$). Zona glomerulosa size was increased in the ATZ group ($48.18 \pm 20.37\mu\text{m}$) ($p<0.0001$). In the zona fascicularis, there was a size decrease in the GLY group ($186.0 \pm 55.37\mu\text{m}$) in comparison to IC ($212.5 \pm 67.32\mu\text{m}$) ($p<0.0001$). Lastly, it was observed an increase in size of the zona reticularis in the MIX group ($609.9 \pm 247.1\mu\text{m}$) ($p<0.0001$). Testosterone levels decreased among GLY ($2.00 \pm 0.81\text{ng/mL}$), ATZ ($2.19 \pm 0.69\text{ng/mL}$) and 2,4-D ($2.06 \pm 1.06\text{ng/mL}$) groups when compared to IC ($4.99 \pm 2.28\text{ng/mL}$) ($p=0.0129$). We can conclude that herbicides – especially when mixed – can affect the adrenal cortex morphology, indicating hormonal dysregulation and glandular dysfunction.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Maternal apical periodontitis impact on insulin resistance and placental morphology in Wistar rats

Authors:

Bruna de Oliveira Alves¹
Ana Carla Thalez Ywabuchi Nobumoto¹
Maria Sara de Lima Coutinho Mattera¹
Bianca Elvira Belardi¹
Anna Clara Cachoni²
Thaís Verônica Saori Tsosura¹
Estêvão Lopes Pereira²
Anielly Gonsalves¹
Lorena Umbelino Rodrigues¹
Camila Conrado França¹
Angela Vermelho Sarraceni¹
Luciano Tavares Angelo Cintra²
Doris Hissako Matsushita¹

Affiliations:

¹ Department of Basic Sciences, São Paulo State University (UNESP), School of Dentistry, Araçatuba, Brazil. (Multicenter Postgraduate Program in Physiological Sciences).

² Department of Preventive and Restorative Dentistry, São Paulo State University (UNESP), School of Dentistry, Araçatuba, Brazil. (Graduate Program in Dental Science).

Keywords: Apical periodontitis. Inflammation. Placenta. Insulin Resistance. Fetal Development.

Abstract:

There is an association between oral inflammation and systemic disease, and that periodontal pathogens cause changes in placental structure. However, there is a paucity of literature on studies investigating the relationship between maternal apical periodontitis (AP), adverse pregnancy outcomes and systemic changes. The aim was investigated insulin resistance (IR) and morphological aspects in the placenta of rats with AP. 24 Wistar rats (2 months old) were assigned to 3 groups: control rats; rats with 1 AP induced in the 1st upper right molar; rats with 4 APs induced in the 1st and 2nd upper and lower right molars. The AP was induced using a carbon steel drill with a 0.1 mm ball at the end. After 30 days of pulp exposure, rats in all groups were mated. The rats were euthanised on the twentieth day of gestation for the following experiments: 1) histological analysis of placenta; 2) glycemia by glucose oxidase method; insulinemia by ELISA method; 3) IR by HOMA-IR calculation. Statistical analysis was performed using analysis of variance (ANOVA) followed by Tukey test ($p < 0.05$). The datas showed: 1) There was an increase in insulinemia and in the HOMA-IR index in the AP1 and PA4 groups compared to the CN group, with AP4 showing a higher value for this index compared to AP1; 2) There was a reduction in the area of the placental labyrinth in the AP4 group compared to the CN group; 3) a reduction in the naso-anal length of fetuses from rats with four f-AP4 lesions compared with fetuses from control f-CN rats; 4) there was no difference in body weight between the groups studied (f-CN, f-AP1 and f-AP4). The datas show that maternal AP is associated with IR and morphological changes in the placenta. Furthermore, these alterations were more pronounced when the number of infection foci increases, highlighting the importance of maternal oral health to prevent systemic changes that impact the intrauterine environment and fetal development. CEUA:0878-2022.

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XXXIX REUNIÃO ANUAL DA FeSB
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Consequences of maternal high-fat/high-caloric diet associated with daily resistance training for placental oxidative stress and inflammatory gene expression

Authors:

Débora Priscila Lima de Oliveira¹
Raíssa Andrade de Araújo Silva²
Débora Kathuly da Silva Oliveira²
Wenícios Ferreira Chaves³
Fernanda Carolina Ribeiro Dias⁴
Kellyanne Rafaella de Oliveira Melo²
Sarah Rachel de Barros Nelo²
Luis Henrique Facunde da Silva²
Josias Gomes Alves Neto²
Márcio Alberto Torsoni³
Thaynan Raquel dos Prazeres Oliveira²
José Antonio-Santos²
Raquel da Silva Aragão²

Affiliations:

¹ Faculdade Pernambucana de Saúde.
² Universidade Federal de Pernambuco.
³ Universidade Estadual de Campinas.
⁴ Universidade Federal do Mato Grosso.

Keywords: Inflammation. Maternal diet. Oxidative stress. Placenta. Pregnancy. Strength exercise.

Abstract:

Physical training could be used to minimize the impact of maternal high-fat/high-caloric ingestion on placental function. Female Wistar rats received control (CD, 3.6 kcal/g, 10% fat) or a high-fat/high-caloric diet (HFD, 4.7 kcal/g, 47% fat) for 3 weeks before and during pregnancy. During the same period, rat performed resistance training 7 to 9 stair climbs at 80% of the maximum carrying capacity, 5 days/week. Euthanasia occurred on the 20th gestational day. Ethical approval 105/2021. Data mean±SD. There was no difference in the placental or labyrinth zone area. In female placenta, SOD activity was decreased (HFD-U 394.8±73.4, CD-T 366.6±120.4, CD-U 546.1±82.4, $p=0.04$ and $p=0.01$, U/mg ptn), and GST activity was increased in HFD-U and CD-T groups (HFD-U 1.6±0.2, CD-T 1.6±0.1, CD-U 0.17±0.02, $p<0.0001$, $\mu\text{mol}/\text{min}/\text{g}$), while both HFD groups had increased *I11b* expression (HFD-U 4.4±1.2, CD-U 1.02±0.2, $p=0.0001$; HFD-T 4.3±0.9, CD-T 1.1±0.1, $p=0.0001$) and HFD-U had increased *I16* expression (HFD-U 22.4±10.1, CD-U 1.02±0.2, $p<0.0001$; HFD-T 4.4±1.3, $p=0.0005$). In male placentas, GST activity was increased in CD-T and in both HFD groups (HFD-U 3.1±0.1, CD-T 2.4±0.1, CD-U 2.0±0.2, $p<0.0001$ and $p=0.04$, HFD-T 2.9±0.2, $p=0.004$ vs CD-T, $\mu\text{mol}/\text{min}/\text{g}$), and total antioxidant capacity was decreased in HFD-T (HFD-T 0.1±0.01, CD-T 0.14±0.01, $p=0.0002$, $\mu\text{M}/\text{Fe}^{2+}$), while both HFD had increased *I11b* expression (HFD-U 10.7±5.3, CD-U 1.0±0.1, $p=0.0009$; HFD-T 12.6±1.4, CD-T 1.4±0.2, $p=0.0004$), and both trained groups had increased *I16* expression (HFD-T 14.6±2.3, CD-T 5.09±0.6, HFD-U 3.7±1.7, $p=0.0001$; CD-T 5.09±0.6, CD-U 1.0±0.2, $p=0.006$). Placental oxidative stress and inflammatory gene expression differently responded to high-fat/high caloric diet and daily resistance training depending on fetal sex.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Effects of Peruvian Maca (*Lepidium meyenii*) on Hematological and Plasma Lipids Markers in Ovariectomized Rats

Authors:

Anna Karolina Kretschmann Florencio de Souza Bagetti¹
Elisângela de Fátima Balzan Valentini¹
Lucas Machado Sulzbacher^{1,2}
Samara Nicole Friske¹
Paula Taís Friske^{1,2}
Giuseppe Potrick Stefani^{1,3,4}
Thiago Gomes Heck^{1,2,5}
Mirna Stela Ludwig^{1,2}

Affiliations:

¹ Physiology Research Group (GPeF), Regional University of the Northwest of the State of Rio Grande do Sul (UNIJUÍ), Ijuí, RS, Brazil.

² Postgraduate Program in Comprehensive Health Care (PPGAIS). Regional University of the Northwest of the State of Rio Grande do Sul (UNIJUÍ), Ijuí, RS, Brazil.

³ Olympic Studies Research Group (GPEO). School of Health and Life Sciences. Pontifical Catholic University of Rio Grande do Sul (PUCRS). Porto Alegre, RS, Brazil.

⁴ Postgraduate Program in Nutrition Sciences (PPGNUT). Federal University of Health Sciences of Porto Alegre (UFCSPA). Porto Alegre, RS, Brazil, Ijuí, RS, Brazil.

⁵ Postgraduate Program in Mathematical and Computational Modeling (PPGMMC), Regional University of the Northwest of the State of Rio Grande do Sul (UNIJUÍ), Ijuí, RS, Brazil.

Keywords: Blood cells. Lipids. Maca. Menopause. Ovariectomy.

Abstract:

Menopause can lead to clinical and metabolic changes. Peruvian maca (MACA, *Lepidium meyenii*) has been shown to be a potential therapy for reducing postmenopausal metabolic effects. We aim to evaluate the effects of MACA consumption on hematological parameters and the lipid profile in the plasma of ovariectomized rats. Wistar rats (n=27, 5 months old) were divided into 4 groups: Control (C, n=5); Ovariectomy (OVX, n=6); Maca (M, n=8); Maca + Ovariectomy (M+OVX, n=8). They were ovariectomized or sham operated (Phase I) and, after 4 weeks (Phase II), received MACA (1 g/kg/day o.v.) for 28 days. CEUA 023/21. After euthanasia, blood cells and plasma lipids were evaluated. Data were evaluated by 1-way-ANOVA, Post Hoc de Tukey or Kruskal-Wallis followed by Dunn's. Regarding hematological parameters, there was no influence of maca consumption in red and white blood cell count, as well as platelets, ($P>0,05$). In the plasma lipid profile (mg/dL), there was no effect on triglycerides and very-low-density lipoprotein ($P>0,05$), but there was a significant difference in the groups OVX and M+OVX, regardless total cholesterol (OVX: $81,67 \pm 10,25$; M+OVX: $84,50 \pm 16,89$) ($P=0,002$), high-density lipoprotein cholesterol (OVX: $66,33 \pm 6,83$; M+OVX: $68,25 \pm 15,36$) ($P=0,008$) and low-density lipoprotein cholesterol (OVX: $28,13 \pm 8,04$; M+OVX: $27,50 \pm 5,34$) ($P=0,006$). These findings indicate that, although estrogen deficiency can affect lipid parameters, MACA consumption was not able to modulate or regulate these markers. Additionally, neither estrogen deficiency state, nor maca consumption influence hematological markers. CNPq, 407329/2016-1, 444286/2024-1, 403136/2024-5, 405546/2023-8, 307926/2022-2 to TGH, CAPES, FAPERGS, UNIJUÍ.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Hypothyroidism impairs glycogen metabolism machinery in the heart of male rats

Authors:

Nathália Pereira de Farias da Silva¹
Juliana Santos Romão¹
Karen de Jesus Oliveira¹

Affiliations:

¹ Universidade Federal Fluminense

Keywords: Glycogen. Glycophagy. Heart. Hypothyroidism.

Abstract: Hypothyroidism is known to affect metabolic processes, but its impact on cardiac glycogen metabolism remains poorly understood. This study investigated the effects of hypothyroidism on glycogen synthesis and glycophagy in the heart of male rats. Male Wistar rats (9-11 weeks old; ~300g), were divided into Euthyroid (Eu, n=9) and Hypothyroid (H, n=9) groups. Hypothyroidism was induced by administering 0.03% Methimazole in drinking water for 21 days. The left cardiac ventricle was harvested for Western blot and RT-qPCR analyses. Serum levels of free T4 and insulin were measured by ELISA. Cardiac glycogen content was assessed using a commercial kit. Data were analyzed by unpaired t-test, with significance set at $p \leq 0.05$. The study was approved by CEUA/UFF nº 2488110221. Hypothyroid rats presented reduced free T4 levels (H: 1.59 ± 0.04 ng/dL vs Eu: 0.12 ± 0.01 ng/dL; $p < 0.0001$) and glycemia (H: 93.6 ± 2.3 mg/dL vs Eu: 104.3 ± 2.1 mg/dL; $p = 0.0031$), with no significant changes in insulin levels (H: 30.00 ± 2.6 ng/mL vs Eu: 23.47 ± 2.6 ng/mL; $p = 0.0962$). Molecular analysis revealed decreased protein expression of pAkt ($p = 0.0093$) in H animals. Interestingly, cardiac glycogen content was increased in hypothyroid animals (H: 0.1241 ± 0.0111 $\mu\text{g}/\mu\text{L}$ vs Eu: 0.0551 ± 0.0051 $\mu\text{g}/\mu\text{L}$; $p < 0.0001$). No difference was found in the protein levels of pGSK3 β ($p = 0.2834$), although *Gsy1* mRNA levels were reduced in H animals ($p < 0.0001$). Protein levels of STBD1, a key protein for glycogen mobilization to lysosomes, were unchanged ($p = 0.7721$). However, pAMPK protein expression was reduced in the H group ($p = 0.009$), which could impair glycogen mobilization. *Gabarap1* mRNA expression ($p < 0.0001$) was also reduced in H animals, reinforcing the suggestion that glycogen mobilization for glycophagy is reduced in hypothyroidism. Thus, hypothyroidism altered cardiac glycogen metabolism in male rats with potential impairment of glycogen mobilization mechanism, which may negatively impact heart homeostasis.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Liraglutide reduces hypothalamic neuroinflammation in obese mice

Authors:

Gabriel Cardoso Santos^{1, 2}
Patrik Saul Zarpellon Barbosa^{1, 2}
Hellen Veida-Silva^{1, 2}
José Antunes Rodrigues^{1, 2}
Lucila Leico K. Elias^{1, 2}

Affiliations:

¹ Universidade de São Paulo, Faculdade de Medicina de Ribeirão Preto
² Departamento de Fisiologia da FMRP/USP

Keywords: Obesity; Hypothalamus; Neuroinflammation; GLP-1; Microglia;

Abstract:

Introduction: Obesity is a chronic disease characterized by a series of metabolic abnormalities that are associated with neuroinflammation, where glial cells, such as astrocytes and microglia, are recruited and actively participate in the initiation of the inflammatory condition. Several treatments are used to reduce food intake and promote weight loss. Glucagon-like peptide 1 (GLP-1) analogues have gained much attention in the current literature, mainly because they exert anti-inflammatory, antidiabetic and anti obesity actions. Therefore, this study aims to analyze the effect of peripheral treatment with liraglutide on hypothalamic neuroinflammation induced by a high-fat diet. **Methods:** C57Bl/6 (Protocol N°1198/2023R1) mice were fed a Chow or HFD diet for 12 weeks and had their body weight and food intake assessed weekly. At the end of the 12th week, the animals were subjected to daily treatment for ten days with liraglutide and, at the end of treatment, body composition was assessed with MiniSPEC and adipose tissues were weighed. Soon after, the animals were anesthetized and subjected to cardiac perfusion and the brains were collected for immunofluorescence of microglia (IBA-1) and p65 NF- κ B in the hypothalamus. **Results:** Our results demonstrated that liraglutide treatment reduced food intake and body weight ($p \geq 0.05$; $p \geq 0.01$, respectively). Liraglutide also reduced the percentage of fat mass and increased the percentage of lean mass and thermogenesis in obese animals ($p \geq 0.05$). In addition, it was also observed that liraglutide treatment in obese animals reduced the hypothalamic expression of microglia and p65 NF- κ B, markers of neuroinflammation. **Conclusion:** Liraglutide reduces food intake and body weight, resulting in decreased hypothalamic neuroinflammation in obese mice.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Full-Spectrum CBD Oil as Adjunct to Insulin Improves Metabolic Outcomes in Type 1 Diabetes

Authors:

José Rodrigo Nascimento Martins¹
Ana Carolina Santos Barboza¹
Ana Luiza De Melo Silva¹
Daiane Maria Queiroz do Carmo¹
Giovanna Gatinho de Sousa Lima¹
Mariana Mendonça Soares¹
Claudete Da Costa Oliveira²
João Gabriel Gouvêa da Silva²
Sandra Rodrigues Mascarenhas¹
Josiane de Campos Cruz¹

Affiliations:

¹ Universidade Federal da Paraíba

² APEPI - Medicinal Cannabis Research and Patient Support Association, Rio de Janeiro, Brasil

Keywords: Antidiabetic agent. Inflammation. Insulin therapy. Metabolism. Oxidative Stress.

Abstract:

Diabetes mellitus is a complex, multisystemic disease that demands insulin therapy and lifestyle change, often combined with oral antidiabetic agents. Treatment adherence is often compromised due to progressive insulin resistance and the need for dose adjustments. Cannabidiol (CBD), a non-psychoactive phytocannabinoid from *Cannabis sativa*, has shown promising effects on glucose metabolism in preclinical studies. However, no studies have investigated CBD as an adjunct to insulin treatment in type 1 diabetes. We hypothesized that full-spectrum CBD oil could improve insulin treatment-induced glycemic control, insulin sensitivity and metabolic outcomes in streptozotocin (STZ)-induced diabetes in rats. Male *Wistar* rats (8–10 weeks) received STZ (65 mg/kg) and were treated for 14 days with CBD oil (30 mg/kg, every 12 h) (n=5) or vehicle (olive oil) (n=5), combined with NPH insulin (5 U/day). Blood glucose was monitored daily (unfasted) and at key timepoints (fasting) before and after STZ, after 7 and 14 days of treatment. At the end, insulin tolerance test was performed, and organs and serum were collected for oxidative stress and cytokine analyses. Data are presented as mean $\pm$ SEM unless stated otherwise. The CBD/insulin group showed reduced unfasted glycemia vs vehicle/insulin (AUC median $\pm$ SD: 727.9 $\pm$ 141.8 vs 1089 $\pm$ 694.8 mg·dL⁻¹·day⁻¹) and fasting blood glucose (135.8 $\pm$ 16.3 vs 296.8 $\pm$ 49.8 mg/dL). Insulin tolerance improved (AUC: 6420 $\pm$ 143.9 vs 3555 $\pm$ 906.2 mg·dL⁻¹·min⁻¹), with higher insulin sensitivity (kITT: 1.628 $\pm$ 0.245 vs 0.746 $\pm$ 0.269 %·min⁻¹). Hepatic malondialdehyde was also lower in the CBD group (0.669 $\pm$ 0.067 vs 1.005 $\pm$ 0.089 nmol·mg protein⁻¹). Although not statistically significant, serum cytokine levels suggest a reduction in TNF- α in the CBD/insulin group (5.188 $\pm$ 0.7481 vs 7.114 $\pm$ 0.9104 pmol·mL⁻¹). These findings support the potential of CBD as an insulin adjuvant in type 1 diabetes - ethics approval: CEUA 6271110324.

Financial support: CAPES, FAPESq, CNPq and APEPI.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Gestational Zika virus infection causes oxidative stress in murine and human placentae: a possible route for antioxidant preventive therapy

Authors:

Verônica Müller de Oliveira NASCIMENTO¹,
Iris Soares ANDRADE¹,
Cherley Borba Vieira de ANDRADE¹,
Rakel Kelly Silva ALVES¹,
Alessandra Cristina Chagas VALIM¹,
Larissa Simões DIAS¹,
Juliana Aranda RODRIGUES¹,
Gabriela Teixeira BARBOSA¹,
Klaus Novaes FONTES¹,
Sharton Vinícius Antunes COELHO²,
Luciana Barros de ARRUDA²,
Enrrico BLOISE³,
Rodrigo Soares FORTUNATO¹,
Tânia Maria Ruffoni ORTIGA¹.

Affiliations:

¹Instituto de Biofísica Carlos Chagas Filho (IBCCF/UFRJ); ²Instituto de Microbiologia Paulo de Góes (IMPG/UFRJ); ³Instituto de Ciências Biológicas (ICB/UFMG).

Keywords: Gestation. Placenta. Oxidative Stress. ZIKV

Abstract:

ZIKV causes Congenital Zika Syndrome (CZS). We investigated whether gestational ZIKV infection would cause oxidative stress (OS) in murine and human placentae, contributing for CZS. Pregnant C57BL/6 mice (8-12 week-old; CEUA: A7/20-036/16) were infected i.v. by ZIKV-BR_{PE243} (5×10^7 PFU; n=22) or Mock (n=28) at gestational day (GD)12.5. At GD18.5, placentae, fetuses and fetal brains were collected and sorted by sex [Female Mock (FM) or ZIKV (FZ), Male Mock (MM) or ZIKV (MZ)]. Infected human placentae (n=12) were obtained from Maternidade Escola da UFRJ (CAAE: 30214214.0.0000.5275). Placentae were intended for IHQ, MET, qPCR, OxyBlot, reduced thiol levels, and antioxidant enzyme activities. Student's t test was used and data were represented as mean $\pm$ SEM. ZIKV enhanced female fetal brains weight (FM:41 $\pm$ 2; FZ:55 $\pm$ 2mg; $p < 0.001$) and decreased placental weight (FM:84 $\pm$ 3; FZ:73 $\pm$ 2mg; $p < 0.05$; MM:94 $\pm$ 3; MZ:82 $\pm$ 2mg; $p < 0.05$). ZIKV increased carbonylated protein levels (FM:0.9 $\pm$ 0.1; FZ:1.15 $\pm$ 0.1a.u.; $p < 0.05$; MM:0.9 $\pm$ 0.0; MZ:1 $\pm$ 0.1a.u.; $p < 0.05$) and nitrotyrosine residues in the labyrinth (FM:16 $\pm$ 0.6; FZ:25 $\pm$ 0.6%; $p < 0.001$; MM:21 $\pm$ 0.2; MZ:25 $\pm$ 0.5%; $p < 0.001$) and junctional zone (FM:11 $\pm$ 1.2; FZ:17 $\pm$ 1.9%; $p < 0.05$; MM:14 $\pm$ 0.5; MZ:16 $\pm$ 0.2%; $p < 0.05$). Degenerated mitochondria and dilated cisterns of GER were found in both labyrinth and junctional zones of FZ and MZ. ZIKV infection did not change thiol levels, SOD, GPx and CAT activities. mRNA of *Nox1* (FM:1 $\pm$ 0.1; FZ:1.3 $\pm$ 0.1 a.u.; $p < 0.05$), *Nox2* (FM:1 $\pm$ 0.2; FZ:1.5 $\pm$ 0.1 a.u.; $p < 0.05$), *Sod2* (FM:1 $\pm$ 0.1; FZ:1.4 $\pm$ 0.1 a.u.; $p < 0.05$), *Sod3* (FM:1 $\pm$ 0.2; FZ:1.7 $\pm$ 0.2 a.u.; $p < 0.05$), *Gpx1* (FM:1 $\pm$ 0.1; FZ:1.4 $\pm$ 0.1 a.u.; $p < 0.05$), and *Gpx3* (FM:1 $\pm$ 0.1 a.u.; FZ:1.4 $\pm$ 0.1 a.u.; $p < 0.05$) were enhanced in placentae of females. In human placentae, ZIKV also increased nitrotyrosine residues (C:25 $\pm$ 2; Z:41 $\pm$ 2%; $p < 0.001$). Our data show that ZIKV infection causes OS in placentae and this could be related to CZS. Our next step is providing pregnant dams an antioxidant therapy to abolish OS and the development of CZS.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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**Title: Reproductive biology of the simultaneous hermaphrodite
Diplectrum formosum (Teleostei: Perciformes)**

Authors:

Graziele Cristine da Silva¹

Talita Sarah Mazzoni²

Affiliations:

¹ Centro de Aquicultura da Unesp – Universidade Estadual Paulista – UNESP.

² Universidade Federal de Alfenas – UNIFAL-MG.

Keywords: Germinal epithelium. Hermaphroditism. Marine fish. Ovo-testis.

Abstract:

Most fish are gonochoristic species. However, hermaphroditism can also be present among Teleostei and can occur simultaneous or sequentially. While sequential hermaphroditism is widespread among fishes, simultaneous hermaphroditism, in which both sexes are functional in the same individual, is characteristic of marine species, especially those that inhabit coral reefs. Although the reproductive cycles of gonochoristic species are better known, the reproductive aspects of simultaneous hermaphrodites is little explored. Like this, in an attempt to understand the reproductive biology of a simultaneous hermaphrodite species, *Diplectrum formosum* was taken as a biological model. The animals were collected in Ubatuba-SP (CEUA: 580-UNESP) and their gonads dissected, fixed in Karnovsky and processed in historesin. The histological sections obtained were stained with metanil yellow, hematoxylin and periodic acid-Schiff. The gonads of *D. formosum* are paired organs, but the histological analyses showed that there is a single gonadal tissue formed by a female and male portion. The single gonad bifurcates into two ovarian lobes anteriorly. Posterior to the point of bifurcation, the ovarian lobes combine to run medial to a broad ring of testicular tissue. Sperm enter into a dorsal sperm sinus, then enter a sperm duct to which it joins just prior to exit through an elongate papillae. Mature eggs enter a posterior ovarian sinus. The ovarian sinus bears numerous elongate ovarian folds, constituting the ovigerous lamellae. Between the female and male gonadal tissue there is an extensive layer of connective tissue forming septa, which segregate the male and female germinal epithelium. In these, functional gametes have been found in the same gonad, however, to date there appears to be no self-fertilization, possibly with mechanisms to prevent this event. These data are important in understanding the relationship between maturation of female and male gonad.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Protective effect of Chrysin on the reproduction of zebrafish exposed to microplastics

Authors:

Mateus Ferreira de Souza¹
Davi Araújo Fernandes¹
Talita Sarah Mazzoni¹

Affiliations:

¹ Universidade Federal de Alfenas – UNIFAL-MG.

Keywords: Infertility. Pollution. Spermatogenesis. Sperm protection.

Abstract:

Microplastics (MPs) have become a global threat causing harm to the health of several species, including humans. Current studies on MPs are limited to describing their effects on several tissues and there is few research on substances capable of mitigating their adverse effects. In an attempt to evaluate the chrysin as a "spermatoprotective" drug against exposure to MPs, we established four experimental groups, with 15 adult males zebrafish per group: control (GC), animals exposed to 0.025 mg/day of chrysin (GCr), animals exposed to 0.5 g/L of MPs (GMP), and animals exposed to 0.5 g/L of MPs + chrysin (0.025 mg/day) (GMPCr). After 30 days of exposure, the males were separated for breeding with females not exposed to MPs and the animals were collected (CEUA - nº 4503250320). Fertilized eggs and viable embryos were evaluated. After spawning, the gonads were dissected, fixed in Karnovsky and processed in historesin. The histological sections were stained with metanil yellow, hematoxylin and periodic acid-Schiff. Histological analyses were performed to evaluate the male germinal epithelium. Untreated females were used for reproduction to evaluate the spermatozoa viability. The quantity of spermatocysts increase in the GMP and a decrease in sperm present in the seminiferous tubules were observed. Vacuolizations in the interstitial tissue and rupture of cysts were also noted. There were no alterations in the germinal epithelium from GCr and GMPCr animals, and the testicular conditions were similar to those of GC. The fertilization rate was 100% for GC, GCr and GMPCr, and 75% for GMP. However, within the first 24 hours post-fertilization (hpf), GMP embryos exhibited delayed development, with 100% mortality at 48hpf. Our results demonstrate that MPs can interfere with spermatogenesis and cause change in the quality of the gametes, leading to delayed embryonic development. However, the chrysin showed spermatoprotective in animals exposed to MPs, ensuring normal reproduction.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Docosaheanoic acid affects prostate cancer microenvironment by reduction of epithelium-to-mesenchymal transition markers and annexin A1 in cancer-associated fibroblasts

Authors:

Lucas Pagliuca Martins¹
Gustavo Matheus Amaro¹
Alana Della Torre da Silva¹
Sebastião Roberto Taboga¹
Sônia Maria Oliani¹
Valéria Helena Alves Cagnon Quitete²
Rejane Maira Góes¹

Affiliations:

¹ Department of Biological Sciences, Institute of Biosciences, Humanities and Exact Sciences, São Paulo State University (UNESP), São José do Rio Preto, São Paulo, Brazil.

² Department of Structural and Functional Biology, Institute of Biology, University of Campinas (UNICAMP), Campinas, São Paulo, Brazil.

Keywords: Annexin A1. Docosaheanoic acid. Prostate cancer. TRAMP mice. Tumor microenvironment.

Abstract:

Prostate cancer is one of the most common types of cancer in men worldwide. Dietary lipids and inflammation play key roles in its development. The omega-3 docosaheanoic acid (DHA) has antitumor action through different mechanisms, including the modulation of inflammatory response. However, the consequences of dietary DHA on the prostate tumor microenvironment (TME) are unknown. Annexin A1 (ANXA1) has been proposed as a target for prostate cancer therapy however remains to be elucidated if such protein is affected by dietary DHA. Here it was investigated how DHA-enriched diet (d-DHA) modulates TME in transgenic adenocarcinoma of the mouse prostate (TRAMP) and ANXA1 tissue expression (CEUA: 5884-1/2021 - UNICAMP). TRAMP mice were divided into four groups (n=10/group) euthanized at 12 or 18 weeks of age, and previously fed for 4 or 10 weeks with standard (C12 and C18) or d-DHA (D12 and D18). The ventral prostates were collected and subjected to morphological, immunohistochemistry and western blotting analyses and blood samples were evaluated using enzyme immunoassay. For both periods, DHA-d increased the expression of ANXA1 in epithelial cells (1.5-fold) but decreased it in cancer-associated fibroblasts (1.6-fold). DHA-d downregulated 4.3-fold TGFB-RII expression only in D12. The expression of smooth muscle α -actin decreased 2.7-fold in D18, when compared to C18 whereas the markers of epithelial-mesenchymal transition (Cadherin-N and Snail) were 2.4-fold downregulated. DHA-d reduced 2-fold the systemic levels of PGE2 and 2.3-fold the protein level of PGTGS2 in D18, in comparison to C18. The present data reveal that anti-tumor effects of DHA on this pre-clinical model, previously described by our group, are due to the modulation of TME with reduction of ANXA1 expression by cancer-associated fibroblasts and epithelium-to-mesenchymal transition markers, highlighting the benefits of DHA intake for prostate cancer management.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: 6-Cyanodopamine as a Novel Endogenous Modulator of Human Vas Deferens Contractility

Authors:

Eric Xavier dos Santos¹
José Britto-Junior¹
Rodrigo Luvizotto²
Antonio Tiago Lima¹
Manoel Odorico Moraes³
Maria Elisabete A. Moraes⁴
Larryn W Peterson⁵
Edson Antunes¹
Adriano Fregonesi^{2,4}
Gilberto de Nucci^{1,3,6}

Affiliations:

¹ Department of Pharmacology, Faculty of Medical Sciences, State University of Campinas (UNICAMP), Campinas, Brazil.

² Department of Surgery, Faculty of Medicine of Jundiaí, Jundiaí, Brazil

³ Division of Urology, Department of Surgery, Faculty of Medical Sciences, State University of Campinas (UNICAMP), Campinas, Brazil.

⁴ Clinical Pharmacology Unit, Drug Research and Development Center, Federal University of Ceará (UFC), Fortaleza, Brazil.

⁵ Department of Chemistry, Rhodes College, Memphis, TN, USA.

⁶ Department of Pharmacology, Institute of Biomedical Sciences, University of São Paulo (USP), São Paulo, Brazil

Keywords: Catecholamines. Smooth muscle. Vas deferens. Epithelial modulation.

Abstract: 6-Cyanodopamine (6-CYD) is a newly discovered endogenous catecholamine that has been identified in cardiac and reproductive tissues such as the rabbit heart, rat ventricles, vas deferens, and in the plasma of patients with chronic kidney disease. This study aims to investigate the functional impact and regulatory mechanisms of 6-CYD in the human epididymal vas deferens (HEVD), a key structure in male reproductive physiology. Segments of HEVD (~1.5 cm) were obtained from patients undergoing vasectomy procedures (protocol 4.468.508). Tissues were incubated with 6-CYD (1, 10, or 100 nM) or left untreated, and then exposed to increasing concentrations of noradrenaline (0.01–300 μ M), dopamine (0.00001–1 mM), or adrenaline (0.01–300 μ M). To investigate potential modulatory pathways involved in 6-CYD release, additional tissue groups were treated with: tetrodotoxin (TTX, 1 μ M), a voltage-gated sodium channel blocker used to inhibit neuronal activity and GKT137931 (1 μ M), a selective inhibitor of NADPH oxidase isoforms NOX1 and NOX. Supernatants from these treatments were collected and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) to quantify 6-CYD levels. Basal 6-CYD release was detected in human vas deferens. This release was unaffected by TTX, suggesting it is independent of neural activity. GKT137931 reduced. Pre-incubation with 6-CYD significantly enhanced vas deferens contractility in response to dopamine and noradrenaline. Notably, 10 and 100 nM 6-CYD increased the maximum response (E_{max}) to dopamine and noradrenaline, while adrenaline-induced contraction was potentiated only at 100 nM. 6-CYD as an important mediator of human vas deferens contractility and demonstrates that its biosynthesis is closely related to glycine availability.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Diabulimia em adolescentes com diabetes tipo 1: Uma revisão narrativa da literatura

Authors:

Eduarda Cristina Pereira dos Santos¹

Affiliations:

¹ CAP UFRJ.

Keywords: Diabulimia. Diabetes tipo 1 (DM1). Distúrbios alimentares.

Abstract:

O diabetes mellitus tipo 1 é o diabetes mais comum na infância e na adolescência e pode estar relacionado a distúrbios alimentares em quem vive com diabetes. Transtornos alimentares impactam em pessoas que vivem com diabetes. A diabulimia é um desses distúrbios. Podendo envolver a omissão de insulina com a finalidade de perda de peso, gerando mau controle do DM1 afetando negativamente os olhos, rins e coração, podendo levar à cetoacidose, amputação de membros e até a morte. Recentemente realizei uma busca que revelou a ausência de uma análise abrangente e atualizada da diabulimia em adolescentes com diabetes. Mesmo com o tema recebendo bastante atenção nos últimos anos, ainda há uma lacuna significativa sobre a prevalência dessa condição em diferentes grupos de adolescentes de acordo com idade, sexo e renda, especialmente em contexto brasileiro. Além disso, os estudos não mostram a influência crescente das redes sociais no comportamento alimentar dos pacientes com essa condição. Dito isto, esse estudo visa preencher essas lacunas oferecendo uma visão mais ampla e atual sobre o tema. Esse trabalho tem o objetivo de realizar uma revisão narrativa resumindo as publicações mais recentes da literatura que respondem a três perguntas selecionadas. Por ser um assunto pouco falado e estudado, mesmo sendo bem relevante, a revisão narrativa fornecerá a compreensão abrangente da diabulimia, permitirá maior visibilidade ao tema e ajudará a identificar áreas que necessitam de mais pesquisas sobre estratégias eficazes de prevenção, sobretudo em contexto brasileiro, podendo futuramente auxiliar em políticas para o Sistema Único de Saúde (SUS).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Antidepressive-like effect of gestrinone in obese female rats

Authors:

Kayky Diniz Daniel¹
Matheus Cordeiro Gadowski²
Ingrid Lais Franco Pontarolo¹
Elisângela Pinotti Conci Costa Rosa¹
Marcelo Machado Ferro¹
Dionízia Xavier Scomparin¹
Rosane Aparecida Ribeiro¹

Affiliations:

¹ Universidade Estadual de Ponta Grossa.

² Unicesumar.

Keywords: Neurological health. Obesity. Synthetic progestin

Abstract:

Obesity is a multifactorial chronic disease which predisposes to depressive and anxiety illnesses. Gestrinone (GES) is a synthetic progestin with antiestrogenic and androgenic properties. Scientific data supports GES use for endometriosis and myomas treatment, but some empirical information has been reported its off-label use against adiposity. As sex steroids regulate neurological health, here we aimed to evaluate the effects of GES on anxiety, memory and depressive-like behavior in obese female rats. Adult female Wistar rats were fed on a high-fat diet (HFD) for 8 weeks to obesity induction. On the following 8 weeks, obese female rats continued to consume HFD and received 3 times a week a gavage of soybean oil containing 0.2% DMSO (HFD group) without or with 0.13 (H0.13), 0.26 (H0.26) or 0.52 mg GES/kg (H0.52). In the last week of the experimental period, all groups of female rats were submitted to elevated plus-maze (EPM), open field (OF) and forced swim test (FST) (CEUA protocol: 23.000077157-5). GES treatment did not alter the percentage of time spent in open arm of EPM or anxiety index in H0.13 ($6.03 \pm 2.20\%$ and 0.80 ± 0.05), H0.26 ($4.18 \pm 1.60\%$ and 0.81 ± 0.04) and H0.52 ($3.24 \pm 1.21\%$ and 0.89 ± 0.04) groups, when compared to HFD ($5.06 \pm 2.34\%$ and 0.82 ± 0.06). Total locomotion was also similar among GES (H0.13 = 62.00 ± 10.64 , H0.26 = 55.50 ± 8.19 and H0.52 = 71.44 ± 14.43) and vehicle treated females (HFD = 59.90 ± 10.27). On the other hand, GES treatment reduced the immobility time during FST in H0.13 (70.07 ± 13.55 s) and in H0.52 females (77.99 ± 13.03 s), but not in H0.26 (95.29 ± 14.72 s), when compared to HFD (128.10 ± 9.57 s), which reflected on an increased swimming time of groups H0.13 (147.9 ± 7.8 s) and H0.52 (153.9 ± 6.3 s), compared to the HFD group (110.2 ± 9.6 s), but not of group H0.26 (125.1 ± 13.0 s). Therefore, we conclude that GES treatment at the doses of 0.13 and 0.52 mg/kg in obese female rats showed an anti-depressive-like effect.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Defensive behavioral reactions in participants with interpersonal violence trauma

Authors:

Sara Domiciano de Jesus Gaspar¹
Bianca Afonso¹
Thayane Fernandes¹
Thayná Alonso¹ Liana Portugal²
Letícia Oliveira³
Mirtes Pereira³
Eliane Volchan⁴
Orlando Fernandes Júnior³
Isabela Lobo¹

Affiliations:

- ¹ Biodiversity and Sustainability Institute, Federal University of Rio de Janeiro, RJ.
² Roberto Alcantara Gomes Institute of Biology, Rio de Janeiro State University, RJ.
³ Department of Physiology, Fluminense Federal University, RJ.
⁴ Carlos Chagas Filho Biophysics Institute, Federal University of Rio de Janeiro, RJ.

Keywords: interpersonal trauma. reaction time. threat Direction. violence

Abstract:

After the perception of a threat, a sequence of motivational defensive behaviors is triggered, described as the “defensive cascade.” In the face of threatening stimuli, open reactions may be activated, characterized by active defense behaviors, or immobility responses, marked by motor suppression. The defensive response varies according to characteristics of the threat, such as its proximity, intensity, and the availability of an escape route. The main objective of this study was to investigate differences in behavioral patterns of the defensive cascade between participants with and without a history of interpersonal violence trauma, using visual stimuli representing scenes associated with violent crimes, in which the direction of the threat relative to the observer modulated the availability of escape. In Experiment I, 69 participants performed a choice reaction time (RT) task, judging the valence of 80 photographs (40 threat stimuli: 20 directed towards and 20 directed away, interspersed with 40 neutral stimuli: 20 directed towards and 20 directed away). In Experiment II, image viewing was free — participants could observe the photographs for as long as they wished, with no specific task ($n = 66$). In both experiments, participants were divided based on whether interpersonal trauma was reported as the most stressful event experienced (CAEE: 92088518.8.0000.5699). Results from both experiments showed main effects of valence, direction and interactions between groups. Threat images led to faster RTs and longer free time, while directed towards images resulted in shorter RTs and shorter free time. Additionally, the group with interpersonal trauma showed faster RTs and longer free time. In conclusion, the direction and valence of the stimulus influence defensive behavior, and the experience of interpersonal trauma appears to induce greater action preparedness.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Systematic Review on the Effects of Valproic Acid (VPA) on Synaptic Plasticity in Animal Models of Autism

Authors:

Rafaela Rezende Herrerias¹ Flávia Araujo Aguiar Duarte¹ Matheus Augusto Arruda Melo³
Victor Rodrigues Santos²

Affiliations:

¹ Neurobiology Laboratory, Institute of Biological Sciences, Federal University of Minas Gerais.

² Department of Morphology, Institute of Biological Sciences, Federal University of Minas Gerais.

³ Graduation Program in Neuroscience, Federal University of Minas Gerais.

Keywords: Autism. Hippocampus. Synaptic Plasticity. Valproic Acid.

Abstract:

This systematic review investigates the effects of valproic acid (VPA) on hippocampal synaptic plasticity in prenatal autism animal models. Searches were conducted in PubMed, Elsevier, and Web of Science using keywords: autism, VPA, synaptic plasticity, hippocampus, and animal models. Exclusion criteria included human studies, non-VPA models, lack of synaptic plasticity analysis, and low methodological quality. Data extraction revealed: 48.15% of studies used 500mg VPA during embryonic exposure, while others varied in dosage (300-600mg) or administration methods; 48.15% employed Wistar rats, with others using Sprague Dawley, Long-Evans, or mice; 37.04% omitted LTP/LTD analysis; 74.07% and 51.85% did not assess dendritic spine morphology or synaptic plasticity, respectively; 74.07% tested males; 62.96% had sample sizes below N=20; hippocampal regions analyzed varied (e.g., dentate gyrus/perforant path: 29.63%, CA1: 29.63%); 50% conducted behavioral tests between P30-P60. Prenatal VPA exposure induced robust synaptic plasticity deficits and autism-like behaviors. However, inconsistent variables (animal model/sex, VPA dose/timing, testing age, methodologies, synaptic processes, and hippocampal regions) hindered cross-study comparability. Standardization of experimental parameters is critical for advancing translational insights.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Neuropsychological and Biomarker Correlates in Mild Traumatic Brain Injury: Acute Cognitive Impairment and Subacute Psychiatric Complications

Authors:

Matheus Augusto Arruda MELO^{1,3}
Agnes Stéphanie da SILVA^{1,3}
Patrícia Fernandes FONTES^{1,2}
Isabella Alves Teixeira de ABREU^{1,2}
Aline Silva de MIRANDA^{1,3}

Affiliations:

¹ Interdisciplinary Laboratory for Medical Investigation (LIIM), School of Medicine, Federal University of Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil.

² Department of Internal Medicine, School of Medicine, Federal University of Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil.

³ Graduation Program in Neuroscience, Federal University of Minas Gerais.

Keywords: Anxiety; Biomarkers; Cognitive deficits; Depression; Mild traumatic brain injury.

Abstract:

This study evaluated cognitive, psychiatric, and biomarker profiles in mild traumatic brain injury (mTBI) patients during acute (24h) and subacute (30 days) phases. Twenty-one patients and 13 healthy controls were included. Neuropsychological tests (MMSE, FAB, Digit Span) revealed acute impairments in direct Digit Span (86.9% below norm), MMSE (34.8%), and FAB (30.4%). At 30 days, deficits persisted in attention/executive functions (reverse Digit Span: 95.6%, inverse Corsi Cubes: 47.8%). Patients exhibited higher HADS scores for anxiety ($p < 0.05$) and depression ($p < 0.05$). Serum biomarkers (IL-4, VEGF) correlated with psychiatric symptoms: IL-4 with anxiety ($\beta = 0.47$, $p < 0.05$) and VEGF with depression ($\beta = 0.52$, $p < 0.05$). Regression analyses identified MMSE ($\beta = 0.885$, $p < 0.001$) and FAB ($\beta = 0.517$, $p < 0.012$) as predictors of cognitive outcomes. Evidence demonstrates that mTBI alters cognitive domains and is associated with psychiatric complications such as anxiety and depression. Biomarkers like IL-4 and VEGF may have prognostic value and aid early detection of these disorders. Results underscore the relationship between cognitive deficits, psychiatric symptoms, and biomarkers post-mTBI (CAA17850513.2.00005149).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Effects of a Visuomotor Exergame on Executive Functions, Sensorimotor Integration, and Self-Regulation in Children with Autism Spectrum Disorder

Authors:

Lauro Grippa Neto¹
João Vitor Galo Esteves^{1,2,3}
Aliny Carvalho Demattê^{1,2}
Felipe Meneguelli Bonone³
Fabrício Bruno Cardoso^{1,3}
Alfred Sholl-Franco^{1,2,3}

Affiliations:

¹ Ciências e Cognição.
² Universidade Federal do Rio de Janeiro.
³ Faculdade CENSUPEG.

Keywords: Autism spectrum disorder. Executive functions. Exergame. Self-regulation. Sensorimotor integration.

Abstract:

Children with Autism Spectrum Disorder (ASD) often present deficits in executive functions, self-regulation, and sensorimotor integration, impacting global development. Exergames have emerged as tools to promote bodily engagement, attentional focus, and behavioral regulation through structured playful tasks. This study evaluated the effects of a visuomotor exergame on executive performance, self-regulation, and sensorimotor integration in children with ASD. The game was a custom browser-based, runner-like application, where children dodged virtual obstacles by moving left, right, or jumping, using facial expressions (e.g., smiling) detected via webcam. The study was approved by the Ethics Committee (approval number: 1.394.198, HUCFF/UFRJ). Sessions were individual, school-based, and supervised by a teacher. Twenty children (aged 7–8; IQ ≥ 70 ; level 1 ASD) were randomly assigned to Control (pre/post assessments only) or Experimental (3x/week, 10-min sessions; 24 total). Three measures were used: (1) STS – Sit-to-Stand, assessing coordination, rhythmic organization, and sensorimotor integration; (2) Self-monitoring scale; and (3) HTKS – Head-Toes-Knees-Shoulders, assessing inhibitory control, working memory, and cognitive flexibility. The experimental group improved significantly: STS by $39.7\% \pm 14.2$, Self-Monitoring by $32.4\% \pm 17.5$, and HTKS by $65.6\% \pm 20.8$ (all $p < 0.01$). The control group showed minor, non-significant gains: STS ($6.8\% \pm 8.6$), Self-Monitoring ($10.9\% \pm 13.5$), HTKS ($16.1\% \pm 14.3$). The experimental group also showed reduced STS variability (CV: 16.7% to 10.2%) and broader response range, notably in HTKS (233%). Findings support the use of visuomotor exergames to enhance self-regulation, sensorimotor integration, and executive functions in ASD multidisciplinary therapeutic programs.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: GPR55 receptor as a modulator of cocaine-induced behavioral and molecular alterations

Authors:

Gabriella Luciana de Oliveira¹
Bruna Lopes Resende¹
Marina Oliveira Boratto¹
Nádia Gomes Rocha¹
Gabriela Reis Cussat¹
Fabrício de Araújo Moreira¹
Antônio Carlos Pinheiro de Oliveira¹

Affiliations:

¹ Department of Pharmacology, Federal University of Minas Gerais, Belo Horizonte, MG.

Keywords: Cocaine. GPR55. Neuroinflammation.

Abstract:

GPR55, a G protein-coupled receptor, has been proposed as a potential "type 3 cannabinoid receptor" due to its similarity to CB1 and CB2 and the ability to bind some of their ligands. GPR55 is expressed in both central and peripheral nervous system regions, including neurons and glial cells, and has been implicated in processes such as learning, memory, and neuroinflammation—all of which are relevant to psychostimulant effects. Although studies suggest a role for GPR55 in amphetamine-related behaviors, its involvement in cocaine-induced alterations remains poorly understood. Objective: To investigate the role of GPR55 in behavioral and glial changes induced by cocaine, with a focus on locomotor sensitization and neuroinflammatory markers. Methods: Male C57Bl/6 mice (9–10 weeks old; CEUA 261/2021) were treated with the GPR55 agonist O-1602 or antagonist ML193 (2.5, 5, or 10 mg/kg, i.p.), combined with cocaine (15 mg/kg, i.p.) administered immediately before each behavioral session. Locomotor activity was assessed using a cocaine sensitization protocol. After the final session, animals were perfused and brains were collected for immunofluorescence analysis of the hippocampus and nucleus accumbens. Iba-1 and GFAP were used as markers of microglial and astrocytic reactivity. Data were analyzed using two-way ANOVA followed by Tukey's post hoc test ($p < 0.05$). Results: O-1602 significantly reduced cocaine-induced hyperlocomotion from the third day of treatment onward, while ML193 had no effect. Cocaine increased GFAP and Iba-1 expression in the dorsal striatum, indicating a neuroinflammatory response; however, no significant differences in glial reactivity were observed between GPR55 agonist-or antagonist-treated groups. Conclusion: GPR55 activation attenuates cocaine-induced locomotor sensitization independently of glial reactivity changes. These findings suggest a neuromodulatory role for GPR55 in cocaine addiction and support further investigation into its interaction with inflammatory and dopaminergic pathways.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: A treadmill exercise session performed during the late consolidation improves recognition memory by hippocampal protein synthesis regulation

Authors:

Gabriela Cristiane Mendes Gomes¹
Karine Ramires Lima¹
Ana Carolina de Souza da Rosa¹
Gabriela Jaques Sigaran¹
Anna Cecília Perretto¹
Pâmela Billig Mello-Carpes¹

¹ Physiology Research Group, Stress, Memory and Behavior Lab, Federal University of Pampa, Uruguai, RS, Brazil.

Keywords: Acute physical exercise. Long-term memory. Recognition memory.

Abstract:

The late consolidation phase is a crucial window for memory persistence modulation and depends on processes like protein synthesis. Acute physical exercise (APE) has been explored as a memory modulator; nevertheless, its specific impact during late consolidation remains unknown. We aimed to investigate the effects and mechanism of APE on the late consolidation of novel object recognition (NOR) memory. Adult male Wistar rats were divided into groups, with or without APE practice: vehicle (VEH); anisomycin (ANI, ribosomal translation inhibitor); and rapamycin (RAPA, mTOR-mediated protein synthesis inhibitor). The procedures followed the Institutional Animal Care and Use Committee (029/2021). Animals in the APE groups were habituated to the treadmill. All animals underwent bilateral cannulation in the CA1 region of the hippocampus. We used the NOR task for memory evaluation, consisting of habituation, training, and tests on days 1, 7, and 14 post-training. 11 hours after training, APE groups performed a 30-minute treadmill exercise. Immediately after APE, or in equivalent time for other groups, animals received drug infusions. The exploration time of the objects was converted into a percentage of the total exploration time and compared to a theoretical mean of 50% using a one-sample Student's t-test. Significance was considered when $P < 0.05$. In the training session, all groups explored both objects similarly ($P > 0.05$). All groups explored the new object for more time in the first test, indicating memory consolidation ($P < 0.05$). However, only APE + VEH showed memory persistence at 7 ($P = 0.001$) and 14 days ($P = 0.002$) post-training. This effect depends on protein synthesis, as inhibition by ANI or RAPA impaired the effects of APE on the 7 ($P = 0.31$; $P = 0.21$) and 14 ($P = 0.05$; $P = 0.06$) day tests. These findings demonstrate that APE 11 hours after NOR acquisition improves memory persistence for up to 14 days by hippocampal protein synthesis modulation.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Metabolomic Insights into the Effects of Brazilian Green Propolis in a Parkinson's Disease Model

Authors:

Kételin Vitória MATIAS¹

Valeria de Cassia Gonçalves¹

Mario Augusto Izidoro²

Fulvio Alexandre Scorza^{1,4}

Frederick Wasinski¹

Josef Finsterer³

Rozana Mesquita Ciconelli⁴

Carla Alessandra Scorza¹

Affiliations:

¹ Disciplina de Neurociência, Departamento de Neurologia e Neurocirurgia, Universidade Federal de São Paulo (UNIFESP), São Paulo, 04039-032, Brazil.

² Laboratório de Espectrometria de Massas, Hospital São Paulo (HSP), São Paulo, 04024-002, Brazil. ³ Neurology and Neurophysiology Center, 1180 Vienna, Austria.

⁴ Departamento de Pesquisa da BP. A Beneficência Portuguesa de São Paulo, São Paulo, 01323-001, Brazil.

Keywords: Metabolomics. Neuroprotection. Parkinson's disease. Propolis. Striatum.

Abstract:

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the selective loss of dopaminergic neurons in the nigrostriatal pathway. Brazilian green propolis exhibits antioxidant, anti-inflammatory, and immunomodulatory properties with neuroprotective potential. This study investigated the metabolic effects of propolis in an experimental PD model induced by bilateral injections of 6-hydroxydopamine (6-OHDA; 12 µg/µl, 1 µl per side) into the striatum of male Wistar rats (n=54, 8 weeks old, 230–300 g). Propolis (200 mg/kg/day, EPP-AF®-C) was administered by oral gavage for 28 consecutive days, starting 24 h after lesion induction. After treatment, animals were euthanized, and brain tissues were collected for immunohistochemical (n=32) and untargeted metabolomic (n=22) analyses. Striatal metabolomic profiling was performed using gas chromatography-mass spectrometry (GC/MS), followed by data preprocessing, normalization, and statistical analysis with MetaboAnalyst 6.0, applying ANOVA, Tukey's post hoc test, and FDR correction (p≤0.05). The results revealed significant modulation of metabolic pathways involved in energy metabolism, lipid homeostasis, neurotransmitter turnover, and redox balance. These findings indicate that propolis exerts a broad biochemical impact on brain metabolism, potentially mitigating PD-associated neurodegenerative mechanisms. The study was approved by the Ethics Committee on Animal Use (CEUA), protocol number 2164030225.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Evaluation of the role of B1 and B2 kinin receptors in the modulation of the inflammatory response induced by lipopolysaccharide (LPS) in astrocyte cell culture

Authors:

Luana Lavezo¹
Mariana Rosolen Tavares¹
Gabriel Rufino Estrela¹
Juliene de Lima Soares Silva¹
Frederick Wasinski¹

Affiliations:

¹ Universidade Federal de São Paulo.

Keywords: B1 and B2 Kinin Receptors. Inflammation. Astrocytes. Lipopolysaccharide. Cytokines.

Abstract:

Astrocytes maintain central nervous system homeostasis, regulate the blood-brain barrier, and actively participate in inflammatory responses. This study aimed to investigate the role of bradykinin B1 (B1R) and B2 (B2R) receptors in calcium signaling and pro-inflammatory cytokines release in primary cortical astrocyte cultures from neonatal C57BL/6 mice (CEUA No. 5501010623) stimulated with LPS. Astrocytes were identified by immunofluorescence for GFAP and S-100. For calcium analysis, cells were loaded with Fluo-4 AM (5 μ M) or Fura-2 AM (5 μ M) and exposed to bradykinin (BK, 1 μ M) with or without selective antagonists (HOE-140, 10 μ M for B2R). ATP (1 μ M) was used as a positive control. In parallel, astrocytes were treated with LPS (100 ng/mL, 24 h) with or without pretreatment with antagonists (10 μ M HOE-140 for B2R or R715 for B1R). The expression of B1R, B2R, and the cytokines IL-1 β , TNF- α , and IL-6 was assessed by RT-qPCR (technical triplicates, n = 3–4), and IL-1 β release was measured by RT-PCR. Data, presented as mean $\pm$ SD, were analyzed by ANOVA followed by Dunnett's post-hoc test (p < 0.05). BK increased Ca²⁺ signaling by 47 $\pm$ 6% above baseline (p = 0.008), an effect reduced by 35 $\pm$ 4% with HOE-140 (p = 0.032), with no significant change observed with desR9BK (DBK). LPS induced a 3.5 $\pm$ 0.4-fold increase in B1R expression (p < 0.001) and a 2.1 $\pm$ 0.3-fold increase in B2R (p = 0.012). IL-1 β expression increased from 12 $\pm$ 2 to 62 $\pm$ 8-fold, TNF- α from 8 $\pm$ 1 to 45 $\pm$ 5, and IL-6 from 15 $\pm$ 3 to 70 $\pm$ 10 (all p < 0.001); these effects were reduced by 60% with co-treatment using the antagonists (p < 0.05). It is concluded that B2R mediates BK-induced Ca²⁺ influx, while B1R, strongly upregulated by LPS, amplifies the inflammatory response. Modulating these receptors may represent a promising therapeutic strategy against neuroinflammation.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Inactivation of the B2 kinin receptor in neurons GABAergic

Authors:

Juliane Silva¹
Luana Lavezo¹
Mariana Rosolen Tavares¹
Frederick Wasinski¹

Affiliations:

¹ Universidade Federal de São Paulo.

Keywords: Dopamine. GABAergic. Kinin B2 receptor. Kallikrein-kinin system.

Abstract:

The kallikrein-kinin system (KKS) is made up of proteolytic enzymes with crucial roles in different organisms, acting in processes such as vasodilation, blood pressure control and pain, via the B1 (B1R) and B2 (B2R) receptors. B1R is related to acute inflammation and B2R to chronic inflammation, both expressed under physiological conditions. Evidence indicates the presence of these receptors in dopaminergic areas of the central nervous system (CNS), associated with motor control, motivation and reward. Our group demonstrated that deletion of B2R in catecholaminergic neurons of the substantia nigra (TH^{ΔB2R}) altered weight and body composition of mice at weeks 5 and 10 ($p < 0.05$), without affecting behavior. To explore the role of B2R in other brain regions, this study (CEUA 5501010623/Unifesp) inactivated B2R in GABAergic neurons (VGAT^{ΔB2R}) of male and female mice, identified by the expression of the vesicular GABA transporter (VGAT). Weight and body composition were assessed at weeks 5, 10 and 15, with no significant differences ($p > 0.05$). Behavioral tests (open field, elevated cross maze, voluntary wheel) and food preference for high-fat diet also showed no changes ($p > 0.05$). All data were expressed as mean $\pm$ SD and analyzed by Student's t-test, considering $p < 0.05$ as statistically significant ($p > 0.05$). In parallel, preliminary data from C57BL/6 mice treated with LPS revealed increased B2R expression in the cortex and striatum after 10h ($p < 0.05$), suggesting its induction during inflammation, since its basal expression is reduced in the CNS. The next steps include subjecting VGAT^{ΔB2R} to an inflammatory insult to investigate the role of B2R in GABAergic neurons in this context.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Evidence that adenosine A_{2A} receptor modulates Ca²⁺ signaling in astrocytes of mice exposed to 24 hours of sustained hypoxia

Authors:

Daniela Accorsi-Mendonça¹
Benedito H. Machado¹

Affiliations:

¹ Departamento de Fisiologia, FMRP-USP

Keywords: Calcium. Astrocyte-neuron interaction. Hypoxia.

Abstract:

Previously we demonstrated that: (1) 24 hours of sustained hypoxia (24h-SH) increased evoked AMPA current amplitude in NTS neurons and (2) the adenosine A_{2A} receptor presents a key role in this effect. Here in we evaluated changes in intracellular Ca²⁺ mobilization in NTS neurons and astrocytes of WT and A_{2A}KO mice exposed to 24h-SH. Male WT and A_{2A}KO mice (6-7 weeks old) were maintained in normoxia (FiO₂: 0.21) or SH (FiO₂: 0.1, 24h, Institutional Ethics Committee Approval #029/2021). Brainstem slices (250 µm thickness) were loaded with Fluo-4 to visualize the Ca²⁺ concentration. For identification, astrocytes were labeled with sulforhodamine 101. Non-NMDA receptors were activated by AMPA superfusion (50 µM). Images were acquired using multiphoton microscopy and processed off-line. The data are presented as peak of fluorescence (%ΔF/F₀) obtained from several regions of interest (ROIs). AMPA superfusion produced a similar increase in Ca²⁺ mobilization in NTS neurons from all groups [WT: 206 ± 23% (31 ROIs/5 slices/4 mice); A_{2A}KO: 193 ± 7% (43 ROIs/6 slices/4 mice)] or exposed to 24h-SH (WT: 154 ± 12% (15 ROIs/3 slices/3 mice); A_{2A}KO: 200 ± 8% (58 ROIs/12 slices/8 mice)). On the other hand, astrocytes from A_{2A}KO after 24h-SH presented an increased in Ca²⁺ mobilization [normoxia: 200 ± 18% (14 ROIs/ 6 slices/ mice); 24h-SH: 244 ± 21% (35 ROIs/ 12 slices/ 8 mice), p=0.0089] than in WT mice [normoxia: 161 ± 12% (25 ROIs/ 5 slices/ 4 mice); 24h-SH: 169 ± 13% (14 ROIs/ 3 slices/ 3 mice)]. In summary, the data show that Ca²⁺ mobilization induced by AMPA stimulation in neurons is not affected by 24h-SH or by the lack of A_{2A} receptors, suggesting that in the increased evoked AMPA current of NTS neurons after SH is not due to changes in intracellular Ca²⁺ mobilization. However, A_{2A} receptors seem to play an inhibitory modulation in the calcium signaling in astrocytes in response to 24h-SH and may have an important role in pathophysiological conditions, such as hypoxia.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Treatments with either Cannabidiol or Cannabigerol reduce Mechanical Hyperalgesia in Rats with Neuropathic Pain

Authors:

William Benneth Canedo de Araújo Leite¹
Aline Aparecida Ferrarese Tiballi Christie¹
Ramos Andrade Leite-Panissi¹

Affiliations:

¹ Department of Psychology, Faculty of Philosophy, Science and Letters of Ribeirão Preto, University of São Paulo.

Keywords: Cannabidiol. Cannabigerol. Hyperalgesia. Neuropathic Pain. Von Frey.

Abstract:

Chronic pain is highly prevalent and debilitating, prompting the search for effective therapies. While cannabidiol (CBD) has been consolidated as an alternative therapy for chronic pain, cannabigerol (CBG) has also shown promise to treat such issues, although underexplored. Therefore, the present study sought to compare the effects of a treatment with CBD and one with CBG, both at 3 mg/kg, in the hyperalgesia induced by constriction of the ischiatic nerve (CCI) in Wistar rats. Experiments were approved by CEUA/FFCLRP (24.1.267.59.2). Measures of mechanical nociceptive thresholds were taken with an electronic von Frey's device in five timestamps: a baseline before CCI, then again seven, 14, 21, and 23 days after the surgery. Animals were divided amongst the conditions CCI-VEIC (n=4), CCI-CBD (n=6), CCI-CBG (n=6), and those false-operated, SHAM-VEIC (n=6), SHAM-CBD (n=6) and SHAM-CBG (n=6). Daily, from the 21st day after surgery, treatments were administered intraperitoneally and injected 60 minutes before testing, with time intervals grounded in the plasmatic absorption of the CBD. Significant interaction was verified between condition (SHAM or CCI) and day of testing (baseline, seven, 14, 21 or 23 days), over mechanical nociceptive thresholds ($F(4, 124) = 10.151$; $p < .001$), which were lower in CCI animals. In CCI-CBD animals, these thresholds on day 14 (pretreatment) and day 23 (third day of treatment) showed a mean difference of -16.74 ± 3.42 (SE), $p = 0.045$, with a mean and SE of 41.80 ± 1.68 on day 14, then 58.54 ± 4 on day 23. Whereas in CCI-CBG animals, mechanical nociceptive thresholds on day 14 and day 23 showed a mean difference of -19.07 ± 3.12 (SE), $p = 0.005$, with a mean and SE of 36.39 ± 3.72 on day 14, then 55.46 ± 1.62 on day 23. While no significant difference in efficacy was observed between CBD and CBG treatments at 3 mg/kg, both increased mechanical nociceptive thresholds and, as such, reduced the hyperalgesia induced by CCI.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Flavonoid rutin as a potential neuroprotective agent in autosomal recessive retinitis pigmentosa

Authors:

Théo Henrique De Lima Vasconcellos ¹
Alexandre Hiroaki Kihara¹

Affiliations:

¹ Neurogenetics Lab, Centro de Matemática, Computação e Cognição, Universidade Federal do ABC, São Bernardo do Campo/SP.

Keywords: Flavonoid. Neurodegeneration. Neuroinflammation. Retina. Rutin.

Abstract:

Retinitis pigmentosa (RP) is an inherited retinal disease that leads to progressive vision loss due to rod photoreceptor degeneration. Regardless of the mutated gene, photoreceptor death by apoptosis, oxidative stress, and neuroinflammation are common outcomes. Currently, RP lacks a universal therapy, with Luxturna® being the only FDA-approved gene therapy for RPE65 mutations. This highlights the need for affordable treatments that benefit a broader patient population. Rutin, a flavonoid found in buckwheat, exhibits anti-apoptotic, antioxidant, and anti-inflammatory properties, making it a potential therapeutic agent for RP. This study evaluated the effects of a single acute subretinal dose of rutin in a mouse model of autosomal recessive RP, focusing on neurodegeneration and neuroinflammation. C3H/HeJRD1 mice (both sexes) were maintained in a 12 h light–dark cycle. Rutin (1 mM) was injected into the right eye at P8 (onset of degeneration), with the left eye receiving vehicle (~1% DMSO in PBS). Retinas were collected at P14 (peak degeneration) for immunofluorescence (n=9). All procedures were approved by CEUA/UFABC (#5037101121). Anti-GFAP was used to assess glial response, while rhodopsin and SOD1 marked photoreceptors and oxidative stress. Morphometric analysis showed that rutin preserved ONL cells in the central area (Vehicle: 5.769 ± 0.444 vs. Rutin: 6.639 ± 0.310 , $P=0.0439$) and thickness (Vehicle: 0.490 ± 0.040 vs. Rutin: 0.617 ± 0.033 , $P=0.0048$). Photoreceptor segments were also better preserved, in a qualitative analysis of the retinas. Although GFAP upregulation is a hallmark of gliosis, here we observed that rutin increased the length of Müller cell processes without raising overall GFAP area coverage (Mean - Vehicle: 130.6 ± 5.8 vs. Rutin: 193.2 ± 9.4 , $P=0.0014$; Max - Vehicle: 193.7 ± 8.6 vs. Rutin: 266.3 ± 8.8 , $P=0.0004$), suggesting a functional reorganization rather than exacerbated gliosis. SOD1 had higher integrated density in the outer retina (Vehicle: 80.21 ± 6.97 vs. Rutin: 97.12 ± 7.85 , $P=0.0233$). Given its multipotential bioactive effects, rutin could be advanced into formulation studies and/or delivery trials, paving the way for a low-cost adjunct therapy for RP. As for the next steps, chronic dose–response studies as well as evaluation of additional neuroinflammatory and functional markers will be implemented to clarify its therapeutic impact on RP.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: High-fat Diet Consumption and Increasing Age Alter Hypothalamic MFSD2a

Authors:

Camila Ayres Cunha Venturini¹
Leandro Kansuke Oharomari¹
Leticia Maria Paula Silva¹
Julia De Lima Boro¹
Jose Rodrigo Pauli¹
Eduardo Rochete Ropelle¹
Adelino Sanchez Ramos da Silva²
Dennys Esper Cintra¹

Affiliations:

¹ State University of Campinas.

² University of São Paulo.

Keywords: Mfsd2a. Dha. Obesity. Hypothalamus. Inflammation.

Abstract:

The hypothalamus is an important brain region for hunger control and energy expenditure. Obesity's systemic low-grade inflammation disturbs hypothalamic functions through blood-brain barrier (BBB) disruptions. Mechanistically, it occurs via tight junctions and/or transmembrane protein alteration, such as MFSD2a. MFSD2a is the major transporter of omega-3 from the blood to the central nervous system and contributes to the maintenance of BBB permeability. However, little is known regarding how diet-induced obesity influences hypothalamic MFSD2a content. In four different study designs, male Swiss mice were utilized to evaluate whether 8/12 weeks of a high-fat diet (HF) inducing obesity, omega-3 (ω 3) source oil intake, central inflammation, saturated fatty acid, and different brain development stages can modify hypothalamic MFSD2a content. All studies were approved by the local ethics committee (CEUA: 6363-1/2023, 6241-1/2023). Obese mice exhibited a higher MFSD2a content than the control group (237%, $p=0.001$), without gene expression differences. Immunofluorescence analyses revealed the presence of MFSD2a in four different hypothalamic areas, with the paraventricular nucleus showing least MFSD2a coverage. Five days of omega-3-rich oil intake could not alter the MFSD2a content or the fatty acids profile. The intracerebroventricular administration of TNF α or palmitic acid did not alter the MFSD2a content. However, the increasing age decreased the MFSD2a content (72%, $p<0,0001$) and exposure time to the HF diet increased (73%, $p=0.0095$) the hypothalamic content of MFSD2a. Altogether, our study demonstrated that the leading ω 3 transporter in the hypothalamus is environmentally and nutrigenomically modulated. Since Mfsd2a plays an important role in the BBB integrity, our results suggest that nutritional interventions can assist in the BBB maintenance, raising the possibility of a coadjuvant in treating neurodegenerative diseases.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Co-Expression Networks Uncover Transcriptional Diversity in Excitatory and Inhibitory Neurons of the Lateral Hypothalamus

Authors:

Allan dos Anjos Monteiro¹
Jackson Cioni Bittencourt¹

Affiliations:

¹ University of São Paulo, Institute of Biomedical Sciences – III, Department of Anatomy, Laboratory of Chemical Neuroanatomy.

Keywords: MCH. Lateral hypothalamic area. Single-cell RNA sequencing. LHA.

Abstract:

The lateral hypothalamic area (LHA) regulates feeding, arousal, and motivated behaviors, exhibiting high cellular diversity. Understanding its molecular architecture is crucial for elucidating neuronal functions. Single-cell RNA sequencing (scRNA-seq) studies reveal functionally distinct LHA populations. We applied high-dimensional weighted gene co-expression network analysis (hdWGCNA) to GABAergic (inhibitory – INH) and Glutamatergic (excitatory – EXC) neurons, identifying co-expression patterns and key regulatory genes contributing to LHA heterogeneity. We analyzed a LHA scRNA-seq dataset, processed using Seurat v5 (R v4.2.2). Low-quality cells and genes were filtered, and cell annotation was performed with ScType. Dimensionality reduction (UMAP/t-SNE) was applied, and gene co-expression networks were constructed using hdWGCNA with soft-thresholding power. Module connectivity (kME) was computed, and differential module eigengenes (DMEs) were compared between sexes using the Mann-Whitney U test. Enrichment analysis was performed using Gene Ontology (GO). Module distribution and co-expression networks were visualized via hdWGCNA. Statistical significance was set at FDR-adjusted $p < 0.05$. We identified 14 GABAergic and 15 Glutamatergic co-expression modules. Network and kME visualization highlighted hub genes, including *Pmch*, *OTP*, and *DPYSL2*, alongside other LHA-associated genes. Sex-based DME comparisons showed significant differences in INH modules M1, M2, M12, and M14, and EXC modules M1 and M3 (FDR-adj. $p < 0.05$). GO enrichment revealed distinct biological pathways, suggesting functional specialization among neuronal subpopulations. Our findings reinforce LHA's role in physiological regulation via distinct neuronal networks. hdWGCNA effectively identified key gene modules and regulatory pathways, highlighting sex differences in gene expression. These insights provide a foundation for exploring sex-specific hypothalamic regulation, warranting further validation.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Effects of a single session of physical exercise on the consolidation and persistence of learning in basic education students

Authors:

Bruna Tarasuk Trein Crespo¹
Ana Luiza Trombini Tadielo²
Pâmela Billig Mello-Carpes³

Affiliations:

¹ Lead author, Universidade Federal do Pampa, Uruguaiiana, RS, Brasil. ² Co-authors, Universidade Federal do Pampa, Uruguaiiana, RS, Brasil. ³ Advisor, Universidade Federal do Pampa, Uruguaiiana, RS, Brasil.

E-mail: brunacrespo.aluno@unipampa.edu.br

Keywords: Learning. Education. Physical exercise. Memory.

Abstract:

Research with animals has shown that various factors influence the consolidation and persistence of learning, including the practice of physical exercise (PE), even a single session. These studies have demonstrated the fundamental role of catecholaminergic neurotransmission systems, which are activated by PE. In this study (Ethics Committee 61470922.6.0000.5323), we investigated the effects of PE on knowledge retention, learning self-perception, and pre-test anxiety in adolescent students, and analyzed the activation of the noradrenergic system indirectly, through salivary alpha-amylase activity. One group of students performed a PE session after a biology class, and a control group did not. Participants were evaluated at three time points (3h, 24h, and 7 days) after the acquisition session. PE facilitated knowledge retention, with students in the PE group scoring higher on the 24h test (7.94 ± 1.26) compared to the control group (6.90 ± 1.47 ; $P = 0.0147$). However, no significant difference was observed after 7 days (PE: 7.02 ± 1.31 vs. control: 6.91 ± 1.44 ; $P > 0.9999$). Learning self-perception was also higher in the PE group in the 7-day assessment ($P = 0.0175$), with an average increase of 1.2 points on a 10-point scale. Pre-test anxiety (IDATE-State) was lower in the PE group at both 3h and 7d (e.g., 3h: PE: 40.2 ± 7.8 vs. control: 45.6 ± 8.2 - $P = 0.0595$), suggesting a potential anxiolytic effect. No significant differences were found in alpha-amylase levels between or within groups ($P > 0.05$). However, as alpha-amylase is an indirect marker and susceptible to multiple confounding factors, caution is warranted in concluding the absence of noradrenergic activation. In summary, a single session of PE appears to enhance short-term learning retention, indicating promising implications for educational strategies and school-based interventions.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Sustained hypoxia increases the amplitude of spontaneous inhibitory currents and reduces the tonic GABAergic inputs in the NTS neurons of C57Bl/6J mice

Authors:

Octávio Augusto de Carvalho MAIA¹
Daniela ACCORSI-MENDONÇA¹
Benedito Honório MACHADO¹

Affiliations:

¹ Department of Physiology, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil.

Keywords: Hypoxia. GABA. Glycine. NTS. Synapse.

Abstract:

It has been well described that hypoxia stimulates the autonomic and respiratory responses via glutamatergic and purinergic signaling in the nucleus tractus solitarius (NTS). Herein we investigated the impacts of sustained hypoxia (SH) on the inhibitory neurotransmission (tonic and phasic) of NTS neurons. Brainstem slices were obtained from the C57Bl/6J male mice (6-7 weeks) after normoxia (FiO₂, 0.21 for 24h) or SH exposure (FiO₂, 0.10 for 24h) and the NTS neuronal activity was recorded using whole cell patch clamp (Institutional ethics committee: CEUA/FMRP-USP #1109/2022R1). SH produced a significant increase in amplitude [SH: 49 ± 5.9 pA (n=33) vs. normoxia: 31.1 ± 3.5 pA (n=22), p= 0.0281] but no changes in frequency [SH: 4.1 ± 0.6 Hz vs. normoxia: 5 ± 0.8 Hz] and half-width (SH: 5.4 ± 0.3 , vs. normoxia: 6.1 ± 0.5 ms) of inhibitory post-synaptic currents (sIPSCs). After picrotoxin perfusion (GABA_A receptor antagonist) the sIPSCs were reduced similarly in both groups: SH: frequency [SH: 1.5 ± 0.2 Hz (n=26) vs. normoxia: 1.2 ± 0.2 Hz (n=16)], amplitude (SH: 14.4 ± 1.3 , vs. normoxia: 15.1 ± 1.8 pA)] and half-width (SH: 2.9 ± 0.2 , vs. normoxia: 3.3 ± 0.2 ms). These sIPSCs were reduced by strychnine (Glycine receptor antagonist) similarly in both groups, SH: frequency [SH: 0.4 ± 0.0 Hz (n=21) vs. normoxia: 0.4 ± 0.1 Hz (n=10)], amplitude (SH: 7.5 ± 1.0 , vs. normoxia: 10.7 ± 1.7 pA) and half-width (SH: 1.7 ± 0.3 , vs. normoxia: 2.7 ± 0.5 ms). Furthermore, picrotoxin produced greater outward shift in the baseline (I_{hold}) in NTS neurons from normoxia than in SH group [ΔI_{hold} : SH: 7.4 ± 2.0 pA (n=15) vs normoxia: 16.7 ± 3.8 pA (n=11), p= 0.0309], indicating a reduction in tonic inhibition on synaptic activity after SH. Our findings suggest that GABA and Glycine mediate the inhibitory modulation on NTS neurotransmission of mice and the stimulation of GABA_A receptors is responsible for the majority of phasic and tonic inhibitory components.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Gestational Chronic Mild Stress Disrupts Sex-Specific Memory in Elderly Offspring

Authors:

Amanda Cristina de Souza¹
Vinicius Schiavinatto Mariano¹
Gabriela Ninin de Carvalho¹
Isabele Cristina Gobo Tibiriça¹
José Antonio Rocha Gontijo¹
Patrícia Aline Boer¹.

Affiliations:

¹ School of Medical Sciences, State University of Campinas, SP, Brasil.

Keywords: Gestational stress. Fetal Programming. Behavioral tests. Mother and Offspring behavioral changes.

Abstract:

Our previous observations showed that gestational stress modulates behavior in a sex- and age-dependent manner, with aged females exhibiting improved memory and depression-like behavior, while males transitioned from a depressive profile in middle age to hyperactive and impulsive behaviors, maintaining long-term memory impairment. To confirm these memory alterations, we conducted the Barnes Maze test at 18 months of age. Pregnant mice were assigned to control (C) and stress (S) groups, with the S group exposed to unpredictable chronic mild stress from gestational days 6 to 21 (CEUA 5774-1). In the memory probe trial, the time spent in the target quadrant was greater in S females (21.59 ± 5.48 vs. 27.88 ± 7.84) and lower in S males (35.51 ± 12.61 vs. 17.60 ± 11.45) compared to the C group. The cognitive score in S females was lower on day 1 (0 ± 0 vs. 0.09 ± 0.03), day 2 (0.01 ± 0.04 vs. 0.03 ± 0.02), and day 3 (0.23 ± 0.31 vs. 0.13 ± 0.22), while in S males, it was lower on day 4 (0.26 ± 0.28 vs. 0.07 ± 0.21) and day 5 (0.32 ± 0.31 vs. 0.14 ± 0.27). The approximation index was similar between females in both groups, but S males showed a greater distance to the target zone on day 2 ($432,948 \pm 140,798$ vs. $550,395 \pm 140,001$), day 3 ($270,122 \pm 177,965$ vs. $431,199 \pm 147,552$), day 5 ($153,292 \pm 109,621$ vs. $239,936 \pm 132,713$), and day 9 ($96,266 \pm 65,590$ vs. $190,120 \pm 132,271$). Our findings confirm that gestational stress induces long-lasting, sex-specific effects on memory performance. While aged females exhibited improved memory despite weaker search strategies, males experienced persistent memory deficits, aligning with previous recognition test results. These results reinforce that gestational stress has lasting, differential impacts on cognitive function, with females demonstrating resilience and males exhibiting sustained impairments into old age.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Impact of early auditory deprivation on memory and long-term potentiation in the hippocampus

Authors:

Mariane Martins Migliaccio¹
Ricardo Maurício Xavier Leão¹

Affiliations:

¹ Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto/SP.

Keywords: Auditory deprivation. Electrophysiology. Hippocampus. LTP. Neurophysiology.

Abstract:

The complete maturation of auditory neurons and circuits occurs only after the auditory canal opens and hearing begins. Evidence suggests that cognitive functions rely on auditory sensory stimuli for full development, but the mechanisms linking hearing loss to higher brain functions remain unclear. This study evaluates the effects of early auditory deprivation on memory and the induction of long-term potentiation (LTP). At postnatal day 14 (P14), coinciding with the opening of the auditory meatus, mice had their auditory canal obstructed with silicone earplugs, increasing auditory thresholds. After 30 days of deprivation (P44) auditory thresholds were measured using Auditory Brainstem Response (ABR). Subsequently, the animals were euthanized for brain tissue dissection, from which hippocampal slices were obtained. Extracellular recordings were performed in CA1 pyramidal and dentate gyrus (DG) granule neurons. LTP was induced using the theta-burst stimulation (TBS) protocol. Another group of animals underwent Object Recognition (NOR) and Object Location tests (OLT). Procedures were approved by the Animal Ethics Committee of FMRP (protocol 1290/2024R1). Thirty days of deprivation increased auditory thresholds in the Plug group compared to the Sham group ($p < 0.0001$). The Plug group also showed reduced discriminative ability in NOR ($p = 0.0005$) and OLT ($p = 0.0362$). The TBS protocol effectively induced LTP in CA1 (control $p = 0.0022$, sham $p = 0.0187$, plug $p = 0.0009$) and DG (sham $p = 0.0130$, plug $p = 0.0160$) in all groups. No significant differences were found between groups in the fEPSP slope before and after LTP induction in the CA1 (before $F = 3.954(2,26)$ / after $F = 1.298(2,23)$) and DG regions (before $p = 0.4530$ / after $p = 0.2880$). The afferent volley amplitude did not increase after LTP induction in the CA1 (control $p = 0.1404$, sham $p = 0.1501$, plug $p = 0.2379$) and DG (sham $p = 0.8061$, plug $p = 0.9732$) regions in any group. However, the afferent volley amplitude, both before ($p = 0.0608$) and after ($p = 0.0547$) LTP induction in the DG of the Sham group, was higher than that of the Plug group. Our results indicate that early auditory sensory deprivation leads to memory impairments, but such impairment is not accounted for by long-term plasticity capacity in the hippocampus.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Study of the enteric nervous system in the distal colon in an animal model of alzheimer's disease

Authors:

Letícia Cipriano Albino¹
Roberta Figueiroa de Souza¹
Marcos Antônio Ferreira Caetano¹
Marina Meira Neta²
Luiz Roberto Giorgetti de Britto²
Patrícia Castelucci¹

Affiliations:

¹ Department of Anatomy.

² Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, São Paulo – SP.

Keywords: Alzheimer's Disease. Brain-gut axis. Enteric nervous system.

Abstract:

In Alzheimer's disease (AD) there is deposition of β -amyloid plaques with predisposition to inflammation with neuronal damage. The compound AD16 is an anti-inflammatory has been used in AD with a reduction in β -amyloid plaques. Studies have demonstrated the link between nitric oxide (NO) and AD and, also alteration of neuronal nitric oxide synthase (nNOS) in the hippocampus of patients with AD. The aim of this research was to study the enteric neurons immunoreactive (ir) nNOS and pan-neuronal β tubulin-III in the distal colon of AD model mice (3xTg-AD) and in animals treated with AD16. C57BL/6 mice (Control group), 3xTg-AD group (AD model) and 3xTg-AD16 group were used and received via gavage for 21 days saline solution and the compound AD16 (1 mg/kg), respectively. Approval of animal use by CEUA, proposal 6567270123. The distal colons were collected, fixed and prepared for double staining of nNOS and β tubulin-III and for histology with H&E staining. The number of nNOS-ir and β tubulin-III-ir neurons per ganglion was counted and the morphology of the distal colon was analyzed. The results demonstrated a significant increase by 24.3% of nNOS-ir neurons/ganglion in the 3xTg-AD group (12.1 ± 0.9) compared to the Control group (9.7 ± 0.7) ($p < 0.05$) and there was a significant decrease by 59% in the 3xTg-A16 group compared to the 3xTg-AD group ($p < 0.05$). There was no significant difference between the Control and 3xTg-A16 groups. There was no significant difference in β tubulin-III-ir neurons/ganglion between the groups. The proportion of nNOS-ir neurons in relation to β tubulin-III-ir were: Control group $45.5\% \pm 4.5\%$, 3xTg-AD group $62\% \pm 3.7\%$ and 3xTg-A16 group $36.4\% \pm 3.5\%$. There were no morphological changes in the groups studied. It is concluded that in the 3xTg-AD group there was an increase in nNOS-ir neurons that could alter intestinal motility. The use of AD16 was able to restore the proportion of nitrergic neurons.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Impacts of ICV administration of CS-6253 in the energy homeostasis of male C57BL/6J mice

Authors:

Natália Stinghen Tonet¹

Ariane Maria Zanesco¹

Licio Augusto Velloso¹

Affiliations:

¹ Obesity and Comorbidities Research Center, University of Campinas (UNICAMP), Campinas, São Paulo 13083-864, Brazil.

Keywords: Energy balance. Hypothalamus. Obesity.

Abstract:

Proopiomelanocortin (POMC) neurons in the mediobasal hypothalamus act as first-order neurons, responding to hormonal and nutrient signals, and promoting reduced hunger and increased energy expenditure. Apolipoprotein-E (apoE) is known to activate POMC neurons, contributing to reduced food intake. In this project we used the peptide CS-6253, an antagonist of the cholesterol transporter in the central nervous system intending to increase the apoE expression. We hypothesized that the CS would lead to an increased apoE expression in the hypothalamus that could activate POMC neurons leading to reduced food intake. We administrated the CS continually using miniosmotic pumps connected to an intracerebroventricular canula in male mice to ensure the central effects of the peptide. Animals were fed with chow or high fat diet (HFD). Body mass and food intake were analyzed for 14 days and at the end of the protocol tissue was collected to gene expression analysis. Body mass was significantly lower in male mice treated with CS in the first days, only in HFD group (CTL 24.37 ± 0.67 ; CS 22.93 ± 0.95 , $p=0.0248$). There were no differences in food intake for both chow and HFD groups. In the moment of euthanasia, fasting glucose and adiposity differ only between diet groups with no effect of the CS treatment. In the gene expression analysis, CS increased inflammatory markers in the hypothalamus (CTL 1 ± 0.91 ; CS 6.47 ± 4.19 , $p=0.0213$) and decreased the Cart gene (CTL 1 ± 0.13 ; CS 0.77 ± 0.04 , $p=0.0185$), related to appetite, energy balance, maintenance of body weight, reward and addiction. In liver and white adipose tissue, CS treatment only modulates the expression of Fasn gene in the chow group (CTL 1 ± 0.39 ; CS 0.38 ± 0.10 , $p=0.0047$). We conclude that CS- 6253 may interact with energy balance in an acute way, losing its large effect with the passing days. More experiments are being conducted to focus on acute administration. CEUA number: 6586-1/2024.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Intracerebroventricular Irisin Treatment Alleviates Hippocampal Inflammation in Diet-Induced Obesity Mice

Authors:

Júlia Cerqueira SANTOS¹
Kelly Cristina Pereira BEM¹
Tassiana Cristina TALPO¹
Guilherme Augusto da Silva NOGUEIRA²
Helena Cristina de Lima BARBOSA¹

Affiliations:

¹ Laboratory of Endocrine Pancreas and Metabolism, Department of Structural and Functional Biology, Biology Institute, University of Campinas.

² Laboratory of Cell Signaling, Department of Medical Pathophysiology, School of Medical Sciences, University of Campinas.

Keywords: Irisin. neurodegenerative diseases. neuroinflammation. obesity.

Abstract:

Obesity is a multifactorial disease and a key risk factor for neurodegenerative disorders. Circulating fatty acids from high-fat diet activate proinflammatory pathways in multiple tissues, leading to chronic inflammation. Irisin, a myokine released during exercise, has gained attention for its potential in reducing inflammatory markers. This study aimed to investigate the effects of irisin on hippocampal inflammation in diet-induced obese mice. Male C57BL/6Junib mice (4 week-old) were fed a standard chow diet (10% fat) or a high-fat diet (45% fat) for 13 weeks (CEUA 5926-1/2021). After this period, the animals underwent brain lateral ventricle cannulation surgery and were divided into 3 groups: CTL-Veh (chow diet, ICV vehicle), HFD-Veh (high-fat diet, ICV vehicle), and HFD-Iri (high-fat diet, ICV irisin 300 ng/day). The treatment was administered for 7 d. At euthanasia, inguinal (iWAT), epididymal (eWAT), and retroperitoneal (rWAT) white adipose tissues were weighed, and the hippocampus was collected. Data normality was assessed using the Shapiro-Wilk test, followed by one-way ANOVA with Tukey's post-hoc test, or Kruskal-Wallis with Dunn's post-hoc test ($P < 0.05$). The HFD-Veh group exhibited higher body weight (33.1 ± 1.8), iWAT (2.5 ± 0.2), eWAT (3.5 ± 0.3), and rWAT (1.2 ± 0.1) relative to the CTL-Veh group (body weight: 25.7 ± 0.7 , iWAT: 0.6 ± 0.05 , eWAT: 0.8 ± 0.09 , rWAT: 0.1 ± 0.02). Hippocampal expression of *Tlr4* (1.5 ± 0.5), *Ifng* (1.6 ± 0.2), and *Bax* (1.3 ± 0.1) was increased in the HFD-Veh group, while no differences were found in *Il6* (1.2 ± 0.3) or *Bcl2* (1.1 ± 0.08) compared to the CTL-Veh group (defined as 1.0 by fold change calculation). In contrast, the HFD-Iri group showed reduced iWAT mass (1.7 ± 0.1) and decreased expression of *Tlr4* (1.08 ± 0.1) and *Il6* (0.8 ± 0.05) compared to HFD mice, with an increase in *Bcl2* expression (1.4 ± 0.1). These findings suggest that irisin administration mitigated hippocampal inflammation and may have contributed to the beneficial metabolic effects.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Impact of Sustained Hypoxia (SH) on the excitatory transmission in the nucleus Tractus solitarius (nTS) neurons of C57Bl/6J mice

Authors:

Mickael S. Luz¹

Daniela Accorsi-Mendonça¹

David D. Kline²

Benedito H. Machado¹

Affiliations:

¹ Department of Physiology, School of Medicine of Ribeirao Preto, University of São Paulo, Ribeirao Preto, SP, Brazil.

² Department of Biomedical Sciences, University of Missouri, Columbia, MO, United States.

Keywords: Excitatory transmission. Excitability. Neuron-astrocyte interaction. GLT-1. GLAST.

Abstract:

We hypothesized that the increased synaptic transmission in the nTS neurons of mice is probably due to changes in glutamate transporters (GLT-1 and GLAST) in astrocytes. We evaluated whether SH increases excitatory transmission and excitability in nTS neurons due to reduced astrocytic modulation. nTS slices were obtained from C57Bl/6J mice after normoxia (N, FiO₂=0.21, 24h) or SH (FiO₂=0.10, 24h). The electrophysiological properties of neurons were assessed using whole-cell patch-clamp and Multi-Electrode Array (MEA). The contribution of GLT-1 and GLAST was examined in the presence of their inhibitors DHK (300μM) and TFB-TBOA (500nM), respectively. The protocols were approved by the Institutional Ethics Committees (073/2021-BR and 42568-US). SH increased AMPA [N:-65±9pA(n=42)vsHM:-95±9pA (n=48)] and NMDA [N:35±7pA (n=12) vsHM: 72±15pA (n=10)] currents in nTS neurons in response to solitary tract (TS) stimulation, suggesting increased excitatory transmission. We evaluated the number of action potentials (AP) fired after TS stimulation (10 Hz). In this case, SH increased AP in nTS neurons [p: 0.0006; N(n=28) and SH(n=27)]. SH increased spontaneous extracellular activity in nTS neurons [N: 12.5±1events/ sec (n=316) vsHM: 24±1events/sec(n=461)]. The astrocytic modulation was evaluated in the presence of GLT-1 and GLAST inhibitors: spontaneous extracellular activity was equally altered by DHK and TFB-TBOA; GLT-1 activity in astrocytes and AMPA current evoked by TS in neurons was not altered by DHK in groups N and SH. AMPA [N:-11±2pA/pF(n=14) vsHM:-17±2pA/pF(n=16)] and NMDA [N:6±1pA/pF (n=14) vsHM: 15±2pA/pF (n=13)] postsynaptic currents were higher after SH. In summary, SH promotes changes in excitatory transmission, spontaneous extracellular activity, and postsynaptic parameters in nTS neurons of mice, but not with changes in glutamate transporters. We conclude that the increase in excitatory transmission in the nTS of SH mice is associated with an elevation of postsynaptic currents.

Financial support: CAPES (#88887.635569/2021-00/#88881.982193/2024-01), FAPESP (#2018/15957-2), CNPq (#309338/2020-4) and NIH (HL166183).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Post-natal development of kv3.1 potassium currents mediated in the fusiform cells in the dorsal cochlear nucleus from mice

Authors:

Mario Henrique Lopes Duarte de Oliveira¹

Alan Eidi Sasaki¹

Ricardo Maurício Xavier Leão¹

Affiliations:

¹ Universidade de São Paulo – Faculdade de Medicina de Ribeirão Preto.

Keywords: Auditory Brainstem. Dorsal Cochlear Nucleus. Electrophysiology. Patch-clamp. Voltage Gated Potassium Channel.

Abstract:

The dorsal cochlear nucleus (DCN) integrates excitatory signals from auditory, somatosensory, and vestibular stimuli, playing a key role in sound source localization, mainly in the vertical plane. Its structure resembles the cerebellum, with three layers: molecular, fusiform, and deep. Fusiform cells are glutamatergic neurons located in the fusiform layer, which integrate auditory and multimodal information from the parallel fibers and send their outputs to the inferior colliculus. Fusiform neurons express a monotonic increase in action potential firing in response to progressive current injections. It has been hypothesized that Kv3 potassium channels, especially Kv3.1, are crucial for controlling the frequency of firing of fusiform neurons. Kv3 channels have fast kinetics and are linked to high-frequency action potential firing. In the calyx of Held, their expression increases after hearing onset, enhancing action potential speed. To test this, electrophysiology experiments of I-V, to see potassium currents, and V-I, to see action potentials, were conducted using the Kv channel blocker TEA (5 mM), analyzing its effects on fusiform cells of Swiss mice before (p7-13) and after (p18-25) hearing onset. Experiments approved under CEUA - 1289/2024R1. No significant differences were found in the amplitude of inhibited potassium currents at +20mV between groups (Pre-hearing: 2403 ± 385.3 pA, n=10, post-hearing: 3083 ± 703.2 pA, n=10; p=0.0892). Likewise, action potential properties remained unchanged, except for firing frequency, which decreased with a larger reduction in animals after the hearing onset (Pre-hearing control: 45.38 ± 9.84 Hz, n=7, Pre-hearing TEA: 23.31 ± 4.73 Hz, n=7; p=0.663. Post-hearing control: 84.26 ± 6.57 Hz, n=9, Post-hearing TEA: 24.04 ± 3.81 Hz n=8; p<0.0001; at 500 pA). These results suggest that Kv3.1 channel expression remains stable across periods, but their role in enabling higher firing frequencies becomes more prominent after hearing onset.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Modulation of Feeding Behavior by K_{ATP} Channels in Serotonergic Neurons of the Dorsal Raphe Nucleus

Authors:

Patrik S. Zarpellon Barbosa¹

Lucila Leico Kagohara Elias²

*Ricardo M. Leão¹

Affiliations:

¹ Universidade de São Paulo – Faculdade de Medicina de Ribeirão Preto.

Keywords: DRN. Food intake. K_{ATP} . Serotonin.

Abstract:

ATP-sensitive potassium (K_{ATP}) channels play a crucial role in coupling metabolic status and neuronal excitability in energetic control and feeding behavior centers. The Dorsal raphe nucleus (DRN) expresses serotonergic (5HT). Evidence shows that 5HT in the DRN participates in the control of feeding behavior. If these neurons express K_{ATP} channels, it is possible that K_{ATP} participates in food intake behavior. To investigate the expression and role of K_{ATP} channels in serotonergic and non-5HT neurons and how modulation of these channels can affect feeding behavior, we employed a combination of Whole-Cell Patch-Clamp technique, immunofluorescence for TpH2 (tryptophan hydroxylase type 2) and Kir6.2 (forming subunit of the K_{ATP} channel), and food intake experiments in DRN cannulated C57BL/6J mice, fasted 12h and satiated animals received tolbutamide and diazoxide respectively (CEUA 1068/2022). Differentiation between 5HT and non-5HT neurons was based on the presence of a "shoulder" during the repolarization phase of the action potential. K_{ATP} activation was induced with diazoxide, while inhibition was achieved using tolbutamide. We observed that 5HT neurons exhibited a shoulder in the repolarization phase of the action potential, distinguishing them from non-5HT neurons. K_{ATP} expression was confirmed by immunofluorescence showing the presence of Kir6.2 in neurons expressing TpH2. Application of diazoxide to 5HT neurons caused hyperpolarization, reduced membrane input resistance, and increased membrane current, effects not observed in non-5HT neurons. Intracranial administration of tolbutamide reduced food intake in the fasting state, while diazoxide increased food consumption in satiated animals. Our findings demonstrate that K_{ATP} channels are predominantly expressed in 5HT neurons in the DRN, and their modulation influences feeding behavior, suggesting that these channels play an important role in food intake.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Adenosine A2B receptors regulate lactate release during brain hypoxia

Authors:

Karla L. RODRIGUES^{1,2}

Alice BRAGA²

Benedito H. MACHADO¹

Aditi KIBE³

Nicholas DALE³

Alexander V. GOURINE²

Affiliations:

¹ Department of Physiology, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil.

² Department of Neuroscience, Physiology and Pharmacology, University College London, London, UK.

³ School of Life Sciences, University of Warwick, Coventry, UK.

Keywords: Adenosine, Adenosine receptors, Astrocytes, Hypoxia, Lactate

Abstract:

Hypoxia, a major stressor, is sensed by astrocytes, which facilitate glycolysis and increase lactate release. Adenosine modulates astrocyte metabolism via A2B receptors expressed by these glial cells. However, little is known about the significance of this signaling mechanism in the response to hypoxia and control of breathing. This study aimed to determine whether adenosine activation of A2B receptors expressed by brainstem astrocytes promotes lactate production under hypoxic conditions. Adult C57Bl/6J (WT), *Adora2b^{flox/flox}* (WT CTRL) and astrocytic A2B knockdown (KD) mice (2–4 months old) were terminally anesthetized with isoflurane. Brainstems were extracted, sectioned into 250 μ m coronal slices. An amperometric lactate biosensor was placed on the brainstem slice. L-lactate (100 μ M) and A2 receptor antagonist ZM241385 (10 μ M) were used in the protocol. Hypoxia was induced by saturating aCSF with nitrogen, lowering PO₂ below 50 mmHg. All animal studies were approved



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Beyond the blindness: Glaucoma-induced circadian dysregulation in the endocrine system of the HPA axis

Authors:

Pietra Souza Barsanele^{1,2}
Leonardo Vinicius de Assis³
Eliz Maria De Oliveira
Furtado^{1,2} Juliano Jefferson Da
Silva⁴ Maristela Oliveira
Poletini⁵ Maria Nathália
Moraes^{1,2}

Affiliations:

- ¹ Programa de Pós-Graduação em Fisiologia, Instituto de Biociências, Universidade de São Paulo.
² Instituto de Ciências Ambientais, Químicas e Farmacêuticas, Universidade Federal de São Paulo.
³ Göteborgs Universitet, Suécia.
⁴ Instituto de Ciências Biomédicas, Universidade de São Paulo.
⁵ Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais.

Keywords: Biological rhythm. Neurodegeneration. Retinal ganglion cell. Corticosterone.

Abstract:

Glaucoma, an optic neuropathy, leads to the degeneration of retinal ganglion cells, which send light information to the suprachiasmatic nucleus (SCN), responsible for synchronizing biological rhythms, like hormone secretion (e.g., corticosterone). Glaucoma mice exhibit disrupted circadian oscillations of light-responsive genes, with a shift in the peak expression of *Avp*, a key efferent SCN signal. As the SCN regulates the temporal oscillation of corticosterone release through the hypothalamic-pituitary-adrenal (HPA) axis, we explored the impact of glaucoma on the circadian molecular components of the HPA axis. Male control and glaucoma mice were maintained in a 12L:12D cycle at 24(±1)°C with food and water *ad libitum*. The SCN, pituitary and adrenal glands were processed for circadian gene expression analysis (N=4-10). Group differences were analyzed by Two-way ANOVA followed by Bonferroni, and rhythmic parameters by CircaCompare. CEUA nº 8143290819. We found increased *c-fos* expression with a 6-hour phase delay (p=0.0122) in the SCN of glaucoma mice. In the pituitary, *Per1* and *Bmal1* expression of glaucoma mice showed an increase in mesor (p<0.0001) and amplitude (p=0.02 and p=0.003, respectively), with a phase advance of 3 and 1 hour (p=0.04 and p=0.01, respectively). *Crh-r1* displayed a 12-hour phase delay (p<0.0001) and reduced mesor (p=0.01) in glaucoma group. In the adrenal, *Per2* and *Bmal1* revealed phase delays of 3 hours (p=0.001) and 1 hour (p=0.04), respectively. Glaucoma mice disrupted *Pomc* and *Mc2-r* oscillatory profiles, while controls-maintained rhythmicity (p=0.01). Plasma corticosterone rhythm was reduced in glaucoma mice compared to controls. Our findings show that glaucoma disrupts circadian HPA axis regulation beyond vision loss, with altered gene expression and corticosterone rhythm, possibly due to SCN dysfunction or pathological signals from retinal degeneration, as increased *c-fos* indicates heightened SCN activation, leading to systemic imbalances.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Interplay Between Sleep Deprivation and the Gut-Brain Axis mediated by the vagus nerve in Feeding Behaviour

Authors:

Luíza Reis Amaral Fonseca¹
João Sérgio Fonseca Guimarães¹
Lívia Larangote Rezende¹
Isadora Maria Lopes de Oliveira¹
Cleiton Lopes Aguiar¹
André Gustavo de Oliveira¹

Affiliations:

¹Universidade Federal de Minas Gerais.

Keywords: Feeding behavior. Gut-brain axis. Sleep deprivation. Vagus nerve.

Abstract:

INTRODUCTION: Feeding behavior encompasses all actions related to the acquisition and ingestion of food, driven by both metabolic demands and sensory-derived pleasure. This complex behavior is modulated by a myriad of internal and external factors, with sleep playing a crucial regulatory role. Sleep orchestrates the balance of neural circuits and peripheral signals, many of which are relayed to the brain via the vagus nerve. Despite growing evidence of sleep influence on feeding behavior, the relationship between sleep, gut-brain axis, and food intake is poorly understood. Here, we investigate how vagal signaling modulates feeding behavior in response to acute sleep deprivation. **METHODS:** Male C57/Bl6 mice (8–10 weeks) were assigned to four groups: Sham, Sham + sleep deprivation (SD), vagotomy (VNX), and VNX+SD. Sleep deprivation was induced via gentle handling for 6 hours (ZT3–ZT9). Feeding behavior was assessed through sucrose and solid food preference tests, and food intake was monitored during SD and 24 hours post-SD. Procedures were approved by the ethical committee (CEUA 280/2018). **RESULTS:** Mice in the VNX+SD group exhibited significantly higher food consumption during sleep deprivation compared to the Sham +SD group (mean consumption: Sham +SD = 0.414g, SD = 0.02244; VNX+SD = 0.6375g, SD = 0.1652; $p = 0.0336$). However, this effect was lost within 24 hours post-SD, with no significant differences observed between groups. Additionally, there were no detected differences in preference for highly palatable food between the experimental groups. No differences were observed in the sucrose preference 24 hours post-SD, despite an overall increased sucrose consumption compared to water in all groups. **CONCLUSION:** Our findings suggest that vagal signaling influences acute feeding during sleep deprivation, but this effect is transient, underscoring the complexity of gut-brain interactions.

FUNDING: This research was supported by CAPES, CNPq, FAPEMIG, and INCT.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: The consumption of cactus flour (*Opuntia ficus-indica*) during aging preserves memory, increases fatty acid levels, and reduces carbonyl levels in brain tissue

Authors:

Renally de Lima MOURA¹
Rita de Cássia Araújo BIDÔ¹
Diego Elias PEREIRA²
Vanessa Bordin VIERA²
Juliana Késsia Barbosa SOARES¹

Affiliations:

¹ Universidade Federal da Paraíba.
² Universidade Federal de Campina Grande.

Keywords: Antioxidants. Fatty acids. Memory preservation. Neuroprotection.

Abstract:

Excessive production of reactive oxygen species (ROS) is toxic to the nervous system, contributing to cognitive aging and neurodegenerative diseases. This study aimed to evaluate the effects of *Opuntia ficus-indica* flour (FOFI) consumption on memory and oxidative stress parameters in the brain tissue of aged rats. The study was approved by the Animal Ethics Committee (protocol no. 0.124.102.022). Fifty male Wistar rats were randomly assigned to five groups (n=10): Adult Control (GCA) and Aged Control (GCI), which received the AIN-93M diet without FOFI; and aged groups PM5, PM10, and PM15, which received the AIN-93M diet supplemented with 5%, 10%, and 15% FOFI, respectively. Memory was assessed using the open field (OF) and object recognition tests, analyzing discrimination (DI) and recognition (RI) indices, in addition to brain fatty acid profile and carbonyl content. Experimental groups showed higher DI at short term (GCA: 0.09 ± 0.14 ; GCI: 0.04 ± 0.09 ; PM5: 0.08 ± 0.04 ; PM10: 0.14 ± 0.03 ; PM15: 0.24 ± 0.04) and long term (GCA: 0.06 ± 0.12 ; GCI: 0.26 ± 0.10 ; PM5: 0.18 ± 0.04 ; PM10: 0.19 ± 0.04 ; PM15: 0.27 ± 0.05), and higher RI at short term (GCA: 0.61 ± 0.06 ; GCI: 0.48 ± 0.05 ; PM5: 0.53 ± 0.03 ; PM10: 0.53 ± 0.05 ; PM15: 0.63 ± 0.02) and long term (GCA: 0.57 ± 0.09 ; GCI: 0.38 ± 0.05 ; PM5: 0.51 ± 0.07 ; PM10: 0.51 ± 0.07 ; PM15: 0.60 ± 0.05), all with $p < 0.0001$. Increased levels of oleic, eicosenoic, arachidonic, and docosahexaenoic acids were observed in experimental groups. Brain carbonyl levels were reduced in PM5 (GCA: 19.90 ± 0.17 ; GCI: 20.81 ± 1.89 ; PM5: 16.70 ± 0.77 ; PM10: 23.02 ± 1.07 ; PM15: 22.33 ± 0.52 ; $p < 0.05$). We demonstrate for the first time that FOFI intake preserves memory, enhances brain fatty acid levels, and reduces oxidative damage in aged rats.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Expression of biochemical markers after hippocampal histamine infusion in the reconsolidation of object recognition memory in Wistar rats

Authors:

André Franco Ludwig¹
Esteban Alberto Gonzalez²
Débora Czarnabay¹
Guilherme Baldo³
Fernando Benetti¹

Affiliations:

¹ Programa de Pós-Graduação em Ciências Biológicas: Fisiologia; Departamento de Fisiologia; Laboratório de Neurofisiologia da Cognição e Desenvolvimento; Universidade Federal do Rio Grande do Sul – UFRGS.

² Programa de Pós-graduação em Genética e Biologia Molecular; Laboratório de Células, Tecidos e Genes, Universidade Federal do Rio Grande do Sul – UFRGS.

³ Programa de Pós-Graduação em Ciências Biológicas: Fisiologia; Departamento de Fisiologia; Laboratório de Células, Tecidos e Genes; Universidade Federal do Rio Grande do Sul – UFRGS.

Keywords: Hippocampus. Histamine. Memory.

Abstract:

The histaminergic system has functions that modulate learning, consolidation and possibly reconsolidation of memory. The aim of the study was to analyze the expression of ERK1 and total CREB in the hippocampus of adult rats previously infused with histamine and/or saline in the intra-CA1 region of the dorsal hippocampus and submitted to an object recognition task (ORT). Brain tissues from Wistar rats collected in a previous study (dorsal hippocampi), approved by the ethics committee for animal use of UFRGS (nº29987), were used. The hippocampi belonged to animals submitted to stereotaxic surgery and ORT, four groups were selected for the current study, (H0') Histamine 0'; (S0') Saline 0'; (H60') Histamine 60'; and (S60') Saline 60'. Where 0': drug infusion and decapitation immediately after the reactivation phase in ORT; and 60': drug infusion and decapitation 60 minutes after the reactivation phase in ORT. Brain tissues were adequately homogenized and analyzed by western blot. Data were expressed as mean and standard deviation, ANOVA test was used for comparisons between multiple groups, with $p < 0.05$ considered significant. For ERK1, the data expressed by arbitrary units (AU) of the percentage in relation to saline 0', showed a statistically significant difference between S0' (1.01 ± 0.08) and H60' (2.90 ± 0.50) ($p = 0.0001$), between S60' (2.03 ± 0.33) and H60' (2.90 ± 0.50) ($p = 0.02$) and between H0' (1.67 ± 0.67) and H60' (2.90 ± 0.50) ($p = 0.001$). For total CREB, a statistically significant difference was observed between S0' (1.11 ± 0.02) and H60' (1.90 ± 0.21) ($p = 0.002$), between S60' (1.36 ± 0.26) and H60' (1.90 ± 0.21) ($p = 0.01$) and between H0' (1.42 ± 0.21) and H60' (1.90 ± 0.21) ($p = 0.03$). Considering the data presented, it is possible to see that there is expression of biochemical markers, such as ERK1 and total CREB in adult rats infused with histamine in the intraCA1 region of the dorsal hippocampus immediately after the ORT reactivation session.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Neurochemical Profile of Hypothalamic PVN Neurons Targeting the DMV in Response to Central Insulin Action

Authors:

Giovanna Castrignano
Camargo¹ Vagner Roberto
Antunes¹

Affiliations:

¹ Department of Physiology and Biophysics, Institute of Biomedical Science, University of São Paulo, SP, Brazil.

Keywords: Dorsal motor nucleus of the vagus. Glucose. Insulin central action. Oxytocin. Paraventricular nucleus of the hypothalamus.

Abstract:

Glucose is essential for cellular functionality, and its control involves hormones such as insulin and glucagon and the autonomic nervous system plays a role in this regulation. The paraventricular nucleus of the hypothalamus (PVN) integrates neuroendocrine responses and projects to the dorsal motor nucleus of the vagus (DMV), influencing liver functions. It is known that oxytocinergic neurons from the PVN project to the DMV and this nucleus is sensitive to the action of this neuromodulator. Previous studies have shown that ICV injection of insulin in the lateral ventricle (LV) reduces the hepatic concentration of glucose, via parasympathetic activation. Here, we aimed to test the hypothesis that central action of insulin activates oxytocinergic PVN neurons that project to the DMV. To test it, we used Wistar rats (300-340g, CEUA nº 8108180324) with guide-cannula in the lateral ventricle for insulin injection (100 nU/μL) or denatured insulin, as control, and injection of Fluorogold into the liver lobe. Animals were anesthetized, perfused with formalin, and the brain removed and further immunohistochemistry procedure with anti-c-Fos (anti-c-Fos, 1:5000) and OT (Anti-Oxytocin, 1:200.000) primary antibody. Qualitative results show significant c-Fos activation in the PVN without a clear colocalization with OT neurons. The control group with icv injection of denatured insulin did not show any c-Fos staining in the PVN. In conclusion, our qualitative findings suggest that central insulin administration activates neurons in the PVN, as indicated by increased c-Fos expression. However, this activation does not appear to be strongly associated with oxytocinergic neurons projecting to the DMV. Future studies employing quantitative analysis and complementary approaches are needed to clarify the precise neuronal populations involved in insulin-mediated signaling within the PVN with projections to DMV.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Neuropeptidergic neurons of the hypothalamus are differentially activated by acute salt loading

Authors:

Pedro Sousa
Soares¹ Vagner
Roberto Antunes¹

Affiliations:

¹ Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, SP, Brazil.

Keywords: Oxytocin. Paraventricular nucleus of the hypothalamus. Salt overload. Supraoptic nucleus. Vasopressin.

Abstract:

The paraventricular nucleus of the hypothalamus (PVN) modulates sympathetic activity, which directly influences blood pressure (BP). Salt overload raises BP in rats, with evidence linking that to chronic activation of the circumventricular organs (CVOs) of the hypothalamus and subsequent activation of the PVN. This study aims to investigate the hypothesis that oxytocin- (OT) and vasopressin-expressing (VP) neurons of the PVN that project to the rostral ventrolateral medulla (RVLM), are differentially activated by acute salt overload. To this end, Wistar rats (300–350g; CEUA nº7025251024) received an injection of the retrograde neuronal tracer FluoroGold™ at 2% (FG) into the RVLM. Subsequently, they received a bolus iv. injection of hypertonic NaCl solution to activate the CVOs and PVN. Hypothalamic slices underwent immunofluorescence reactions to visualise FG, OT (GP-anti-OT, 1:200.000) or VP (Rb-anti-VP, 1:20.000) peptides, and c-Fos (Rb-anti-cFos, 1:5000 or GP-anti-cFos, 1:5000), a neuronal activation marker. Retrograde tracer's visualisation has not yet been successful, but immunofluorescence analyses reveal differential activation of the anterior, intermediate, and posterior PVN, as well as the supraoptic nucleus (SON), another key BP regulatory region, by acute salt loading. Qualitative analysis of OT and VP neurons shows the highest c-Fos colocalization in the intermediate PVN, while posterior and anterior regions exhibit significantly less. OT+ and VP+ neurons in the SON also appear to be differentially activated, with the highest colocalization with c-Fos in its intermediate and posterior portions. Our findings suggest that the PVN and SON neurons exhibit subregion- and phenotype-dependent sensitivities to acute salt loading, which raises the possibility of an equally differential contribution to BP regulation under such conditions, a hypothesis that warrants further investigation in future studies.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Effects of adenosine triphosphate administration in OVLT neurons on blood pressure and heart rate of rats

Authors:

Grasielly Menezes
Lima¹ Vagner
Roberto Antunes¹
Paula Magalhães
Gomes¹

Affiliations:

¹ Department of Physiology and Biophysics, Institute of Biomedical Sciences,
University of São Paulo

Keywords: ATP. Hypertension. OVLT. Blood pressure. P2 receptor.

Abstract:

Hyperosmolality-induced neurogenic hypertension involves the activation of hypothalamic and brainstem nuclei, leading to sympathetic overactivity and increased blood pressure (BP). The circumventricular organs (CVOs), such as the organum vasculosum of the lamina terminalis (OVLT), detect changes in osmolarity and influence BP via projections to the paraventricular nucleus of the hypothalamus (PVN). Recent studies have identified purinergic P2X7 receptors in the OVLT, suggesting the involvement of purinergic signaling at this nucleus level in the neural control of BP. The aim of this study was to investigate the cardiovascular effects of adenosine triphosphate (ATP) injected into the OVLT. Wistar rats (12 weeks old; CEUA ICB/USP: #7737080323) were implanted with a guide cannula targeting the OVLT under anesthesia with isoflurane (2–5%). After recovery, BP and heart rate (HR) were monitored via a femoral artery catheter under urethane anesthesia (1.2 g/kg). ATP (1.2 nmol – 30 nL) was microinjected into the OVLT, and its effects were assessed before and after pharmacological antagonism of P2 receptors with PPADS (10 μ M – 30 nL). Preliminary findings indicated that ATP administration in the OVLT resulted in an increase in BP (Δ MAP: +10 mmHg, n=1). Two minutes after P2 receptor antagonism with PPADS in the OVLT, ATP was injected again (Δ MAP: +12 mmHg, n=1). No changes were observed in HR. These findings suggest that the activation of P2 receptors by ATP in the OVLT affects BP, possibly involving the activation of the PVN-brainstem circuitry. However, further experiments are needed to confirm this hypothesis.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Physiological responses reveal that COVID-19 impacts psychological health

Authors:

Letícia de Oliveira MEDEIROS^{1,2}
Mário Gabriel Teixeira MILANO¹
Fabrício Júnio Mendes SANTOS¹
Abner Ramos de CASTRO¹
Nádia SHIGAEF³
Luis Aureliano IMBIRIBA⁴
Anaelli Aparecida NOGUEIRA-CAMPOS^{1,2}

Affiliations:

¹ Laboratório de Neurofisiologia Cognitiva (LabNeuro), Departamento de Biofísica e Fisiologia, Instituto de Ciências Biológicas, Universidade Federal de Juiz de Fora (UFJF) - Juiz de Fora/MG.
² Programa de Pós-Graduação em Ciências da Reabilitação e Desempenho Físico-Funcional, Universidade Federal de Juiz de Fora (UFJF) - Juiz de Fora/MG.
³ Departamento de Psicologia, Universidade Federal de Juiz de Fora (UFJF) - Juiz de Fora/MG.
⁴ Escola de Educação Física e Desportos, Universidade Federal do Rio de Janeiro (UFRJ) - Rio de Janeiro/RJ.

Keywords: Emotion. Mental Health. Neurophysiological. Psychological Trait.

Abstract:

Evidence suggests that COVID-19 has caused neurological and physiological effects. Given the high social impact that this pandemic has recently unveiled on the population's lives, an effect on mental health is still expected. This study assessed whether psychological aspects related to mental health and life quality would impact physiological responses by heart rate variability (HRV) in COVID-19 patients. This observational study was approved by the local ethics committee (CAAE: 52499121.8.0000.5147). It included 18 participants of both sexes (83.33% women, over 18 years old; mean age of 43.44 ± 13.94 years) diagnosed with COVID-19, recruited around 12.72 ± 4.36 months after infection. ECG recording was used to register the HRV. Moreover, the following questionnaires: TAS-20, SRQ-20, DASS-21, Mini-SPIN, and WHOQOL-BREF were applied to figure out the psychological trait. Data were analyzed using the JASP 0.15 with a significant level at 5%. HRV analysis showed an RMSSD value of 7.36 ± 4.04 ms and an LF/HF ratio of 3.12 ± 4.24 . Psychological assessments revealed significant cognitive and emotional changes: 61.11% had alexithymia (TAS-20), 83.33% had proven mental distress (SRQ-20), 27.77% had severe/very severe depression, anxiety, and stress (DASS-21), and 22.22% exhibited traits of social phobia (Mini-SPIN). Multiple linear regression (forward method) showed a strong impact of 10 independent psychological variables (4 domains of WHOQOL-BREF, SQR-20, TAS-20, 3 domains of DASS-21, Mini-SPIN) on the LF/HF ratio ($p=0.002$; adjusted $R^2=0.71$), which estimates the balance between the sympathetic and parasympathetic nervous systems. In addition, the difficulty in identifying emotions (TAS-20) was strongly associated with reduced parasympathetic activity (RMSSD) ($p=0.006$). These results suggest that COVID-19 infection impacts mental health and cardiac autonomic activity, with evidence for bidirectional causality.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Risk and Protective Factors for Suicidality in the Homeless Population of Belo Horizonte

Authors:

Ianny Avila^{1,2}

Lucas De Aguiar Wan^{1,2}

Jéssica Nascimento Rodrigues^{1,2}

Sordaini Maria Caligiorne^{1,2}

Frederico Duarte Garcia^{1,2,3,4}

Affiliations:

¹ Núcleo de Pesquisa em Vulnerabilidade à Saúde da Universidade Federal de Minas Gerais.

² Departamento de Psiquiatria da Faculdade de Medicina, Universidade Federal de Minas Gerais.

³ Programa de Pós-graduação em Neurociências, Universidade Federal de Minas Gerais.

⁴ Programa de Pós-graduação em Medicina Molecular, Universidade Federal de Minas Gerais.

Keywords: Mental Health disorders. Suicidality. Homeless Population.

Abstract:

The prevalence of suicidal ideation among homeless people is remarkably higher than among the general population, as shown by a meta-analysis from 2019. The objective of this study is to unravel the risk and protective factors for suicidality in the homeless population, using data from the Third and fourth Census of the Homeless Population of Belo Horizonte, reviewed and approved by the Research Ethics Committee of the Federal University of Minas Gerais (C.A.A.E. 21727513.5.0000.5149), which included an assessment of suicidality, mental health, and their correlations. For this, a subsample of 123 subjects were randomly assigned to perform the Brazilian version of the Mini Neuropsychiatric Interview (MINI) questionnaire. People experiencing homelessness were defined as subjects under extreme poverty; reporting broken or fragile family ties; living in unconventional and irregular housing; using public spaces or degraded areas as living accommodation and for provisional purposes in a temporary or permanent manner; and/or subjects housed in shelters for overnight stays. A multivariate analysis was performed using the chi-square test of independence to investigate which categorical variables were correlated. Our data showed a statistically significant correlation between living accompanied on the streets and positive suicidality. Surprisingly, no relationship was found between staying in touch with family and lower suicide rates, and owning a pet was the only factor that showed a significant association with lower odds of suicide. Also, a significant relationship between suicidality and presence of psychotic syndrome, generalized anxiety disorder and illegal drug use was found. A possible explanation for a higher prevalence of suicidal ideation among homeless people could be the greater magnitude of mental disorders and higher level of substance use among them. Biological theories suggest that substance use represents a vulnerability to suicide ideation and behaviour.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Therapeutic hypothermia for the treatment of neonatal hypoxic-ischemic brain injury in male and female rats

Authors:

Ricardo Ribeiro Nunes^{2,5}
Isadora D'ávila Tassinari^{2,5}
Bianca Büchele Martins, B.B¹
João Vitor Miotto Tauffer¹
Janaína Zang^{2,5}
Jefferson Teodoro Araujo¹
Carlos Alexandre Netto^{2,3}
Ana Helena Paz^{2,4,5}
Luciano Stürmer de Fraga^{1,2,5}

Affiliations:

- ¹ Departamento de Fisiologia-ICBS-UFRGS.
- ² PPG Fisiologia-UFRGS.
- ³ Departamento de Bioquímica-UFRGS.
- ⁴ Departamento de Ciências Morfológicas-UFRGS.
- ⁵ Centro de Pesquisa Experimental-HCPA.

Keywords: Behavior. Neonatal hypoxia-ischemia. Neuroprotection. Therapeutic hypothermia.

Abstract:

Neonatal hypoxia-ischemia (HI) is one of the main causes of mortality in newborns. Therapeutic hypothermia (TH) is the only treatment available to reduce associated neurological impairments, however it has some limitations: diminished efficacy in severe cases and restricted time window to be applied. This study evaluated the effects of TH in brain injury and behavioral performance in neonatal rats submitted to HI. Male and female Wistar rats at the 7th post-natal day (PND7) were divided in four experimental groups: SHAM, SHAM+TH, HI and HI+TH. The induction of HI consisted in the occlusion of the right common carotid artery followed by exposure to an hypoxia atmosphere containing 8% oxygen during 90 min. TH was applied by reducing pups body temperature to 32°C during 5 hours. Animals were euthanized at either PND8 or PND21 for brain injury volumes evaluation in Nissl stained slices. Olfactory discrimination test (PND14) and cylinder test (PND20) were evaluated. Results were compared by Kruskal-Wallis followed by Dunn. Severe brain injury (~60%), was observed in both sexes 24h or 2 weeks after HI induction, but TH partially protected the brain only at PND21 in males (K=17.09, p<0.001). In the olfactory discrimination test, females showed increased latency to reach home bedding and spent less time in this area, a condition that was reverted by TH (latency: K=12.11, p=0.007; time: K=9.75, p=0.021). Males did not show any effects of HI or TH in olfactory discrimination. Animals submitted to HI showed impaired use of the contralateral forepaw in the cylinder test, a condition that was not ameliorated by TH (males: 31.93, p<0.001; females: 25.64; p<0.001). TH efficacy in neuroprotection ranged from inefficient to partially efficient depending on outcome and sex. Our results reinforce the inherent variability in TH effects, its limitations on severe brain injuries and the impact of sexual dimorphism in neonatal HI outcomes. Ethics Approval: CEUA/HCPA #2022-0598.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Role of lactate in the inflammasome NLRP3 signaling pathway after neonatal hypoxia-ischemia

Authors:

Isadora D'Ávila Tassinari^{2,5}
Ricardo Ribeiro Nunes^{2,5}
Bianca Büchele Martins,
B.B¹ João Vitor Miotto
Tauffer¹ Janaína Zang^{2,5}
Nícolas Heller Ribeiro¹
Nadja Schröder^{1,2}
Carlos Alexandre Netto^{2,3}
Ana Helena Paz^{2,4,5}
Luciano Stürmer de Fraga^{1,2,5}

Affiliations:

- ¹ Departamento de Fisiologia-ICBS-UFRGS.
- ² PPG Fisiologia-UFRGS.
- ³ Departamento de Bioquímica-UFRGS.
- ⁴ Departamento de Ciências Morfológicas-UFRGS.
- ⁵ Centro de Pesquisa Experimental-HCPA.

Keywords: Brain injury. Lactate. Neonatal hypoxia-ischemia. Neuroinflammation.

Abstract:

Neonatal hypoxia-ischemia (HI) is a major cause of neurological deficiencies in newborns. Lactate (LAC) is a preferred metabolite in the brain of neonates and has already demonstrated neuroprotective potential in HI animal models, probably acting as an energetic substrate. Attention has been drawn to the signaling roles of lactate through the GPR81/HCAR1 receptor, inhibiting the inflammasome NLRP3/caspase-1/IL-1 β pathway involved in chronic inflammation. However, this supposed action of lactate in the inflammatory response in neonatal HI is unclear. Therefore, this study focused on evaluating the long-term influence of the administration of lactate after neonatal HI in the inflammasome pathway in adulthood. Male and female Wistar rats (n=2-4) at the 7th post-natal day (PND7) were submitted to neonatal HI (permanent occlusion of the right common carotid artery followed by exposure of the animals to a hypoxic atmosphere with 8% oxygen for 60 min maintaining a body temperature of 37°C). The animals were divided in four experimental groups: HI (submitted to HI), HI+LAC (submitted to HI and receiving lactate), SHAM (fictitious surgery) and SHAM+LAC (fictitious surgery and receiving lactate). Lactate (1g/kg) was administered intraperitoneally 30min and 2h after hypoxia. Animals were euthanized at PND60 and the right hippocampus was collected for the analysis of the gene expression of NLRP3, caspase-1 and IL-1 β . Results were expressed as a percentage in relation to control and analyzed by Student's t test. No alterations on the expression of NLRP3 or caspase-1 were observed in either sexes. However, mRNA levels of IL-1 β increased in the HI+LAC males (t=3.50, p=0.039), indicating that sexual dimorphism seems to modulate the expression of this cytokine. More studies are necessary to describe the possible involvement of lactate in the NLRP3 and, consequently production of IL-1 β temporally hypoxic-ischemic conditions in neonates. Ethics approval: CEUA/HCPA: 2021-0586



XXXIX REUNIÃO ANUAL DA FeSB
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Exercício agudo reduz neurotoxicidade induzida por 6-OHDA e possíveis implicações para estágios iniciais da Doença de Parkinson experimental

Authors:

Larissa LAZARINI¹
Bruna Berto GATTINIBONI¹
Edson Antônio FIORENZA NETO¹
Karin Almeida da SILVA¹
Luiz Felipe MARQUEZE¹
Ricardo Corrêa CUNHA²
Ricardo A. PINHO¹

Affiliations:

¹ Laboratório de Bioquímica do Exercício em Saúde, Programa de Pós-Graduação em Ciências da Saúde, Pontifícia Universidade Católica do Paraná, Curitiba, Paraná, Brasil.

² Departamento de Educação Física, Universidade Positivo, Curitiba, Paraná, Brasil.

Keywords: Neurotoxicidade. 6-OHDA. Exercício físico. Doença de Parkinson.

Abstract:

A neurotoxicidade induzida pela 6-hidroxidopamina (6-OHDA) no bulbo olfatório mimetiza os estágios iniciais da Doença de Parkinson (DP) ao promover degeneração seletiva de neurônios dopaminérgicos e ativando estresse oxidativo e processos inflamatórios que se propagam para outras estruturas cerebrais. O exercício físico (EF) tem sido investigado como potencial intervenção neuromodulatória capaz de atenuar os efeitos neurotóxicos. Este estudo objetivou avaliar o efeito agudo do EF, em diferentes intensidades, sobre esta neurotoxicidade difusa da 6-OHDA. Ratos Wistar machos de 90 dias receberam uma única dose de 6-OHDA (12µg/µl) no bulbo olfatório esquerdo ou veículo (solução salina 0,9%), e após 12 dias realizaram um teste de velocidade máxima (Vmax). Posteriormente, foram subdivididos em Sham e DP. Os animais foram exercitados a 50% da Vmax ou a 80% da Vmax. 24h após uma única sessão exercício físico, foram imediatamente eutanasiados para análise de mediadores inflamatórios no estriado e perfil redox no córtex cerebral, através de análises por kits multiplex, teste de Bradford e Elisa, analisando os mediadores inflamatórios no estriado, perfil redox e expressão proteica no córtex cerebral. Níveis de MDA foram significativamente maiores tanto no grupo DP 50% Vmáx vs. Sham (P= 0,0344); Média Dif.= - 0.082 (95%CI: -0,159 to -0,0056); como também DP 50% Vmáx vs 80%Vmáx (P= 0.001) Média dif. = 0,1322 (95%CI: 0.05525 to 0.2092). IL1- α teve níveis significativamente maiores no grupo Sham vs. DP (P<0.001); Média Dif.=202.1(95%CI: 52.79 to 351.4). IL1- β com níveis significativamente maiores no grupo DP 80% Vmáx vs Sham (P<0.021); Média Dif.=160.9 (95%CI: 23,95 to 297,9). IL6 teve níveis significativamente maiores no grupo Sham vs. DP (P<0.011); Média Dif.= 3088 (95%CI: 714.1 to 5461). TNF- α com níveis significativamente maiores no grupo DP 80% vs 50% Vmáx (P=0,038); Média Dif.= 27.53 (95%CI: -54.07 to -0.983). Não foram observadas diferenças significativas na expressão de BDNF, c- FOS e CREB. Em conjunto, os resultados demonstram que animais sob efeitos da 6-OHDA e submetidos a diferentes intensidades de esforço físico de forma aguda apresentam alterações no equilíbrio redox estriatal bem como na resposta inflamatória cerebral cortical. Apesar das alterações observadas, a expressão de proteínas associadas à neuroplasticidade permaneceu inalterada. Esses achados destacam a complexidade da interação entre exercício físico e neuroinflamação em modelos de doença de Parkinson e ressaltam a necessidade de mais estudos que aprofundem os mecanismos envolvidos e as possíveis implicações terapêuticas do exercício em diferentes estágios da doença. Este projeto foi aprovado pelo Comitê de Ética no Uso de Animais (CEUA), em conformidade com as diretrizes nacionais vigentes para experimentação animal.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Study of Inhibitors of DNA Binding Protein ID1 and ID3 in Pitt-Hopkins Syndrome, a neuropsychiatric disorder in the autistic spectrum

Authors:

Breno Braga Hernandez¹
Fabio Papes¹

Affiliations:

¹University of Campinas – Institute of Biology - Department of Genetics, Evolution, Microbiology and Immunology.

Keywords: Autism. Differentiation. Neuron. Progenitor. Proliferation.

Abstract:

Pitt-Hopkins Syndrome is a genetic disease in the autistic spectrum caused by haploinsufficiency of the Transcriptional Factor 4 gene (TCF4). This disorder is characterized by profound intellectual disability, severe motor delay and absence of speech. Our previous work determined that patient-derived neural progenitor cells exhibit deficits in proliferation. We also showed that patient-derived brain organoids display aberrant content of neural progenitors. Although it was determined that the Wnt signaling pathway is diminished in these cells, possibly contributing to their lower proliferation rate, how the mutations in TCF4 lead to Wnt dysregulation is unknown. In this project, we have knocked down TCF4 using antisense oligonucleotides (ASOs) on neuroblastoma cell line SH-SY5Y and patient-derived neurons. Furthermore, we checked whether this genetic manipulation causes changes in the expression of bHLH proteins ID1 and ID3, which have been shown to be higher in patient-derived neural progenitor cells (NPCs). NPCs were cultured until they reached 70% confluency, followed by removal of FGF-2 for two weeks to induce neuronal differentiation. Neurons were then treated for two subsequent days with 100 uM of each of three Locked Nucleic Acid (LNA)-containing ASOs designed based on the TCF4 coding sequence. One day after the last treatment; total RNA was extracted and RT-qPCR was conducted with TaqMan Probesets for the following target genes: TCF4, ID1, ID3 and WNT3. We could confirm that ASO-induced knock down of TCF4 was successful; importantly, we could also verify that the lower expression of TCF4 is correlated with higher expression of ID1 and ID3 and diminished WNT3 expression. Our results shed light onto an autism spectrum disorder and may provide future targets for pharmacological therapy. This study was approved by the Ethics Committee on Human Subjects (CEP) at UNICAMP, under approval number CAAE: 86136925.6.0000.5404.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: The NMDA receptor antagonist MK-801 inhibits the sensitization of salt intake independent of alterations in salt taste

Authors:

Aline Aparecida Zenatti¹
Emilson Donizete Pereira Junior¹
Patricia Maria de Paula¹
Jose Vanderlei Menani¹
Carina Aparecida Fabricio de Andrade¹
Laurival Antonio de Luca Junior¹

Affiliations:

¹ Department of Physiology and Pathology, School of Dentistry, São Paulo State University, UNESP, Araraquara-SP, Brazil

Keywords: Behavioral sensitization. Body-fluid balance. Dehydration. Palatability. Sodium appetite.

Abstract:

The intraperitoneal injection of N-methyl-D-aspartate (NMDA) receptor antagonist dizocilpine, MK-801, inhibits sodium appetite sensitization in rats (Hurley & Johnson, 2013). The anti-sensitizing effect of the drug has been determined by the disappearance of the enhancement in salt *intake*, a stronger behavioral response to repeated sodium depletions. We donot know whether the inhibitory effect of MK-801 on salt intake enhancement relates to alterations in salt *taste*. The objective of the present study was to investigate whether MK-801 (0.15 mg/kg) associated with sodium depletion influences the future of salt taste, as determined by video recording of somatic motor orofacial responses to intraoral infusion of salt (0.3 M NaCl), in adult male rats (n = 11-12). The drug was paired with episodes of rapid-onset (1-h latency) sodium appetite in response to sodium depletion, that is, in response to a combination of furosemide with low-dose captopril (Furo/Cap). Each pairing of MK-801 (or its vehicle) + Furo/Cap was performed every other three days, three times. In a post-pairing trial, conducted 3 days after the last pairing, we evaluated whether the pairing would affect salt taste in rats now treated with only Furo/Cap. History of MK-801 versus vehicle produced no alterations in orofacial responses to 0.3 M NaCl in this post-pairing trial. For example, hedonic responses in euhydrated condition, similar in both groups (14 ± 1 vs. 22 ± 5 , respectively, $p > 0.05$), increased the same (five-fold average) in response to Furo/Cap. Aversive responses, also similar in both groups in euhydrated condition (22 ± 2 vs. 38 ± 10 , $p > 0.05$), was not altered in either group by Furo/Cap. The MK-801 + Furo/Cap did not enhance 0.3 M NaCl intake compared with vehicle (4.1 ± 0.8 ml/h vs. 11.7 ± 1.0 ml/h, respectively; $p < 0.05$). The results suggest that MK-801 affects sodium appetite sensitization independently of alterations in salt taste. CEUA FOAR: 42_2017. Funding: CNPq 306833/2021-2.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Cafeteria Diet Induces Obesity and Increases Anxious Behavior in Male Rats

Authors:

Tayná Nery Banckes¹
Matheus Felipe Zazula¹
Marcelo Machado Ferro²

Affiliations:

¹ Laboratório de Plasticidade Morfofuncional, Departamento de Biologia Celular, Universidade Federal do Paraná – Curitiba, Paraná, Brasil.

² Laboratório de Neuropsicobiologia, Departamento de Biologia Geral, Universidade Estadual de Ponta Grossa – Ponta Grossa, Paraná, Brasil.

Keywords: Anxiety-like behavior. Cafeteria diet. Obesity. Ultra-processed diet.

Abstract:

Obesity is a multifactorial disease strongly associated with the consumption of ultra-processed foods. Studies have indicated a bidirectional relationship between obesity and mental disorders such as depression and anxiety. This study investigated whether early exposure to a cafeteria (CAF) diet, a model that mimics human consumption of highly palatable, energy-dense, and nutritionally poor foods, could induce anxiety and/or depression-like behaviors in male rats. Sixteen male Wistar rats were used and, at postnatal day 30, were randomly assigned to either a control group (CON), receiving standard chow, or a CAF group, fed the cafeteria diet for ten weeks. Body weight and caloric intake were monitored regularly from weaning onward. Behavioral testing included the open field (OF), elevated plus maze (EPM), and forced swim test (FST), to evaluate locomotion, anxiety-like, and depressive-like behaviors, respectively. After euthanasia by decapitation, mesenteric, retroperitoneal, and perigonadal fat pads were collected, summed, and expressed as g/100 g body weight (CEUA 0575718/2021). Plasma levels of total cholesterol, glucose, and triglycerides were also measured. Data were analyzed using Student's *t*-test or the Mann-Whitney *U* test ($p \leq 0.05$). Compared to the CON group, CAF rats showed significantly greater body weight gain ($p = 0.0183$), higher caloric intake from the third week onward ($p < 0.05$), increased fat accumulation ($p < 0.001$), and elevated plasma glucose levels ($p = 0.0003$). In the OF test, CAF animals displayed reduced locomotor activity ($p = 0.0013$) and fewer entries into the center area ($p = 0.0283$). In the EPM, CAF rats spent less time in the open arms ($p = 0.0415$) and made fewer open-arm entries ($p = 0.0146$). No significant differences were observed in the FST ($p > 0.05$). These findings demonstrate that the cafeteria diet induced obesity, metabolic alterations, and increased anxiety-like behavior, without evidence of depressive-like behavior. This highlights the negative impact of early exposure to ultra-processed foods on both metabolic and emotional health.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Investigating the role of ephrin receptors in the neuronal migration process in a monogenic autism spectrum disorder

Authors:

Nicolas Gasparine Figueiredo¹

Fabio Papes¹

Affiliations:

¹ Universidade Estadual de Campinas (UNICAMP).

Keywords: Autism. Neurodevelopmental Disorders. Rare Genetic Diseases. Neuronal Migration. Neurobiology.

Abstract:

Autism Spectrum Disorders (ASD) are neurodevelopmental conditions characterized by behavioral, cognitive and motor impairments. Pitt-Hopkins Syndrome (PTHS) is an ASD caused by *de novo* mutations in the *TCF4* gene. In neurons and organoids derived from PTHS patients, we observed reduced expression of genes encoding chemotactic molecules that regulate migration, including Ephrins (*EFN* genes) and their receptors (*EPH* genes). In this project, we have utilized in vitro 2D cultures of neurons derived from PTHS patients to investigate the link between impaired neuronal migration and changes in *EPH* gene expression. We analyzed time-lapse microscopy images from PTHS and controls neuronal 2D cultures to quantify migration. Moreover, we employed qPCR and immunostaining to assess the expression of neuronal migration markers, as well as *EPH* family members. Our results revealed a statistically significant reduction of migration marker *CRMP1* and *EPHA* subfamily ephrin receptor genes. These findings indicate impairments in neuronal migration, potentially driven by dysregulation of *EPHA* signaling pathways. We have also performed *knockdown* of selected *EPHA* genes, followed by evaluation of neuronal migration. Our study provides insight that will help elucidate the molecular and cellular mechanisms underlying this disease and may contribute to the development of targeted therapies. This project was approved by the Research Ethics Committee on Human Subjects (CEP) at UNICAMP, under CAAE approval number 86136925.6.0000.5404.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Evaluation of microglial cell response after rutin treatment in an retinitis pigmentosa animal model

Authors:

Gabriela Maria Badin¹
Théo Henrique de Lima Vasconcellos¹
Alexandre Hiroaki Kihara¹

Affiliations:

¹ Neurogenetics Lab, Centro de Matemática, Computação e Cognição, Universidade Federal do ABC, São Bernardo do Campo/SP.

Keywords: Flavonoid. Neurodegeneration. Neuroinflammation. Retina. Rutin.

Abstract:

The retina, a crucial component of the central nervous system, is susceptible to various degenerative diseases that can lead to vision loss. Retinitis pigmentosa (RP) is an inherited disorder characterized by the progressive degeneration of rod photoreceptors, driven by mechanisms such as apoptosis, oxidative stress, and neuroinflammation. Currently, treatment options are limited, with Luxturna®— a gene therapy targeting RPE65 mutations—being the only FDA-approved therapy, underscoring the need for broader, more accessible alternatives. Rutin, a flavonoid found in buckwheat, exhibits anti-apoptotic, antioxidant, and anti-inflammatory properties, making it a potential therapeutic agent for RP. This study evaluated the effects of a single acute subretinal dose of rutin in a mouse model of autosomal recessive RP. C3H/HeJRD1 mice (both sexes) were maintained on a 12:12h light-dark cycle. Rutin (1 mM) was injected into the right eye at P8 (the onset of degeneration), with the left eye receiving vehicle (~1% DMSO in PBS). The entire retinas were collected at P14 (the peak of degeneration) and flat-mounted for immunofluorescence (n = 5). All procedures were approved by CEUA/UFABC (#5037101121). Anti-Iba1 was used to assess the microglial response. Qualitative analyses were conducted, observing the central and peripheral retinal regions, along with the inner portion (corresponding to the GCL-IPL) and the outer portion (corresponding to the OPL-ONL), noting differences across the regions, with a more significant presence of microglia displaying a stellate morphology in the outer portion of vehicle, in contrast to apparently more amoeboid microglia in the treated retinas in the central region. Also, the counting of IBA1-positive cells was performed. These findings suggest that rutin may modulate microglial activation, warranting further investigation with expanded sample sizes and complementary analyses.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Biodegradable hydrogel membranes as treatment after spinal cord injury

Authors:

Sophia Sthefanie MOURA²

Marília Inês MÓVIO¹

Cayo Antônio Soares de ALMEIDA^{1,2}

Alexandre Hiroaki KIHARA¹

Affiliations:

¹ Laboratório de Neurogenética, Centro de Matemática, Computação e Cognição, Universidade Federal do ABC.

² Universidade Municipal de São Caetano do Sul.

Keywords: Biomaterials. Inflammation. Neuroscience. Spinal Cord Injury.

Abstract:

The spinal cord, located within the vertebral column, is essential for communication between the body and the brain, controlling movement and reflexes. Spinal cord injuries caused by trauma such as transections, hematomas, compressions, or penetrating objects can lead to severe complications. After the injury, secondary damage occurs due to dysregulated biochemical processes and the breakdown of the blood-spinal cord barrier (BSCB), including edema, cystic cavities, ischemic imbalance, neuroinflammation, and neurotransmitter dysregulation. These processes exacerbate the initial damage, leading to glial scar formation and necrosis. Rodent models are effective for study and reproducibility. Advances in biomaterials, such as hydrogels and bioscaffolds, have offered new therapeutic approaches, promoting tissue regeneration, controlled drug release, and BSCB reconstruction. The selection of the ideal biomaterial depends on its physicochemical properties, and personalized treatment is necessary due to the complexity of spinal injuries. This study evaluated the effectiveness of a sodium alginate hydrogel applied to the injured spinal cord of C57BL/6J mice, analyzing its impact on tissue regeneration and motor function using the Basso Mouse Scale (BMS). The animals were divided into two groups: one with untreated injury and one treated with the biomaterial. The protocol was approved by the Animal Ethics Committee (CEUA/UFABC - 8171271023). The results showed that the hydrogel significantly improved motor function, sensitivity, and balance, with reduced inflammation and enhanced healing, without signs of rejection. Neural reconnection was favored, indicating control of secondary injury and glial scarring. It is concluded that the hydrogel promotes near-normal motor recovery. Future advancements may include combinations with stem cells and drug delivery systems. Optimizing biomaterials will be essential for more effective and personalized treatments for spinal cord injuries.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: KLK6 Expression in a Neonatal Asphyxia Model: Implications for Neuroinflammation and Hypoxic-Ischemic Encephalopathy

Authors:

Luana Silva de Lima¹
Gabrielle Aguiar Mota¹
Daiana Alves Correia Lima¹
Débora Sterzeck Cardoso¹
Pamela Pinheiro Martins¹
Bruna Petrucelli Arruda¹
Alexandre Hiroaki Kihara²
Sílvia Honda Takada¹

Affiliations:

¹ LABORATORY OF NEUROHISTOLOGY/UFABC, SÃO BERNARDO DO CAMPO - SP – BRASIL.

² NEUROGENETICS LABORATORY/UFABC, SÃO BERNARDO DO CAMPO - SP – BRASIL.

Keywords: Perinatal asphyxia. Hippocampus. Neurodevelopment. Blood-brain barrier.

Abstract:

Perinatal asphyxia is a leading cause of neonatal mortality, with around 1 million deaths annually. Survivors may develop long-term motor and cognitive impairments, posing significant public health challenges. This condition, characterized by the failure to establish breathing at birth, can result from events such as pulmonary collapse, fetal hemorrhage, and metabolic disorders. The brain is particularly vulnerable to oxygen deprivation, leading to hypoxic-ischemic encephalopathy (HIE), which triggers cell death, oxidative stress, excitotoxicity, and neuroinflammation. Among the mediators involved, vasoactive molecules like kallikrein play a role in increasing blood-brain barrier permeability, potentially exacerbating neuroinflammation. KLK6, a key kallikrein isoform in the central nervous system, is involved in blood-brain barrier integrity, extracellular matrix degradation, cell migration, and inflammation in neurodegenerative diseases. However, its role in HIE remains unexplored. Neonate Wistar rats (P1) underwent neonatal anoxia (100% nitrogen, 25 min, 37°C), while controls were kept under the same conditions without air exchange. At P7, brain slices were analyzed via immunofluorescence to assess KLK6 in hippocampal regions, and Western blot evaluated its protein levels (CEUA: 8984211122). This pilot study precedes research on resveratrol's effects in asphyxiated animals. Western blot detected a 38 kDa KLK6 glycosylated form, but no differences were observed between groups ($N = 7$, $T(14) = 0.25$, $P = 0.81$). Histological analysis, despite stronger KLK6 labeling in CA3, showed no significant differences in CA1, CA3, or DG regions ($N = 5$, $F(1,24) = 0.11$, $P = 0.74$). These findings suggest that glycosylated KLK6 may not play a role in HIE physiopathology at P7. Further studies should investigate its involvement at earlier time points, as well as potential neuroprotective effects of resveratrol in modulating KLK6 expression and function in this context.

Financial support: FAPESP (20/16268-6 SHT; 2024/00357-0 GAM) and UFABC.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Evaluation of the effect of pharmacological blockade of TLR4 in the medial prefrontal cortex and small intestine of mice subjected to maternal immune activation combined with perinatal asphyxia

Authors:

Pamela Pinheiro Martins¹
Mélany Ferreira Vido¹
Ludmila Araujo da Rocha¹
Taís Fernandes de Oliveira¹
Helen Indalencio Gomes¹
Débora Sterzerck Cardoso¹
Bruna Petrucelli Arruda¹
Vinicius De Andrade Oliveira²
Alexandre Hiroaki Kihara³
Sílvia Honda Takada¹

Affiliations:

¹ Universidade Federal do ABC (UFABC), Laboratório de Neurohistologia, Centro de Matemática, Computação e Cognição, São Bernardo do Campo, SP, Brazil.

² Universidade Federal do ABC (UFABC), Centro de Ciências Naturais e Humanas, São Bernardo do Campo, SP, Brazil.

³ Universidade Federal do ABC (UFABC), Laboratório de Neurogenética, Centro de Matemática, Computação e Cognição, São Bernardo do Campo, SP, Brazil.

Keywords: Brain-Gut. Maternal immune activation. Neuroinflammation. Perinatal asphyxia. TLR4.

Abstract:

Maternal immune activation (MIA) triggered by infection during pregnancy is a risk factor for premature birth and perinatal asphyxia, both of which impact organs like the brain and gut. Their interaction through immune pathways may contribute to neurodevelopmental disorders. This study evaluated whether pharmacological inhibition of TLR4 with TAK-242 could mitigate the effects of a double-hit model combining MIA and perinatal asphyxia in mice. Pregnant C57BL/6 females received LPS (50 µg/kg, i.p.) on gestational day 14. Pups (1.8–2.5 g) were exposed to asphyxia in 100% nitrogen for 12 min. Groups included controls (VCV), double-hit (LAV), and treated (LAT; TAK-242, 3 mg/kg, i.p., for 4 days) (CEUA: 8306100221). Acute (24h) and juvenile (P36) brains and small intestine (SI) samples were collected for immunofluorescence, Fluoro-Jade C, western blot, and developmental assessments. MIA reduced maternal weight ($p < 0.0001$) and litter size ($p = 0.0025$). In pups, increased cell death ($p = 0.0261$), microglial activation ($p < 0.0001$), and elevated TLR4 and ARG1 expression in PFCm and SI were observed in LAV vs. controls. At P36, ARG1 ($p = 0.0084$) and iNOS ($p = 0.0095$) remained elevated in LAV. Somatic changes included reduced head size ($p < 0.050$) and altered body length ($p < 0.050$). Sensorimotor impairments in LAV included deficits in acceleration ($p = 0.0152$), handgrip ($p = 0.0095$), vibrissae placement ($p = 0.0016$), and startle reflex ($p = 0.0011$), alongside reduced sociability ($p = 0.0142$). TAK-242 treatment attenuated several of these alterations. These findings indicate that MIA combined with perinatal asphyxia affects brain-gut development, leading to lasting functional deficits, and that TLR4 inhibition is a promising therapeutic strategy.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Effect of maternal neglect on emotional behavior in male and female rats

Authors:

Aline Ananias de Lima¹
Maria Eduarda Santana do Nascimento³
Wilkson Rafael de Miranda Santos³
Fernando Antônio Bezerra Filho³
Swane Miranda Alves²
Sandra Lope de Souza^{1,2}

Affiliations:

¹ Graduate Program in Neuropsychiatry and Behavioral Sciences - UFPE, Recife-PE, Brazil.
² Graduate Program in Nutrition - UFPE, Recife-PE, Brazil.
³ Department of Nutrition - UFPE, Recife-PE, Brazil.

Keywords: Maternal deprivation. Early-life stress. Emotional behavior.

Abstract:

In addition to providing nutrition, breastfeeding fosters mother-infant bonding during a pivotal period of neurological development, playing a protective role in emotional maturation. In contrast, early maternal neglect has been associated with long-lasting behavioral consequences. This study examined the effects of maternal separation and early weaning on emotional behavior in Wistar rats. Approved by the Animal Ethics Committee (protocol no. 0036/2024), the study involved 25 rats (13 males and 12 females), randomly assigned to a neglect group (NG) or control group (CG). NG animals were separated from their mothers for 3 h/day from postnatal day (PND) 2 to 14 and weaned on PND-15; CG animals were weaned on PND-30. Body weight was recorded weekly. Sucrose consumption was assessed on PND-35 and 140 using a two-bottle choice test (water vs. 2% sucrose) over 24 h for 5 consecutive days. Anxiety-like behavior was evaluated on PND-59 and 159 using the elevated plus maze; each animal was filmed for 5 minutes to quantify time spent in open arms. Data were expressed as mean $\pm$ SD and analyzed using two-way ANOVA with Bonferroni post hoc test (GraphPad Prism v.8; significance set at $p \leq 0.05$). No significant differences in body weight or sucrose intake were observed between groups. However, adult NG animals spent less time in open arms than CG (males: $*p=0.0399$; females: $*p=0.0183$), suggesting increased anxiety-like behavior. These results indicate that early maternal neglect did not induce anhedonia or alter physical development, but promoted anxiety-related behavior in adulthood, reinforcing the hypothesis that early-life adversity increases vulnerability to emotional disturbances.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: The role of sleep in the acquisition of cocaine conditioned place preference

Authors:

Igor Menezes Gonçalves Rego¹
Alan Patrick Silva Gusmão¹
Lis Marques de Carvalho e Vieira Santos¹
Rafaela Schuttenberg Polanczyk¹
Luka Rodrigues Alves¹
Ikaro Jesus da Silva Beraldo¹
Francisco de Assis Carvalho do Carmo¹
Renan Augusto Viana Mendes¹
Fernandes¹
João Pereira Leite²
Fabricio de Araujo Moreira¹
Cleiton Lopes Aguiar¹

Affiliations:

¹ Universidade Federal de Minas Gerais.

² Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo.

Keywords: Sleep. Memory. Cocaine. Addiction.

Abstract:

Conditioned place preference (CPP) is a classic paradigm for studying substance abuse, showing a strong spatial association with the effects of psychoactive substances such as cocaine. There is evidence in the literature of a bi-directional relationship between cocaine abuse and sleep. Given the importance of the process of consolidating contextual memories, sleep may represent an important window of opportunity for the formation of associative memory linked to addiction. The aim of this work is to investigate the role of CPP post- acquisition sleep for the consolidation of cocaine-induced place preference in rats. Electrodes were implanted in adult male Wistar rats in the mPFC and CA1 region of the hippocampus, as well as an electromyogram (EMG) for movement recordings and the fixation of reference and ground on the occipital bone for cocaine sleep characterization. Also, a second group of rats underwent a CPP protocol for 6 days, which consisted of exposing the animal to the entire apparatus for 15 minutes and quantifying time spent in both compartments. For the next 4 days, the animals were alternatively treated with either cocaine (15 mg/kg) or saline via intraperitoneal injection, with each substance always associated with the same compartment. On the last day, the animals were once again allowed to freely explore the entire apparatus, and the time spent on each compartment was once again quantified. To test the role of sleep for the CPP consolidation, a subset of rats were sleep-deprived for 3 hours after each conditioning session. Initially, we characterized the behavioral and electrophysiological effects of cocaine (15 mg/Kg). All protocols were previously approved by CEUA UFMG (58/2025). Interestingly, after the well-described acute effects induced by cocaine, namely: hyperlocomotion and oscillatory patterns typical of arousal state, animals eventually slept and showed a striking increase in the delta band power in the medial prefrontal cortex (mPFC). We found a statistically significant increase in delta power between groups in the mPFC [SAL(D2) vs COCA-15(D2): +17,98%; Two-way ANOVA; treatment x time: $F(2, 13) = 37,52$, $p < 0,0001$, post hoc Tukey test, SAL(D2) vs COCA-15(D2); $p < 0,0001$. We have also successfully implemented the CPP protocol in our lab, with animals in the cocaine group showing a preference for the compartment paired with cocaine [Treatment) vs Compartment: +11,65%; Compartment: +35,53%; Two-way ANOVA; treatment x time: $F(2, 13) = 37,52$, $p < 0,0001$, post hoc Tukey test, SAL(D2) vs COCA-15(D2); $p < 0,0001$. We have also successfully implemented the CPP protocol in our lab, with animals in the cocaine group



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

showing a preference for the compartment paired with cocaine [Treatment) vs Compartment: +11,65%; Compartment: +35,53%; Two-way ANOVA; treatment x compartment: $F(1, 36) = 8,192$, $p = 0,0070$; compartment: $F(1,36) = 24,29$, $p < 0.0001$, post hoc Tukey test, NP Control vs P Coca; $p = 0,0004$, NP Coca vs P Coca; $p < 0,0001$, P Control vs P Coca, $p = 0,0247$. Next, we've tested if sleep deprivation for 3 hours after each conditioning session would be able to affect conditioning acquisition. This timeframe was chosen in light of the fact that animals subjected to cocaine in the previous experiment would display hyperlocomotion for ~3 hours after injection, during which there was an absence of sleep (sleep deprivation by gentle handling took place 3 hours after injection in both groups, at the start or sleep rebound in the cocaine group). The analysis of this experiment is still ongoing. In conclusion, we have successfully characterized the effects of cocaine on sleep as well as implementing a CPP behavioral protocol. Additional analysis is ongoing to ascertain the effects of sleep deprivation in the acquisition of place preference.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: The effect of Maternal Neglect on the body and reflex development of male rats

Authors:

Maria Eduarda Santana do Nascimento¹
Wilkson Rafael de Miranda Santos¹
Fernando Antônio Bezerra Filho¹
Swane Miranda Alves²
Aline Ananias de Lima³
Sandra Lopes de Souza^{2,3}

Affiliations:

¹ Department of Nutrition - UFPE, Recife-PE, Brazil.

² Graduate Program in Nutrition - UFPE, Recife-PE, Brazil.

³ Graduate Program in Neuropsychiatry and Behavioral Sciences - UFPE, Recife-PE, Brazil.

Keywords: Maternal neglect. Reflex-ontogeny. Stress.

Abstract:

The maturation of neural connections in the offspring through maternal stimuli will occur mainly through learning mechanisms. However, maternal neglect in early life is recognized as an early stress factor that negatively interferes with neurodevelopment, with negative consequences that have repercussions throughout life. Therefore, the aim of the research was to evaluate, in animals subjected to maternal neglect, body growth through weight measurements and murinometrics, and nervous system development through the ontogenesis of reflexes. The research was approved by the ethics committee (protocol nº 0036/2024). The male Wistar rats were used, subdivided into the neglect group (NG) (the animals were subjected to maternal separation from postnatal day (PND) 2 to day 14, followed by early weaning on PND-15) and the control group (C) weaned on PND-30. During the neonatal phase, from PND-2 to PND-20, the following were individually assessed: body weight; murine measurements were taken using a caliper and millimetre ruler; the ontogeny of reflexes was assessed using specific instruments for each reflex. According to the analysis, it was observed that body weight was lower ($p < 0.001$) in the NG group on PND-5 to 20 than in the C group. When the murineometric measurements of the NG group were analyzed, they were smaller than those of the C group, in relation to the anteroposterior axis of the skull ($p < 0.001$) at PND-8 and PND-14 to 20, the laterolateral axis of the skull ($p < 0.001$) at PND-8 and 17, the longitudinal axis ($p < 0.001$) between PND-5 to 20 and the caudal axis ($p < 0.001$) at PND-11 and 17 to 20. In the ontogeny of reflexes, there was a delay in negative geotaxis ($p < 0.001$) in the NG group compared to the C group. It was concluded that maternal neglect reduced body weight at the same time as murineometric measurements. It also delayed the negative geotaxis reflex.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Carotid Bodies Mediate Sympathetic Control of Inflammation During Systemic LPS Challenge

Authors:

Tales Ferruccio Vicentini Rosin¹
Livia Eduarda Cabrera Corrêa¹
Rodrigo Silva de Moraes¹
Isabela Paula Leirão¹
Lais Medeiros Cardoso¹
Fernanda Campos de Medeiros¹
Débora Simões de Almeida Colombari¹
Eduardo Colombari¹
Fernanda Gonçalves Basso¹
Daniel Breseghello Zoccal¹
Pedro Lourenço Katayama¹

Affiliations:

¹ Department of Physiology and Pathology, School of Dentistry, São Paulo State University, Araraquara, São Paulo, Brazil.

Keywords: Carotid body. Inflammation. Neuroimmune. Sympathetic nervous system.

Abstract:

Peripheral systemic inflammation activates brain circuits that modulate innate immunity. While the sensory pathways mediating this body-brain communication are not fully understood, recent evidence indicates that the carotid bodies, which sense blood composition, can detect elevated TNF-alpha levels. This detection recruits brain pathways that increase sympathetic activity, a counterregulatory mechanism against further TNF-alpha production. To investigate the role of carotid bodies in broader systemic inflammation, we used a lipopolysaccharide (LPS)-induced model where multiple inflammatory mediators are elevated in the blood. Ethical approval was obtained (Araraquara School of Dentistry, UNESP; 09/2024). Male Sprague-Dawley Holtzman rats (300-385 g) underwent sham surgery (SHAM) or bilateral carotid body excision (CB-X) and, after 7 days, received LPS (0.5 mg/kg, 300 µL, i.p.) or sterile saline (0.9% NaCl, 300 uL, i.p.). In the rostral ventrolateral medulla (RVLM), a region enriched in premotor sympathetic neurons, LPS activated significantly more neurons in SHAM (25 ± 5 c-FOS+ cells/section; $n = 3$) compared to CB-X rats (14 ± 2 c-FOS+ cells/section; $n = 3$; $p < 0.05$, Student's t-test). Consistently, LPS induced a greater increase in splanchnic sympathetic nerve activity in SHAM than in CB-X rats ($n = 2$ -3/group). Furthermore, CB-X rats treated with LPS exhibited significantly higher plasma TNF-alpha levels (5680 ± 1186 pg/mL, $n = 6$) compared to all other groups (sham + saline: 26 ± 23 pg/mL, $n = 6$; CB-X + saline: 120 ± 159 pg/mL, $n = 6$; sham + LPS: 2085 ± 1156 pg/mL, $n = 6$; $p < 0.05$, one-way ANOVA). These findings suggest that the carotid bodies act as sensors of systemic inflammation, recruiting the sympathetic nervous system to dampen the inflammatory response.

Financial support: FAPESP, CNPq.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: OVLT-PVN purinergic pathway contributes to blood pressure control during acute hyperosmotic stimulation

Authors:

Giovanna Arielle de Oliveira¹
Paula Magalhães Gomes¹
Vagner Roberto Antunes¹

Affiliations:

¹ Universidade de São Paulo.

Keywords: Neurogenic hypertension. Organum Vasculosum of the Lamina Terminalis. Purinergic signaling.

Abstract:

Hyperosmolality-induced hypertension involves OVLT activation, which projects monosynaptically to the PVN— that expresses purinergic (P2) receptors. Previous studies indicate that PVN sympathoexcitation depends on P2 receptor activation, and hyperosmotic stimuli trigger ATP release, partly from astrocytes. We hypothesize that an acute hyperosmotic stimulus in the OVLT elevates blood pressure (BP) via ATP release in PVN neurons. To test this, we evaluated: 1) neuronal activation (c-Fos) and purinergic phenotype (VNUT) in OVLT-projecting PVN neurons, and 2) the effect of P2 antagonism on BP. Adult male Wistar rats (10 weeks; CEUA ICB/USP: #7737080323) were divided into two protocols: 1) triple-labeling with Fluoro-Gold injected into the PVN combined with VNUT and c-Fos immunofluorescence after i.v. infusion of hyperosmotic (3M NaCl, n=4) or isotonic (0.154M, n=6) solution; and 2) OVLT microinjections of 3M NaCl (50 nL) before and after PVN PPADS (10 μ M, 100 nL/side), with blood pressure (BP) recorded via femoral artery catheter (n=5). Data are mean $\pm$ SE; $p < 0.05$ by one-way ANOVA and Student's t-test. Immunoreactive neurons for VNUT were observed in the dorsal cap and lateral margins of the OVLT and were colocalized with c-Fos+ cells projecting to the PVN. While only a small number of c-Fos-expressing neurons were observed in the OVLT of rats treated with isotonic saline (5 ± 15 cells), a significantly higher number of c-Fos+ neurons were found in the OVLT of rats treated with hypertonic saline (55 ± 15 cells). Stimulation of the OVLT with 3M NaCl elicited a significant increase in BP, an effect that was attenuated after bilateral injection of PPADS in the PVN (Δ MAP: NaCl OVLT: $+10 \pm 1$ mmHg vs. NaCl OVLT post-PPADS PVN: $+2.4 \pm 1.3$ mmHg, $p < 0.0001$). Collectively, our findings show that hyperosmotic NaCl stimulation activates an OVLT-PVN purinergic pathway that contributes to blood pressure regulation during acute osmotic challenges. Financial Support: FAPESP 2023/04290-5.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Maternal Consumption of a High-sucrose or High-fat Diet Alters Cognition and Memory Behavior and Hippocampal Transcriptome in Offspring

Authors:

Mayara de Nóbrega Baqueiro¹
Aline Franco de Campos¹
Suleyma Oliveira Costa¹
Guang Han²
Luciana Caroline Paulino do Nascimento¹
Marcio Alberto Torsoni¹
Michael Ross^{2,3}
Mina Desai^{2,3}
Adriana Souza Torsoni¹

Affiliations:

¹ School of Applied Sciences, University of Campinas (UNICAMP); Limeira, SP, Brazil.

² The Lundquist Institute at Harbor-UCLA Medical Center.

³ Department of Obstetrics and Gynecology, David Geffen School of Medicine, University of California Los Angeles at Harbor-UCLA, Torrance, CA, USA.

Keywords: Cognition. Sucrose. Fat. Hippocampus. Memory. Offspring.

Abstract:

The Western Diet, characterized by high levels of saturated fats and simple sugars such as sucrose, is associated to metabolic disorders that trigger neuroinflammation and adversely impact cognition. This dietary pattern compromises the hippocampus, particularly the CA3 region, which is crucial for learning and memory, especially associative memory. This study aimed to evaluate the effects of maternal high-sucrose and high-fat diet on the memory, behavior and CA3 transcriptome of offspring. **Methodology:** C57BL/6 female mice (CEUA UNICAMP #5636-1/20) were fed during pregnancy and lactation a control diet (CT 10% fat, 7% sucrose, n=6-9), a high sucrose diet (HS 10% fat, 35% sucrose, n=13-18), or high-fat diet (HF 45% fat, 17% sucrose, n=5-6). After birth, the litter size was adjusted to 3 females and 3 males and the offspring were weaned onto control diet. At 12 weeks, the Novel Object Recognition (NOR) test was performed to assess memory. Following testing, offspring were euthanized and the hippocampal CA3 area was sampled for RNA sequencing (transcriptome).

Results: Female, but not male offspring of HS dams exhibited a greater distance covered and a lower discrimination ratio of the new object compared to those from CT dams, while no differences were observed in the offspring of female HF dams compared to CT. In males, offspring of HF dams showed a lower discrimination ratio of the new object than both CT and HS groups. Transcriptome analyses of the CA3 region revealed that HS female offspring showed upregulation of *TERT1* and *CXCR4* genes, which mediate stem cell proliferation and cytokine response interaction, while HF male offspring showed upregulation of *POMC* and *MC4R* genes. These findings suggest that exposure to high levels of sucrose or fat during development can have distinct and harmful effects on behavior and memory in both female and male offspring.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Early acoustic deprivation does not substantially affect the electrophysiological properties of fusiform neurons of the dorsal cochlear nucleus (DCN) of mice

Authors:

Alan Eidi SASAKI¹
Mario Henrique Lopes Duarte de OLIVEIRA¹
Natalia BOARETTO¹
Nikollas Moreira BENITES²
Ricardo Mauricio Xavier LEÃO¹

Affiliations:

¹ Faculdade de Medicina de Ribeirão Preto – USP.
² University of South Florida.

Keywords: Auditory brainstem. Fusiform neurons. Neurophysiology. Persistent sodium current. Whole-cell patch-clamp.

Abstract:

The auditory system is responsible for processing sound information received from the environment. The peripheral auditory system receives stimuli and translates them into electrical signals as action potentials (APs) in the auditory nerve, which reaches central nuclei. Cochlear nuclei constitute the first processing center of auditory information in the central nervous system. Fusiform neurons present in the dorsal cochlear nucleus (DCN) exhibit active states (firing spontaneously) and silent states. These states are controlled by expression of persistent sodium currents (INaP). Active states develop after hearing onset (P14) along with a refinement of APs. We tested the hypothesis that auditory experience triggers the appearance of active states and changes in AP firing. We used a model of acoustic deprivation consisting of a fast cure silicone gel earplug applied at P14, which increases hearing thresholds by 30-40 dB. Experiments approved by FMRP ethics committee for animal experimentation - CEUA (1288/2024R2). Swiss mice (P14) were divided into control (no manipulation), sham (manipulated, no plug) and plug groups. At P21±2, sham and plug groups were tested for plug effectiveness using Auditory Brainstem Response (ABR). At this age, all groups were euthanized for brainstem dissection and DCN slices. Next, whole-cell patch clamp was performed for voltage-clamp (I-V) and current-clamp (V-I) recordings. Plug effectiveness was confirmed using ABR. I-V comparison at -45mV showed no significant change of INaP across all groups. AP parameters (V-I) showed no significant change between sham (n=21) and plug (n=18) groups, but there was significant difference between control (n=14) and sham groups in some parameters. Our data suggests that auditory experience is not necessary to drive maturation of APs nor the establishment of active states of fusiform neurons. The differences between control and sham mice suggest that some parameters could be sensitive to anesthesia.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Sympathetic denervation aggravates hyperalgesia but does not affect synovial TNF- α levels in zymosan-induced temporomandibular joint inflammatory pain in rats

Authors:

Livia Eduarda Cabrera Corrêa¹
Tales Ferruccio Vicentini Rosin¹
Débora Simões de Almeida Colombari¹
Daniel Breseghello Zoccal¹
Eduardo Colombari¹
Pedro Lourenço Katayama¹

Affiliations:

¹ Department of Physiology and Pathology, School of Dentistry, São Paulo State University, Araraquara, São Paulo, Brazil.

Keywords: Inflammation. Inflammatory pain. Neuroimmune interactions. Sympathetic nervous system.

Abstract:

Previous research suggests the sympathetic nervous system (SNS) modulates pain and inflammation. This study investigated the SNS role in zymosan-induced inflammatory pain of the temporomandibular joint (TMJ) in rats. Experiments were performed on male Holtzman rats (~300 g) and were approved by the Ethical Committee in Animal Experimentation of the Araraquara School of Dentistry, São Paulo State University (09/2024). We first conducted neuroanatomical experiments and confirmed that the sympathetic neurons innervating the TMJ reside in the ipsilateral superior cervical ganglion (SCG). Then, we subjected a group of animals to left SCG surgical removal (SCG-X) to eliminate the sympathetic innervation of the ipsilateral (left) TMJ. After 7 days, SHAM and SCG-X rats had their baseline TMJ mechanical nociceptive threshold assessed by an electronic Von Frey. Rats then received either zymosan (1 mg in 40ul sterile saline) or sterile saline (40 ul) into the left TMJ and the nociceptive threshold was reassessed 24 hours later. Multiple comparisons by 2-way ANOVA showed that SCG-X or saline treatments alone had no effects ($p > 0.05$). Zymosan treatment decreased the mechanical nociceptive threshold (hyperalgesia) in both SHAM and SCG-X rats compared to their counterparts treated with saline ($p < 0.05$). Remarkably, SCG-X rats with zymosan had lower ($p < 0.05$) nociceptive threshold (55.3 ± 9.3 g; $n=9$) than SHAM with zymosan (91.9 ± 20.7 ; $n=9$). We then hypothesized that the absence of SNS could exacerbate the zymosan-induced inflammation aggravating hyperalgesia. TNF- α levels were quantified by ELISA in the TMJ synovial fluid of SHAM and SCG-X rats 24 hours after zymosan or saline injections. TNF- α levels were similar in SHAM and SCG-X rats injected with zymosan (SHAM: 14.02 ± 5.5 pg/mL vs SCG-X: 13.21 ± 2.1 pg/mL; $n=6-7$, $p > 0.05$). Our data indicate that the SNS restricts the TMJ hyperalgesia in the zymosan-induced inflammatory pain model, independent of local TNF- α .

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Screen use in children aged 12 to 42 months attending daycare with an enriched environment

Authors:

Letícia Henrique Leite da Silva¹
Maria Eduarda Rodrigues Alves dos Santos^{1,2}
Ana Patrícia da Silva Souza^{1,2}
Karollainy Gomes da Silva^{1,2}
Ana Beatriz Januário da Silva^{1,2}
José Maurício Lucas da Silva^{1,2}
Mayara Luclécia da Silva^{1,2}
Robson Feliciano da Silva²
Aline Ananias de Lima¹
Georgiane Brandão Rodrigues da Silva²
Erica Helena Alves da Silva²
Antonietta Claudia Barbosa da Fonseca Carneiro¹
Waleska Maria Almeida Barros^{1,2}
Sandra Lopes de Souza¹

Affiliations:

¹ Graduate Program in Neuropsychiatry and Behavioral Sciences, Center for Medical Sciences - UFPE, Recife - PE, Brazil.

² Integrated Center of Neuroscience Technologies (CITENC), Osman Lins University Center - UNIFACOL, Vitória de Santo Antão - PE, Brazil.

Keywords: Development. Toddler. Childcare.

Abstract:

Screen use in early childhood has been associated with problems in overall development, especially impairments in social and self-regulation skills. Enriched environments are combinations of stimuli that allow exploration, socialization, imagination, and other actions that aid in adequate development. The objective of this study is to observe the frequency of screen use, types of screens used, and average screen time in children aged 12 to 42 months attending daycare centers with an enriched environment. This study is an excerpt from a longitudinal study approved by the Research Ethics Committee of the Federal University of Pernambuco under opinion No. 7.447.703, under resolution 466/12. Seventeen children with a mean age of 32.25 months were evaluated before being placed in an enriched daycare environment (T0) and after 6 months of placement (T1) through a semi-structured questionnaire addressed to guardians with the following questions regarding screen use: 1-Use of screens (1-yes, 2-no), 2- Type of screen (1-television, 2-cell phone, 3-computer, 4-tablet), 3- Screen time (in minutes). At T0, 16 children (94.1%) used screens according to reports from guardians, while only one did not. The frequency remained the same at T1, however, the child who did not use screens started to use them, while another who used screens stopped using them. Regarding the type of screen most frequently reported by those responsible, it was television (T0: 70.6%, n=12; T1: 64.7%, n=11), followed by cell phones (T0: 23.5%, n=4; T1: 23.5%, n=4) at both times. A reduction in the average screen time was also observed, being 91.43±15.78 minutes at T0, while 56.53 minutes±11.05 at T1. Thus, it is possible to observe a reduction in screen time after 6 months of submission to the enriched environment. Future perspectives include investigating these results after 1 year of submission and the effect of the enriched environment on this outcome.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Effects of Dance on Daily Activities of Children with Autism Spectrum Disorder: Randomized Controlled Triple-Blind Clinical Trial

Authors:

Lívia Maria Ribeiro Rosário¹
Ana Flávia Felicioni de Oliveira²
Daniele Monique Galdino Silva³
Kevin Douglas Feliciano de Paiva³
Adriana Teresa Silva Santos⁴
Luciana Maria dos Reis⁴

Affiliations:

- ¹ Institute of Experimental Biology, Federal University of Alfenas – MG.
² Institute of Movement Sciences, Federal University of Alfenas – MG.
³ Department of Physical Therapy, South of Minas University Center, Brazil.
⁴ Institute of Movement Sciences, Federal University of Alfenas – MG.

Keywords: Dance. Functionality. Music Therapy. Autism Spectrum Disorder. Rehabilitation.

Abstract:

Autism Spectrum Disorder (ASD) is a pervasive neurodevelopmental condition characterized by persistent deficits in communication and social interaction, along with restrictive and repetitive behavioral patterns. Since sensory abnormalities can negatively affect the development and performance of functional skills, independence in adulthood may be compromised. In light of this, dance has been investigated for its potential to reduce core symptoms of autism, foster social interaction, and improve functional independence. This study aimed to analyze the effects of dance on the functional skills related to daily activities in children with ASD. A randomized, controlled, triple-blind clinical trial was conducted with 26 children aged five to ten years diagnosed with ASD at the Varginha Foundation for Individuals with Special Needs, Varginha/MG, Brazil. Participants were assigned to either an experimental group (EG, n = 14), which received dance therapy (fourteen sessions lasting 30 to 50 minutes, twice per week) combined with multiprofessional care, or a control group (CG, n = 12) receiving only multiprofessional care. The study was approved by the Human Research Ethics Committee of Universidade Federal de Alfenas (approval no. 59074822.6.0000.5142). To assess outcomes, the Childhood Autism Rating Scale and the Pediatric Evaluation of Disability Inventory – Computer Adaptive Test (PEDI-CAT) were applied before and after the intervention, and at follow-up. Data were analyzed using SPSS® version 20.0 (t-test, Mann-Whitney, ANOVA), with a significance level of 5% ($p < 0.05$). The EG showed significant improvements in the PEDI-CAT Daily Activities domain between pre- and post-intervention ($p = 0.00$; 95%CI: -4.57 to -2.13) and pre-intervention and follow-up ($p = 0.00$; 95%CI: -3.87 to -1.27). Therefore, dance combined with multiprofessional therapies enhanced the functional abilities of children with ASD.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Effects of chronic exposure to glyphosate-based herbicides on behavior and short-and long-term memory of female and male Wistar rats

Authors:

Luã Henrique Urrutigaray^{1,2}
Welerson dos Reis^{1,3}
Anna Karolina Kretschmann Florencio de Souza Bagetti^{1,3}
Diovana Gelati de Batista^{1,4}
Eduarda Bordignon^{1,5}
Maria Eduarda Todendi^{1,5}
Laura Schleder Corrêa^{1,6}
Samara Cristine Knebel^{1,6}
Thiago Gomes Heck^{1,3,6}

Affiliations:

¹ Grupo de Pesquisa em Fisiologia (GPeF), Universidade Regional do Noroeste do Estado do Rio Grande do Sul (UNIJUÍ).

² Curso de Psicologia, Universidade Regional do Noroeste do Estado do Rio Grande do Sul (UNIJUÍ).

³ Curso de Medicina, Universidade Regional do Noroeste do Estado do Rio Grande do Sul (UNIJUÍ).

⁴ Programa de Pós-Graduação em Modelagem Matemática e Computacional, Universidade Regional do Noroeste do Estado do Rio Grande do Sul (UNIJUÍ).

⁵ Curso de Biomedicina, Universidade Regional do Noroeste do Estado do Rio Grande do Sul (UNIJUÍ).

⁶ Programa de Pós-Graduação em Atenção Integral à Saúde, Universidade Regional do Noroeste do Estado do Rio Grande do Sul (UNIJUÍ).

Keywords: Glyphosate. Memory. Pesticides.

Abstract:

Introduction: Glyphosate (GLY) is the most widely used herbicide, extensively used in agriculture. While it is considered safe by different regulatory agencies at typical exposure levels, studies have suggested that glyphosate can induce oxidative stress and neuroinflammation, thus contributing to the development of neurobehavioral disorders.

Objectives: We investigated whether chronic low-dose GLY exposure alters memory and behavior in male and female Wistar rats. **Methods:** Adult (4 months-old), female (F) and male (M) Wistar rats (n=24) were divided equally into 4 groups for 12 weeks: Controls (F and M groups), and F-GLY and M-GLY groups, which received Glyphosate ~0.5 mg/kg/day in water (weekly adjusted) during 12 weeks, representing the acceptable daily intake (ANVISA). In the last week, the rats were placed (8-12 a.m.) in a controlled environment with two objects for familiarization, and 5h and 24h after that, one of the objects was changed to test short and long recognition memory, respectively. Data were tested for normality and compared by two-way ANOVA (P<0.05, GraphPad). **Results:** In the short-term object recognition test, the groups showed no differences in time of exploring new/old objects (seconds) (F=44.6±28.6, F-GLY=44.1±30.9, M=83.1±48.6 and M-GLY= 60.6±49.4, P=0.144). Similar results were observed in the long-term object recognition test (F=70.7±51.2, F-GLY= 84.8 ± 23.3, M= 120.0±96.5 and M-GLY= 89.8±85.3, P=0.356). In the Open Field test, there was no difference in movement time (F=156.6±39.6, F-GLY=155.1±28.3, M=136.8±96.5 and M-GLY=163.3±70.8, P=0.817), and males spent more time in the center (F=39.6±30.6, F-GLY=25.3±13.2, M=76.08±88.3 and M-GLY=96.0±71.3, P=0.038) and in the lateral than females (F=260.3±30.6, F-GLY=274.6±13.2, M=223.8±88.3 and M-GLY=204.1±71.5 P=0.038), independently of the GLY exposure.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Conclusion: Chronic exposure to glyphosate at low doses did not alter behavior and memory performance in Wistar rats, independently of the sex. Ethics Committee: CEUA nº 028/24.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Melatonin promotes significant reduction in TNF- α levels in the hippocampus of rats with apical periodontitis

Authors:

Anna Clara Cachoni¹
Louise Elias Carvalho²
Ana Carla Thalez Iwabuchi Nobumoto²
Bruna de Oliveria Alves²
Maria Sara de Lima Coutinho Mattera²
Bianca Elvira Belardi²
Estêvão Lopes Pereira²
Lorena Umbelino Rodrigues²
Luciano Tavares Angelo Cinta¹
Doris Hissako Matsushita²

Affiliations:

¹ Department of Preventive and Restorative Dentistry, São Paulo State University (UNESP), School of Dentistry, Araçatuba, Brazil.

² Department of Basic Sciences, São Paulo State University (UNESP), School of Dentistry, Araçatuba, Brazil.

Keywords: Apical Periodontitis; Neuroinflammation; Melatonin; Cognitive Dysfunction

Abstract:

Chronic inflammation of the teeth, such as Apical Periodontitis (AP) and Periodontal Disease (PD), has been associated with systemic disorders, including cognitive deficits. AP is an inflammation of the apex of the tooth root, usually resulting from bacterial infection, which triggers a systemic increase in pro-inflammatory cytokines. Among them, TNF- α stands out for activating inflammatory pathways, such as the JNK signaling cascade, promoting neuroinflammation and the accumulation of β -amyloid(A β), one of the main neuropathological markers of Alzheimer's disease. Despite this relationship, few studies investigate the direct correlation between AP and changes in the brain inflammatory pathway. Melatonin (MEL) has antioxidant and anti-inflammatory properties, in addition to a potential neuroprotective effect. Evidence suggests that MEL can modulate TNF- α /JNK signaling. Therefore, this study aimed to evaluate whether induced PA compromises cognitive function in rats and whether treatment with MEL can prevent such alterations. For this purpose (CEUA FOA Process: 292-2024), 32 Wistar rats (2 months) were assigned to four experimental groups (n=8): 1) Control (CN); 2) Control treated with MEL (CNMEL); 3) Induced AP (AP); and 4) Induced AP and treated with MEL (APMEL). PA was induced by exposure of the pulp tissue to the oral environment and, after 30 days, the animals received daily treatment with MEL (5 mg/kg, orally) for 60 days. After the experimental period, the modulation of the TNF- α , the β -amyloid content and JNK inflammatory pathway in the hippocampus was analyzed by western blotting. Statistical analysis was performed by two-way ANOVA, followed by the Bonferroni test (p<0.005). The results showed that AP promoted an increase in TNF- α in the hippocampus and melatonin reversed this change CN:13,5848; AP:24,3521; CNMEL:12,6864; APMEL:15,594. We conclude that AP promotes inflammation in the hippocampus and melatonin may be an effective adjuvant treatment in reducing neuroinflammation.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Virtual Reality and aspects of sleep and cognition in overweight/obese children in Northeastern Brazil

Authors:

Ana Patrícia da Silva SOUZA^{1,2,3}
Maria Eduarda Rodrigues Alves dos SANTOS^{1,3}
Ana Beatriz Januário da SILVA^{1,3}
Karollainy Gomes da SILVA^{1,3}
Beatriz Machado SILVA⁴
José Maurício Lucas da SILVA^{1,2,3}
Mayara Luclécia da SILVA^{1,3}
Aline Ananias de LIMA¹
Williclecia Walkiria Dias FERREIRA³
Érica Helena Alves da SILVA³
Robson Feliciano da SILVA³
Letícia Henrique Leite da Silva¹
Antonietta Cláudia Barbosa da Fonseca CARNEIRO^{1,2,3}
Sandra Lopes de SOUZA¹
Waleska Maria Almeida BARROS^{1,2,3}

Affiliations:

¹ Postgraduate Program in Neuropsychiatry and Behavioral Sciences, Center for Medical Sciences, Federal University of Pernambuco, Recife, Pernambuco, Brazil.

² Department of Physiotherapy, Health Sciences Center, FACOL University Center (UNIFACOL), Vitória de Santo Antão, Pernambuco, Brazil.

³ Integrated Center for Neuroscience Technologies, FACOL University Center (UNIFACOL), Vitória de Santo Antão, Pernambuco, Brazil.

⁴ Laboratory of molecular biology in infectious diseases - department of parasitology - Instituto Aggeu Magalhães – Fiocruz, Recife, Pernambuco, Brazil.

Keywords: Children. Cognition. Exergames. Sleep. Virtual reality.

Abstract:

Virtual Reality is a resource that has been considered strategic and relevant for adopting a more active lifestyle and may be helpful for overweight or obese children. The objective was to evaluate the effect of the Virtual Reality Games protocol on cardiometabolic variables, sleep, and cognitive performance in overweight/obese children from public schools. The sample consisted of children aged 5 to 9 years old. They underwent anthropometric measurements, blood collection, application of the pediatric sleep scale, and the Mini-Mental State Examination test. Ethics committee number: 5.586.951, Federal University of Pernambuco. The virtual reality protocol is conducted at school, twice a week, for 40 minutes, featuring games that simulate sports and dance, utilizing the Xbox Kinect. To date, 54 children with a mean age of 7 ± 1.45 years, in four schools, were evaluated, of which 25 (46.29%) were female. A descriptive analysis of the data was performed. Initially, the children were allocated into two groups: Control (C), $n=25$, and Intervention (I), $n=29$. BMI (kg/m^2) C 20.48 ± 2.3 , I 20.65 ± 2.3 . In the final evaluation, high total cholesterol levels were observed in both groups (C $164.84 \text{ mg}/\text{dl} \pm 18.05$; I $160.20 \text{ mg}/\text{dl} \pm 20.99$). Sleep time was within the recommended standard (C $10.3 \text{ h} \pm 1.85$ and I $9.58 \text{ h} \pm 1.31$). However, regarding the levels of daytime sleepiness, the control group presented a higher average (10.32 ± 4.64) compared to the initial evaluation (9.8 ± 4.75). In the cognitive



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

evaluation, groups C (25.7 ± 7.4) and I (22.9 ± 6.3) increased the averages of the general totals; however, in the attention and calculation domain, the averages demonstrated a low score for C 5.4 ± 3.6 and I 2.9 ± 3.4 . Vitamin B12 levels were analyzed only in the initial evaluation, and the average was 388.87 ± 155.16 (pg/mL). Data collection is ongoing. To date, it has been possible to identify averages in cardiometabolic and cognitive variables that are different from the values considered adequate, indicating possible dysfunctions or risk conditions.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Evaluation of the involvement of TLR4 receptors in nociception induced by SARS-COV-2 peptide 3 in mice

Authors:

Rafaela Silva dos Santos^{1, 2, 3}
Giovane Galdino de Souza^{1, 2, 3}

Affiliations:

¹ Federal University of Alfenas (UNIFAL-MG).

² Center for Experimental Biology (CBIOEX).

³ Pain Immunobiology Laboratory (LabNID).

Keywords: SARS-CoV-2. Microglia. TLR4 Receptor.

Abstract:

The 2019 coronavirus (COVID-19) is a highly transmissible infectious disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which has caused severe health, economic, and developmental issues in countries around the world, leading, in extreme cases, to the death of thousands of people. Glial cells are involved in the modulation of pain caused by this inflammation, which, when activated, can increase the expression of receptors that activate intracellular pathways, culminating in the release of pró-inflammatory mediators. The Spike protein on the surface of the S1 subunit of the virus is responsible for binding to the Toll-like receptor 4 (TLR4), which functions to recognize molecular patterns associated with damage and pathogens (DAMPs and PAMPs). In this way, the present study, under the protocol 0010/2021, investigated the participation in nociception induced by the peptides (PSPD2003) present in the SARS-CoV-2 Spike protein. The peptides were synthesized and donated in collaboration with the Chemistry Institute of the São Paulo State University, Araraquara-SP campus. Male C57BL/6 mice, weighing between 20 and 25g, were used for this purpose. The von Frey filament test was used to evaluate the nociceptive threshold. To induce nociception, peptide 3 was administered intrathecally (i.t.). The role of the TLR4 receptor was investigated using the LPS-RS antagonist and inhibitors of the MAPK/p38/NFkB pathway. To evaluate the role of microglia, the minocycline inhibitor was used. Blocking the TLR4 receptor, the MAPK/p38/NFkB signaling pathway, and inhibiting microglia resulted in the reversal of nociception ($p < 0,001$). The results suggest the involvement of TLR4 and the MAPK/p38/NFkB signaling pathway in microglia in nociception induced by peptide 3 from the SARS-CoV-2 Spike protein.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Oxidative balance in the brainstem in the context of cancer

Authors:

Vanessa Lima de Souza^{1, 2, 3}
Tiago Lacerda Ramos^{3,4}
Débora Eduarda da Silva Fidelis^{3,4}
Tamara Stefany de Miranda Melo^{1,5}
Nataly da Silva Souza^{1,5}
Matheus Santos de Sousa Fernandes^{3,4}
Fabrício Oliveira Souto^{3,4}
Cláudia Jaques Lagranha^{1,2}

Affiliations:

¹ Laboratory of General, Molecular and Exercise Biochemistry (LABMEX-UFPE). ² Graduate Program in Neuropsychology and Behavioral Sciences (UFPE).
³ Keizo Asami Institute, Federal University of Pernambuco.
⁴ Graduate Program in Biology Applied to Health, Federal University of Pernambuco.
⁵ Undergraduate student in physical education.

Keywords: Brainstem. Cancer. Oxidative Stress.

Abstract:

Introduction: Cancer is a multifactorial disease driven in part by oxidative stress, resulting from an imbalance between pro-oxidant and antioxidant systems, which promotes DNA damage, genomic instability, and tumor progression. This study aims to analyze the modulation of oxidative balance in the brainstem under cancer conditions. **Methods:** Male C57BL/6 mice from the iLIKA/UFPE vivarium will be maintained in an experimental vivarium (22°C ± 2) with Nuvelab CR1 diet and ad libitum water. At 45 days, they were divided into 2 groups: G1 (without cancer) and G2 (with cancer). In the fourth week, G2 received 300 µL of sarcoma 180 (10⁶ cells). At 90 days, the animals were euthanized for mitochondrial and oxidative analyses in the brainstem. CEUA-UFPE (0051/2023). The results were performed using the Welch t-test and the Mann-Whitney test (p < 0.05). **Results:** Indicators of mitochondrial function, oxidative stress and antioxidant defenses were evaluated. Mitochondrial functionality showed a significant difference in NAD⁺/NADH levels, p=0.039, but did not show a significant difference in citrate synthase levels, p=0.279. Lipid peroxidation and protein oxidation were lower in G2, with values of p=0.072 and 0.004, respectively. SOD activity was not significant between the groups, p = 0.613, while CAT was significant with p = 0.021, and GST did not present statistical differences, p = 0.249. The REDOX state (GSH/GSG) also did not show significant differences with p = 0.696 but showed significant changes in total thiol levels (p = 0.003). Conclusion: The brainstem of animals with cancer showed metabolic alterations and greater antioxidant defense, suggesting neuroprotective adaptation against oxidative stress.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Impacts of enriched environment on the modulation of oxidative balance in the hypothalamus in the context of cancer

Authors:

Vanessa Lima de Souza^{1, 2, 3}
Débora Eduarda da Silva Fidelis^{3,4}
Tiago Lacerda Ramos^{3,4}
Maria Eduarda de Lima^{1,5}
Matheus Santos de Sousa Fernandes^{3,4}
Fabrício Oliveira Souto^{3,4}
Cláudia Jaques Lagranha^{1,2}

Affiliations:

- ¹ Laboratory of General, Molecular and Exercise Biochemistry (LABMEX-UFPE).
² Graduate Program in Neuropsychology and Behavioral Sciences (UFPE).
³ Keizo Asami Institute, Federal University of Pernambuco.
⁴ Graduate Program in Biology Applied to Health, Federal University of Pernambuco.
⁵ Undergraduate student in biological sciences.

Keywords: Cancer. Enriched Environment. Hypothalamus. Oxidative Stress.

Abstract:

Introduction: Cancer is a multifactorial disease characterized by abnormal cell growth and influenced by oxidative stress, which impacts the brainstem and metabolic functions. This study examines how an enriched environment (EE) modulates mitochondrial function and oxidative balance in the hypothalamus of a cancer model. **Methods:** Male C57BL/6 mice from the iLIKA/UFPE vivarium will be kept in an experimental vivarium (22°C ± 2) with Nuvilab CR1 feed and ad libitum water. At 45 days, they will be distributed in: (1) Standard Environment (SE) (4-6/cage, 13×20×30 cm) and (2) enriched environment (EE) (6-10/cage, 18×34×41 cm) with inanimate objects capable of enhancing psychomotor stimuli. There will be two groups: SE with cancer, EE with cancer. In the fourth week, both groups received 300 µL of sarcoma 180 (10⁶ cells). At 90 days, the animals were euthanized for mitochondrial and oxidative analyses in the hypothalamus. CEUA-UFPE (0051/2023). Performed using Welch's t-test and the Mann-Whitney test (p < 0.05). **Results:** The indicators of mitochondrial function, oxidative stress, and antioxidant defenses were evaluated. Mitochondrial functionality showed no significant differences between the groups. NAD⁺/NADH, p=0.05, and citrate synthase, p=0.34. Lipid peroxidation and protein oxidation were lower in EE, with p values of 0.005 and <0.0001, respectively compared to SE. SOD activity increased in EE compared to SE p = 0.01, while CAT and GST showed no statistical differences (p=0.40 and 0.59). The REDOX state (GSH/GSG) was higher in SE compared to EE, p = 0.006, with no significant changes in total thiols (p=0.22). **Conclusion:** EE attenuated oxidative stress in the hypothalamus, mitigating biomolecular damage by increasing the activity of enzymatic antioxidant markers.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Enhancing Quality of Life: Lithium as a Neuroprotective Agent in Chemotherapy-Induced Neuropathy

Authors:

Matheus F. Itaborahy¹
Isadora Z. L. F. Feng¹
Uri F. Vieira-Machado¹
Izabela B. da Silveira¹
Thalita M. Valverde²
Guilherme M. J. Costa²
Jennifer D. S. Guimarães¹
Daniela Laet-Souza³
Carlos Malamut⁴
M. Alessandra F. Martins¹
Júlia M. Marques¹
Julia Rezende-Ribeiro¹
Vladimir Gorshkov⁸
Frank Kjeldsen⁸
Maria Eduarda S. Favalessa¹
Izabella F. Acipreste¹
Thiago Verano-Braga¹
Hernandes F. Carvalho⁷
André G. Oliveira¹
Marlon Lemos Dias⁵
Alfredo M. Goes³
Barbara E. Ehrlich⁶
M. Fátima Leite¹

Affiliations:

¹ Departamento de Fisiologia e Biofísica, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

² Departamento de Morfologia, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

³ Departamento de Bioquímica e Imunologia, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

⁴ Centro de Desenvolvimento de Tecnologia Nuclear (CDTN/CNEN), Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

⁵ Centro de Pesquisa de Medicina de Precisão, Instituto de Biofísica Carlos Chagas Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

⁶ Department of Pharmacology, Yale University School of Medicine, New Haven, CT USA.

⁷ Departamento de Biologia Estrutural e Funcional, Universidade Estadual de Campinas, Campinas, SP Brazil.

⁸ Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense M, Denmark.

Keywords: Chemotherapy. Neuropathy. Calcium signaling. Quality of life.

Abstract:

Background: The growing population of cancer survivors, due to advancements in cancer therapies, faces increasing prevalence of chemotherapy-induced peripheral neuropathy (CIPN) and cognitive impairment (CICI), which reduce post-treatment quality of life. Antibody-drug conjugates (ADCs) are being developed to target cancer cells and avoid noxious adverse effects. However, despite the efforts, some of these drugs continue to present a high prevalence of neuropathy. A drug often employed in approved ADCs is Monomethyl Auristatin E (MMAE), a microtubule-based agent. The aim of this study was to investigate the potential



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

use of lithium, commonly used in neurological dysfunctions, to alleviate MMAE-induced neuropathy. Methods: We developed a model of MMAE-induced CIPN and CICI and used behavior and sensory tests to analyze its sensory and cognitive effects. We also explored calcium signal, protein levels and tumor progression upon treatments. (CEUA:201/2023) Results: MMAE impaired short-term memory, shown in the Novel Object Recognition Test ($p > 0.05$, familiar vs. novel object) while lithium preserved it ($p > 0.001$). MMAE reduced capsaicin-induced licking time ($p < 0.001$), and lithium preserved it similar to the control ($p > 0.05$). MMAE led to DRG axon myelin thinning ($5.9 \pm 2.0 \mu\text{m}$ vs. $7.9 \pm 1.8 \mu\text{m}$; $p < 0.0001$), prevented by lithium ($7.6 \pm 1.9 \mu\text{m}$; $p > 0.05$). Intra-epidermal nerve fiber density also dropped with MMAE treatment (0.551 ± 0.025 branches/ mm^2 , $p < 0.0001$ vs. saline), and lithium preserved it (1.446 ± 0.267 ; $p > 0.05$ vs. saline). MMAE reduced Ca^{2+} signaling amplitude in DRG (109.3 ± 5.2 vs. 119.2 ± 5.2 ; $p < 0.01$), which lithium prevented (121.2 ± 10.4 ; $p > 0.05$ vs. saline). Proteomics showed MMAE altered expression of key proteins linked to Ca^{2+} signaling, inflammation, and nerve repair ($p < 0.05$), mostly normalized by lithium ($p > 0.05$). Conclusion: This study shows that lithium is a potential strategy to prevent chemotherapy-induced neuropathies, improving quality of life of cancer survivors.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Epigenetic mechanisms regulating bdnf expression that underlie the relationship between early-life stress and cognitive function across sexes

Authors:

Beatriz Cristina Weidle Vuaden¹
Caroline Rodrigues Guedes¹
Layza Kowalski¹
Nadja Schröder¹
Maria Noêmia Martins De Lima¹

Affiliations:

¹Universidade Federal do Rio Grande do Sul, Instituto de Ciências Básicas da Saúde, Laboratório de Neurobiologia das Disfunções da Memória.

Keywords: BDNF. Cognition. Epigenetics. Maternal deprivation.

Abstract:

Exposure to early-life stress may increase susceptibility to cognitive impairments later in life. Maternal deprivation (MD), an early-life stress model, impairs memory in adult rats in a highly heterogeneous way. Epigenetic regulation of gene expression may explain this phenomenon. This study aimed to investigate whether epigenetic modulation of BDNF expression (via non-coding RNAs) underlies recognition memory impairment in adult maternally deprived rats. Male (M) and female (F) rats underwent MD from postnatal day 1 to 14. In adulthood, they were tested in the novel object recognition (NOR) task to identify individuals with worse and better cognitive performance in comparison to controls. After 15 days, rats were re-exposed to the NOR task (to induce a new memory consolidation phase) and euthanized 3h later. Prefrontal cortex (PFC) and hippocampus (HIP) samples were collected to analyze BDNF expression (ELISA) and non-coding RNAs affecting BDNF (PCR). The study was approved by CEUA/UFRGS (approval number 41993). M and F rats subjected to MD showed heterogeneous performance in the NOR task, allowing identification of inferior (IL) and superior (SL) learners. The percentage of SL was similar between sexes ($p=0.977$, chi-square). In females, no association between estrous cycle phase and memory performance was found ($p=0.375$, chi-square). BDNF levels in the HIP did not differ between groups (M, $p=0.374$; F, $p=0.140$, ANOVA). However, IL males showed decreased BDNF in the PFC compared to non-deprived and SL groups ($p<0.001$, Tukey post hoc). This effect was not observed in the PFC of females ($p=0.787$, ANOVA). Understanding the mechanisms related to persistent alterations observed in adult life induced by early stressful circumstances could contribute to developing a therapy to improve cognitive performance in IL subjects.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Assessment of working memory in rats subjected to chronic sleep deprivation treated with statins

Authors:

Maria Luisa Dourado¹

Gabriela Larissa Lima da Silva²

Stephane da Silva Alves²

Kevin Silva Muller³

Arthur Gasparindo Moreira¹

José de Anchieta de Castro e Horta-Junior^{1,2}

Affiliations:

¹ São Paulo State University (Unesp), Institute of Biosciences, Botucatu.

² São Paulo State University (Unesp), Medical School, Botucatu.

³ University of Campinas (Unicamp), Institute of Biology (IB), Campinas.

Keywords: Atorvastatin. Radial arm maze. Rosuvastatin. Simvastatin. Spatial memory.

Abstract:

Chronic sleep deprivation is a rising problem in society. Sleep is associated with memory consolidation and cognitive performance. Statins are drugs used for hypercholesterolemia treatment. They're hydrophilic or lipophilic drugs that present a diversity of pleiotropic effects, among them neuroprotection. This work aims to observe the effect of rosuvastatin, atorvastatin and simvastatin on the working memory of Wistar rats subjected to chronic sleep deprivation through the 8-arm radial maze test (CEUA 1414-2022). Young adult male Wistar rats were distributed in 8 groups (n=15): control group (CG) without sleep deprivation and treated with vehicle; sleep deprivation group (DG), with a maximum of 6 hours of sleep per day and treated with vehicle; sleep deprivation+rosuvastatin groups, receiving a single daily dose of 2.1mg/kg (DGR-) and 20mg/kg (DGR+) of the drug; sleep deprivation+atorvastatin groups, receiving a single daily dose of 4.2mg/kg (DGA-) and 20mg/kg (DGA+) of the drug; sleep deprivation+simvastatin groups, receiving a single daily dose of 4.2mg/kg (DGS-) and 20mg/kg (DGS+) of the drug. After 28 days of treatment, the animals performed the working memory test in an 8-arm radial maze, with single exposure. The measured variables included latency to first arm visit (L1A), latency to visit all arms (LVA), mean visits per arm (MVA), number of partial entries (PE), full entries (FE), time spent in the center of the maze (TC), total errors (TE) and errors after the first FE per arm (EAFE), considering $\alpha=0.05$. It was observed that the pharmacological intervention didn't have a significant effect, although the effects of sleep deprivation were very significant in TC (GC=39±10, GP=30±4.3, p=0.0107), LVA (GC=341.4±93.89, GP=264.10±107.5, p=0.0144), MVA (GC=1.54±0.34, GP=1.92±0.44, p=0.0071), TE (GC=5.08±3.96, GP=7.87±3.56, p=0.0133) and EAFE (GC=5.00±3.69, GP=7.93±3.65, p=0.0133). This shows that the drugs couldn't reduce the memory loss caused by chronic sleep deprivation.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Retinal Dystrophin Deficiency Drives Flash Electrophysiological Alterations in Duchenne Muscular Dystrophy Mouse Models

Authors:

André Muarício Passos Liber¹
Mirella Telles Salgueiro Barboni²
Jan Kremers³
Cyrille Vaillend¹

Affiliations:

¹ Université Paris-Saclay, CNRS, Institut des Neurosciences Paris Saclay, 91190 Gif-sur-Yvette, France.

² Department of Ophthalmology, Semmelweis University, Budapest, Hungary.

³ Section for Retinal Physiology, University Hospital Erlangen, Erlangen, Germany.

Keywords: Duchenne muscular dystrophy. Dystrophins. Electrophysiology.

Abstract:

Duchenne muscular dystrophy (DMD) is an X-linked disorder caused by mutations in the DMD gene, leading to absence of full-length dystrophin, Dp427, and smaller dystrophins in brain and retina (Dp260, Dp140, Dp116 and Dp71) depending on the mutation location. Here, we determined if mutations responsible for loss of one or several dystrophins have distinct effects on retinal physiology. We analyzed dark-adapted (DA) and light-adapted (LA) flash electroretinograms (ERG) in adult mdx mice lacking Dp427; mdx2Cv mice lacking Dp427 and Dp260; and dmd-null mice lacking all dystrophins, compared to their respective WT littermate (ethics committee 40546) and to ERGs previously recorded in mdx52 mice (lacking Dp427, Dp260 and Dp140). ERG responses were marginally affected in mdx mice (a-waves: $p=0.013$; b-waves $p=0.009$), suggesting limited alteration of ribbon synapse transmission. In contrast, the b-wave amplitudes showed increasing reductions in mdx2cv, mdx52 and dmd-null mice ($p<0.001$), with significant increases in b-wave implicit times ($p<0.001$), while oscillatory potentials were homogeneously reduced in these models ($p<0.001$), suggesting alterations in the inner retina due to the absence of Dp260. In dmd-null mice the DA a-waves were larger, while DA b-waves were smaller, showing an electronegative response as observed in patients. ERGs were preserved under LA conditions in mdx and mdx2Cv mice, suggesting a role for Dp427 and Dp260 in rod photoreceptor pathway. In contrast, they were reduced in mdx52 mice ($p<0.001$), and further more in dmd-null mice ($p<0.001$), suggesting roles for Dp140 and Dp71 in cone photoreceptor to ON-bipolar cell pathway, in line with hypotheses derived from previous localization and functional studies. The statistical differences among DMD mouse models show that mutations closer to the 3' end of dmd-gene and affecting Dp140 and Dp71 lead to a more severe ERG phenotype similar to that reported in patients, and highlight the distinct roles of the different retinal dystrophins. ERGs can reflect the severity of central dysfunction in DMD.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Multicomponent training prevents memory deficits induced by early neurodegeneration related to Alzheimer's Disease

Authors:

Pedro Luiz Erves Ribeiro¹
Gabriela Cristiane Mendes Gomes¹
Larissa de Almeida Dias¹
Anna Cecília Perretto Vieira de Souza¹
Ben-Hur Souto das Neves¹
Pâmela Billig Mello-Carpes¹

Affiliations:

¹ Universidade Federal do Pampa, Laboratório de Estresse, Memória e Comportamento, Uruguaiana, RS, Brasil.

Keywords: Alzheimer. Exercise. Memory.

Abstract:

Alzheimer's disease (AD) is a neurodegenerative disorder marked by amyloid- β accumulation, oxidative damage, and neuronal loss, leading to memory decline and behavioral changes. Early AD affects the Locus Coeruleus (LC), a region important for attention and stress responses via noradrenergic projections. Given the limited efficacy of drugs in slowing AD, exercise has gained interest as a non-pharmacological strategy. We evaluated the effects of multicomponent training (MT) in preventing cognitive deficits in a LC early neurodegeneration model. We used 47 male Wistar rats ($n = 7-8/\text{group}$), half of them underwent MT for 6 weeks (running, strength, and cognitive exercises) and half not (Sed). Then, stereotaxic surgery was performed using 6-hydroxydopamine (6-OHDA) injections to induce LC lesions, associated with ascorbic acid (AA) to avoid 6-OHDA oxidation. Behavioral tests included object recognition (OR), inhibitory avoidance (IA), and olfactory discrimination (OD). (CEUA/UNIPAMPA 009/2023). In OR training, all groups explored both objects similarly ($P > 0.05$). In the test, Sed+Saline and MT groups spent more time exploring the new object, indicating memory consolidation (Sed+Saline: $P = 0.0068$; MT+Saline: $P = 0.0230$; MT+6-OHDA: $P = 0.0212$; MT+AA: $P = 0.0028$). Conversely, Sed+6-OHDA ($P = 0.7630$) and Sed+AA ($P = 0.1724$) explored both equally, indicating memory impairment. In OD, Sed groups showed no preference (Sed+Saline: $P = 0.9688$; Sed+6-OHDA: $P = 0.3828$; Sed+AA: $P = 0.0781$), while MT+Saline ($P = 0.0078$) and MT+6-OHDA ($P = 0.0234$) preferred the familiar compartment, suggesting improved discrimination. MT+AA showed no preference ($P = 0.1328$). In IA training, no differences were found ($P > 0.05$). In the test, only MT+Saline increased latency ($P = 0.0313$), suggesting memory formation. These results indicate that MT prevents LC-related memory deficits and enhances olfactory and aversive memory, supporting its potential as a non-pharmacological strategy to mitigate cognitive decline in AD.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Effects of maternal ultra-processed foods on neuronal expression of serotonin transporter in offspring of rats

Authors:

Nathalia Carla de Andrade Pereira¹
Isabeli Lins Pinheiro^{1,2}
Livia Maria de Lima Leôncio^{3,4}
Bruno Rafael Lima Silva³
Sthefany Júlia Alves do Monte⁴
Dayane Silva do Nascimento¹
Antonyel Silva Gonçalves Melo³
Ligia Cristina Monteiro Galindo^{1,5}

Affiliations:

¹Universidade Federal de Pernambuco, Programa de Pós-graduação em Nutrição, Atividade Física e Plasticidade Fenotípica.
² Universidade Federal de Pernambuco, Centro Acadêmico de Vitória, Cursos de Educação Física.
³ Universidade Federal de Pernambuco, Centro de Ciências da Saúde.
⁴ Universidade Federal de Pernambuco, Programa de Pós graduação em Nutrição.
⁵ Universidade Federal de Pernambuco, Departamento de Anatomia.

Keywords: Obesity. Phenotypic plasticity. Serotonin. Westernized diet. Ultra-processed food.

Abstract:

During the critical periods for development, the highest caloric energy intake can be associated with late morphofunctional, physiological and metabolic changes in the organisms. The use of diets with ultra-processed foods (UPF) during these periods can even affect the balance of neurotransmission systems, such as serotonergic. This study aimed to evaluate the neuronal expression of the serotonin transporter (SERT) in the nuclei of the hypothalamus and brainstem in the offspring of rats subjected to an ultra-processed diet during lactation. Wistar rats were allocated into the following groups: the Control Diet Group (CD), which received only a standard vivarium diet (Nuvilab CR-1; 3.47 kcal/g), and the Ultra- Processed Food Diet Group (UPF), which received a diet (Nuvilab CR-1 + High-Calorie Food; 4.12 kcal/g) during lactation. The SERT-IR neurons in the brainstem and hypothalamus of the offspring were quantified on the 31st DPN. Student's t-test was used ($p \leq 0.05$). The ultra-processed diet did not alter the total number of SERT-IR neurons in the arcuate nucleus (Arc) of the hypothalamus or in the dorsal, medial, and posterior medial subnuclei of the brainstem raphe. In the median raphe nucleus, there was higher immunoreactivity to SERT in the ultra-processed group (DC: 90; IIQ 68-110.9; $n=4$ versus DAU: 132; IIQ 123.5-137.5; $n=3$, $p \leq 0.02$). Therefore, the use of an ultra-processed diet during lactation was associated with a reduction in the number of SERT-immunoreactive neurons in isolated nuclei of the raphe and hypothalamus (lateral portion) and an increase in SERT immunoreactivity in the median raphe nucleus of the ultra-processed group. The project was approved by CEUA-UFPE (process 0083-2021).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: The modulatory effect of novelty on the persistence of reconsolidated recognition memory depends on the activation of protein kinase a in the dorsal hippocampus of rats

Authors:

Ana Carolina de Souza da Rosa¹
Karine Ramires Lima¹
Gabriela Cristiane Mendes Gomes¹
Anna Cecília Perretto¹
Giulia Azevedo Martinez¹
Bruno Pereira Coura¹
Pâmela Billig Mello-Carpes¹.

Affiliations:

¹ Physiology Research Group; Federal University of the Pampa, Rio Grande do Sul.

Keywords: Reactivation. Object Recognition. Persistence. Exposure to Novelty. PKA.

Abstract:

Exposure to novelty has been studied and used to improve recognition memory through reconsolidation, also modulating persistence. Therefore, we investigated the involvement of dopaminergic D1/D5 receptors and the PKA pathway in the modulation of novelty on the persistence of reconsolidated memory (CEUA/UNIPAMPA 028/2022). Wistar rats were divided into 5 groups (n=14–31/group): (i) vehicle; (ii) novelty; (iii) novelty+SCH23390 (D1/D5 antagonist); (iv) novelty+Rp-cAMPS (PKA inhibitor); (v) SKF38393 (D1/D5 agonist). Animals underwent stereotaxic surgery for cannulation of the CA1 region of the hippocampus and the Object Recognition (OR) task, which included a reactivation session with a familiar object (A) and a novel object (C) 24h after acquisition. Novelty groups were exposed to novelty for 5 min after reactivation. Drugs/vehicle were infused afterward or at an equivalent time. The memory persistence test with a novel object (D) was performed 7 days after reactivation. Differences were statistically significant when $P < 0.05$. At reactivation, all groups explored the novel object for more time ($p < 0.0001$). In the 7-day OR test, group (i) explored both objects similarly ($p > 0.05$), showing reactivation alone doesn't promote persistence of reconsolidated memory. Group (ii) showed persistence for objects A, B, and C ($p = 0.0156$ for A+D; $p = 0.0378$ for B+D; $p = 0.0119$ for C+D). Group (v) showed persistence for A and B ($p = 0.0111$ for A+D; $p = 0.0039$ for B+D), but not C ($p = 0.3778$ for C+D), similar to group (iii) ($p = 0.0175$ for A+D; $p = 0.0313$ for B+D; $p = 0.6163$ for C+D). In the investigation of the PKA pathway, we observed that group (iv) did not show persistence for object A ($p = 0.5859$) or C ($p = 0.8993$), however, persistence for object B continued to occur ($p = 0.0057$). Therefore, we suggest that exposure to novelty, in the temporal window of reconsolidation, facilitates OR memory reconsolidation and its persistence via PKA-dependent mechanisms in the hippocampus.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Early life stress, L-DOPA and their interplay have distinct effects in the behavior of prepubertal female and male mice

Authors:

Anna Clara Silva Braz¹
Ana Luíza de Araújo Lima Reis¹
Paula Bertu Valverde¹
Sofia Caetano Avritzer¹
Lorena Terene Lopes Guerra¹
Muiara Aparecida Moraes¹
Laila Blanc Arabe¹
Bruna Lopes Resende¹
Nathália Mylena de Senna Cruz¹
Bruno Rezende Souza¹

Affiliations:

¹ Departamento de Fisiologia e Biofísica - ICB – UFMG.

Keywords: Behavioral. Early-life stress. Dopamine. Maternal separation. Neurodevelopment.

Abstract:

Early-life stress is linked to neuropsychiatric disorders, though not all exposed individuals develop mental illnesses. Since dopamine alterations are also associated with these disorders, we examined how elevated dopamine combined with early-life stress affects. We used C57/BL6 female and male mice, which were subjected to the maternal separation (MS) protocol for 3 hours/day and/or L-DOPA administration from the P2 to P14. The experimental groups were: Naïve; Saline; L-DOPA; MS; MS + Saline; MS + L-DOPA. At P28, the animals were subjected to a series of behavioral tests, including the Locomotor Box (LB), Open Field Test (OFT), Light-Dark Box (LDB), Elevated Plus Maze (EPM), and Forced Swim Test (FST). At P32, the animals were euthanized. All tests were approved by the CEUA protocols: 86/2016 and 201/2019. All data were analyzed using a two-way ANOVA followed by Tukey's post hoc test. MS stress increases exploratory and locomotor activity in females, with differences in both sex ($p=0.019$) and stress factor ($p=0.071$). In the OFT, MS females travelled a greater distance than naïve ($p=0.032$). In the LDB, MS males spent less time in the light ($p=0.007$). MS males spent less time in the light than naïve ($p=0.014$), and there were more transitions in naïve males than control females ($p=0.009$). Males move less between the light/dark compartments ($p=0.016$). In the EPM, MS males spent less time in the open arms ($p=0.029$). In the FST, MS showed more immobility time than naïve males ($p=0.040$) and MS females ($p=0.014$), and more overall immobility than females ($p<0.001$). L-DOPA females showed increased locomotor behavior ($p=0.017$). Overall, MS + L-DOPA showed fewer behavioral effects than MS, suggesting that dopamine might have a protective role during early-life stress. The study highlights the complexity of the interaction between early-life stress and dopamine in the development of behavior, a sex-specific difference. Acknowledgement CNPq, FAPEMIG, CAPES, ISN and PRPq.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Impact of Cannabidiol on Novel Object Recognition Test After Traumatic Brain Injury in Rats

Authors:

Clauder Oliveira Ramalho^{1,2}
Luisa Brandão Carneiro^{1,2}
Amanda Reis Flores¹
Pedro Henrique Cassaro Lirio³
Polyana Lima Meireles Dalpiaz⁴
Alline Cristina de Campos³
Francisco Silveira Guimarães³
Lívia Carla de Melo Rodrigues¹

Affiliations:

¹ Programa de Pós-Graduação em Ciências Fisiológicas, Universidade Federal do Espírito Santo (UFES), Vitória (ES, Brasil).
² Centro Universitário Multivix, Vitória (ES, Brasil).
³ Departamento de Farmacologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto (SP, Brasil).
⁴ Departamento de Morfologia, Universidade Federal do Espírito Santo (UFES), Vitória (ES, Brasil).

Keywords: Cannabidiol. Cognition. Traumatic brain injury.

Abstract:

Traumatic brain injury (TBI) is a leading cause of mortality, resulting in cognitive and physical deficits that affect quality of life. Despite its high prevalence, effective pharmacological treatments remain limited. Cannabidiol (CBD), known for its neuroprotective, anti-inflammatory, and antioxidant properties, might be a potential therapeutic agent for TBI-related cognitive deficits. This study aims to investigate whether CBD can attenuate recognition memory impairments in a rat model of TBI. Seventy-four Wistar rats were divided into four groups: SHAM/VEH, TBI/VEH, SHAM/CBD, and TBI/CBD. TBI was induced using a new weight-drop model (UFES Animal Ethics Committee 03/2022). CBD (20 mg/kg), dissolved in saline and Tween 80, was administered intraperitoneally for three consecutive days post-TBI, while the vehicle groups received only saline with Tween 80 (VEH). Behavioral performance was evaluated using the Novel Object Recognition Test (NORT) and statistical analysis was performed using two-way ANOVA and Tukey post-hoc test, with $p < 0.05$. No differences were found in short-term object recognition memory for the different groups ($p > 0.05$), with recognition indexes ranging from 47.14 ± 6.85 (TBI/CBD $n = 16$) to 52.74 ± 7.51 (TBI/VEH $n = 15$). However, in long-term assessment, the TBI/VEH group (37.35 ± 5.64) showed significant impairment in NORT compared to SHAM/VEH ($n = 15$, 67.53 ± 7.85 , $p < 0.05$). Animals treated with CBD 20 mg post TBI (46.43 ± 6.51) presented a better recognition index compared to TBI/VEH ($p < 0.05$), suggesting a beneficial effect of CBD. These findings suggest that CBD treatment may have a positive effect on long-term memory in this TBI model. Further research is necessary to better understand the potential of CBD on cognitive functions following TBI.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Single-Cycle Binge Drinking During Pregnancy Alters Offspring's Motor Neurodevelopment in Rats in a Sex-dependent Manner

Authors:

Isabella Maia Carmo e Silva¹
Tiago Monteiro Batista¹
Chan David Ribeiro¹
João Carlos do Nascimento Martins¹
Rafael Hendrick Freitas de Paula¹
Luan Nascimento Pereira de Amorin¹
Maria Vitoria Oliveira Rebelo¹
Maria Luisa Lopes da Silva¹
Enzo Gabriel Lacerda Rodrigues¹
Jofre Jacob da Silva Freitas²
Sabrina de Carvalho Cartágenes¹
Luanna de Melo Pereira Fernandes^{1,2}

Affiliations:

¹ Laboratory of Neuropharmacology and Behaviour – LANEFC, Center for Biological and Health Sciences, State University of Pará (UEPA), Belém, Pará, Brazil.

² Amazon Preclinical Studies Center, Center for Biological and Health Sciences, State University of Pará (UEPA), Belém, Pará, Brazil.

Keywords: Binge drinking. Heredity. Motor neurons. Pregnancy.

Abstract:

Alcohol exposure during pregnancy and lactation impairs fetal neurodevelopment, especially in central nervous system regions linked to cognitive, motor, and executive functions. However, it remains unclear whether a single binge episode early in pregnancy affects offspring motor function and spinal cord morphology. This study aimed to investigate the motor neurodevelopment of male and female offspring after a single binge drinking cycle during pregnancy. It was approved by the Ethics Committee on Animal Use (CEUA/UEPA), under n. 11/2022. Wistar female pregnant rats received intragastric gavage with either distilled water or ethanol at 3.0 g/kg (20% w/v) on gestational days 6, 7, and 8. After birth, the offspring (n = 48) were subjected to behavioral and histological analyses on postnatal day 40, including the open-field test and motoneuron counting. The ANY-maze software was used to access the total distance traveled (TDT), average speed (AS), and rotations number (RN) parameters at open field test. After the animals were perfused and the spinal cord was processed, sectioned and stained with HE, and motoneuron counted at cervical, thoracic, and lumbar segments. Female offspring showed impaired at spontaneous locomotor activity (TDT: $p=0.0054$; AS: $p=0.0029$; RN: $p=0.0006$) and reduced cervical motoneurons number ($p=0.0094$) without changes in thoracic or lumbar regions. On the other hand, the male offspring showed no altered motor parameters analyzed (all $p>0.05$), and there were no differences of motoneuron number in cervical, thoracic or lumbar regions in spinal cord (all $p>0.05$). Single-cycle prenatal binge alcohol led to sex- and region-specific effects, with females showing both motor neurodevelopment and histological impairments with motoneurons loss, while males did not. Single-cycle prenatal binge alcohol exposure led to sex- and medullary segment-dependent effects, with females showing both motor neurodevelopmental impairment and motoneuron loss in the cervical segment, while males did not. These findings thus demonstrate the vulnerability of female offspring to early prenatal alcohol exposure and its neurodevelopmental consequences.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: The efficacy of GLP-1 receptor agonists in the treatment of cognitive impairments in preclinical models of diabetes mellitus: a systematic review and meta-analysis

Authors:

Rafael Hendrick Freitas de Paula^{1,2}
Isabella Maia Carmo e Silva²
Chan David Ribeiro^{1,2}
Bruno Kauê Rodrigues Vilhena¹
Luan Nascimento Pereira de Amorim^{1,2}
Enzo Gabriel Lacerda Rodrigues^{1,2}
Maria Vitoria Oliveira Rebelo²
João Carlos do Nascimento Martins^{1,2}
Sabrina de Carvalho Cartágenes²
Luanna de Melo Pereira Fernandes²

Affiliations:

¹ Undergraduate medical student, Center of Biological Sciences and Health, University of Pará State, Belém, Pará, Brazil.

² Laboratory of Neuropharmacology and Behavior, Center of Biological Sciences and Health, University of Pará State, Belém, Pará, Brazil.

Keywords: Behavior. Cognition. Diabetes mellitus. GLP-1 receptor agonists.

Abstract:

There is strong evidence that type-2 diabetes mellitus (T2DM) is associated with reduced cognitive function. However, the underlying pathophysiological mechanisms remain unclear. Glucagon-like peptide-1 (GLP-1) is a hormone involved in metabolic regulation and exerts central nervous system effects. GLP-1 receptor agonists (GLP-1 RAs) are proposed as potential therapies for cognitive dysfunction, though their effects remain controversial. This study aimed to evaluate the impact of GLP-1 RAs on cognition in animal models of T2DM through a systematic review and meta-analysis. We searched PubMed, Embase, Web of Science, and OVID for in vivo experimental studies published up to February 2025 comparing GLP-1 RAs with placebo. Primary outcomes were behavioral performance measures from the Morris Water Maze (MWM), Y-Maze (YM), and Novel Object Recognition (NOR) tests. Data were analyzed using Mean Difference (MD) and Standardized Mean Difference (SMD) with 95% confidence intervals (CI), following Cochrane recommendations. A total of 44 studies involving 1,103 animals were included; 548 received GLP-1 RAs. Significant cognitive improvements were observed in intervention groups: reduced MWM Escape Latency (MD -14.3; 95% CI -18.7/-9.9; $p = <0.001$; $I^2 = 98.3\%$) and MWM Pathway Length (SMD -0.9; 95% CI -1.5/-0.3; $p = 0.002$; $I^2 = 60.7\%$) in rats and mice GLP-1 Ras-treated. Increased MWM Time Spent in the Target Quadrant (SMD 1.5; 95% CI 1-2; $p < 0.001$; $I^2 = 77.4\%$), MWM Frequency of Crossing (MD 2.1; 95% CI 1.5-2.7; $p < 0.001$; $I^2 = 85.1\%$), NOR Index (MD 18.5; 95% CI 7.5-29.4; $p < 0.001$; $I^2 = 93.5\%$) and YM Alternation Percentage (MD 16.2; 95% CI 0.6-31.7; $p = 0.041$; $I^2 = 97.6\%$). No significant difference was found in YM Total Arm Entries (MD 9.3; 95% CI -6.3/25; $p = 0.243$; $I^2 = 93.4\%$). GLP-1 RAs significantly improved cognitive performance in T2DM models without motor damage, as evidenced by enhanced behavioral test results, supporting their therapeutic potential in cognitive impairment.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Brain reactivity in victims of interpersonal violence and its relationship with peritraumatic tonic immobility

Authors:

Thayane Ferreira von Helde¹
Sara Domiciano de Jesus Gaspar¹
Bianca Afonso Soares da Silva¹
Letícia de Oliveira²
Mirtes Garcia Pereira Fortes²
Filipe Braga³
Eliane Volchan⁴
Isabel de Paula Antunes David²
Isabela Villarinho de Paula Lobo¹

Affiliations:

¹ Instituto de Biodiversidade e Sustentabilidade, Universidade Federal do Rio de Janeiro, RJ.
² Departamento de Fisiologia e Farmacologia, Universidade Federal Fluminense, RJ.
³ Instituto de Ciências Médicas, Universidade Federal do Rio de Janeiro, Macaé/RJ.
⁴ Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, RJ.

Keywords: Defensive behavior. ERP. Interpersonal trauma. LPP. Violence.

Abstract:

The detection of danger signals triggers adaptive responses essential for survival. Studies using EEG show that the Late Positive Potential increases with arousing stimuli. This neural marker is linked to emotional salience, but it is not clear if defensive responses, such as peritraumatic tonic immobility, are related to LPP. The objective of this study is to investigate brain reactivity to pictures of firearms in traumatized subjects. The experiment involved a final sample of 56 participants. Approved by the Research Ethics Committee of UFRJ (CAAE: 92088518.8.0000.5699). The task presenting pictures from four categories: a) in which a firearm was directed-away to the observer; b) in which a non-lethal object was directed-away to the observer; c) with firearm directing-toward to the observer; d) with a non-lethal object directing-toward to the observer. EEG signals were recorded to investigate LPP. Additionally, participants completed questionnaires on trauma history and peritraumatic tonic immobility. ANOVA indicated a significant main effect for the valence, with greater LPP amplitude for violent images than for neutral ones. There was an interaction between region and orientation, with higher LPP for directed images in the central region. Tonic immobility was positively associated with LPP in response to directed violent images (controlling for valence and orientation) in the central region. In summary, the results suggest that violent images, especially when directed, enhance LPP amplitude. Furthermore, the greater the peritraumatic tonic immobility, the higher the LPP in response to directed violent images. These findings indicate an interaction between threat perception and adaptive mechanisms, influencing defensive behaviors.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Environmental Enrichment Effects on Anxiety-/Depression-Like Behaviors in Rats Submitted to Osmotic Stress

Authors:

Bruna Pedroso Oliveira Jungles Gonçalves dos Reis^{1,2}

Ana Júlia Amaral Moreira¹

Luiz Fernando Camargo Silva^{1,2}

Beatriz Suzim Severino¹

Mariana Demarchi¹

Ana Caroline Gonçalves¹

Bárbara Holanda¹

Felipe Debori¹

Gabriela Lumi Yabu¹

Lucila Leico Kagohara Elias³

José Antunes-Rodrigues³

Wagner Luis Reis^{1,2}

Affiliations:

¹ Neuroendocrinology Laboratory. Physiological Science Department. Biological Science Center.

² Multicenter Graduated Program in Physiological Science. Federal University of Santa Catarina (UFSC). Florianópolis, Santa Catarina, Brazil.

³ Department of Physiology. School of Medicine of Ribeirão Preto. University of São Paulo. Ribeirão Preto, São Paulo, Brazil.

Keywords: Anxiety behavior. Environmental enrichment. Osmotic stress.

Abstract:

Environmental enrichment (EE) enhances brain plasticity through physical, cognitive, and social stimuli. Also, it improves behavior and reduces stress, anxiety and cognitive decline. Still, hydroelectrolytic imbalance evokes humoral and neurosecretory responses, affecting thirst and behaviors. Thus, this study investigated EE effects on anxiety-/depression-like behaviors during osmotic stress. Male post-weaning Wistar rats were housed for 5 weeks in EE cages with rotating sets of objects or in standard cages (SC). Next, 24 or 48 hours before experiments, they underwent to osmotic stimulation (water deprivation, 24WD and 48WD, respectively) or remained normohydrated (NH) as control group. (CEUA/UFSC nº1072240523). According to the different behavioral tests, open field test showed no differences in crossings, periphery time spent or center percentage time among EE groups (EE-NH, -24WD and -48WD), compared to their respective SC groups. However, EE increased the number of rearings in NH (33.0 ± 4.5) and 24WD (23.6 ± 5.3) groups related to SC-NH (22.3 ± 4.2) and SC-24WD (13.1 ± 3.5), respectively ($P < 0.05$ for both). In elevated plus maze test, SC-24WD rats ($52.0 \pm 3.8\%$, $P < 0.05$) spent more time in open arm compared to SC-NH group ($26.8 \pm 4.6\%$), however, no differences were observed between SC and EE groups during osmotic stimulation. Instead, SC-24WD (9.1 ± 1.4) and EE-24WD (8.3 ± 0.9) rats exhibited more risk behavior than their respective SC-NH (1.1 ± 0.3) and EE-NH (2.0 ± 0.3) groups ($P < 0.05$ for both). Nonetheless, none of EE groups showed influence risk behavior compared to SC rats. Overall, these findings suggest that EE may play an important role in exploratory behavior, as evidenced by increased rearing. On the other hand, anxiolytic responses of EE were slight evident in WD model, however, further behavioral tests will be performed to investigate EE's influence during osmotic stress.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Ketamine exhibits a long-lasting antidepressant effect in the early weaning model in male mice

Authors:

Elifrances Galdino de Oliveira¹
Mayara Victória de Souza Barbosa¹
Diógenes Afonso de Lima¹
Eduardo Carvalho Lira¹ Sandra Lopes
de Souza² Dayane Aparecida Gomes¹

Affiliations:

¹ Departamento de Fisiologia e Farmacologia - Universidade Federal de Pernambuco.

² Departamento de Anatomia - Universidade Federal de Pernambuco.

Keywords: Depression. Early weaning. Ketamine.

Abstract:

Ketamine, an anesthetic, has recently been repurposed for the treatment of major depressive disorder, a debilitating condition that affects 185 million people worldwide. In animal models of depression-like behavior, ketamine showed antidepressant effects that lasted up to 13 days. However, it is not known whether ketamine has longer-lasting antidepressant effects. Thus, the aim of this study was to test whether the antidepressant effects of ketamine extend from adolescence (40 days of age) to adulthood (80 days of age) in a mouse model of early weaning. To determine whether early weaning induces depressive-like behavior, male and female mice (*Mus musculus*, Swiss) were weaned at 14 days (early weaning, EW group) or 21 days (CTRL group) of age. The groups were tested for depression-like behavior in the tail suspension test (TST) at 40 and 80 days of age. Ethics Committee approval protocol number 112/2022. In males, the EW group (n=10) showed increased immobility in the TST at 40 (CTRL: 12,8±7,9; EW: 92,5±39,5; p<0,0001) and 80 days (CTRL: 52,1±19,4; EW: 112,3±49,8; p=0,001) compared to the CTRL group (n=12). In females, the EW group (n=10) showed an increase in immobility at 40 days (CTRL: 5,0±7,2; EW: 41,2±18,5; p<0,0001), but not at 80 days (CTRL: 55,5±34,3; EW: 82,2±60,8; p=0,385), compared with the CTRL group (n=12). Thus, females were not used to verify the long-lasting antidepressant effect of ketamine. In order, to test the duration of the antidepressant effect of ketamine, a new cohort of early-weaned male mice was administered ketamine (10 mg/kg) at 39 days of age (EW+KET group). Compared with the EW group, the EW+KET group (n=10) exhibited reduced immobility at 40 (EW: 92,5±39,5; EW+KET: 39,7±15,6; p=0,0001) and 80 days (EW: 112,3±49,8; EW+KET: 66,7±29,7; p=0,01). In conclusion, our results are unprecedented in demonstrating that ketamine administration has an antidepressant effect that persists for 40 days in males in the mouse model of early weaning.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Influence of environmental enrichment on the thirst during water deprivation

Authors:

Bruna Pedrosa Oliveira Jungles Gonçalves dos REIS^{1,2}
Luiz Fernando Camargo da SILVA^{1,2}
Ana Julia Amaral MOREIRA¹
Beatriz Suzim SEVERINO¹
Mariana DEMARCHI¹
Ana Caroline GONÇALVES ¹
Bárbara HOLANDA ¹
Felipe DEBORI¹
Gabriela Lumi YABU¹
Lucila Leico Kagohara ELIAS³
José ANTUNES-RODRIGUES³
Wagner Luis REIS^{1,2}

Affiliations:

¹ Neuroendocrinology Laboratory. Physiological Science Department. Biological Science Center.

² Multicenter Graduated Program in Physiological Science. Federal University of Santa Catarina (UFSC). Florianopolis, Santa Catarina, Brazil.

³ Department of Physiology. School of Medicine of Ribeirao Preto. University of São Paulo. Ribeirao Preto, São Paulo, Brazil.

Keywords: environmental enrichment, osmotic stimulation and thirst

Abstract:

Water deprivation (WD) is the main stimulus to activate central mechanisms for body fluid volume and osmotic regulation, which in turn, increases water intake. Still, the effects of environmental enrichment (EE) on emotional and cognitive behaviors are well known, including reduction of anxiety and stress. However, the influence of EE on the thirst was never been evaluated. Thus, this work investigated the effects of EE on water intake in basal condition or WD response. Male Wistar rats with 21 days old were housed for 5 weeks in standard cages (SC) or EE cages with different sets of playful objects (changed weekly). To induce osmotic stimulation, animals were subjected to 24h or 48h WD, and subsequently placed in a metabolic cage with water access to measure water intake over 120 minutes. Normohydrated (Normo) rats in SC or EE were used as control. (CEUA/UFSC n. 1072240523). Initially, over 5 weeks evaluation, animals in EE did not show any weekly difference in water intake, total weight or weight gain when compared to SC group. Secondly, after osmotic stimulation, an increase in hematocrit was observed in SC-24WD ($50.8 \pm 0.5\%$) and SC-48WD ($46.8 \pm 0.4\%$) groups compared to the SC-Normo group ($42.8 \pm 0.3\%$, $P < 0.05$ for both). Conversely, only EE-48WD group ($50.9 \pm 0.9\%$, $P < 0.05$) showed higher hematocrit than EE-Normo group ($44.2 \pm 1.2\%$). Interestingly, over 120min water intake measurements, it was observed that EE-24WD animals ($20.2 \pm 0.8\text{ml}/100\text{g}$, $P < 0.05$) had a greater water intake compared to SC-24WD group ($15.3 \pm 1.3\text{ml}/100\text{g}$). In contrast, the EE-48WD group ($26.2 \pm 2.8\text{ml}/100\text{g}$, $P < 0.05$) showed reduction of water intake compared to SC-48WD group ($30.2 \pm 2.1\text{ml}/100\text{g}$). These results demonstrate that EE itself did not affect water intake over time. However, EE animals when submitted to 24h or 48h WD showed opposite effects on thirst. So far, the anxiolytic effects of EE could be involved during chronic osmotic stimulation (48WD), attenuating demand and consumption of water.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Anxiolytic and antidepressant effects of ketamine treatment on behavioral changes during adolescence and adulthood induced by an early-life immune activation model

Authors:

Mayara Victória de Souza Barbosa¹
Elifrances Galdino de Oliveira¹
Diógenes Afonso de Lima¹
Eduardo Carvalho Lira¹
Sandra Lopes de Souza²
Dayane Aparecida Gomes¹

Affiliations:

¹ Departamento de Fisiologia e Farmacologia, Universidade Federal de Pernambuco.
² Departamento de Anatomia, Universidade Federal de Pernambuco.

Keywords: Anxiety. Depression. Early life adversity. Immune activation. Ketamine.

Abstract:

In recent decades, ketamine has emerged as a therapy for treatment-resistant depression, demonstrating antidepressant effects within hours of administration. However, the duration of the antidepressant effect remains to be elucidated. The aim of this study was to investigate the effects of ketamine treatment on behavioral changes induced by early immune activation. Female mice (*Mus musculus*, Swiss) were administered saline (CTRL group, n=12) or lipopolysaccharide (EIA group, n=12) - 100 µg/kg intraperitoneally on postnatal day (PND) 14. In addition, one group (EIA+KET, n=10) was treated with a single dose of ketamine (10 mg/kg) on the PND 39. The open field (OF) and tail suspension tests (TST) were performed in adolescence (PND 41 and 42) and adulthood (PND 81 and 82). Ethics approval protocol number 112/2022. In the OF, EIA showed reduced time in the center (CTRL: 60.36±12.5; EIA: 41.78±13.62; p=0.0021) and entries in the center (CTRL: 67.83±15.09; EIA: 41.17±14.92; p=0.0003) compared to CTRL in adolescence; EIA+KET showed an increase in time in the center (EIA+KET: 62.88±20.10; p=0.0088) and entries in the center (EIA+KET: 58.80±13.94; p= 0.0230) compared to EIA in adolescence. There was no difference in time in the center (CTRL: 59.5±27.55; EIA: 41.74±16.9; p=0.0702) and entries into the center (CTRL: 61.00±25.14; EIA: 46.58±20.55; p=0.1383) in adulthood. In TST, EIA showed increased immobility compared to CTRL in adolescence (CTRL: 5.07±7.28; EIA: 59.13±11.65; p <0.0001) and adulthood (CTRL: 55.31±34.35; EIA: 101.5±35.28; p=0.0037). In adolescence, EIA+KET showed reduced immobility (EIA+KET: 7.84±10.41; p <0.0001) compared to EIA; in adulthood, EIA+KET showed increased immobility compared to CTRL (EIA+KET: 105.03±42.51; p=0.0105). In conclusion, EIA induces depression-like behavior in adolescence and adulthood, and anxiety-like behavior in adolescence. Single-dose ketamine treatment has acute anxiolytic and antidepressant effects, but not long-lasting ones.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Paternal anabolic steroid exposure promotes autism-like behavior in adult mice offspring

Authors:

Raphael da Silva Lau¹
Breno Reis Corrêa Arigoni¹
Victor Hugo de Maria Paura de Souza¹
Guilherme Ferreira Bueno¹
Fernando da Silveira Ribeiro Rocha²
Bruno Henrico Bertoni de Mendonça¹
Lívia Carvalho Dantas¹
Liliani Aparecida Sereno Fontes de Medeiros¹
Jonas Xavier Torres¹
Yasmin Cavalcante de Sousa¹
Brena Barcellos Luiz¹
Amanda da Silva Salgado¹
Wellington da Silva Côrtes¹
Roberto Laureano-Melo¹

Affiliations:

¹ Universidade Federal Rural do Rio de Janeiro.

² Universidade Leonardo Da Vinci.

Keywords: Autism. Anabolic-androgenic steroids. Paternal programming. Offspring, mice.

Abstract:

Paternal environmental factors before conception and during sperm development may influence the offspring's health later in life. This study aimed to investigate whether paternal exposure to anabolic-androgenic steroids (AAS) before conception predisposes mouse offspring to autism spectrum disorder (ASD)-like behavior. For this purpose, male Swiss mice were randomly divided into two groups: the control group received peanut oil, while the treated group was administered testosterone propionate (7.5 mg/kg, s.c.) twice a week for five weeks. After this period, these males were mated, and their offspring underwent a behavioral test battery at 70 days of age, including the open field test, object recognition task, three-chamber social approach test, and light-dark box test. At the end of the experiment, the hippocampus was dissected for RNA analysis. The work was registered with the CEUA under number 012282/2017. Our results indicate that paternal AAS treatment induces long-lasting behavioral alterations in both female and male offspring, including increased anxiety-like behavior, impaired memory, and deficits in social interaction. Additionally, we observed a significant increase in hippocampal Gabra2 expression exclusively in male offspring. A strong effect of paternal AAS treatment during preconception period was verified in Gad1 and Bdnf expression. These findings suggest that paternal AAS exposure may program neurodevelopmental vulnerabilities in offspring, contributing to ASD-like phenotypes.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Evaluation of the effect of nanoparticles containing bioactive peptides from hardened beans in the prevention of neuroinflammation and oxidative stress

Authors:

Elisabete Borges da Silva¹

Stéfanny Duarte Costa¹

Thayss Ghabriella Fernandes Gomes Cardoso Viturino da Silva¹

Nickson Ribeiro dos Santos¹

Juliana Vila Verde Ribeiro²

Richard Douglas Rodrigues Ferreira²

Jullia Ingrid Rodrigues Martins²

Carlos Henrique Xavier Custódio²

Karla De Aleluia Batista³

Affiliations:

¹ Centro Universitário Uniaraguaia.

² Universidade Federal de Goiás-UFG.

³ Instituto Federal de Goiás- Campus Goiânia Oeste.

Keywords: Bioactive molecules. Nanoparticles. Neuroinflammation. Neurodegenerative diseases. Peptides.

Abstract:

Neuroinflammation and oxidative stress, associated with neurodegenerative diseases, have been widely studied, especially after COVID-19, which left brain sequelae in many patients. Thus, the search for natural compounds encapsulated in nanoparticles, capable of modulating these processes in the central nervous system, is essential to develop therapeutic and preventive alternatives. Therefore, this study aims to evaluate the effects of nanoparticles containing common bean peptides (NP@PV3) in preventing damage related to neuroinflammation and oxidative stress in animals subjected to liposartan (LPS). For this study, the work was accepted by CEUA of UFG under number 119/23. Wistar rats were anesthetized with ketamine and xylazine (10/100 mg/kg) and underwent stereotaxic surgery for central injection of LPS (300 ng) and PV3 (300 ng). After surgery, the animals were allowed to recover for 5 days before performing open field behavior tests, elevated plus maze, memory test for identifying new objects, and spatial location tests. After the behavior tests, the animals were housed in metabolic cages for 24 hours and then euthanized for urine, organ, and serum collection for biochemical analyses. The findings show that NP-CS@PV3 nanoparticles were efficient in normalizing serum creatinine and urinary urea levels to values observed in control animals without inducing inflammation. Treatment with NP-CS@PV3 nanoparticles reduced C-reactive protein levels, indicating a reduction in the inflammatory process in the body. In behavioral tests, PV3 and NP-CS@PV3 reduced anxiety without causing damage to locomotion and improved cognitive ability in these animals. Therefore, we can conclude that the use of nanoparticles with bioactive peptides is an excellent biotechnological alternative for the treatment of neurodegenerative diseases.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Levels of vitamin B12, cognitive aspects, and gross motor coordination in children

Authors:

Maria Eduarda Rodrigues Alves dos Santos^{1,2}
Ana Patrícia da Silva Souza^{1,2}
Karollainy Gomes da Silva^{1,2}
Ana Beatriz Januário da Silva^{1,2}
Beatriz Machado Silva³
José Maurício Lucas da Silva^{1,2}
Mayara Luclécia da Silva^{1,2}
Robson Feliciano da Silva²
Letícia Henrique Leite da Silva¹
Aline Ananias de Lima¹
Georgiane Brandão Rodrigues da Silva²
Erica Helena Alves da Silva²
Antonietta Claudia Barbosa da Fonseca Carneiro¹
Waleska Maria Almeida Barros^{1,2}
Sandra Lopes de Souza¹

Affiliations:

¹ Graduate Program in Neuropsychiatry and Behavioral Sciences, Center for Medical Sciences - UFPE, Recife - PE, Brazil.

² Integrated Center of Neuroscience Technologies (CITENC), Osman Lins University Center - UNIFACOL, Vitória de Santo Antão - PE, Brazil.

³ Laboratory of molecular biology in infectious diseases - department of parasitology - Instituto Aggeu Magalhães - Fiocruz, Recife, Pernambuco, Brazil.

Keywords: Cobalamin. Cognition. Motor Skills. Vitamin B12.

Abstract:

Neurodevelopment requires the interaction of multiple factors, with a proper balance of essential macronutrients and micronutrients being fundamental. In this context, vitamin B12 plays a key role as a coenzyme in various biochemical processes critical to the health of the nervous system. Therefore, this study aimed to investigate the correlations between vitamin B12 levels and nutritional, cognitive, and motor factors in children attending public schools. This cross-sectional study was conducted in public schools in the municipality of Vitória de Santo Antão, Pernambuco, Brazil, involving children aged 5 to 9 years. The research was approved by the Human Research Ethics Committee of the Federal University of Pernambuco (CAAE: 68693823.5.0000.5208). The participants underwent a sociodemographic questionnaire, anthropometric assessments, a food frequency questionnaire, blood collection, a gross motor coordination test (Körperkoordinationstest für Kinder - KTK), and screening for suggestive signs of cognitive alterations using the Mini-Mental State Examination (MMSE). A total of 124 children were evaluated, with a mean age of 6.8 years (± 1.3). According to nutritional status, 26 children (21%) were classified as underweight, 65 (52.4%) as eutrophic, and 33 (26.6%) as overweight or obese. The mean vitamin B12 levels in these respective groups were 366 pg/mL (± 187), 373 pg/mL (± 127), and 348 pg/mL (± 160). Correlation analyses between vitamin B12 levels and the studied variables revealed a positive correlation with household income ($r = 0.209$, $p = 0.026$), total MMSE score ($r = 0.250$, $p = 0.043$), and the language domain of the same instrument ($r = 0.319$, $p = 0.009$). In conclusion, in this sample of children aged 5 to 9 years enrolled in public schools, vitamin B12 levels were positively associated with socioeconomic factors and cognitive performance. However, no significant correlations were observed with nutritional status or gross motor coordination.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: The Extract of Pequi (*Caryocar coriaceum*) Shows Anticonvulsant Effects in Adult Zebrafish (*Danio rerio*)

Authors:

Katarine Ferreira da Silva¹
Igor de Codes Soares¹
Gabriel Martins de Araújo Sousa¹
Paula Ticiani Alves de Souza¹
Ariel Falanga de Oliveira¹
Lucca Moura de Freitas¹
Mário Guilherme de Paula Sales¹
Cassia Taiane Viana Moraes²
Caroline Pereira dos Santos Rocha¹
Vitória Isabele Gomes Ferreira¹
Paula Alexandre de Freitas²
Saulo Chaves Magalhães¹
Jonas Araujo de Sousa¹
Gabriela Alves do Nascimento¹
Keciany Alves de Oliveira¹

Affiliations:

¹ Higher Institute of Biomedical Sciences, State University of Ceará-UECE.

² Postgraduate Program in Nutrition and Health, State University of Ceará-UECE.

Keywords: Anticonvulsant. Epilepsy. Pequi. Zebrafish.

Abstract:

Epilepsy is a prevalent neurological disorder characterized by recurrent seizures and often accompanied by comorbid conditions such as anxiety, highlighting the need for novel therapeutic agents with neuroprotective properties. Pequi pulp (*Caryocar coriaceum*) contains several bioactive compounds that may provide health benefits, including potential neuroprotection. This study evaluated the ethanolic extract of pequi pulp (EEPP) in adult zebrafish (*Danio rerio*) to determine acute toxicity, sedative potential, and anticonvulsant activity. All procedures were approved by the Ethics Committee on Animal Use at UECE (protocol 04009489/2023). Zebrafish (n=6 per group) were orally administered 20 μ L of EEPP (0.01, 0.1, or 1.0 mg/mL), diazepam (DZP; 10 mg/mL; positive control), vehicle (3% DMSO), or received no treatment (*Naive*). Acute toxicity was evaluated over 96 hours, with no mortality observed at any concentration (CL₅₀ > 1.0 mg/mL). Sedative effects were assessed using the open-field test, based on the number of line crossings. While DZP significantly reduced locomotor activity ($q = 13.97$, $p < 0.0001$ vs. *Naive*; $q = 13.23$, $p < 0.0001$ vs. Vehicle), all EEPP doses showed no significant locomotor reduction ($q = 13.51$; $q = 13.65$; $q = 13.90$, all $p < 0.0001$ vs. DZP), suggesting no sedative effect. Anticonvulsant activity was evaluated using pentylenetetrazol (PTZ, 30 mM) to induce seizures. All EEPP doses showed a delay in seizure onset comparable to DZP ($q = 1.370$; $q = 0.3675$; $q = 0.7350$, $p > 0.05$ vs. DZP), while DZP significantly delayed seizures compared to control ($q = 8.353$, $p < 0.0001$). These results indicate that EEPP is non-toxic, does not induce sedation, and presents anticonvulsant activity in zebrafish, supporting its potential use as a neuroprotective and antiepileptic agent.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

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XXXIX REUNIÃO ANUAL DA FeSBE
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XVI CONGRESSO DA SBBN
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8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Effects of supplementation with *Spirulina in natura* on metabolic parameters and brain morphology in rats treated during lactation: sex-specific analysis

Authors:

Marcos Maciel Fèlix¹
Gabriela Gonçalves da Conceição Antonucci^{1,2}
Giovana da Fonseca Strauss¹
Luan Cristian da Silva³
Valquíria Pereira de Medeiros²
Ana Eliza Andreazzi¹

Affiliations:

¹ Department of Biophysics and Physiology, Institute of Biological Sciences, Federal University of Juiz de Fora, Juiz de Fora, Brazil.
² Department of Biochemistry, Institute of Biological Sciences, Federal University of Juiz de Fora, Juiz de Fora, Brazil.
³ Department of Biology, Institute of Biological Sciences, Federal University of Juiz de Fora, Juiz de Fora, Brazil.

Keywords: Hypothalamus. Morphology. *Spirulina*.

Abstract:

Natural *spirulina* has recently emerged as a promising dietary supplement. In addition to improving individual metabolic parameters—such as food intake and adiposity—it may also reduce inflammation in various brain regions, particularly within the hypothalamus. This study aimed to investigate the transgenerational effects of natural spirulina supplementation during lactation on body weight, food intake, adiposity, and cellular density in the hypothalamic tissue of rats, focusing on the F1 generation. Animals were divided into two groups: F1CV and F1CS (CEUA/UFJF Protocol 025/2023). The F1CV group consisted of offspring from the F0 generation that received an oral saline solution, while the F1CS group consisted of offspring whose F0 generation received natural *spirulina* (400 mg/kg) diluted in saline. Feed intake was measured three times per week after weaning. Final body weight, perigonadal, and retroperitoneal fat were collected and weighed during euthanasia. Brains were removed post-mortem and fixed for histological analysis using standard hematoxylin and eosin staining. Statistical analysis was conducted using GraphPad Prism v.10.1. Results indicated a significant reduction in food intake and retroperitoneal fat in the F1CS group compared to the F1CV group, as well as reduced perigonadal fat in F1CS males. Histological examination revealed that natural *spirulina* did not alter cellular density in the ventromedial hypothalamic region. These findings suggest that *spirulina* supplementation during lactation can effectively reduce food consumption and adiposity, supporting its potential as an affordable and accessible strategy for metabolic regulation.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Cognitive performance across different dystrophins in Duchenne muscular dystrophy: evidence from WASI assessments

Authors:

Sarah Leonardo DIAS¹,
Mirella Telles Salgueiro BARBONI^{1,2},
Alódia BRASIL¹,
Karlla Danielle Ferreira LIMA³,
Clara Gontijo CAMELO³,
Marco Antônio Veloso de ALBUQUERQUE ³,
Edmar Zanoteli ³,
Dora Fix Ventura¹.

Affiliations:

¹ Department of Experimental Psychology, Institute of Psychology, University of Sao Paulo, Brazil.

² Department of Ophthalmology, Semmelweis University, Budapest, Hungary.

³ Department of Neurology, University of São Paulo, São Paulo/São Paulo, Brazil.

Keywords: Cognition. DMD. Dp140. Dp71. QI. Duchenne. Duchenne Muscular Dystrophy. Intelligence

Abstract:

Duchenne muscular dystrophy (DMD) is a neuromuscular disease frequently associated with cognitive impairment, particularly in patients with mutations affecting the shorter dystrophins, example Dp140 and Dp71. This study aimed to investigate the relationship between genotype and cognitive phenotype in DMD. Thirty-six patients were assessed using the Wechsler Abbreviated Scale of Intelligence (WASI). Patients were grouped based on the dystrophin presumably affected: Group 1 (Dp427), Group 2 (Dp427+Dp260), Group 3 (Dp427+Dp260+Dp140+Dp116), and Group 4 (Dp427+Dp260+Dp140+Dp116+Dp71). ANOVA revealed significant group differences for full-scale IQ ($F(3,12) = 4.44, p = 0.026$), verbal IQ ($F(3,11.4) = 5.67, p = 0.013$), and performance IQ ($F(3,14.4) = 4.82, p = 0.016$). Tukey post-hoc tests showed significantly poorer performance in Group 4 compared to Groups 1 and 2. Group 4 had the lowest IQ scores (FSIQ = 66, VIQ = 65, PIQ = 69), suggesting a more severe cognitive impact when Dp71 is affected. These findings reinforce existing evidence that Dp71 deficiency plays a critical role in the cognitive phenotype of DMD, particularly regarding neurodevelopmental impairments.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: The potential action of Oleoylethanolamide on fear memory reconsolidation

Authors:

Vanessa Marieli Ceglarek^{1,2}
Debora Czarnabay^{1,2}
Jéssica Antunes da Silva²
Guilherme Mascarenhas Fontes²
Gustavo Provensi³
Maria Beatrice Passani⁴
Sara Cristina Sagae⁵
Fernando Benetti^{1,2}

Affiliations:

¹ Programa de Pós-graduação em Ciências Biológicas – Fisiologia, Departamento de Fisiologia, Instituto de Ciências Básicas da Saúde (ICBS) da Universidade Federal do Rio Grande do Sul (UFRGS), Rua Ramiro Barcelos, 2600 (90035-003), Porto Alegre/RS, Brasil.

² Laboratório de Neurofisiologia da Cognição e do Desenvolvimento (NeuroCoDe), ICBS, UFRGS.

³ Dipartimento di Scienze della Salute, Università di Firenze, Florence, Italy.

⁴ Dipartimento di Neuroscienze, Psicologia, Area del Farmaco e Salute del Bambino, Università di Firenze, Florence, Italy.

⁵ Programa de Pós-graduação em Ciências Farmacêuticas; Laboratório de Fisiologia Endócrina e Metabólica (LAFEM), Centro de Ciências Biológicas e da Saúde da Universidade Estadual do Oeste do Paraná (Unioeste), Cascavel/PR, Brasil.

Keywords: CA1. Contextual fear conditioning. OEA. Protein inhibitors.

Abstract:

The maladaptation of fear memory has been linked to stress-related disorders, a global health problem that urgently requires new therapeutic strategies. Oleoylethanolamide (OEA), an endogenous lipid mediator, has demonstrated anxiolytic and pro-cognitive properties, emerging as a promising candidate for therapeutic intervention. The present study, approved by ethical committee (n.33836), for the first time investigated the effects of OEA on fear memory reconsolidation - memory reactivation and modification process. Using the contextual fear conditioning paradigm in rats, intraperitoneal (i.p) doses of OEA (1, 5, or 10 mg/kg) or saline solution (CTL) were administered immediately after memory reactivation (n=9- 10/group) and difference mean compared. Retention tests were performed 3 or 24h post-treatment. The 10mg-OEA group showed a significant reduction in freezing behavior at 24h compared to all other groups (vs.: CTL=13.52, $p<0.001$; 1mg=8.63 and 5mg=8.96, $p<0.05$). A similar effect was observed at 3h ($p<0.05$), indicating a rapid onset of action; however, the effect was more pronounced at 24h (vs.: 3h- CTL=29.92; 3h-OEA=27.05; 24h-CTL=23.80; $p<0.0001$), suggesting modulation of synaptic plasticity pathways. To explore underlying mechanisms, Anisomycin (ANI), a protein synthesis inhibitor, or rapamycin (RAPA), an mTOR pathway inhibitor, was infused immediately after i.p of OEA or saline (n=15- 17/group) into CA1 region of the Hippocampus. ANI blocked OEA's effects by demonstrating dependence on de novo protein synthesis, whereas RAPA did not alter outcomes, indicating a mechanism independent of or downstream from mTORC1 signaling (OEA vs.: CTL=33.93; ANI=51.64; OEA-ANI=41.56; RAPA= 45.43; $p<0.0001$; OEA-RAPA=20.81, $p=0.02$; OEA-Rapa vs.: RAPA=24.63; ANI=30.83; OEA-ANI=20.75; $p<0.01$). These novel findings showed OEA's ability to modulate the reconsolidation of fear memory, suggesting an alternative mechanism to mTORC1 for exploring new therapeutic interventions.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Clinical and Molecular Profile of Duchenne Muscular Dystrophy in a Single Center in Brazil

Authors:

Karlla Danielle Ferreira Lima¹
Sarah Leonardo Dias²
Marco Antônio Veloso Albuquerque¹
Clara Gontijo Camelo¹
Alódia Brasil²
Dora Fix Ventura²
Edmar Zanoteli¹

Affiliations:

¹ Department of Neurology, Medical School of the University of São Paulo, São Paulo, Brazil.

² Department of Experimental Psychology, Institute of Psychology, University of São Paulo, Brazil.

Keywords: DMD. Genotype. Intellectual disability. Molecular profile. Neurodevelopment.

Abstract:

Duchenne muscular dystrophy (DMD) is an X-linked recessive neuromuscular disorder caused by mutations in the DMD gene, leading to progressive muscle degeneration and weakness, early loss of ambulation, and death from respiratory or cardiac complications. This retrospective cross-sectional study evaluated the clinical profile of DMD patients followed at a Brazilian reference center between 2000 and 2024. A total of 143 medical records were reviewed. Patients had a mean age of 11 years (range 2–23). The average age of symptom onset was 3 years, with diagnosis confirmed at 6 years. The mean initial CPK level was 12,936. The most common initial symptoms were falls (40%), motor delay (15%), and gait disturbances (10%); 3% presented with language delay and 5% with asymptomatic enzyme elevation. Regarding genotype, 62% had exon deletions, 14% duplications, 10% nonsense mutations, 10% frameshift variants, and 4% splice-site changes. Corticosteroids were started at a mean age of 6.9 years: 70% used deflazacort, 25% prednisone/prednisolone, and 5% had missing data. Loss of ambulation occurred in 33% (mean age 9.6 years). Neurodevelopmental disorders were present in 39%: 32% had intellectual disability, 13% ADHD, 6% autism spectrum disorder, 2% oppositional defiant disorder, and 13% had more than one condition. Only two patients had epilepsy, one with an additional genetic variant. This cohort revealed a delayed diagnosis compared to international data and highlighted a high prevalence of neurodevelopmental disorders. The molecular profile, with 76% deletions/duplications, aligns with global findings.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Catecholaminergic Modulation of LEAP2-Mediated Behavioral and Thermogenic Responses to Aversion in Rats

Authors:

Stefanne Madalena Marques¹
Isac Cardoso dos Santos¹
Hanniely Thais Menezes dos Santos¹
Juliana Vila Verde Ribeiro¹
Michelle Mendanha Mendonça²
Eduardo Colombari²
Rodrigo Mello Gomes¹
Gustavo Rodrigues Pedrino¹
Carlos Henrique Xavier¹

Affiliations:

¹ Instituto de Ciências Biológicas. Universidade Federal de Goiás. Goiânia - GO. Brazil.

² Faculdade de Odontologia de Araraquara. Universidade Estadual Júlio Mesquita Filho UNESP. Araraquara, SP. Brazil.

Keywords: GHRSR. LEAP2. Ghrelin. Dopamine. Catecholamines.

Abstract:

Liver-Expressed Antimicrobial Peptide-2 (LEAP2) was described as an endogenous inverse agonist of the growth hormone secretagogue receptor (GHSR), acting at the binding site for the orexigenic gastric hormone ghrelin. We recently demonstrated the GHSR involvement in cardiovascular, renal, and psychogenic reactivity; the mechanisms involved include an attenuation of hyperadrenergic stimulation and of β -adrenoreceptors mediated responses. Considering literature reports implicating GHSR in dopaminergic pathways governing ingestive behaviors and reward, we investigated the role of catecholaminergic signaling in LEAP2-mediated behavioral and thermoregulatory responses to aversive stimuli. Adult male Wistar rats implanted with cannulas into lateral encephalic ventricle were tested across behavioral, physiological, and metabolic paradigms, including panic-like behavior, locomotion, ingestive behavior, exercise performance, and thermoregulation (CEUA-UFG 071/23). Prior to testing, animals received pretreatment (i.p.) with tyrosine hydroxylase inhibitor AMPT (200mg/kg), dopaminergic antagonist chlorpromazine (CLP - 2mg/kg), or vehicle, followed by intracerebroventricular injections of LEAP2 (6nmol/2uL) or saline. LEAP2 reduced aversion-induced thermoregulatory responses and exerted anxiolytic and anti-panic effects. Dopamine receptor antagonism attenuated LEAP2 influence on aversive and ingestive behaviors, while the catecholaminergic depletion by AMPT were implicated in LEAP2-induced tail skin vasoconstriction and exercise-related CO₂ production. The absence of AMPT or CLP effects on treadmill-related oxygen consumption suggests the involvement of additional pathways. These findings highlight the role of catecholaminergic signaling and dopamine receptors in LEAP2 modulation of physiological responses in defense reactions mediated by central nervous system, supporting its potential role as a neurotherapeutic agent.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Non-Toxic and Non-Sedative Anxiolytic Effects of *Syzygium cumini* (Jamelão) Extract in Adult Zebrafish (*Danio rerio*)

Authors:

Cassia Taiane Viana Moraes²
Igor De Codes Soares¹
Gabriel Martins de Araújo
Sousa¹ Paula Ticiani Alves de
Souza¹ Ariel Falanga de
Oliveira¹
Lucca Moura de Freitas¹
Mário Guilherme de Paula Sales¹
Gabriela Alves do Nascimento¹
Paula Alexandre de Freitas¹
Saulo Chaves Magalhães¹
Maria Rayane Correia de Oliveira²
Luís Sérgio Fonteles Duarte¹
Nilberto Robson Falcão do
Nascimento¹ Ariclécio Cunha de
Oliveira¹
Keciany Alves de Oliveira¹

Affiliations:

¹ Higher Institute of Biomedical Sciences, State University of Ceará-UECE.

² Postgraduate Program in Nutrition and Health, State University of Ceará-UECE.

Keywords: Anxiety. *Syzygium cumini*. Toxicity. Zebrafish.

Abstract:

Anxiety disorders represent a frequent concern in mental health, requiring the development of safe and effective therapies. In this context, *Syzygium cumini* (L.) Skeels, popularly known as jamelão, exhibits various biological properties (anti-inflammatory, antidiabetic, and antioxidant activities). The present study evaluated the 96-hour acute toxicity, possible sedative effect, and anxiolytic-like action of an ethanolic/methanolic extract of *Syzygium cumini* (EEMJ) in adult zebrafish (*Danio rerio*). All procedures were approved by the Ethics Committee on Animal Use (CEUA/UECE, protocol nº 04009489/2023), all results were expressed as mean $\pm$ SEM and analyzed by one-way ANOVA followed by Tukey's test ($p < 0.05$), and the animals were submitted to a 96-hour acute toxicity test, the open field test, and the light-dark test. Adult zebrafish (60–90 days; $n = 6/\text{group}$) were orally treated (20 μL) with EEMJ (0.01, 0.1, or 1.0 mg/mL), diazepam (DZP; 10 mg/mL), vehicle (3% DMSO), or received no treatment (*Naive*). No mortality was observed at any EEMJ concentration ($\text{LC}_{50} > 1.0 \text{ mg/mL}$). In the open field test, EEMJ at all doses did not reduce locomotor activity compared to *Naive* ($q = 1.940$; $q = 0.9472$; $q = 1.849$, $p > 0.05$) and vehicle ($q = 1.669$; $q = 0.6766$; $q = 1.579$, $p > 0.05$), whereas DZP significantly decreased locomotion ($q = 20.25$, $p < 0.0001$ vs. *Naive*; $q = 19.98$, $p < 0.0001$ vs. Vehicle). In the light-dark test, EEMJ (0.01, 0.1, 1.0 mg/mL) significantly increased time in the light zone relative to *Naive* ($q = 10.48$; $q = 13.31$; $q = 12.56$, $p < 0.0001$) and vehicle ($q = 11.37$; $q = 14.21$; $q =$, $p < 0.0001$), showing an anxiolytic-like effect comparable to DZP (differences not significant vs. DZP: $q = 4.108$; $q = 1.274$; $q = 2.027$, $p > 0.05$). These findings suggest that EEMJ is non-toxic, does not induce sedation, and exhibits anxiolytic potential in zebrafish, indicating promise as a natural therapeutic option for anxiety.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Maternal acetaminophen treatment during pregnancy induces autism-like behavior in adult mice offspring

Authors:

Diego de Oliveira Peixoto¹
Amanda da Silva Salgado²
Raphael da Silva Lau²
Brena Barcellos Luiz²
Yasmin Cavalcante de Sousa²
Jonas Xavier Torres²
Lívia Carvalho Dantas²
Liliani Aparecida Sereno
Fontes de Medeiros²
Wellington da Silva Cortes²
Roberto Laureano-Melo²

Affiliations:

¹ Centro Universitário de Barra Mansa.

² Departamento de Ciências Biológicas, Instituto de Ciências Biológicas e da Saúde, Universidade Federal Rural do Rio de Janeiro.

Keywords: Acetaminophen. Autism spectrum disorder. Memory. Pregnancy. Sociability.

Abstract:

Acetaminophen (ACET) is one of the most commonly used drugs during pregnancy worldwide. However, recent studies have reported associations between prenatal acetaminophen exposure and neurobehavioral problems in children. This study aimed to investigate whether maternal exposure to ACET, at both low and high doses, predisposes mouse offspring to autism spectrum disorder (ASD)-like behaviors. Pregnant female mice were randomly assigned to three groups: a control group that received saline, and two treatment groups that received ACET (50 or 150 mg/kg, *p.o.*) once daily from gestational day 8 to 16. After birth, male offspring underwent a behavioral test battery at 70 days of age, including the open field test, object recognition task, three-chamber social approach task, light-dark box test, and step-down avoidance test. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test, assuming a 95% confidence interval ($p < 0.05$). Rout's test was used to detect outliers. The study was approved by the Animal Use Ethics Committee. Maternal ACET treatment impaired both absolute and relative discrimination indexes in the object recognition task ($F_{2,33} = 3.97$, $p = 0.02$ and $F_{2,33} = 4.89$, $p = 0.01$, respectively). Offspring also displayed sociability deficits, as evidenced by reduced social preference and social novelty indexes in the three-chamber task ($F_{2,30} = 6.82$, $p = 0.003$ and $F_{2,31} = 3.63$, $p = 0.03$, respectively). Moreover, impaired aversive memory was observed in the step-down avoidance test ($F_{2,60} = 5.35$, $p < 0.001$). These findings suggest that maternal ACET exposure may program neurodevelopmental vulnerabilities in offspring, contributing to ASD-like phenotypes. Further studies are needed to elucidate the potential effects of maternal ACET exposure on epigenetic inheritance, the expression of ASD-related genes, and morphological changes during corticogenesis.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Statin-induced recovery of hippocampal neuronal activity and inflammatory regulation in chronically sleep-deprived rats

Authors:

Gabriela Larissa Lima da
Silva¹ Higor Hernany Dias¹
Kevin Silva Muller²
Stephane da Silva
Alves¹ Arthur
Gasparindo Moreira³
Tatiane de Camargo
Martins¹ Pedro Tadao
Hamamoto Filho¹
José de Anchieta de Castro e Horta-Junior^{1,2}

Affiliations:

¹ São Paulo State University (Unesp), Medical School, Botucatu.

² University of Campinas (Unicamp), Institute of Biology (IB), Campinas.

³ São Paulo State University (Unesp), Institute of Biosciences, Botucatu.

Keywords: Neuronal activation, FOS protein, Cytokines, Neuroprotection.

Abstract:

Chronic sleep deprivation (SD) leads to neuroinflammation and cognitive impairments, particularly in the hippocampus. Statins are known for their neuroprotective and anti-inflammatory effects. The aim of this study was to investigate the impact of simvastatin, atorvastatin, and rosuvastatin on hippocampal activity and inflammatory responses in SD rats. Five groups of Wistar rats ($n = 7/\text{group}$) were used: CG (control), SDV (SD + vehicle), SDS (SD + simvastatin), SDA (SD + atorvastatin), and SDR (SD + rosuvastatin). SD was performed using the multiple platform method for 45 consecutive days, concomitant with the administration of the drug (20 mg/kg/day). Rats were tested 70 minutes after a working memory task in the Barnes maze, and brains were processed for FOS immunohistochemistry. Cytokine levels (IL-4, IL-6, IL-8, IL-10, IL-17, TNF- α , TGF- β) were measured by ELISA. We considered $p < 0.005$ as statistically significant. In the CA1 region, SDV showed fewer FOS-positive cells (14.8 ± 7.1) than CG (39.1 ± 22.7 , $p = 0.0450$). Atorvastatin (SDA) significantly increased FOS-positive cells (101.8 ± 89.6) compared to SDV ($p = 0.0391$). In the inferior dentate gyrus, SDV (120.4 ± 56) had more cells than CG (38.9 ± 10.6 , $p = 0.0033$). No differences were found in the CA3 or superior dentate gyrus. IL-8 levels were higher in the simvastatin (SDS) and rosuvastatin (SDR) groups compared to CG ($p = 0.0008$, $p = 0.0029$) and atorvastatin ($p = 0.0019$, $p = 0.0083$). TGF- β levels were reduced in SDS and SDA compared to CG ($p = 0.0428$, $p = 0.0450$). No significant differences were observed in IL-4, IL-6, IL-10, IL-17, or TNF- α . These results indicate that atorvastatin may reverse sleep deprivation-induced changes in CA1 neuronal activity and modulate TGF- β levels. Furthermore, simvastatin also modulates inflammatory cytokines by influencing IL-8 and TGF- β levels, while rosuvastatin modulates IL-8.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Relationship Between Birth Weight and Oxidative-Antioxidant Balance in Early Childhood

Authors:

Karollainy Gomes da Silva^{1,2}
Maria Eduarda Rodrigues Alves dos Santos^{1,2}
Ana Patrícia da Silva Souza^{1,2}
Ana Beatriz Januário da Silva^{1,2}
José Maurício Lucas da Silva^{1,2}
Mayara Lucélia da Silva^{1,2}
Robson Feliciano da Silva²
Letícia Henrique Leite da Silva¹
Georgiane Brandão Rodrigues da Silva²
Aline Ananias de Lima¹
Erica Helena Alves da Silva²
Antionietta Claudia Barbosa da Fonseca Carneiro¹
Waleska Maria Almeida Barros^{1,2}
Sandra Lopes de Souza¹

Affiliations:

¹ Graduate Program in Neuropsychiatry and Behavioral Sciences, Center for Medical Sciences - UFPE, Recife - PE, Brazil

² Integrated Center of Neuroscience Technologies (CITENC), Osman Lins University Center - UNIFACOL, Vitória de Santo Antão - PE, Brazil.

Keywords: Birth weight. Child development. DOHaD. Oxidative stress.

Abstract:

The first 1000 days of life are defined as a critical developmental period during which experiences in this phase may play a significant role in programming metabolic and health outcomes. The Developmental Origins of Health and Disease (DOHaD) hypothesis posits an inverse relationship between birth weight and long-term metabolic health, as adverse intrauterine events can induce permanent alterations in the function of metabolic organs. These outcomes are often linked to fetal programming, which leads to lasting modifications in infant growth patterns and metabolism. Oxidative stress, characterized by an imbalance between the production of reactive oxygen species (ROS) and antioxidant defense capacity, is associated with pathological processes, including adverse fetal growth. Although studies suggest an interaction between oxidative stress and birth weight, evidence on this correlation in early childhood remains scarce. Therefore, this study aimed to analyze concentrations of oxidative and antioxidant biomarkers in preschoolers and evaluate their relationship with birth weight. This cross-sectional study involved 18 children with a mean age of 2.80 years (± 0.72). Blood samples were collected to quantify oxidation markers (malondialdehyde [MDA] and protein carbonyls) and antioxidants (total thiol [SH]). Results demonstrated a significant positive association between carbonyl and SH concentrations ($p=0.001$; $r = 1.000^{**}$). Birth weight showed a negative correlation solely with SH levels ($Rho = -0.559$; $p=0.024$). Additionally, children over 3 years exhibited significant differences in MDA concentrations compared to younger participants ($p^*=0.0449$). Notably, higher antioxidant (SH) levels were observed in low-birth-weight children, suggesting a potential compensatory mechanism to mitigate the deleterious effects of oxidative substrates associated with intrauterine growth restriction. This phenomenon, however, may entail long-term energetic trade-offs, compromising competing metabolic functions. Ethical approval: UFPE Human Research Ethics Committee (CAAE: 80950424.5.0000.5208).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Role of irisin in the brains of irradiated mice: effects on behavior, neuronal plasticity and neuroinflammation

Authors:

Luciana Penedo de Melo Teixeira¹
Jhon Brandon Felix Ferreira¹
Isaías dos Santos Silva¹
Heitor Gabriel de Souza Saraiva¹
Maria Eduarda Gomes Moreira¹
Cherley Borba Vieira de Andrade¹
Maurício Moacir da Silva Borges¹
Brenda Akemi Nagagata¹
Leslie Pereira¹
Jemima Fuentes Ribeiro da Silva¹
Samara Cristina Ferreira-Machado¹

Affiliations:

¹ Universidade do estado do Rio de Janeiro – UERJ.

Keywords: Irisin. Neuroinflammation. Brain. Irradiation.

Abstract:

Radiotherapy is effective against brain tumors but may lead to late side effects, such as cognitive deficits linked to neuroinflammation. Physical exercise has been proposed as a promising non-pharmacological strategy due to its neuroprotective effects. During exercise, molecules like irisin are released and have been associated with neuroplasticity and neuronal survival. Studies suggest that this myokine may help attenuate neurodegenerative processes; however, its effects on irradiated brains remain poorly explored. Therefore, it is essential to investigate whether physical exercise can mitigate radiation-induced neurological damage. (CEUA-003/2022) A total of 60 male Swiss mice were used and assigned to the following groups: sedentary control (SC), irradiated sedentary (SI), exercise control (EC), and irradiated exercise (EI). Following irradiation, the exercised groups underwent treadmill running for 3 months. Two behavioral tests (Open Field and Object Location Task) were conducted. Immunofluorescence analysis was performed to assess FNDC5/irisin, synaptophysin, and TNF- α expression. Ninety days after irradiation, all groups showed a similar exploratory profile in the Open Field test, with no locomotor or exploratory impairments. In the Object Recognition test, the SI group exhibited cognitive decline compared to the SC group; however, this decline was not observed in the exercised groups. The EI groups demonstrated performance like the control group, indicating significant neurological improvement ($p < 0.05$). Molecular analyses revealed a significant increase in FNDC5/Irisin and Synaptophysin expression in the exercised groups, along with reduced TNF- α expression, supporting the observed improvements in behavioral outcomes. Three months of physical exercise after irradiation attenuated the cognitive deficit, possibly mediated by irisin. Molecular changes support this improvement, and other pathways are under investigation.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Histomorphometry of AgNORS in the CA1 hippocampal area of *rattus norvegicus* with Alzheimer's disease and subjected to progressive resistance training

Authors:

Amanda Soares Coura¹

Laura Kaori Menegussi Nakano¹

Lilium Midori Ide¹

Affiliations:

¹ Universidade Federal de São João del-Rei (UFSJ).

Keywords: Streptozotocin. Histochemistry. Memory.

Abstract:

Alzheimer's disease (AD) is characterized by neurodegeneration associated with the formation of senile beta-amyloid plaques and neurofibrillary tangles composed of hyperphosphorylated tau protein, resulting in impaired memory and motor skills. The hippocampus, essential for memory, is one of the main areas affected by the disease and is divided into different subareas, emphasizing CA1 due to its key role in consolidation and connection with the entorhinal cortex. Physical activity has been described as an essential non-drug preventive and neuroprotective factor. Therefore, the study was developed to evaluate the neuroplasticity of pyramidal neurons of hippocampal CA1 in *Rattus norvegicus* Wistar subjected to progressive resistance training (PRT) and an experimental model of AD. Approved by CEUA/UFSJ under protocol No 1032130223, in stage 1 (n = 32), PRT consisted of the ladder climbing model with weights attached to the proximal portion of the animal's tail, for five consecutive days, in four weeks; subsequently, transient induction of AD occurred by streptozotocin i.c.v. bilaterally administered; and in stage 2, the animals were euthanized after 30 days of AD induction for brain collection and histological processing using the AgNOR technique. The animals were divided into the following groups: exercised and AD-induced (TRPDA; n=6); non-exercised and AD-induced (CTRDA; n=8); exercised and not AD-induced (TRPSH; n=4); non-exercised and not AD-induced (CTRSH; n=5). As result, CTRDA presented a lower number of nucleoli per nucleus ($7.516 \mu\text{m}^2$; $5.253 \mu\text{m}^2$; $77 t=2.573$; $p=0.05$) and a smaller nucleolar area ($8.675 \mu\text{m}^2$; $5.613 \mu\text{m}^2$; $56 \mu\text{m}^2$; $t=2.862$; $p=0.02$) compared to CTRSH, allowing to confirm the action of STZ in reducing proliferative activity and neural synthesis in the transient Alzheimer's model, without, however, identifying the possible neuroprotective action with the experimental training model and histochemical technique used.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Investigation of migration defects in neurons derived from patients with a monogenic autism spectrum disorder

Authors:

Pedro Kenzo Kobaiashi Forti¹

Fabio Papes¹

Affiliations:

¹ Department of Genetics, Evolution, Microbiology and Immunology, Institute of Biology, University of Campinas (UNICAMP).

Keywords: Autism. Migration. Neurodevelopment. Pitt-Hopkins.

Abstract:

Pitt-Hopkins Síndrome (PTHS) is a genetic disease in the autistic spectrum caused by haploinsufficiency of the Transcription Factor 4 gene (TCF4). This disorder is characterized by motor delay, intellectual disability, and absence of speech. Our previous studies showed that patient-derived cells and organoids exhibit impaired neural progenitor proliferation and neuronal differentiation. Moreover, our preliminary data suggest that the neural tissue from these patients display neuronal migration defects. This study aims to characterize the migration profile of PTHS patient-derived cells by performing scratch assay and live-cell imaging experiments. Neuronal Progenitor Cells (NPCs) derived from patients and same-sex parents were cultivated and differentiated into neurons. Then, in a saturated scratch assay plate, we created a gap by removing a streak of cells from the plate floor. Next, we imaged the neuronal cultures every 12 hours for 10 days to analyze how cells migrate to fill the gap. After image scoring using specialized software, we concluded that some PTHS cell lines show a severe reduction in migration rate, as determined by the number of cells occupying the gap, as well as by measuring their migration speed. We raise the hypothesis that the severity of the migration defects observed in each cell line is correlated with the type of genetic mutation found in the TCF4 gene. Our results shed light onto the cellular and molecular pathophysiology of an autism spectrum disorder.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Behavioral effects of maternal separation stress are sex-specific and distinct in prepuberty and early adulthood

Authors:

Bruna Lopes Resende^{1,2,5}
Caio Dias Massardi^{1,2}
Sofia Campos F Vieira^{1,2}
Matheus Magno Rosa de Assis^{1,2}
Anna Clara Silva Braz^{1,2}
André Gustavo de Oliveira^{1,3}
Gustavo Batista de Mezes^{1,4}
Antônio Carlos de Oliveira Pinheiro^{1,5}
Bruno Rezende de Souza^{1,2}

Affiliations:

¹ Universidade Federal de Minas Gerais.

² Laboratório de Neurodesenvolvimento e Evolução, Departamento de Fisiologia e Biofísica, Instituto de Ciências Biológicas.

³ Laboratório de Circuitos Fisiológicos, Departamento de Fisiologia e Biofísica, Instituto de Ciências Biológicas.

⁴ Center for Gastrointestinal Biology, Departamento de Morfologia, Instituto de Ciências Biológicas.

⁵ Laboratório de Neurofarmacologia, Departamento de Farmacologia, Instituto de Ciências Biológicas.

Keywords: Neurodevelopment. Stress. Behavior. Adolescence. Early-life.

Abstract:

Complex interactions among the organism and its environment form the foundation for behavioral patterns observed in adulthood. Most psychiatric disorders emerge before adulthood, with higher incidence of mood, anxiety and schizophrenia during adolescence – when neurodevelopmental processes occur more intensely, with synaptic pruning being one of the most relevant for refining neuronal communication. Notably, early-life stress exposure is a risk factor for the onset of these disorders. The hypothesis of this study is that early-life stress interacts with neurodevelopmental processes and leads to different behavioral outcomes in adolescent and adult mice. To test this hypothesis, male and female mice were subjected to maternal separation stress (MS) from P1 to P14. From P28 or P58, all animals underwent a battery of behavioral tests for assessment of motor/exploratory and anxiety-like behaviors (CEUA 99/2022). We observed that during adolescence, male mice exposed to MS exhibit hyperlocomotion in the open field test, along with an increased latency to first explore the dark compartment in the light/dark box; while females lost the preference for social stimuli characteristic to adolescence. The “stress exposure” factor was associated with less time in the dark compartment in the light/dark box, fewer entries into the closed arms and center of the elevated plus maze, as well as fewer crossing between regions. Further, the “sex” factor itself was related to a basal behavioral difference in the light/dark box. When the same tests were performed in adult mice, the only remaining alteration was the influence of exposure to stress in the elevated plus maze. In our study, we observed a shift in early-life stress response from adolescence to adulthood, thus emphasizing the relevance of studying different developmental phases, and highlighting the significance of considering experiences that are particular to formative periods when discussing social well-being and health.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Evaluation of cerebral neovascularization and neuroinflammation in spontaneously hypertensive rats fed a high-fat diet and treated with sitagliptin

Authors:

Lucas Silva Bastos¹
Isaias dos Santos Silva²
Péricles Fabrício Gomes Filho²
Paulo Rogerio Silva de Paiva Júnior¹
Heitor Gabriel de Souza Saraiva²
Maria Eduarda Gomes Moreira da Silva²
Mauricio Moacir da Silva Borges²
Luciana Penedo de Melo Teixeira²
Jhon Brandon Felix Ferreira²
Samara Cristina Ferreira Machado²
Leslie Pereira²
Jemima Fuentes Ribeiro da Silva²
Cherley Borba Vieira de Andrade²
Aluana Santana Carlos¹

Affiliations:

¹ Universidade Iguaçu – UNIG, Nova Iguaçu – RJ – Brasil. ² Universidade do Estado do Rio de Janeiro – UERJ.

Keywords: Hypertension. Neuroinflammation. Neovascularization. Rats. Sitagliptin.

Abstract:

Systemic arterial hypertension (SAH) and obesity are conditions associated with metabolic, inflammatory, and cardiovascular alterations. Studies suggest that DPP-4 inhibitors, such as sitagliptin, exert antioxidant and tissue-protective effects, potentially improving mitochondrial function, vascularization, and neuroinflammation. This study evaluated the effects of sitagliptin on neovascularization and neuroinflammation in spontaneously hypertensive rats (SHR) fed a high-fat diet. Animals were divided into three groups (n=8): SHR control, SHR with high-fat diet (HF), and SHR-HF treated with sitagliptin (1 mg/kg/day) starting at 150 days of age. Diets were prepared according to the American Institute of Nutrition protocol. At 180 days, animals were euthanized for blood and brain collection. Systolic and diastolic blood pressure, lipid profile, serum insulin, and expression of IBA-1, GFAP, IB-4, and VEGFA in the hypothalamus were assessed. Sitagliptin significantly reduced both systolic and diastolic blood pressure and improved dyslipidemia and hyperinsulinemia in treated animals. Cryostat sectioning and immunohistochemistry are ongoing for neuroinflammatory analysis. We conclude that sitagliptin improved metabolic and cardiovascular parameters, with potential benefits in modulating neuroinflammation in a model of hypertension and high-fat diet.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Maternal separation disrupts olfactory memory via ERK/CREB/ α 7-nAChR signaling in the olfactory bulb and hippocampus

Authors:

Débora Czarnabay^{1,2,3,4}
Leo Meira Martins^{1,5}
Patricia Pranke^{1,3,4}
Fernando Benetti^{1,2,4}

Affiliations:

¹ Programa de Pós-graduação em Ciências Biológicas: Fisiologia, Departamento de Fisiologia, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul – UFRGS – Rua Sarmento Leite, 500 (90050-170), Porto Alegre/RS, Brazil.

² Laboratório de Neurofisiologia da Cognição e Desenvolvimento, Departamento de Fisiologia – Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Rua Sarmento Leite, 500 (90050-170), Porto Alegre/RS, Brazil.

³ Hematology and Stem Cell Laboratory, Faculty of Pharmacy, Universidade Federal do Rio Grande do Sul, Av. Ipiranga, 2752 (90610-000), Porto Alegre/RS, Brazil.

⁴ Instituto de Pesquisas com Células-tronco, Porto Alegre/RS, Brazil.

⁵ Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul – UFRGS, Porto Alegre/RS, Brazil.

Keywords: Cholinergic receptors. CREB. ERK hippocampus. Maternal separation. Nest odor learning.

Abstract:

Maternal behavior is crucial for infant development, providing physical, emotional, and social support. Early-life adverse events, such as maternal separation (MS), disrupt brain development, increasing the risk of psychiatric disorders and cognitive impairments. This study investigated the effects of repeated MS (3 h/day during postnatal days 1–10 [PND1–10]) on olfactory memory and related biomarkers (ERK/CREB pathway, α 7-nAChR) in the olfactory bulb (OB) and hippocampus (HP) of rat pups. Pregnant Wistar rats ($n = 64$) were housed individually in the UFRGS sectorial vivarium. From PND1, pups were separated from their dams for 3 h/day (MS group); controls remained with their mothers (non-separated group, NS) (Project #29716). One male and one female pup per litter were tested. Behavioral data are expressed as mean $\pm$ SEM. MS impaired offspring behavior, significantly reducing time spent in the nest area (NS: $57.33 \pm 3.14\%$; MS: $39.96 \pm 4.46\%$; $p = 0.012$) and increasing latency to locate maternal odor during memory tests. Separated pups also showed deficits in odor preference (first-choice paradigm; $p < 0.05$). At the molecular level, MS downregulated CREB ($p = 0.0216$) and α 7-nAChR ($p = 0.0152$) expression in the OB and HP, suggesting ERK signaling pathway disruption. These findings demonstrate that disrupted mother-offspring bonding alters olfactory memory development, likely via impaired ERK/CREB/ α 7-nAChR signaling, highlighting how early-life stress compromises sensory-cognitive integration.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Maternal separation disrupts fear memory consolidation via AChE/CREB/Na⁺/K⁺-ATPase pathways: Rescue by galantamine treatment

Authors:

Débora Czarnabay^{1,2,3,4}
Leo Meira Martins¹
Guilherme Fontes Mascarenhas²
Angela TS Wyze⁵
Fátima Guma⁵ Guilherme Baldo^{1,6}
Patricia Pranke^{1,3,4}
Fernando Benetti^{1,2,4}.

Affiliations:

¹ Programa de Pós-graduação em Ciências Biológicas: Fisiologia, Departamento de Fisiologia, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul – UFRGS – Rua Sarmento Leite, 500 (90050-170), Porto Alegre/RS, Brazil.

² Laboratório de Neurofisiologia da Cognição e Desenvolvimento, Departamento de Fisiologia – Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Rua Sarmento Leite, 500 (90050-170), Porto Alegre/RS, Brazil.

³ Hematology and Stem Cell Laboratory, Faculty of Pharmacy, Universidade Federal do Rio Grande do Sul, Av. Ipiranga, 2752 (90610-000), Porto Alegre/RS, Brazil.

⁴ Instituto de Pesquisas com Células-tronco, Porto Alegre/RS, Brazil.

⁵ Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul – UFRGS, Porto Alegre/RS, Brazil.

⁶ Laboratório de Terapia Gênica - Departamento de Fisiologia – Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Rua Ramiro Barcelos, 2600 (90035-003), Porto Alegre/RS, Brazil.

Keywords: Aversive memory. Cholinergic receptors. CREB. Maternal separation. Mitochondrial potential.

Abstract:

Maternal separation (MS) during the neonatal period induces long-lasting behavioral and neurochemical alterations. This study examined MS effects on aversive memory, acetylcholinesterase (AChE) activity, Na⁺/K⁺-ATPase function, and molecular markers (α7-nAChR, CREB, pCREB, mitochondrial mass/potential) in the hippocampus (HP) and medial prefrontal cortex (mPFC) of adult male Wistar rats treated with Galantamine (Gal). Pregnant Wistar rats (n = 64) were individually housed. From postnatal day 1 (PND1), pups were separated from dams for 3 h/day (MS group); controls remained with mothers (non-separated, NS) (Project #29716). In adulthood, males received saline or Gal (1 mg/kg) before inhibitory avoidance training. Memory was tested 24 h later (latency to descend, *t*_{max} = 300 s). AChE/Na⁺/K⁺-ATPase activities were measured in HP, mPFC, and perirhinal cortex (PCe); other markers were assessed via flow cytometry (*p* < 0.05). MS impaired fear memory (NS-Sal: 206.3 ± 35.49 s, n = 15; MS-Sal: 99.50 ± 37.03 s, n = 12; *p* = 0.0494), an effect reversed by Gal (MS-Gal: 155.7 ± 42.24 s, n = 10; *p* = 0.0086 vs. MS-Sal). MS increased AChE activity in HP/PCe (NS: 1.186 ± 0.044; MS: 1.388 ± 0.108; *p* < 0.05), while Gal normalized HP AChE levels. Na⁺/K⁺-ATPase activity decreased in HP (NS: 174.5 ± 6.23; MS: 145.3 ± 4.42; *p* = 0.0034) but not in mPFC/PCe; Gal restored this activity (MS-Gal: 156.2 ± 11.13 vs. NS: 150.6 ± 3.96; *p* = 0.6241). MS reduced basal CREB,



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

pCREB, and $\alpha 7$ -nAChR levels, with deficits persisting post-task, whereas Gal rescued CREB/pCREB expression ($p = 0.0086$). Mitochondrial potential was lower in HP of MS rats (NS: 171.6 ± 8.41 ; MS: 134.2 ± 5.75 ; $p < 0.05$). These findings demonstrate that MS-induced memory deficits involve HP-specific AChE hyperactivity, Na^+/K^+ -ATPase dysfunction, and impaired CREB signaling, all of which were reversed by Galantamine, highlighting its therapeutic potential for early-life stress-related cognitive deficits.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Anxiolytic Effects of Carvacrol in Adult Zebrafish (*Danio rerio*): Behavioral Assessment Using Light-Dark and Open Field Tests

Authors:

João Marcos Rodrigues da Silva¹
Igor de Codes Soares¹
Gabriel Martins de Araújo Sousa¹
Paula Ticiani Alves de Souza¹
Ariel Falanga de Oliveira¹
Lucca Moura de Freitas¹
Mário Guilherme de Paula Sales¹
Caroline Pereira dos Santos Rocha¹
Paula Alexandre de Freitas¹
Maria Rayane Correia de Oliveira²
João Gabriel Leite da Silva¹
Gabriela Alves do Nascimento¹
Saulo Chaves Magalhães¹ Keciany
Alves de Oliveira¹

Affiliations:

¹ Higher Institute of Biomedical Sciences, State University of Ceará-UECE.

² Postgraduate Program in Nutrition and Health, State University of Ceará-UECE.

Keywords: Anticonvulsant. Carvacrol. Epilepsy. Zebrafish.

Abstract:

Anxiety disorders are the most frequent symptoms of chronic stress among psychiatric conditions, affecting around 25% of the population across all age groups. In this context, compounds derived from medicinal plants may exhibit anxiolytic potential. Carvacrol (2-methyl-5-(1-methylethyl)-phenol) is a monoterpenic phenol compound, found mainly in oregano oil (*Origanum vulgare*), and is known for its anti-inflammatory and antioxidant activities. This study aimed to evaluate the anxiolytic potential of carvacrol (CVC) in adult zebrafish (ZFa). The animals were treated orally with 20 μ L of CVC (0.0001, 0.0005, 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, or 1.0 mg/mL), or with Vehicle (3% DMSO; 20 μ L; *p.o.*), or with a positive control treated with Diazepam (10 mg/mL; *p.o.*). An untreated group was included (Naive). After one hour, fish were subjected to the open field test (to assess locomotor activity) and the light-dark test (to measure anxiety-like behavior). Data were analyzed using ANOVA and Tukey's test, with statistical significance at $p < 0.05$. The protocol was approved by the UECE Animal Ethics Committee (CEUA- 31032.004795/2023-86). CVC at concentrations of 0.0001–0.01 mg/mL did not significantly affect locomotion, but differed from diazepam ($q = 6.302$ – 9.082 , $p < 0.001$). Diazepam reduced activity compared to naive and vehicle groups ($q = 7.783$, $p < 0.0001$; $q = 5.653$, $p < 0.0001$). In the light-dark test, CVC (0.0001, 0.001, 0.01 mg/mL) showed anxiolytic-like effects ($q = 6.291$ – 9.738 , $p < 0.0001$ vs. control), similar to those observed with diazepam. These findings suggest that carvacrol has significant anxiolytic activity in zebrafish, supporting its potential as a natural anxiolytic agent.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Rosuvastatin attenuates the negative effects on aversive memory of sleep-deprived rats subjected to the elevated plus maze

Authors:

Ygor Manata Jacobina¹
Gabriela Larissa Lima da Silva¹
Stephane Alves da Silva¹
Kevin Silva Muller²
Arthur Gasparindo Moreira³
José de Anchieta de Castro e Horta-Junior^{1,2}

Affiliations:

¹ São Paulo State University (Unesp).
² Institute of Biosciences, Botucatu (IBB).

Keywords: Cognition. Memory. Neuroprotection. Statins.

Abstract:

The Chronic Sleep restriction, a current scenario as well as hypercholesterolemia, results in consequences for the memory consolidation process. Rosuvastatin is a lipid-lowering drug that, in the nervous system, influence certain neural processes. The objective of this study is to evaluate the effects of rosuvastatin on the aversive memory of Wistar rats subjected to chronic sleep deprivation by the 2 exposure protocol of the elevated plus maze (EPM). 60 animals were used, divided into 4 groups (CEUA-1414/2022, N=15), organized as: CG (control group: vehicle without sleep restriction); DG (deprivation group: vehicle and sleep restriction); RG+ (rosuvastatin group: sleep restriction + 20mg/kg) and RG- (rosuvastatin group: sleep restriction + 4.2mg/kg). The method used for sleep deprivation was the adapted multiple platform, performed daily for 45 consecutive days, with the EPM being applied on the 25th day. In the first exposure to EPM, DG presented a greater number of entries into the open arm than CG ($p=0.0001$), RG+ ($p=0.0115$) and RG- (<0.0001); and in the comparative analysis between the exposures, DG>CG ($p<0.001$). Regarding the number of entries into the closed arm, DG>CG ($p=0.016$); RG+ ($p=0.0009$) and RG- ($p<0.0001$); and in the relative analysis, the same occurs between DG and CG ($p=0.0114$). In the time of head dips, there is no significance between CG and DG, only between DG and RG+ ($p=0.0029$) and RG- ($p<0.0001$), in which DG presents a higher index; in the comparative analysis, DG>CG ($p=0.0020$). In the open arm time, only the DG and RG-relationship ($p=0.0042$) presents significance, in which DG>RG-, as well as in the analysis of the closed arm time ($p<0.0001$). In head dip frequency, DG>CG ($p=0.0255$), and DG>RG- ($p=0.0120$). In the center time, DG>RG+ ($p=0.0032$) and DG>RG- ($p=0.0119$). These results indicate impairment of aversive memory in DG rats in relation to CG, as well as suggest attenuation of such negative effects in the groups treated with rosuvastatin.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Dose-Dependent Effects of Simvastatin on Barnes Maze Performance in Sleep-Restricted Rats

Authors:

Stephane da Silva Alves^{1,2}
Gabriela Larissa Lima da Silva^{1,2}
Kevin Silva Muller^{1,2}
Verônica de Araujo Parducci^{1,2}
Arthur Gasparindo Moreira^{1,2}
José de Anchieta de Castro e Horta Junior^{1,2}

Affiliations:

¹ Universidade Estadual Paulista (UNESP).
² Instituto de Biociências de Botucatu (IBB).

Keywords: Entorhinal cortex. Neuroprotection. Sleep deprivation. Spatial memory. Simvastatin.

Abstract:

Sleep is crucial for body homeostasis, and chronic sleep deprivation can impair spatial memory mediated by the entorhinal cortex (EC). This study evaluated the neuroprotective effects of simvastatin (4.2 mg/kg and 20 mg/kg) in male Wistar rats subjected to 18-hour daily sleep deprivation (modified multiple platform method) for 45 consecutive days, with parallel simvastatin or vehicle treatment. Animals were divided into four groups (n=15 each): control (GC), sleep-deprived (GP), sleep-deprived with 4.2 mg/kg (GPS-) or 20 mg/kg (GPS+) simvastatin. Behavioral testing in the Barnes maze occurred from day 40 to 45 (5 testing days with 3 daily trials), (CEUA 1414/2022). The escape box position remained fixed within daily trials but changed between days. Sessions were recorded and analyzed using MATLAB R2024B/ZebTrack to assess latency, search strategies, distance traveled, and time spent in target areas. FOS immunohistochemistry in the EC revealed significantly reduced neuronal density in the medial EC of GPS- (40.18 ± 7.073) versus GC (66.69 ± 7.360 ; $p=0.0328$), with no other significant differences. Data were analyzed with one-way ANOVA/Tukey (parametric) or Kruskal-Wallis/Friedman/Dunn tests (non-parametric; $\alpha=0.05$), excluding outliers (ROUT method, $Q=2\%$). GPS- showed lower latency in the second trial (13.57 ± 3.561) versus GPS+ (22.56 ± 2.193 ; $p=0.0219$) and more direct searches, suggesting better adaptation. GP spent less time in the target area (20.84 ± 1.319) than GC (35.9 ± 3.411 ; $p=0.00081$), indicating memory impairment. Despite reduced medial EC activation, GPS- matched GC's behavioral performance, implying enhanced neural efficiency. Low-dose simvastatin may mitigate sleep deprivation effects on spatial memory, while the high dose showed no clear benefits.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Ramped Plus Maze as a Behavioral Test for Anxiety in Zebrafish Larvae (*Danio rerio*)

Authors:

Eduarda Maria Silva de Souza¹
Matheus Magno Rosa de Assis¹
Bruno Rezende de Souza¹

Affiliations:

¹ Department of Physiology and Biophysics, Institute of Biological Sciences, Federal University of Minas Gerais, MG, Brazil.

Keywords: Anxiety. Behavior. Ramped Plus Maze. Larvae. Zebrafish.

Abstract:

From the simplest organisms to the most complex, the ability to avoid aversive stimuli and situations is crucial for survival. Approaching desirable situations and avoiding undesirable ones are fundamental tasks, observable in behavioral responses and biochemical changes. Avoidance behavior may be linked to imminent danger or the anticipation of future events based on stimuli. However, these responses are not always tied to natural homeostasis and may be associated with pathological processes. Anxiety disorders are a global public health challenge, affecting about one-third of the world's population, with Brazil having the highest prevalence. While heredity and genetic factors contribute to anxiety disorders, environmental variables play a predominant role, highlighting the need to understand their neurobiological origins and physiological responses. This study investigates the viability of the Ramped Plus Maze (RPM) as a new behavioral test to assess anxiety in zebrafish larvae. Adapted from the elevated plus maze used in rodents, the RPM presents a height-based conflict, resembling established anxiety tests. Larvae were tested in the RPM and Light/Dark (LD) test at different water column heights (1.1cm, 1.3cm, 1.5cm) and developmental stages (5, 14, 21 days post-fertilization). Results show that water height influences behavior, with 1.3cm optimal at 5dpf and 1.5cm at 14 and 21dpf. Preliminary findings suggest the RPM is a promising tool for assessing anxiety in zebrafish larvae, offering insights into the emergence of anxiety-like behavior during development. Validating RPM could advance behavioral neuroscience research, enabling drug screening and genetic studies to better understand anxiety disorders. This study, approved by the Ethics Committee (CEUA protocol 192/2023), was supported by Capes, CNPq, and Fapemig.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Dissociating the contribution of C1 and DMV neurons to glycemic challenge responses in rats

Authors:

Deborah de Paula Romeu¹

Leticia Urçulino da Silva¹

Giovanna Castrignano Camargo¹

Vagner Roberto Antunes¹

Affiliations:

¹ University of São Paulo, Department of Physiology and Biophysics, Institute of Biomedical Sciences.

Keywords: C1 neurons. Dorsal motor nucleus of the vagus. Glucoprivation. Hyperglycemia. Parasympathetic nervous system.

Abstract:

Hindbrain catecholaminergic neurons coordinate responses to glucose deficits, but their role in hyperglycemia is less understood. The parasympathetic efferents to the liver and the pancreas originate from preganglionic neurons from the dorsal motor nucleus of the vagus (DMV), which also receives excitatory inputs from the catecholaminergic C1 neurons in the ventrolateral medulla (VLM), being reasonable to suppose that this C1-DMV connection comprises a circuitry that controls glucose homeostasis. Here we sought to assess which glycemic challenge may recruit this circuitry. Male Wistar rats (CEUA #9141070319) were exposed to either glucoprivation with 2-deoxy-d-glucose (2DG; 400mg/kg; ip; n=3), glucose overload (3.6g/kg; ip; n=4) or a vehicle (NaCl 0,9%; ip; n=4). After 90 min, rats were anesthetized and perfused to immunoreaction for c-FOS, a marker of acute neuronal activation. Cells were counted using Icy Bioimage software. Data was analyzed by one-way ANOVA with Sidak post-hoc test or Student's t tests to compare 3 and 2 groups, respectively. Both stimuli, 2DG and glucose overload, led to FOS+ staining in the C1 neurons and in the DMV, and 2DG resulted in the highest detection of FOS in the DMV (48 ± 12 cell/section, vs. 21 ± 10 for glucose overload and 0.3 ± 0.25 for the vehicle; $p=0.01$). To determine whether DMV activation depends on C1 inputs, challenges were repeated 14 days after depleting C1 neurons using bilateral injection of anti-dopamine-beta-hydroxylase-saporin (DBH-SAP; 2.4 ng/100nL) into the VLM. DBH-SAP depleted nearly 50% of C1 neurons, but we found no significant difference in FOS+ staining in the DMV compared to the rats with intact C1, neither by 2DG ($p=0.2234$) nor glucose overload ($p=0.4989$). Our results suggest that both C1 and DMV neurons are activated by glycemic challenges, to a greater extent during glucose shortages than surpluses, but parasympathetic activation does not seem to depend exclusively on inputs from C1 neurons to the DMV.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Comparative Module-Based Analysis Identifies Conserved Pathways Involved in Amyloidogenic Regulation and Neurodegeneration in Magnocellular Neurons

Authors:

Allan dos Anjos Monteiro¹
Victor Jardim Duque¹
João Victor Silva Nani¹
André de Souza Mecawi¹

Affiliations:

¹ Paulista School of Medicine - Federal University of São Paulo - Department of Biophysics – Laboratory of Molecular Neuroendocrinology.

Keywords: Magnocellular neurons. Alzheimer disease. Neurodegenerative disease. Vasopressin. Oxytocin.

Abstract:

Magnocellular neurons (MCNs) are key neuroendocrine regulators through oxytocin (OXT) and vasopressin (AVP) release. Interestingly, MCNs show notable resilience to neurodegeneration. We applied high-dimensional weighted gene co-expression network analysis (hdWGCNA) to single-cell/nucleus RNA sequencing data from MCNs across five species (*Homo sapiens* (Hs), *Macaca fascicularis* (Mf), *Callithrix jacchus* (Cj), *Mus musculus* (Mm), and *Rattus norvegicus* (Rn)) to identify conserved transcriptional modules associated with neurodegenerative and amyloid-related pathways. Differential Module Eigengenes (DMEs) in AVP/OXT subclusters were compared via MAST; gene overlap was visualized with a Venn diagram. Functional enrichment with Gene Ontology (GO), KEGG, and Reactome was performed for module-specific and conserved genes. GO analysis revealed enrichment ($p_{\text{adj}} < 0.05$) in pathways related to negative regulation of amyloid fibril formation, negative regulation of amyloid precursor protein biosynthesis (Mf, Mm), regulation of β -amyloid formation, and positive regulation of β -amyloid clearance. Among genes enriched in AVP/OXT MCNs, *PSEN2*, *BACE1*, *APOE*, and *CLU* emerged as key regulators of amyloid processing and Alzheimer's disease pathogenesis. In humans, *APOE* notably promotes β -amyloid clearance, highlighting its translational relevance. These pathways were significantly enriched in AVP- or OXT-expressing MCNs as evidenced by DMEs analysis. KEGG and Reactome analyses of conserved genes inter-species confirmed enrichment in neurodegenerative pathways. The identification of these conserved amyloidogenic pathways across species and MCN subtypes reveals intrinsic neuroprotective mechanisms likely underlying MCN resilience to neurodegeneration. These findings deepen understanding of MCN function and highlight those neurons as promising targets for neurodegenerative and amyloid-related therapies, as well as for a better understanding of the pathophysiology of neurodegenerative diseases.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Acute effects of Transcutaneous Vagus Nerve Stimulation in women with Fibromyalgia: a randomized, crossover, simulation-controlled clinical trial

Authors:

Marcos Lisboa Neves¹
Lauriana Urquiza Nogueira¹
Iara Gonçalves Teixeira¹
Guilherme Fleury Fina Speretta¹

Affiliations:

¹ UFSC Florianópolis, PPG em Neurociências, Brasil.

Keywords: Autonomic nervous system. Chronic pain. Cognitive function. Heart rate variability.

Abstract:

Fibromyalgia (FM) is a chronic condition characterized by widespread pain, physical fatigue, sleep impairment and cognitive decline, mainly affecting women. Its etiology has not yet been elucidated, but evidence points to central nervous system sensitization, leading to hyperalgesia and autonomic disorders. This randomized and crossover study (CAAE 81840824.0.0000.0121) evaluated the acute effect of transcutaneous auricular vagus nerve stimulation (taVNS) on pain, cognition, and heart rate variability (HRV) in women with fibromyalgia. Following the American College of Rheumatology criteria, 14 women diagnosed with FM were randomized to receive 30 minutes of taVNS (100 Hz, 250 μ s, and 1 and 6 mA) or sham taVNS (0 mA) in the left auricle, with a 15-day interval between interventions. Behavioral assessments were performed using the Numerical Pain Rating Scale (NPRS) and the Widespread Pain Scale (WPS); cognitive tests such as the Trail Making Test (TMT), Stroop and the Paced Auditory Serial Addition Test (PASAT), besides the analysis of HRV, with pain as the primary outcome. Twelve women (46.7 ± 11.7 years of age) completed the study. The taVNS was not superior to Sham in NPRS ($p=0.48$; DM -0.42; IC 95% -1.67 to 0.84); WPS ($p=0.19$; DM -1.67; IC 95% -4.31 to 0.98); TMT-A ($p=0.37$; DM 2.25; IC 95% -3.09 to 7.59); TMT-B ($p=0.21$; DM 13.33; IC 95% -8.81 to 35.48); Stroop-A ($p=0.71$; DM -0.42; IC 95% -2.86 to 2.03); Stroop-B ($p=0.21$; DM 0.83; IC 95% -0.54 to 2.21); Stroop-C ($p=0.83$; DM 0.42; IC 95% -3.72 to 4.56); PASAT ($p=0.68$; DM -0.83; IC 95% -5.20 to 3.53); and HRV (time and interaction): SDNN ($p=0.43$; $F(1, 8) = 0.675$), RMSSD ($p=0.27$; $F(1, 8) = 1.426$), Low Frequency (nu) ($p=0.29$; $F(1, 8) = 1.273$) and High Frequency (nu) ($p=0.29$; $F(1, 8) = 1.285$). In conclusion, taVNS via the left auricle does not evoke acute responses in pain, cognition and HRV in women with fibromyalgia.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Nanoencapsulated Oleoylethanolamide Restores Short-Term Spatial Memory in Alzheimer's Disease Mice

Authors:

Camila Fonseca Balcewicz¹

Camila Maria Coltri¹

Sara Cristiana Sagae Schneider¹

Affiliations:

¹ State University of Western Paraná.

Keywords: Streptozotocin-intracerebroventricular. Phosphatidylcholine. Y-maze.

Abstract:

With over 55 million people affected globally, Alzheimer's disease (AD) is the leading cause of dementia worldwide. This progressive neurodegenerative illness has increasing prevalence and economic impact, yet few effective therapeutic options are available. Experimental models, such as intracerebroventricular (ICV) injection of streptozotocin (STZ), mimic the sporadic form of AD, allowing for the study of potential therapies. An experimental AD model was established in 40 male C57BL/6 mice (100-112 days, 25-30g), conforming to ethical guidelines (protocol nº 20-21). The mice were divided into five groups (n=8/group): CTL (saline icv + saline ip), STZ (streptozotocin icv to induce AD + saline ip), STZ+OEA (STZ icv + OEA ip), STZ+OEA NANO (STZ icv + nanoencapsulated OEA ip), and STZ+NANO (STZ icv + nanocapsule ip). AD was induced via icv STZ administration (3µL, 3mg/kg). Fifteen days later, a 7-day treatment with OEA (20mg/kg) was initiated. Afterward, the animals were subjected to the Y-maze test, verifying the alternation rate and total number of entries. In the alternation rate, the STZ (56.66±10.06) and/or STZ+NANO (48.42±12.18) groups significantly decreased this rate compared to the CTL group (73.11±6.268, $p<0.05$). Conversely, STZ+OEA (84.93±8.613) and STZ+OEA NANO (72.38±8.785) increased this rate compared to STZ ($p<0.05$), achieving similar performance to CTL. In locomotor activity, STZ (17.25±2.866) and/or STZ+NANO (9.50±3.117) had a decreased total number of entries compared to CTL (25.25±2.435, $p<0.05$). Among the treatment groups, only STZ+OEA NANO (25.50±3.251) showed a significant increase in entries compared to STZ ($p<0.05$) and similar performance to CTL, while STZ+OEA (20.50±4.504) did not significantly differ from STZ. Therefore, the present project, in resonance with previous studies, revealed that treatment with OEA nanoencapsulated in PLC significantly restored cognitive deficits in short-term spatial memory induced by ICV STZ infusion.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Utilization of Oleoylethanolamide Associated with Phosphatidylcholine Nanoparticles: Effect on Glycemic Metabolism in an Experimental Model of Alzheimer's Disease

Authors:

Camila Maria COLTRI¹

Maria Júlia Todero¹

Gabriela Cardoso Posteraro¹

Ana Beatriz Azevedo de Oliveira¹

Maria Vitória Rebelo¹

Camila Fonseca Balcewicz¹

Vanessa Marieli Ceglarek²

Sara Cristina Sagae Schneider¹

Affiliations:

¹ State University of Western Paraná.

² State University of Rio Grande do Sul.

Keywords: Alzheimer's. Oleoylethanolamine. Glycemic metabolismo.

Abstract:

Alzheimer's Disease (AD) is the leading cause of dementia, but its relationship with glycemic metabolism is poorly understood. To investigate this, AD was induced in 40 male C57BL/6 mice (100-112 days, 25-30g), according to ethics (protocol nº 20-21). The animals were divided into four groups (n=10/group): CTL (saline icv + saline ip), STZ (intracerebroventricular streptozotocin for AD induction + saline ip), STZ+OEA (STZ icv + OEA ip), and STZ+OEA NANO (STZ icv + nanoencapsulated OEA ip). STZ was administered icv (3µL, 3mg/kg). After 15 days, the animals received 7-day treatment with OEA (20mg/kg). To evaluate glycemic metabolism, the insulin tolerance test (ITT) was performed with administration of regular insulin ip (0.75 UI/kg) and glucose dosage at 3, 6, 9, 12, 15, and 18 minutes, generating glycemic curves. Data were analyzed by the KiTT index. In the ITT, the CTL group showed progressive reduction of glycemia (124.8±41.91 mg/dL). In contrast, the STZ+SAL group showed a less accentuated response (140.7±19.84 mg/dL) and early recovery, indicating insulin resistance. The STZ+OEA (120.0±23.96 mg/dL) and STZ+OEA NANO (123.7±38.62 mg/dL) groups had intermediate curves, closer to CTL. The analysis of KiTT confirmed these findings. The index was significantly reduced in the STZ+SAL group (2.001±2.368) compared to CTL (5.103±0.6385, p<0.05), confirming insulin resistance. Treatment with OEA, especially STZ+OEA NANO (4.835±1.341), improved KiTT, bringing its values closer to the control group levels. In conclusion, icv STZ administration leads to insulin resistance, and treatment with OEA, especially nanoencapsulated, is effective in attenuating this condition, indicating a potential therapy.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Effects of progressive resistance training on astrocytic and neuronal density in *Rattus norvegicus* Wistar induced to Alzheimer's disease

Authors:

Amanda Mendes Silva¹
Amanda Soares Coura¹
Liliam Midori Ide¹

Affiliations:

¹ Universidade Federal de São João del-Rei (UFSJ); Departamento de Ciências Naturais (DCNAT).

Keywords: Glial fibrillary acidic protein. Hippocampal formation. Memory. Pyramidal neurons. Streptozotocin.

Abstract:

Alzheimer's disease (AD) is characterized by neurodegeneration with beta-amyloid protein and senile plaques, associated with intense synaptic loss due to activation of microglia and astrocytes, and neuronal degeneration. The hippocampal formation is one of the main areas affected by the disease and plays a fundamental role in memory. Considering the controversies regarding the non-pharmacological preventive action of progressive resistance training (PRT), research was developed to evaluate the astrocytic and neuronal density in male *Rattus norvegicus* Wistar submitted to training, followed by the induction of transient AD by bilateral icv injection of streptozotocin. The PRT involved the use of force by climbing a ladder with progressively heavier weights attached to the animal's tail. Thirty-two animals were divided into four groups: trained and AD (TRAD, n=7), untrained and AD (UTAD, n=8), trained and subjected to sham surgery, sham (TRSH, n=8), and untrained and sham (UTSH, n=8). After euthanasia, the brain was removed and processed for evaluation of astrocytic immunoreactivity to glial fibrillary acidic protein (GFAP) and quantification of pyramidal neurons. The experimental protocols were approved by CEUA/UFSJ (No. 2581281123). The data were statistically analyzed with the Kruskal-Wallis and followed by Dunn's test, with a significance level of 0.05. The results demonstrate higher astrocytic density in TRSH (23.63 ± 4.72) as compared to UTSH (19.38 ± 6.48) and UTAD (20.88 ± 4.39) in CA3 and CA1 ($X^2=18.5$; $X^2=15.60$, respectively). In dentate gyrus ($X^2=20.83$) there was an increase in astrocytes in UTAD to TRAD (18.25 ± 2.82 ; 31.87 ± 7.90) and an increase in relation to UTSH (14.88 ± 4.64); in addition, the increasing in TRSH (29.13 ± 5.30) to UTSH. There was also a reduction in the density of pyramidal neurons in trained to untrained animals ($X^2=20.4$). In conclusion, PRT increases astrocytic density while reducing pyramidal cell density in the analyzed regions.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Physiological adaptations to long-term circuit resistance training: cardiovascular risk modulation in aging

Authors:

Marcelo Pereira de Lima¹
Renata Miyabara^{1,2}
Lujain Fouad Khalaf³
Shady Salah Bagady³
Saed Fawaz Raddawi³
José Antônio Silva Jr.⁴
Ovidiu Constantin Baltatu^{1,3}
Luciana Aparecida Campos¹

Affiliations:

¹ Center of Innovation, Technology, and Education (CITE), Anhembí Morumbi University, São José dos Campos, SP, Brazil.

² UNINOVAFAPI University Center – Afya Educacional, Teresina, PI, Brazil.

³ College of Medicine, Alfaisal University, Riyadh, Saudi Arabia.

⁴ Postgraduate Program in Medicine, UNINOVE, São Paulo, SP, Brazil.

Keywords: Aging. Cardiovascular risk factors. Circuit resistance training. Exercise physiology. Health promotion

Abstract:

Physical exercise plays a crucial role in chronic disease prevention and health promotion among older adults. This study evaluated the long-term effects of a multidisciplinary physical exercise program on cardiovascular health parameters in older adults over a six-year period. The intervention involved circuit resistance training incorporating aerobic, strength, balance, and flexibility exercises, supervised by a multidisciplinary team. Sixty participants (n=30 intervention, n=30 sedentary controls, mean age 70.2±5.4 years) completed the study. The Active Life program demonstrated significant improvements in cardiovascular risk factors, including systolic arterial pressure (difference: 10.5±2.4 mmHg, 1.5-6.0 years), triglycerides-HDL-C ratio (starting at 2.5 years), triglyceride levels (33.9±5.8 mg/dL), and fasting glucose (16.1±4.9 mg/dL) compared to the sedentary group. These findings support the effectiveness of sustained circuit resistance training in managing cardiovascular risk factors in older adults, with program success attributed to its multidisciplinary approach and social engagement components. Ethics Committee Approval: CAAE: 11818919.8.0000.5492

Financial Support: Anima Institute - AI (research professor fellowships to OCB and LAC).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Physical Activity Improves Mood States in Military Personnel During a Ship Expedition to Antarctica

Authors:

Marcelo Teixeira de Andrade¹
João Pedro Cruz Cerqueira¹
Gabriel Jesus de Santana¹
Wellington Douglas de Souza²
Dawit Albieiro Pinheiro Gonçalves²
Michele Macedo Moraes³
Rosa Maria Esteves Arantes⁴
Thiago Teixeira Mendes¹

Affiliations:

¹ Exercise Physiology and Health Laboratory, Universidade Federal da Bahia, Salvador, Brazil.

² Sports Training Center, School of Physical Education, Physiotherapy and Occupational. Therapy, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil;

³ Department of Applied Physiology and Kinesiology, University of Florida, Gainesville, Florida, United States.

⁴ Department of Pathology, Institute of Biological Sciences, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

Keywords: Confined environments. Expeditionary stress. Linear mixed models. Psychological well-being. Sleep quality.

Abstract:

Antarctic expeditions aboard a ship pose significant challenges for military personnel due to extreme conditions, isolation, and prolonged confinement (ICE), negatively impacting psychological wellbeing. We evaluated changes in mood state among military personnel during an Antarctic expedition. Twenty-two military personnel (19 men, three women) from the crew of the Polar Ship Almirante Maximiano (H41) participated in the study (EC UFMG: 19092819.8.0000.5149). They completed the Brunel Mood Scale and the Pittsburgh Sleep Quality Index (PSQI) at 0, 30, and 120 days. The Total Mood Disturbance Index (TMD) was calculated by summing the scores for tension, depression, anger, fatigue, and confusion, then subtracting vigor. The global PSQI was computed using its seven components. A linear mixed model was fitted with a random slope effect. Independent variables were age, sex, physical activity, expedition duration, and PSQI Global. The dependent variable was TMD. The best-fit model indicated that longer expeditions increased TMD ($\beta=0.22$; $p<0.01$), suggesting a progressive negative ICE impact. Poorer sleep correlated with higher TMD ($\beta=1.04$; $p<0.01$), highlighting its role in emotional regulation. Conversely, the interaction between expedition duration and physical activity ($\beta=-0.17$; $p<0.01$) showed that exercise mitigated ICE's adverse effects. Specifically, every additional 10 days of expedition led to a 1.7-point TMD reduction among physically active personnel, suggesting a protective effect. These findings highlight that prolonged confinement worsens mood, particularly in those with poorer sleep. However, physical activity was a protective factor, underscoring the need for strategies to support mental and physical well-being during long-term missions.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Impact of vegetarian diets on the muscle recovery process after exercise-induced muscle damage in trained men

Authors:

Andressa Roehrig Volpe-Fix¹
Beatriz Rugila Salvalággio¹
Bruno Moreira Silva¹
Ronaldo Vagner Thomatieli-Santos¹

Affiliations:

¹ Universidade Federal de São Paulo (UNIFESP).

Keywords: Exercise. Strength. Recovery. Vegetarianism.

Abstract:

Vegetarian diets are becoming popular among active individuals, and little is known about their impact on muscle recovery. This study aims to evaluate muscle strength and rating of perceived exertion (RPE) in omnivorous, lacto-ovo-vegetarian, and strict vegetarian subjects (Ethic Committee of UNIFESP/HSP –06217P/2021). 36 trained men, aged 20-45 years, were divided into omnivorous-OMN(n=12), lacto-ovo-vegetarian-LOV(n=13) and strict vegetarians-SV(n=11) and were submitted to a muscle damage protocol in an isokinetic dynamometer. A maximal voluntary contraction test was made before (B), immediately after (IA), and 24h (24hA) after exercise as well as RPE. Using repeated measures ANOVA test to perform statistical analysis, significant differences were observed over time regarding peak torque ($F=33.79$; $p<0.001$; $\eta^2=0.148$) (B) vs (IA) ($p<0.001$) and (B) vs (24hA) ($p<0.001$) and RPE, ($F=0.922$; $p=0.408$; $\eta^2=0.014$) (B) vs (IA) ($p<0.001$), and (B) vs (24hA) ($p<0.001$) without significant differences between groups in both. Although numeric differences between groups were observed in percentage of loss strength - SV($22.5\%\pm 25.7$), OMN($20.1\%\pm 19.6$), LOV($18.6\%\pm 20.4$) - percentage of strength recovery - OMN($3.87\%\pm 8.76$), SV($0.3\%\pm 11.9$), LOV(-0.146 ± 14.4) - and percentage of perceived recovery - SV($27.1\%\pm 23.2$), LOV($8.62\%\pm 37.8$), OMN($5.25\%\pm 23.2$) - this differences were not significant. Since no significant differences between groups were found in any of the parameters evaluated, we concluded that adopting a vegetarian pattern don't impact the muscle recovery process.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Time-restricted feeding combined with exercise training promotes metabolic improvements, reduces adiposity, and fat accumulation in the liver of obese mice

Authors:

Guilherme Correia ANTONIO¹
Beatriz de Souza PITOLI¹
Ana Paula Azevêdo MACÊDO¹
Guilherme Soderini ERLICH¹
Amanda Abreu AVELINO¹
Gustavo Almeida IASNISWSKI¹
Larissa Moreira DIAS¹
Guilherme Domingos BRISQUE¹
Alisson Luiz da ROCHA¹
Adelino Sanchez Ramos DA SILVA²
Dennys Esper CINTRA^{3,4}
Eduardo Rochete ROPELLE^{1,4}
José Rodrigo PAULI^{1,4}

Affiliations:

¹ Laboratory of Molecular Biology of Exercise, University of Campinas (UNICAMP), Limeira, Sao Paulo, Brazil.

² School of Physical Education and Sport of Ribeirao Preto (EEFERP/USP), University of Sao Paulo, Ribeirao Preto, Sao Paulo, Brazil; School of Medicine of Ribeirão Preto, University of Sao Paulo (USP), Ribeirao Preto, Sao Paulo, Brazil.

³ Laboratory of Nutritional Genomics, University of Campinas (UNICAMP), Limeira, Sao Paulo, Brazil.

⁴ Obesity and Comorbidities Research Center (OCRC), University of Campinas, Campinas, Sao Paulo, Brazil.

Keywords: High-fat diet. Obesity. Metabolism. Exercise. Intermittent fasting.

Abstract:

Metabolic dysfunction-associated steatotic liver disease (MASLD), a condition characterized by the accumulation of fat in the liver, is estimated to be the most common liver disease worldwide. Being that obesity is an important risk factor and contributor to the development of MASLD, and, therefore, weight reduction can improve this disease. Previous studies in preclinical models of diet-induced obesity and fatty liver disease have shown the independent benefits of exercise training and time-restricted feeding (TRF) in preventing weight gain and hepatic fat accumulation. Here, we are investigating the combined effect of TRF and physical exercise on obesity and MASLD in Swiss obese mice induced by the ingestion of a high-fat diet. Before starting the experiment, the study was approved by the Animal Use Ethics Committee (CEUA 6318-1/2023). The TRF consists of 12 hours of food access in the active phase, while the exercise training protocol was aerobic, resistance, or combined (aerobic + resistance), with four weekly sessions applied at the end of the mice's light cycle. Thus, TRF was evaluated separately and combined with these different exercise training protocols. Analyses related to body mass, physiological, histological, and molecular were performed during and at the end of the experiment. Statistical analysis was performed using GraphPad Prism 9.1, with two-way ANOVA and Tukey's post-test ($p < 0.05$). The results revealed that the high-fat diet induced obesity (Control 42 ± 3 ; Obese 55 ± 5 grams), metabolic disorders (i.e. fasting blood glucose, Control 130 ± 8 ; Obese 270 ± 12 mg/dL), and greater fat accumulation in the liver (i.e. hepatic triacylglycerols, Control 0.8 ± 0.2 ; Obese 5.1 ± 1.2 mg/g of liver tissue). On the other hand, interventions with TRF and TRF combined with different physical exercise protocols were able to alleviate the impacts of a high-fat diet. The results reveal that TRF



**XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO**

**8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP**

combined with physical exercise had more significant effects than TRF alone. Mice that underwent combined interventions (TRF+different types of exercise) showed reduced weight and body adiposity, improved insulin sensitivity and fasting blood glucose, and this was accompanied by less accumulation of liver fat (with significant reduction of lipogenic genes). This demonstrates synergism in the combined action and could be a new intervention strategy to combat obesity, metabolic, and liver diseases. As a conclusion, it was found that TRF alone and mainly combined with physical training have synergistic and complementary effects, mitigating the effects of feeding a high-fat diet on obesity and MASLD.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Exercício físico atenua alterações metabólicas e renais em camundongos com doença renal diabética experimental

Authors:

Camille Tozato¹
Amanda Kruguer¹
Larissa Marques¹
Ricardo Pinho¹

Affiliations:

¹ Laboratório de Bioquímica do Exercício em Saúde, Programa de Pós-Graduação em Ciências da Saúde, Pontifícia Universidade Católica do Paraná, Curitiba, Paraná, Brasil. ²Departamento de Educação Física, Universidade Positivo, Curitiba, Paraná, Brasil.

Keywords: Doença renal do diabetes, exercício físico, inflamação, metabolismo

Abstract:

A Doença Renal Diabética (DRD), resultante da hiperglicemia persistente no Diabetes Mellitus, leva a alterações renais como lesões glomerulares, inflamação, disfunções metabólicas e aumento do estresse oxidativo. Nesse contexto, os efeitos do exercício físico têm sido investigados mas ainda não estão completamente elucidados. Este estudo teve como objetivo avaliar os efeitos do exercício físico regular sobre indicadores clínicos, anatomopatológicos e inflamatórios em um modelo experimental de DRD. Após aprovação pela Comissão Ética para Uso de Animais da UFPR (nº 23075.020691/2024-75), 48 camundongos C57BL/6, com 60 dias de idade, foram distribuídos em grupos Sham e DRD, com e sem treinamento. Os animais foram alimentados com dieta padrão ou dieta hiperlipídica (DHL) por 16 semanas, sendo que, na oitava semana, receberam uma dose única de estreptozotocina (STZ, 45 mg/kg). A seguir, foram submetidos a oito semanas de treinamento físico (3 a 4 sessões/semana, 13 a 17 m/min). Durante o período experimental, o consumo calórico e glicemia foram mensurados além do teste de tolerância à glicose. Quarenta e oito horas após a última sessão de exercício, os animais foram eutanasiados e os rins coletados para posterior análises. Os resultados revelaram um aumento significativo da massa corporal no grupo DRD (48,35g±2,88) em comparação ao grupo Sham exercitado (27,50g±0,98). A ingestão calórica foi significativamente maior no grupo Sham na semana 1 (3,55g±2,13, p<0,0001) com redução gradual e sem variação significativa ao longo do estudo quando comparado aos outros grupos, porém um aumento significativo foi observado no grupo Sham+Ex na semana 25 (1,47g±0,11, p<0,05). O perfil metabólico mostrou glicemia elevada no grupo DRD (285mg/dL±93,33) submetido à dieta hiperlipídica e STZ, com hiperglicemia sustentada no grupo DRD+Ex aos 30 (545mg/dL±74) e 60 (496mg/dL±87,28) minutos. A análise histopatológica renal mostrou um aumento significativo da área glomerular no grupo DRD (5782,79µm²±492,94) comparado aos grupos controle (3818,76µm²±301,61) e exercitados (5119,00µm²±841,58). A imunoexpressão de NGAL e TNF-α nos túbulos renais do grupo DRD-Ex foi de baixa intensidade, enquanto a IL-1β apresentou imunomarcagem de moderada a alta intensidade em todos os grupos. Em conclusão, o exercício físico regular atenuou distúrbios metabólicos, inflamatórios e alterações renais, sugerindo efeito nefroprotetor e reforçando seu potencial como estratégia não farmacológica no manejo da DRD.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Effects of Short-Term Wet Static Apnea Training on Well-Being, Internal Load, Recovery, and Physical Performance of Para Swimmers

Authors:

Lucas Siqueira-Moraes^{1,2,3}
Carolina Batista Simões^{1,2,3}
Wellington Douglas de Souza^{1,2,3}
Gabriel Adler Gomes-Martins^{1,2,3}
Christopherson Dias Nascimento⁴
Gustavo Ramos Dallas Bernardina^{4,7}
Felipe Alencar de Carvalho⁵
Mauro Heleno Chagas^{4,7}
Luciano Sales Prado^{3,4}
Andressa Silva^{4,8}
Marco Túlio de Mello^{4,8}
Glauber da Silva⁶
Dawit Albieiro Pinheiro Gonçalves^{1,2,3}

Affiliations:

¹ Ergogenic and Systemic and Molecular Physiology Research Group (ErgoFis), School of Physical Education, Physiotherapy and Occupational Therapy, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

² Section of Sports Physiology (SFE), Sports Training Center (CTE), Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

³ Exercise Physiology Laboratory (LAFISE), School of Physical Education, Physiotherapy and Occupational Therapy, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

⁴ Sports Training Center (CTE), Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

⁵ School of Physical Education, Physiotherapy and Occupational Therapy, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

⁶ Department of Physiology and Biophysics, Institute of Biological Sciences, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

⁷ Biomechanics Laboratory (BIOLAB), School of Physical Education, Physiotherapy and Occupational Therapy, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

⁸ Center for Studies in Psychobiology and Exercise (CEPE), School of Physical Education, Physiotherapy and Occupational Therapy, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

Keywords: Internal training load. Para-swimming. Recovery. Sports performance. Wet static apnea training.

Abstract:

This study aimed to investigate the effects of short-term wet static apnea training (WSAT) on the well-being, internal load, recovery, and performance of para-swimmers. Five para swimmers from the Sports Training Center at the Universidade Federal de Minas Gerais participated in a 5-week study performing high external swimming training loads and maximal strength training in weeks 1, 2, 4, and 5, and recovery in week 3 with low-intensity and low-volume swimming. After each swimming session, five sessions of WSAT were performed in week 4. Pre-session measurements included resting heart rate variability (rHRV), well-being parameters, perceived recovery status (PRS), and countermovement jump (CMJ) height, while post-session measurements included session rating of perceived exertion (sRPE). Apnea in different conditions and 100-meter swimming time trial were recorded before and after WSAT. Approval Research Ethics Committee number: 6.311.124. WSAT increased weekly PRS (WPRS) at week 5 compared to week 2 ($p = 0.034$; $\eta^2 = 0.703$), suggesting enhanced recovery



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

perception. Specifically, athletes showed higher weekly RPE (WRPE) and session RPE (WsRPE) in weeks 4 and 5 compared to week 3 ($p = 0.017$, $\eta^2 = 0.805$; $p = 0.047$; $\eta^2 = 0.805$), indicating higher perceived exertion during weeks with high training loads. However, no significant changes were observed in rHRV, CMJ, and 100-meter swimming times. Our preliminary findings suggest that short-term WSAT can positively affect perceived recovery in para swimmers. WSAT may need longer and/or more intense apnea training protocols to induce significant adaptations in physiological markers or physical performance.

Funding: Pro-Reitoria de Pesquisa - Federal University of Minas Gerais, Conselho Nacional de Desenvolvimento Científico e Tecnológico (N.444769/2023- 4 and N. 405140/2021-5), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Secretaria Especial do Esporte do Ministério da Cidadania (Governo Federal, Brazil - N.58000.008978/2018-37 and N. 71000.056251/2020-49), Comitê Paralímpico Brasileiro, Centro de Treinamento Esportivo e Fundação de Apoio ao Ensino, Pesquisa e Extensão.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Effects of physical training and/or a carbohydrate-restricted and high-protein diet on muscle morphometric parameters and irisin/FNDC5 expression of obese rats

Authors:

Samuel Santos Bittencourt PEREIRA¹
Júlia de Oliveira BORGES¹
Luciano Evangelista dos Santos FILHO¹
Grazielle Prates Lourenço dos Santos BITTENCOURT¹
Thiago Macêdo Lopes CORREIA¹
Amanda Alves de ALMEIDA¹
Maiara Raulina de Jesus DIAS¹
Regiane Ribeiro DIAS¹
Douglas Santos BATISTA¹
Karine Chaves SILVA¹
Rafael PEREIRA²
Amélia Cristina Mendes de MAGALHÃES¹

Affiliations:

¹ Universidade Federal da Bahia.

² Universidade Estadual do Sudoeste da Bahia.

Keywords: Irisin; High-protein diet; Muscle; Obesity; Exercise training.

Abstract:

Obesity induces metabolic and muscular alterations that contribute to comorbidities and adverse health outcomes. Exercise training (ET) and dietary interventions are key strategies for the treatment of obesity. Our study is based on the existing gap in the isolated or combined effects of using a carbohydrate-restricted high-protein diet and/or ET in an animal model of diet-induced obesity on muscle morphometric parameters and irisin/FNDC5 expression. Twenty-eight male Wistar rats with obesity induced by hypercaloric diet (HCD) were divided into 4 groups (n=7): Hypercaloric diet and sedentary (HCS), Hypercaloric diet + TR (HCT), Carbohydrate-restricted and hyperprotein diet and sedentary (HPS) and Carbohydrate-restricted and hyperprotein diet (DHP) + TR (HPT). After 12 weeks of the protocol, serum was collected, and the gastrocnemius deposit was collected. Serum irisin was measured by ELISA. Histological slides were used for morphometric analysis and FNDC5 protein expression using immunohistochemistry. The data was analyzed by Two Way ANOVA followed by the Bonferroni post-test ($p < 0.05$). Approval by the CEUA of IMS/CAT-sinUFBA (No. 053/2017). TR significantly increased serum irisin (HCS: 5.8 ± 1.1 ; HCT: 10.6 ± 3.2 ; HPS: 5.2 ± 1.6 ; HPT: 14.8 ± 6.7 , $p = 0.0006$). FNDC5 immunolabeling was modulated by both TR and diet (HCS: 6.3 ± 0.9 ; HCT: 31.9 ± 1.3 ; HPS: 8.4 ± 0.8 ; HPT: 49.8 ± 2.4 , p_{Diet} and $p_{\text{TR}} < 0.001$). The same was observed for muscle weight (HCS: 0.4 ± 0.05 ; HCT: 0.5 ± 0.04 ; HPS: 0.5 ± 0.04 ; HPT: 0.6 ± 0.04 , $p_{\text{Diet}} = 0.0033$ and $p_{\text{TR}} = 0.0274$). Both interventions increased fiber cross-sectional area (HCS: 2692 ± 264 ; HCT: 3247 ± 334 ; HPS: 3661 ± 309 ; HPT: 3764 ± 213 , $p_{\text{Diet}} < 0.001$ and $p_{\text{TR}} = 0.0053$). However, only the diet increased the number of myonuclei (HCS: 2.2 ± 0.1 ; HCT: 2.8 ± 0.2 ; HPS: 3.2 ± 0.5 ; HPT: 3.0 ± 0.2 , $p_{\text{Diet}} < 0.0001$). Our results showed that the high-protein diet and/or TR effectively promoted beneficial effects on gastrocnemius morphofunctional parameters and irisin/FNDC5 expression.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Effects of high-intensity interval training versus continuous aerobic training on nitro-oxidative stress markers in female rats with cisplatin-induced hepatotoxicity

Authors:

Bruna Santos SILVA¹
Fernanda Santos Portela SAMPAIO^{1,2}
Lara Fabiana Luz MALHEIRO^{1,3}
Caroline Assunção OLIVEIRA^{1,3}
Érika Azenathe Barros MERCÊS^{1,2}
Lais Mafra de BENEDICTIS^{1,3}
Ana Jullie Veiga FERNANDES¹
Júlia Mafra de BENEDICTIS¹
Júlia Spínola ÁVILA¹
Patrícia da Silva OLIVEIRA
Amélia Cristina Mendes de MAGALHÃES
Telma de Jesus SOARES
Fabrício Freire de MELO
Liliany Sousa de Brito AMARAL^{1,2,3}

Affiliations:

¹ Universidade Federal da Bahia.
² Programa de Pós-Graduação em Biociências.
³ Programa de Pós-Graduação em Ciências Fisiológicas.

Keywords: Cisplatin. Liver. Oxidative stress. Exercise Training.

Abstract:

Cisplatin (CP) is a widely used chemotherapeutic agent, but its application is limited by cytotoxic effects, including hepatotoxicity. This study aimed to compare the effects of high-intensity interval training (HIIT), light-intensity (LIT) and moderate-intensity (MIT) continuous training on oxidative and nitrosative stress markers in female rats with CP-induced hepatotoxicity. Thirty-five Wistar rats were divided into five groups (n=7): sedentary control (C+S); CP and sedentary (CP+S); CP and LIT (CP+LIT); CP and MIT (CP+MIT); and CP and HIIT (CP+HIIT). After 8 weeks of training, animals received CP (5 mg/kg, i.p.) and were euthanized 7 days later. Liver samples were analyzed by ELISA and immunohistochemistry. The study was approved by the CEUA of IMS/CAT-UFBA (No. 056.2018). The CP+S group showed significantly increased expression of 4-HNE (271.3±47.28), a marker of lipid peroxidation, and 8-OHdG (684.3±52.27), a marker of oxidative DNA damage, compared to the C+S group (205.9±36.23 and 88.5±23.0, respectively) (p<0.05). Although CP treatment increased hepatic antioxidant enzyme levels compared to C+S—glutathione peroxidase (116.9±19.8 vs. 67.0±10.1), superoxide dismutase (9.83±0.94 vs. 6.41±1.07), and catalase (289.0±47.2 vs. 120.3±14.6)—these levels were significantly higher in the CP+HIIT group (204.1±20.5; 16.64±0.91; and 437.3±47.3, respectively) (p<0.05). Likewise, HIIT led to higher expression of Nrf2 (701.6±91.8) compared to the CP+S group (378.6±47.1) (p<0.05). In terms of nitrosative stress, CP significantly increased nitrotyrosine immunostaining compared to the C+S group (37.95±16.26 vs. 4.0±1.79) (p<0.05). However, HIIT substantially reduced nitrotyrosine levels (9.24±7.10) compared to CP+LIT (24.03±15.53) and CP+MIT (13.18±4.05) (p<0.05). These findings suggest that exercise training, particularly through the HIIT protocol, offers cytoprotective effects against CP-induced hepatotoxicity by enhancing antioxidant defense mechanisms and reducing oxidative and nitrosative stress.

Financial support: Fundação de Apoio à Pesquisa e Extensão (FAPEX-UFBA).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Effects of Aerobic Exercise on Immune Cell Profile of Perivascular Adipose Tissue and Vascular Function in Obese Female Mice

Authors:

Teresa Maria da Ré¹ Guilherme Alves dos Santos¹ Luiza Helena Rossi da Silva¹ Lilian de Oliveira Coser² Silvio Roberto Consonni³ Maria Andréia Delbin¹

Affiliations:

¹ Laboratório de Biologia Vascular, Departamento de Biologia Estrutural e Funcional, Instituto de Biologia, Universidade Estadual de Campinas (UNICAMP) – Campinas, SP, Brazil.

² Laboratório de Regeneração Nervosa, Departamento de Biologia Estrutural e Funcional, Instituto de Biologia, Universidade Estadual de Campinas (UNICAMP) – Campinas, SP, Brazil.

³ Laboratório de Citoquímica e Imunocitoquímica, Departamento de Bioquímica e Biologia Tecidual, Instituto de Biologia, Universidade Estadual de Campinas (UNICAMP) – Campinas, SP, Brazil.

Keywords: Aerobic exercise training. Perivascular adipose tissue. Females. Obesity. Vascular reactivity.

Abstract:

Obesity alters the secretory profile of perivascular adipose tissue (PVAT), impairing vascular function. However, the mechanisms of PVAT dysfunction in obesity are not fully understood. Aerobic exercise may provide an important intervention by directly interfering with the phenotype and secretory pattern of PVAT. This study evaluated the effects of aerobic exercise on vascular function, senescence, immune cell profile, and phenotype of thoracic aorta PVAT (tPVAT) in obese female mice (C57BL6/Junib). Animals were divided into sedentary (SD), obese sedentary (OB/SD), and obese trained (OB/TR) groups (CEUA: 5849-1/2021). Obesity was induced by a 16-week high-fat diet, and moderate aerobic exercise training started in the 9th week and continued until the 16th week on a treadmill. Vascular function was assessed in the thoracic aorta with (PVAT+) and without (PVAT-) PVAT to acetylcholine (ACh), thromboxane A₂ (U46619), and serotonin (5-HT). Flow cytometry evaluated immune cells, and β -galactosidase activity quantified vascular senescent. Potency (pEC_{50}) and maximum response (E_{MAX}) to ACh were reduced in PVAT+ ($55 \pm 5\%$; -6.19 ± 0.2) compared to PVAT- ($69 \pm 4\%$; -6.89 ± 0.2) rings in OB/SD group. The E_{MAX} was restored in OB/TR PVAT+ ($72 \pm 2\%$) compared to PVAT- ($73 \pm 2\%$) rings. No difference was observed in responses to U46619. E_{MAX} and pEC_{50} to 5-HT were reduced in PVAT+ ($6 \pm 1mN$; -5.67 ± 0.3) compared to PVAT- ($8 \pm 1mN$; -6.47 ± 0.1) rings in SD group. This anticontractile effect was absent in OB/SD but partially restored in OB/TR. Flow cytometry revealed an increase in T lymphocytes, especially NK cells, in OB-SD ($28.6 \pm 1.4\%$) and a reduction in OB/TR ($0.2 \pm 0.1\%$). Vascular β -galactosidase activity was increased in OB/SD associated with a profound phenotypic change of tPVAT. Exercise increased adiponectin and partially mitigated phenotypic change of tPVAT. These data provide insight into the role of exercise as a therapeutic approach in obesity and the influence of PVAT on vascular health in female.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Interactive effect of a plant-based omega-3 fatty acid intake and exercise training on hippocampal GPR120 of obese mice

Authors:

Leandro Kansuke Oharomari¹
Camila Ayes Cunha Venturini¹
Leticia Maria Paula Silva¹
Julia de Lima Boro¹
José Rodrigo Pauli¹
Eduardo Rochete Ropelle¹
Adelino Sanchez Ramos da Silva²
Dennys Esper Cintra¹

Affiliations:

¹ State University of Campinas

² University of São Paulo

Keywords: Ala. Exercise. Ffar4. Obesity. Cognition.

Abstract:

GPR120 is recognized as a lipid sensor and can mediate the potential health benefits of the polyunsaturated fatty acid omega-3. Omega-3 and exercise training have been tested to mitigate cognitive decline since both can display neurotrophic effects. On the other hand, obesity is associated with a higher risk of developing neurodegenerative diseases. However, how the intake of a plant-based omega-3 source combined with exercise can influence hippocampal GPR120 in obesity is unknown. Thus, four-week-old male Swiss mice were initially divided into control (standard chow, N=18) and high-fat diet (31% lard, N=72, HF). After eight weeks (the obesity-induced period), animals in the HF group were subdivided into four groups (N=18): high-fat sedentary, HF + chronic moderate continuous exercise, HF + flaxseed oil (1/3 lard substitution, FS), and HF + exercise and FS. After four weeks of interventions, all animals were submitted for several behaviors and glucose-metabolism-related tests. The HF diet successfully induced an obese phenotype followed by increased anxiety behavior. Exercise alone partially blunted body weight gain caused by the HF diet and exhibits better glucose metabolism only when combined with FS intake. GPR120 was abundantly found throughout all main hippocampal areas (Dentate Gyrus, *Cornus Ammonis* 1-2-3). Interestingly, obesity increased total hippocampal GPR120 content and, more specifically, in the DG. However, neither intervention was able to alter the hippocampus's function, inflammatory markers, GPR120 protein content, and DG volume. Therefore, in obese mice, the interactive effect of four weeks of FS intake and exercise was restricted to the peripheral system. In conclusion, understanding hippocampal GPR120's roles in obesity may elucidate new physiopathology pathways of obesity-induced cognitive decline and open the door to new therapeutic targets focusing on lipid sensors. This study was approved by the local ethics committee (CEUA-6230-1).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Association of decorin levels and cardiometabolic benefits in the context of post-cardiopulmonary exercise test in endurance athletes

Authors:

Antônio Alves de Fontes-Junior¹
Ana Paula Renno Sierra²
Cesar Augustus Zocoler de Sousa¹
Maria Fernanda Cury-Boaventura¹

Affiliations:

¹ Interdisciplinary Post-graduate Program in Health Sciences, Institute of Physical Activity and Sports Sciences, Cruzeiro do Sul University.

² School of Physical Education and Sport, University of São Paulo.

Keywords: Cardiometabolic Adaptations. Cardiopulmonary Test. Decorin. Endurance Exercise. Myokines.

Abstract:

This study investigated the relationship between circulating decorin levels and cardiometabolic parameters in amateur marathon runners after a cardiopulmonary exercise test (CPET). Decorin, a myokine derived from proteoglycans, has been suggested as a mediator of the cardiometabolic effects induced by endurance exercises, such as marathons. Fifty-one male marathon runners (± 41.5 years) participated, who performed the CPET between one and three weeks before the São Paulo International Marathon. The protocol consisted of a progressive treadmill test with a 1% incline, starting at 8 km/h and increasing by 1 km/h every minute until exhaustion. During the test, participants were monitored with a 12-lead electrocardiogram, and gas exchange was measured by indirect calorimetry. VO₂ max was determined according to the American College of Sports Medicine protocol. After the race, blood samples were collected to measure decorin levels by enzyme-linked immunosorbent assay (ELISA). The Ethics Committee of Cruzeiro do Sul University, Brazil (License No. 3.895.058) approved the study. The results showed negative correlations between decorin levels and parameters such as VCO₂ ($r = -0.3894$, $p = 0.0047$), respiratory quotient (RQ) ($r = -0.3254$, $p = 0.0198$), PETCO₂ ($r = -0.4201$, $p = 0.0021$), carbohydrate oxidation ($r = -0.317$, $p = 0.0234$), and the percentage of carbohydrate oxidized ($r = -0.3193$, $p = 0.0224$) at the anaerobic threshold. Negative correlations were also observed at critical resting and peak levels ($r = -0.478$, $p = 0.0004$; $r = -0.3953$, $p = 0.0041$), and positive correlations with VE/VCO₂ at different points of the test (AT: $r = 0.5258$, $p < 0.0001$; CR: $r = 0.503$, $p = 0.0002$). These findings suggest that decorin plays an important role in post-exercise cardiometabolic adaptations, influencing ventilatory efficiency and energy metabolism. The results contribute to the understanding of molecular mechanisms in endurance exercise and their implications for cardiometabolic health.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Skeletal Muscle Adaptations to Fluoroquinolone Treatment and Voluntary Running in Obesity Prevention

Authors:

João Pedro Mazzi¹
Niedson Correia de Lima Junior¹
Gerson Profeta de Souza^{1,2}
Linnéa Bergenholm³
Gavin O'Mahony³
Jeremie Boucher³
Letícia de Souza Figueiredo¹
Gabriel Palermo Ruiz^{1,2}
Marcelo Alves da Silva Mori¹

Affiliations:

¹ Department of Biochemistry and Tissue Biology, University of Campinas, Campinas, Brazil.

² Department Cellular and Molecular Physiology, Joslin Diabetes Center, Harvard Medical School, Boston, MA, USA.

³ Medicinal Chemistry, Research and Early Development, Cardiovascular, Renal and Metabolism (CVRM), BioPharmaceuticals R&D, AstraZeneca, Gothenburg, Sweden.

Keywords: Fluoroquinolone. Metabolism. Obesity. Running wheel. Skeletal muscle.

Abstract:

Introduction: Obesity is a major global health challenge, closely linked to metabolic disorders such as type 2 diabetes. While weight loss strategies often lead to muscle mass reduction, recent findings from our group indicate that some fluoroquinolone compounds can attenuate weight gain and improve glycemic homeostasis while preserving muscle mass. Considering the crucial role of striated skeletal muscle in metabolism, we aimed to investigate the combined effects of fluoroquinolone treatment and voluntary running exercise on muscle adaptations in an obese mouse model. **Methods:** To comprehend the relationship between fluoroquinolones and skeletal muscle, we utilized male C57BL/6J mice (CEUA: 6579-1/2024) fed on a high-fat diet (HFD) for 10 weeks to induce obesity. Afterwards, they received fluoroquinolone treatment for 10 weeks, remaining on HFD, with or without running wheel access. Muscle function was assessed by grip strength and fatigue resistance (treadmill exhaustion test). Muscle adaptations were analyzed through histology, gene expression of fiber-type markers, and structural remodeling. **Results:** Fluoroquinolone treatment attenuated weight gain, increased grip strength, and improved treadmill fatigue resistance. Mice with voluntary wheel access exhibited enhanced performance in running parameters when treated with fluoroquinolone. Groups treated with fluoroquinolone showed increased expression of genes associated with muscle development, function, and structural remodeling. These results suggest that our compounds can lead to adaptations that contribute to improved metabolic efficiency. **Conclusion:** These findings suggest that fluoroquinolone compounds can modulate skeletal muscle adaptations that could contribute to obesity prevention and metabolic health. Further investigations will explore the molecular mechanisms underlying these changes.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Effects of aerobic physical training over Achilles tendon on obese female mice

Authors:

Sara Luiza Custódio de Souza¹
Camila Alves Lage Santos¹
Camila Beatriz Risos da Vitória²
Teresa Maria da Ré²
Maria Andréia Delbin²
Petrus Pires Marques³
Flávia da Ré Guerra¹

Affiliations:

¹ Universidade Federal de Alfenas.

² Universidade Estadual de Campinas. ³ Universidade José do Rosário Vellano.

Keywords: Inflammation. Obesity. Physical activity. Tendinopathy.

Abstract:

Obesity is an excess of body fat that causes damage to health. Current studies suggest a close link between obesity and the onset of chronic tendinopathy. Physical exercise has been shown to promote positive adaptations to obesity and tendinopathy. The study used female C57BL6/JUnib mice 4-5 weeks old (CEUA/UNICAMP: 5849-1/2021), divided into 4 experimental groups: sedentary control (SD), trained control (TR), sedentary obese (OB/SD), and trained obese (OB/TR). Obesity was induced by a high-fat diet for 16 weeks (6.2 kcal/gram: 32% carbohydrates, 20.3% proteins and 38% lipids). After 8 weeks of standard or high-fat diet, the TR and OB/TR groups began aerobic exercise training on a treadmill at moderate intensity (50-60% of maximum capacity), 5 days/week, 1 hour/day, for 8 weeks. The animals were euthanized and tendon samples were removed for subsequent morphological (Hematoxylin-eosin, Toluidine Blue and Pricosirius staining) and biomechanical analyses. The biomechanical tests were carried out on a Tetstar II model machine using an increase in load, at a constant displacement rate of 20 mm/min, using a 1KN load cell until the tendon was ruptured. The SD group showed lower cellularity and disorganization of the tenocytes. The TR and OB/TR groups showed an increase in glycosaminoglycans in the tension region. The obese groups had more disorganized and less compact collagen. There was impairment of the tissue's biomechanical properties: the OB/TR group exhibited an 18.99% increase in maximum strength compared to the OB/SD group. The tendon cross-sectional area in the OB/SD group was 86.03% smaller than in the TR group. Thus, aerobic exercise promoted significant structural and functional improvements in the tendon, demonstrating its effectiveness in restoring the tissue's properties. Physical exercise improved tendon composition, indicating that physical activity can help restore its morphological and biomechanical properties.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Effect of inorganic nitrate on microvascular function, angiogenesis and muscle endurance in healthy adults

Authors:

Carlos Alberto de Souza Filho¹
Thiago da Silveira Alvares¹

Affiliations:

¹ Multidisciplinary Center UFRJ-Macaé, Federal University of Rio de Janeiro (UFRJ-Macaé).

Keywords: Cardiovascular system. Endothelial dysfunction. Physical exercise.

Abstract:

The function of the vascular endothelium is regulated by the balance in the synthesis of vasodilator and vasoconstrictor compounds. An imbalance in this production leads to endothelial dysfunction, characterized by a reduction in the capacity of the endothelium to perform its functions, with some of the causal factors being decreased production and lower bioavailability of nitric oxide. In addition, low arginine concentration, reduced expression of the endothelial nitric oxide synthase enzyme, and the occurrence of oxidative stress are other factors that favor the occurrence of endothelial dysfunction. Due to this dysfunction, the skeletal muscles receive less blood supply, compromising the delivery of oxygen and other nutrients. This fact leads to a reduced capacity to perform daily activities. The present study aims to investigate whether the acute ingestion of beetroot juice can increase plasma concentrations of nitrate and nitrite, induce angiogenesis, improve microvascular function, and consequently favor muscular resistance. This study will be a randomized crossover study, and will involve individuals aged between 18 and 45 years of both sexes (Ethics committee approval - CAAE: 75851523.0.0000.5699). The pre-intervention consists of baseline analyses such as blood pressure, a 5-minute vascular occlusion test, and a forearm fatigue resistance test. During the intervention, the volunteers will ingest 140 mL of beetroot juice or 140 mL of placebo, and will subsequently undergo vascular analyses and muscle resistance analyses. It is expected that the ingestion of beetroot juice may promote both an increase in plasma nitrate and nitrite concentrations and an increase in angiogenesis, which as a consequence will promote an improvement in microvascular function, and this in turn will favor an increase in resistance to muscle fatigue, enabling a better ability to perform activities of daily living, in addition to a better ability to perform physical exercises.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Muscle tissue adaptations after eccentric exercise injury: Morphological, biochemical and functional aspects

Authors:

Mariana Baptista¹
Isabella Gomes¹
Matheus Bertanha Fior¹
Jurandyr Pimentel Neto¹
Lara Caetano Rocha-Braga¹
Adriano Polican Ciena¹

Affiliations:

¹ Laboratory of Morphology and Physical Activity (LAMAF), Institute of Biosciences, São Paulo State University (UNESP), Rio Claro, São Paulo, Brazil.

Keywords: Eccentric contraction. Muscle injury. Protein extraction. Skeletal striated muscle. Triceps brachii.

Abstract:

Muscle injuries compromise muscle function, which causes damage to cellular structures and triggers the local inflammatory process. Eccentric exercise can be used as a model for the study of muscle injuries since it causes excessive stretching of muscle fibers, which results in the activation of inflammatory proteins. The aim of the present study was to analyze the adaptations in the morphological, biochemical, and functional components of the triceps brachii muscle after an eccentric exercise injury model. Fifty adult male *Wistar* rats were equally distributed into groups: Control (C) (n=10) and Injury (L) (n=40), subjected to the injury protocol (subgroups PI0H, PI24H, PI4D, and PI7D). The protocol was performed on a treadmill and consisted in 18 sets/5 min at a speed of 16 m/min and an inclination of -16° (CEUA - nº 2223 - 25/2023). For functional analysis, joint edema and grip strength tests were assessed. Cellular components were analyzed using light microscopy (Hematoxylin-Eosin and Picrosirius staining). Protein extraction and quantification of IL-6, IL-10, and TNF- α (ELISA) were performed for protein analysis. The results obtained were subjected to one-way ANOVA statistical analysis in the GraphPad Prism software ($p < 0.05$). Group C presented homogeneous results, with a smaller circumference of the humeroradial joint, a smaller relative area of myonuclei and associated connective tissue, and a lower concentration of IL-6. In comparison, PI0H demonstrated a larger circumference and diameter of the joint ($p = 0.045$). Group PI24H obtained a higher amount of IL-6 ($p = 0.0062$). PI4D also presented a significant increase in IL-6 ($p = 0.0263$), while PI7D demonstrated a larger area ($p = 0.0001$) and fractal dimension ($p = 0.0002$) of the associated connective tissue, in addition to an increase in circumference about edema ($p = 0.0009$). We conclude that the eccentric contraction protocol promotes changes in the associated connective tissue and IL-6, increased joint edema, and decreased strength.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Effects of curcumin supplementation associated with strength training on muscle thickness, maximal strength and training biomarkers: a randomized, double-blind clinical trial

Authors:

Luiz Eduardo Dias Diniz¹
Carolina Kei Furusato de Souza¹
Gabriel Alan Luvizon Coelho de Souza¹
Leonardo Moreira dos Santos²
Giuseppe Potrick Stefani^{1,3}

Affiliations:

¹ Olympic Studies Research Group (GPEO). School of Health and Life Sciences. Pontifical Catholic University of Rio Grande do Sul (PUCRS). Porto Alegre, RS, Brazil.

² Polytechnic School. Pontifical Catholic University of Rio Grande do Sul (PUCRS). Porto Alegre, RS, Brazil.

³ Postgraduate Program in Nutrition Sciences (PPGNUT). Federal University of Health Sciences of Porto Alegre (UFCSPA). Porto Alegre, RS, Brazil.

Keywords: Curcumin. Hypertrophy. Resistance training. Supplementation.

Abstract:

Human studies on the effect of curcumin extract associated with strength training have not yet been fully investigated. This double-blind, randomized clinical trial aimed to evaluate the effects chronic curcumin supplementation on muscle thickness, maximal strength, and training-related biomarkers in healthy individuals. Eighteen volunteers were randomly assigned to the curcumin group (500 mg with 5.0 mg of piperine per day) or the placebo group (500 mg of cornstarch per day). Both groups followed the same strength training protocol, performed at least three times a week for 12 weeks, from 85 to 95% of maximum strength. Skeletal muscle assessment by ultrasound and maximal strength testing (one repetition maximum) were performed at baseline and the end of the study. Food consumption was assessed at weeks 1, 6, and 12 using energy availability. The Research Ethics Committee approved the project under number 6.755.757. Biceps muscle thickness increased in the curcumin group compared to baseline (placebo: -1.61 ± 2.12 mm; curcumin: $+2.58 \pm 2.87$ mm; $P=0.016$, effect size: 1.66 – huge effect), as well as increased vastus lateralis muscle thickness (placebo: -1.56 ± 4.54 mm; curcumin: $+4.23 \pm 4.28$ mm; $P=0.041$, effect size: 1.32 – huge effect). There was an increase in maximum strength in the bench press (placebo: $+4.18 \pm 2.67$ kg; curcumin: $+9.75 \pm 6.36$ kg; $P=0.038$, effect size: 1.14 – huge effect) and barbell curl (placebo: $+1.03 \pm 1.69$ kg; curcumin: $+5.12 \pm 3.22$ kg; $P=0.006$, effect size: 1.59 – huge effect) compared to the beginning of the study. Testosterone-to-cortisol ratio did not alter comparing the two groups (placebo: $+1.51 \pm 5.72$ ng.dL⁻¹/μg.dL⁻¹; curcumin: $+4.08 \pm 8.30$ ng.dL⁻¹/μg.dL⁻¹; $P=0.497$). The results indicate that curcumin supplementation can enhance muscular adaptations to strength training, increasing muscle thickness and maximum strength. These findings are not related to anabolic biomarkers, further studies are needed to better understand the underlying mechanisms.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Effects of curcumin supplementation associated with strength training on inflammatory profile, biochemical biomarkers and skeletal muscle mass in strength training practitioners: A randomized, double-blind clinical trial

Authors:

Luiz Eduardo Dias Diniz¹
Carolina Kei Furusato de Souza¹
Gabriel Alan Luvizon Coelho de Souza¹
Leonardo Moreira dos Santos²
Giuseppe Potrick Stefani^{1,3}

Affiliations:

¹ Olympic Studies Research Group (GPEO). School of Health and Life Sciences. Pontifical Catholic University of Rio Grande do Sul (PUCRS). Porto Alegre, RS, Brazil.

² Polytechnic School. Pontifical Catholic University of Rio Grande do Sul (PUCRS). Porto Alegre, RS, Brazil.

³ Postgraduate Program in Nutrition Sciences (PPGNUT). Federal University of Health Sciences of Porto Alegre (UFCSA). Porto Alegre, RS, Brazil.

Keywords: Cortisol. Curcumin. Interleukin-6. Interleukin-10. Resistance training. Supplementation.

Abstract:

Strength training is widely recognized for its benefits on overall health, including anti-inflammatory effects and improved quality of life. Additionally, dietary supplements, such as curcumin, have attracted attention for their antioxidant and anti-inflammatory properties, with evidence suggesting potential effects in shaping muscle recovery and exercise-induced inflammation. This study aims to explore the effects of curcumin supplementation on inflammation, body composition, and biomarkers in strength training participants. Eighteen strength-trained volunteers were randomly assigned to the Curcumin group (500mg + 5mg piperine per day) or Placebo group (500 mg cornstarch per day). Both groups followed the same strength training protocol, performed at least 3 times a week for 12 weeks. Body composition assessments and blood samples were collected at baseline and the end of the study. Total skeletal muscle mass increased in the Curcumin group compared to the Placebo group (placebo: $+0.16 \pm 0.44$ kg; curcumin: $+0.97 \pm 0.79$ kg; $P=0.033$, effect size: 1.26 – huge effect). Regarding biochemical parameters, cortisol levels decreased in the Curcumin group compared to the placebo group (placebo: $+6.94 \pm 8.50$ $\mu\text{g/dL}$; curcumin: -1.07 ± 4.67 $\mu\text{g/dL}$; $P=0.034$, effect size: 1.16 – huge effect). Interleukin-6 (IL-6) decreased in the Curcumin group compared to the Placebo group post-experiment (placebo: $+4.05 \pm 4.35$ pg/mL ; curcumin: -0.66 ± 1.96 pg/mL ; $P=0.015$, effect size: 1.39 – huge effect), interleukin-10 (IL-10) increased (placebo: -6.95 ± 4.87 pg/mL ; curcumin: $+5.15 \pm 2.15$ pg/mL ; $P=0.001$, effect size: 3.21 – huge effect), as well as IL-6/IL-10 ratio improved in Curcumin group (placebo: -0.77 ± 0.70 $\text{pg.mL}^{-1}/\text{pg.mL}^{-1}$; curcumin: $+0.23 \pm 0.21$ $\text{pg.mL}^{-1}/\text{pg.mL}^{-1}$; $P=0.001$, effect size: 1.93 – huge effect). Our results indicate that curcumin supplementation can enhance skeletal muscle hypertrophy, as well as promote positive metabolic changes (anti-inflammatory environment) in response to strength training.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Effects of Physical Training and Cocoa Flavanols on Memory and Neurotrophic Factors in a D-Galactose-Induced Aging Model

Authors:

Daniel Massote de Melo Leite¹
Bruno Pereira Melo^{1,2}
Júlia Sousa Ramos¹
Camila Berbert Gomes¹
César Augusto da Silva Caetano³
João Gabriel da Silveira Rodrigues¹
Danusa Dias Soares¹

Affiliations:

¹ Exercise Physiology Laboratory, School of Physical Education, Physiotherapy and Occupational Therapy, Universidade Federal de Minas Gerais, UFMG, Belo Horizonte, Brazil.

² University Center, UNITOP - PALMAS/Tocantins, Brazil.

³ School of Pharmacy – Universidade Federal de Minas Gerais, UFMG, Belo Horizonte, Brasil.

Keywords: Ageing. BDNF. Cocoa. D-Galactose. Combined Training.

Abstract:

Aging is associated with progressive and irreversible declines in cognitive and physical functions, yet its underlying mechanisms remain incompletely understood. Animal models, such as D-galactose-induced aging in rodents, offer valuable insights into the cellular and molecular processes driving these impairments. Recent studies suggest that interventions like physical training and polyphenol-rich diets (e.g., cocoa flavonols) may attenuate age-related deficits. However, the combined effects of these interventions on memory and neurotrophic factors, have not been fully explored. In this study (approved by UFMG's Ethics Committee (161/2020). Wistar rats were administered daily injections of D-galactose (150 mg/kg) for eight weeks to induce an aging phenotype. The animals were then randomized into four groups (n=8–9 per group): Sedentary with placebo (REST-PLA), sedentary with cocoa flavonol supplementation (REST-COCOA), trained with placebo (CT-PLA), and trained with cocoa (CT-COCOA). The training protocol consisted of ladder climbing and treadmill running at 60% Vpeak, with cocoa flavonol supplementation provided via gavage at a dose of 45 mg/kg/day, 50 minutes prior to exercise or rest. Following the intervention, cognitive function was assessed using the Novel Object Recognition test. Two-way ANOVA compared the effects of supplementation and activity, followed by post-hoc Student's t-test ($p \leq 0.05$). The REST COCOA group restored the cognitive deficit induced by D-gal in the STM discrimination index (0.70 ± 0.04) vs. REST PLA (0.45 ± 0.07) (Interaction $p < 0.05$; Suppl $p < 0.05$). Subsequently, the animals were euthanized, and tissue samples from the hippocampus and frontal cortex were collected for analysis of the neurotrophic factor BDNF. The BDNF levels in the hippocampus were increased in the CT (PLA) group (130.1 ± 8.6) vs. REST (PLA) (105.3 ± 7.5) (Interaction $p \leq 0.04$). In conclusion, cocoa flavonol supplementation effectively improved memory and CT promoted modulation of the BDNF in the hippocampus, in this ageing model.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: High-fat diet induces higher adipose levels, lower muscle mass and strength and glucose resistance in female mice

Authors:

Lucas Antônio da Silva Dias^{1,3}
Gustavo de Oliveira Zanetti^{1,3}
Gabriel Valasco de Oliveira Santos^{1,2,3}
Felipe Vergetti Melo Teixeira¹
Pedro William Martins Pessôa^{1,3}
Larissa Nardy Gomes³
Carolina Batista Simões^{1,2,3}
Gabriel Adler Gomes-Martins^{1,2,3}
Luiz Carlos Carvalho Navegantes⁴
Erick Prado de Oliveira⁶
Camila Fernanda Costa e Cunha Moraes Brandão⁵
Dawit Albieiro Pinheiro Gonçalves^{1,2,3}

Affiliations:

¹ Ergogenic and Systemic and Molecular Physiology Research Group (ErgoFis), School of Physical Education, Physiotherapy and Occupational Therapy, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

² Sports Physiology Section (SFE) - Sports Training Center (CTE) of Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

³ Exercise Physiology Laboratory (LAFISE), School of Physical Education, Physiotherapy and Occupational Therapy, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

⁴ Department of Physiology, Ribeirão Preto Medical School, Universidade de São Paulo, SP, Brazil.

⁵ Department of Physical Education, Universidade Estadual de Minas Gerais, Divinópolis, MG, Brazil.

⁶ Universidade Federal de Uberlândia, Uberlândia, MG, Brazil.

Keywords: Obesity; Female; High-fat Diet; Physical Performance

Abstract:

Obesity is a multifactorial epidemic that affects people worldwide, with higher prevalence in women. Obesity may affect insulin sensitivity that induces several metabolic disturbances negatively affecting protein metabolism. The endocrine-metabolic impairment may cause muscle atrophy, which in turn may lead to a decrement in physical performance, i.e., an ergolytic effect. This study aimed to evaluate the effects of a high-fat diet (HFD) on physical performance, body mass (BM) and tissue mass (TM) of female mice. Eighty CD1 female mice were randomly divided in 4 groups: 20 mice fed with a commercial chow (AIN-93G, CM), 20 mice fed with a HF-control diet (handmade AIN-93G, C), 40 mice fed with a HFD (35% lipid) for 12-18 weeks were further divided in those who reached 40g of BM (n=11, HF≥40g) or not (n=27, HF<40g). Before and after diet interventions, all groups underwent a Glucose Tolerance Test (GTT), and Grip Strength Test (GST) and an Incremental Load Test (ILT) to assess strength and aerobic performance, respectively. Vaginal lavage was done to identify the estrous cycle. Animal Research Ethics Committee/UFMG: 204/2024. HF≥40g showed higher BM (~34%) and white adipose TM (~322%), lower strength (~32%) and muscle TM of Tibialis Anterior, Extensor Digitorum Longus and Plantaris (~24%, ~25%, ~21%) after HFD than all other groups. HF≥40g had smaller soleus and Triceps Suræ TM than CM and HF<40g (~18%, ~12%) and smaller Gastrocnemius TM than C (~25%). No difference was found in time to fatigue and maximum speed for ILT. The area under the curve of glucose response in GTT from



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

HF $\geq$ 40g was higher than other groups (~54%). The estrous cycle analysis showed higher prevalence in diestrus (~42%) and metestrus (~31%) but no difference among groups. HFD impaired GTT and induced sarcopenic obesity, i.e., loss of muscle mass and strength and higher BM and adipose TM, in female mice. Ongoing analysis will determine HFD effects on protein synthesis.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Longer Sleep Duration Explains Less Perceived Exertion in Para-Athletes During High Thermal Strain Training Sessions

Authors:

Gabriel Adler Gomes-Martins^{1,2,3,4}
Marcelo Teixeira Andrade⁵
Wellington Douglas de Souza^{1,2,3,4}
Lucas Siqueira-Moraes^{1,2,3,4}
Carolina Batista Simões^{1,2,3,4}
Carlos Makleyton Caetano Schuchter^{4,6}
Maria Clara Nogueira Ferreira^{1,3}
Marco Túlio de Mello^{4,6}
Andressa Silva^{4,6}
Dawit Albieiro Pinheiro Gonçalves^{1,2,3,4}

Affiliations:

¹ Section of Sports Physiology (SFE), Sports Training Center (CTE), Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

² ERGOgenic and Systemic and Molecular Physiology Research Group (ErgoFis), School of Physical Education, Physiotherapy and Occupational Therapy, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

³ Exercise Physiology Laboratory (LAFISE), School of Physical Education, Physiotherapy and Occupational Therapy, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

⁴ Sports Training Center (CTE), Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

⁵ Psychology Program, Faculdade de Minas (FAMINAS), Belo Horizonte, MG, Brazil.

⁶ Center for Studies in Psychobiology and Exercise (CEPE), School of Physical Education, Physiotherapy and Occupational Therapy, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

Keywords: Para athletes. Sleep. Thermal stress. Training load.

Abstract:

Internal load (IL) consists of the psychophysiological responses of the athlete to the work performed (external load; EL). Rating of perceived exertion (RPE), a valid tool to assess IL, may be affected by athlete's well-being, particularly sleep, and weather conditions. In 2023, Minas Gerais state presented the highest temperature ever recorded in Brazil, and outdoor sports performance could be impaired. We evaluated 26 para-athletes (17 men and 9 women; 26.4 ± 7.8 years) with physical (17), visual (6), and intellectual (3) impairments. Thermal strain (TS) was estimated from the wet bulb globe temperature (WBGT) index of each training session recorded by a National Institute of Meteorology weather station. Before each session, para-athletes reported the duration and quality of sleep (7-point scale, 1 - "very, very good" to 7 - "very, very bad"). After each session, para-athletes reported their RPE (aRPE; 10-point scale, 1 - "rest" and 10 - "maximum"). The coach's RPE (cRPE; i.e., EL) was also recorded. A mixed linear model was conducted with the independent variables: day of year, sleep duration, sleep quality, cRPE, and WBGT, and the dependent variable: aRPE. Research Ethics Committee of UFMG (27518619.4.0000.5149). During the season, WBGT varied between 20.1 from to 41.4°C. The best model included WBGT ($\beta=0.14$; $p=0.10$), WBGT vs. cRPE ($\beta=0.04$; $p<0.01$), and WBGT vs. sleep duration ($\beta=-0.02$; $p<0.01$). An increase of 1°C in WBGT explained a 0.14 a.u. increase in aRPE and an increase in WBGT associated with cRPE explained an elevation of 0.04 a.u. in aRPE. Furthermore, a rise in WBGT (1°C) associated with an increase in sleep duration had an inverse effect, explaining reductions (0.02 a.u.) in the aRPE. Increases in aRPE are explained by the rise in WBGT index either isolated or associated with cRPE (i.e. EL). As expected, a longer sleep duration mitigates the increases in high TS-induced aRPE (i.e. IL).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Effects of systemic vibratory therapy on sarcopenia parameters of frail older adults: preliminary findings

Authors:

Ana Carolina Coelho de Oliveira¹
Fernanda Cristina Lima de Oliveira¹
Luelia Teles Jaques de Albuquerque¹
Gabriela Ribeiro Riggi Rangel Pereira¹
Julia dos Santos Nascimento¹
Marcia Cristina Moura Fernandes¹
Marco Antonio de Souza Gama¹
Liszt Palmeira de Oliveira¹
Mario Bernardo-Filho¹
Danúbia da Cunha de Sá-Caputo¹

Affiliations:

¹ Universidade do Estado do Rio de Janeiro

Keywords: Body composition. Elderly. Functionality. Muscle strength. Whole body vibration.

Abstract:

During aging, besides increased vulnerability and reduced muscle reserve, changes in body composition occur, including muscle mass loss, reduced functionality, and strength, due to sarcopenia. Systemic vibratory therapy (SVT) through whole-body vibration exercise (WBVE) on a vibration platform (VP) has shown benefits in improving physical-functional independence in older. This study aimed to evaluate the effects of SVT on the criteria of sarcopenia through skeletal muscle mass (SMM), physical-functional performance (PFP), and muscle strength (MS) in frail older. This non-randomized clinical trial, approved by the ethics committee (CAAE 30649620.1.0000.52.59). Twenty older frail adults were allocated into two groups: standing (n=10; age 67.60±6.86 years; BMI 30.48±5.15 kg/m²) or seated (auxiliary chair with feet in the VP) (n=10; age 68.81±4.11 years; BMI 31.87±4.63 kg/m²). WBVE was performed on an alternating VP at frequencies from 5 to 14 Hz (increased by 1 Hz every 2 sessions), peak-to-peak displacements (PPD) of 2.5, 5.0, and 7.5 mm, over 20 sessions (2 per week). Each session had 1-min work and 1-min rest periods at each PPD, forming a sequence repeated 3 times (sessions 1–10) or 4 times (sessions 11–20). SMM was assessed by bioimpedance, PFP by Timed Up and Go (TUG), and MS by handgrip strength before and after intervention. Paired and unpaired t-tests were performed using GraphPad Prism 9. Data presented as mean ± standard deviation. Difference was considered at p<0.05. The results demonstrate the SMM index (25.59±6.56[pre]/26.44±6.955[post], p=0.16); (21,73±2,17[pre]/21,81±2.67[post], p=0.84), MS (30.37±19.28[pre]/26.77±8.76[post], p=0.43; 18.57±11.16[pre]/ 19.87±10.47 [post], p=0.37), and TUG time (11.66±1.88[pre]/11.78±4.12[post], p=0.94; (17.90±10.90[pre]/13.65±3.92[post], p=0.11) in groups standing and seated respectively, no significant differences were found within or between groups. While the elderly individuals analyzed are frail according to Fried's subjective assessment, they did not meet all the criteria of sarcopenia diagnosis before or after the SVT. However, the seated group demonstrated worst performance in tests and a greater tendency toward sarcopenia, even after SVT. Further research is needed to explore these parameters.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Treinamento físico e suplementação com probióticos reduzem rigidez arterial em animais hipertensos por diferentes mecanismos

Authors:

Juliana Barbosa GOMES¹

Thiago José Dionísio³

Rafael Antunes Nicoletti^{1,2}

Carlos Ferreira dos Santos³

Sandra Lia Amaral^{1,2}

Affiliations:

¹ Departamento de Educação Física, Faculdade de Ciências, Universidade Estadual Paulista (UNESP) - Bauru, Brasil.

² Programa Interinstitucional de Pós-Graduação em Ciências Fisiológicas, PIPGCF, UFSCar/UNESP, São Carlos, Brasil.

³ Departamento de Ciências Biológicas, Faculdade de Odontologia, Universidade de São Paulo (USP) - Bauru, Brasil.

Keywords: Microbiota intestinal. Rigidez arterial. Treinamento combinado.

Abstract:

Rigidez arterial, que pode ser determinada pelo aumento na deposição de colágeno, é uma alteração que reduz a complacência dos vasos, e considerada uma preditora de eventos cardiovasculares futuros. Estudos demonstram que o desequilíbrio da microbiota intestinal pode estar associado ao aumento da rigidez arterial e a melhora dessa disbiose é capaz de atenuar esse aumento. No entanto, poucos estudos avaliam a importância da microbiota na rigidez arterial. Assim, este estudo avaliou os efeitos do treinamento físico combinado (TFC) e da suplementação com probióticos na rigidez arterial de ratos espontaneamente hipertensos (SHR). Quarenta e oito SHR (250g) foram distribuídos em: sedentário (S), sedentário probiótico (SP), treinado (T) e treinado probiótico (TP). Oito ratos wistar (250g) foram usados como controle. Os SHR foram tratados com probiótico (Yakult®, 2ml/65g via gavagem) ou água e submetidos ao TFC (esteira e escada, 60% da capacidade máxima, 5 d/sem, em dias alternados, 60 dias) ou mantidos sedentários. Foram analisadas velocidade da onda de pulso (VOP), razão da expressão gênica de bactérias (firmicutes e bacteroidetes - F/B) nas fezes e produção de colágeno-1 na aorta dos animais. Os dados foram analisados por *student-T test* e ANOVA de um caminho, com post-hoc de dunnet's ($p < 0,05$). No início, a VOP dos SHR (+31%), a produção de col-1 (+191%) e a razão F/B (+335%) estavam maiores no grupo S, vs W ($p < 0,05$). Ao final, os animais tratados com probiótico ou treinados apresentaram menores valores de VOP (-12%), acompanhados de menores valores de COL-1 na aorta (P, -36%, T, -28% e TC, -30%), vs S ($p < 0,05$). No entanto, somente os animais treinados (T, -47% e TP, -44%) apresentaram menor razão F/B, S. Estes resultados sugerem que o TFC reduz rigidez arterial, acompanhado de redução de col-1 e da razão F/B nas fezes dos animais. A redução de VOP induzida pela suplementação com probiótico acontece por mecanismos independentes da razão F/B nas fezes dos animais.

Aprovação na Comissão de ética: (CEUA) da Faculdade de Ciências da UNESP, Bauru. (# nº 43/2024).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Effects of systemic vibratory therapy on body fat mass and waist-to-hip ratio in adults with obesity: preliminary results

Authors:

Jennyfer Silva Mazini^{1,2}
Gabriel Siriano Damasceno dos Santos^{1,2}
Ana Gabriellie Valério-Penha^{1,3}
Alessandra Andrade do Nascimento¹
Ana Carolina Coelho-Oliveira¹
Aline Reis-Silva^{1,2}
Thaís Porto Amadeu⁴
Mario Bernardo-Filho¹
José Alexandre Bachur⁵
Danúbia da Cunha de Sá-Caputo^{1,2,3}

Affiliations:

¹ Laboratório de Vibrações Mecânicas e Práticas Integrativas, Departamento de Biofísica e Biometria. Instituto de Biologia Roberto Alcântara Gomes and Policlínica Universitária Piquet Carneiro, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brazil.
² Programa de Pós-Graduação em Ciências Médicas, Faculdade de Ciências Médicas, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brazil.
³ Programa de Pós-Graduação em Fisiopatologia Clínica e Experimental, Faculdade de Ciências Médicas, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brazil.
⁴ Faculdade de Ciências Médicas, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brazil.
⁵ Universidade de Franca, São Paulo, SP, Brazil.

Keywords: Body composition. Chronic disease. Metabolic health. Obese. Whole body vibration.

Abstract:

Obesity is a chronic condition characterized by excessive body fat mass (BFM) accumulation, associated with an increased waist-to-hip ratio (WHR), which represents risks for metabolic diseases. In this context, systemic vibratory therapy (SVT) is a physical exercise modality that may be considered an option for managing this population. The aim was to evaluate the effect of SVT on BFM and WHR in adults with obesity. This is a randomized, double-blind, interventional, longitudinal clinical trial, with ethical approval (CAAE 30649620.1.0000.5259). Twenty-four adults with obesity were randomized into three groups: side-alternating vibratory platform (AVP) (n=9, age 44.88±8.41 years; BMI 34.32±2.55 kg/m²); vertical vibratory platform (VVP) (n=5, age 49.80±9.33 years; BMI 37.60±1.68 kg/m²); and sham (SG) (n=10, age 40.50±9.07 years; BMI 35.94±3.04 kg/m²). The AVP and VVP groups used a frequency of 30 Hz, peak-to-peak displacement of 2.5 mm, and an amplitude low, respectively. Each session consisted of 1-min of vibration in semi-squat position, followed by 1-min of rest (no vibration), repeated 15 times, totaling 29 minutes, twice a week for 6 weeks. In SG, the vibrating platform is off. BFM and WHR were assessed using bioelectrical impedance analysis before and after the interventions. SPSS version 20 was used for statistical analysis. The pre-test data analysis was performed using MANOVA, while the post-test analysis employed factorial repeated measures MANOVA. Significance set at p<0.05. Data are expressed as mean differences ± standard error of the mean difference. The within-group analysis suggests a significant reduction in BFM (-14.611±5.293; p<0.01) and WHR (0.055±0.025; p<0.03) in the AVP group. On the other hand, no significant changes were observed in the VVP group (BFM: -8.950±7.10; p>0.22; WHR: 0.001±0.034; p>0.97) or in the SG group (BFM: -6.465±5.021; p>0.21; WHR: -0.005±0.024; p>0.83). Between-group analysis did not indicate statistical differences (p>0.60). The results indicate that only the AVP showed a significant reduction in BFM and WHR, suggesting that the decrease in excess fat may positively affect the WHR. However, further studies are necessary to confirm these findings.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Funding: Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Role of extracellular calcium in fatigue resistance of type-II skeletal muscle fibers during repeated submaximal stimulation

Authors:

Matheus Chiaradia Greguer¹ Paulo Guimarães Gandra¹

Affiliations:

¹ LabMusc, Departamento de Bioquímica e Biologia Tecidual, Instituto de Biologia, Universidade Estadual de Campinas- UNICAMP.

Keywords: Extracellular calcium. Fatigue. Skeletal muscle.

Abstract:

During skeletal muscle fiber stimulation, free intracellular calcium levels are primarily regulated by calcium release and reuptake by the sarcoplasmic reticulum. However, calcium exchange between the intracellular and extracellular environments may also contribute to calcium dynamics during repeated stimulation. Previous studies suggest that extracellular calcium is not essential for fatigue resistance during short bouts of high-frequency stimulation in muscles predominantly composed of type IIb fibers. In contrast, we hypothesized that extracellular calcium plays a critical role in supporting contractile function in type IIa fibers during prolonged periods of submaximal stimulation. To test this hypothesis, force generation was measured in bundles of 5 to 10 muscle fibers isolated from the mouse flexor digitorum brevis (FDB), which is composed mainly of type IIa fibers. Fiber bundles were stimulated once every 4 seconds for 30 minutes at a frequency of 30 Hz under two conditions: in Tyrode's solution containing 1.8 mM Ca^{2+} or in calcium-free Tyrode's solution. All procedures were approved by the Ethics Committee on the Use of Animals at the State University of Campinas (CEUA-UNICAMP). After 12 minutes of stimulation, force production was significantly lower in bundles stimulated in calcium-free medium compared to those in the presence of extracellular calcium ($27.18 \pm 5.48\%$ versus $45.40 \pm 10.68\%$ of the first contraction respectively; $p = 0.0230$). After 30 minutes, force declined further to $12.01 \pm 2.54\%$ of the initial value in the calcium-free group, while it remained at $45.42 \pm 2.39\%$ in the calcium-containing condition ($p < 0.0001$ for the difference between groups). These results indicate that extracellular calcium is important for maintaining contractile function in type IIa fibers during prolonged submaximal stimulation, likely by supporting calcium influx and helping to maintain sarcoplasmic reticulum calcium content available for release during stimulation.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Acute effects of combined systemic vibratory therapy and osteopathic manipulative treatment on parasympathetic activity in individuals with obesity: preliminary results

Authors:

Andréa Ferreira-Silva^{1,2}
Daniel Batouli-Santos^{1,2}
André Luiz Bandeira Dionizio Cardoso^{1,2}
Danúbia da Cunha de Sá-Caputo^{1,2}
Mario Bernardo-Filho¹

Affiliations:

¹ Laboratório de Vibrações Mecânicas e Práticas Integrativas, Departamento de Biofísica and Biometria, Instituto de Biologia Roberto Alcântara Gomes and Policlínica Universitária Piquet Carneiro, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ 20950-003, Brazil.

² Programa de Pós-Graduação em Fisiopatologia Clínica e Experimental, Faculdade de Ciências, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ 20550-900, Brazil.

Keywords: Autonomic nervous system. Exercise. Heart rate variability. Whole-body vibration.

Abstract:

Obesity is a disease characterized by excess body fat, associated with cardiovascular risk and autonomic nervous system (ANS) imbalance. Heart rate variability (HRV) is a noninvasive measure of the ANS. Systemic vibratory therapy (SVT), in which subjects are exposed to mechanical vibration (MV) generated by a vibrating platform (VP), has been investigated for its potential effects on autonomic modulation and cardiovascular function. This study investigates the acute effect of combined SVT and osteopathic manipulative treatment (OMT) on parasympathetic activity in obese individuals. This is a clinical trial with twenty obese individuals (15 women, 5 men) randomized into two groups: a control group (CG) and combined treatment (SVT+OMT). During SVT sessions individuals were positioned standing (VP base), with knees flexed at 130° (MV-frequency of 30Hz, peak-to-peak displacement of 2.5mm, 9 bouts of 1-min, separated by 1-min rest period, for 18-min), followed by OMT in supine position for 17-min. The CG followed the same protocol with VP off. HRV was analyzed before and after the interventions using the *root mean square of successive RR interval difference* (RMSSD) index. Protocol was approved by the Ethics Committee CAAE 30649620.1.0000.5259. Statistical analysis performed using R 4.4.1, evaluating anthropometric variables, and RMSSD using repeated measures and one-way ANOVA. Difference was considered at p -value <0.05 . In the baseline did not indicate difference between the groups regarding age (GC-51.6 $\pm$ 16.1/ SVT+OMT-53.4 $\pm$ 10.1years, $p=0.768$) / body mass (GC-98.4 $\pm$ 30.5/ SVT+OMT-91.5 $\pm$ 17.7kg, $p=0.570$), stature (GC-160.8 $\pm$ 7.9/ SVT+OMT-163.1 $\pm$ 9.2cm, $p=0.971$), body mass index (GC-37.9 $\pm$ 9.3/SVT+OMT-34.4 $\pm$ 5.9 kg/m², $p=0.544$). In the intergroup ($p=0.247$) and intragroup ($p=0.649$) RMSSD index analysis, the observed findings did not present significant differences. In conclusion, the CG, and the combined treatment (SVT+OMT) did not alter the HRV in these individuals.

Financial Support: This research was supported by the *Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)*, *Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ)* and *Coordenação de aperfeiçoamento de Pessoal de Nível Superior (CAPES)* under grant 001.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Effects of Aerobic Exercise and Semaglutide on Lipid Profile in a Classical Menopause Model

Authors:

Bruna Selau¹
Rafaella Sanfelice Normann¹
Leonardo Marensi Bastos²
Gustavo Kasper Cubas¹
Rodrigo de Oliveira Corrêa da Cunha Herrmann¹
Marjoriane de Amaral¹
Jorge Felipe Argenta Model¹
Luiz Carlos Kucharski¹
Anapaula Sommer Vinagre¹

Affiliations:

¹ Departamento de Fisiologia, Instituto de Ciências Básicas da Saúde (ICBS), Universidade Federal do Rio Grande do Sul (UFRGS).

² Departamento de Farmacologia, Instituto de Ciências Básicas da Saúde (ICBS), Universidade Federal do Rio Grande do Sul (UFRGS).

Keywords: Aerobic exercise. Lipid profile. Menopause. Semaglutide.

Abstract:

Menopause increases visceral obesity, leading to dyslipidemia with an altered lipid profile. The current literature presents several pharmacological and non-pharmacological strategies to treat this condition, such as semaglutide (SEM), a GLP-1 receptor agonist, and aerobic exercise (EXE), respectively. However, the SEM and EXE interaction under this context remains unknown, justifying the need for further studies investigating these factors' metabolic effects. In this study, 38 female Wistar rats underwent ovariectomy and were divided into 4 distinct experimental groups (n=9-10): sedentary (SED) + Saline (SAL), SED + SEM, EXE + SAL, and EXE + SEM. The exercise protocol consisted of a treadmill running at moderate intensity (60% VO₂ max) for 20 minutes per day, 5 days a week, for 4 weeks. The groups received either SEM (0.3 mg/kg, s.c.) or SAL (0.9%, s.c.) once a week. Blood samples were collected for lipid profile analysis at the end of the experiment. This project was approved by CEUA/UFRGS (#44415). Data are represented as mean ± standard error and were analyzed by Two-Way ANOVA followed by Tukey's post hoc test. Plasmatic triglycerides (TGL) in SEM groups (65.87±0.138 mg/dL) decreased compared to SAL groups (94.37±4.97 mg/dL; p<0.001). Total cholesterol decreased in EXE groups (81.75±2.68 mg/dL) compared to SED groups (95.27±3.52 mg/dL; p=0.018), and the same occurred with LDL cholesterol (EXE groups: 59.05±2.53 mg/dL; SED groups: 72.45±3.56 mg/dL; p=0.024). ANOVA analysis of HDL cholesterol identified an interaction between SEM and EXE factors: rats from the EXE + SEM group (4.77±1.67 mg/dL) showed decreased HDL cholesterol levels compared to the SED + SEM group (8.66±3.08 mg/dL; p=0.0078). In conclusion, the preliminary results suggest that aerobic exercise reduced total and LDL cholesterol, while semaglutide lowered TGL. The interaction between these factors influences HDL cholesterol levels, requiring further studies for better understanding.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Effects of a high-fat diet on glucose responses and on physical performance in female mice evaluated at different times of the day

Authors:

Gabriel Valasco de Oliveira Santos^{1,2,3}
Lucas Antônio da Silva Dias^{1,3}
Gustavo de Oliveira Zanetti^{1,3}
Felipe Vergetti Melo Teixeira¹
Pedro William Martins Pessôa^{1,3}
Larissa Nardy Gomes³
Carolina Batista Simões^{1,2,3}
Gabriel Adler Gomes-Martins^{1,2,3}
Luiz Carlos Carvalho Navegantes⁴
Erick Prado de Oliveira⁶
Camila Fernanda Costa e Cunha Moraes Brandão⁵
Dawit Albieiro Pinheiro Gonçalves^{1,2,3}

Affiliations:

¹ Ergogenic and Systemic and Molecular Physiology Research Group (ErgoFis), School of Physical Education, Physiotherapy and Occupational Therapy, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

² Section of Sports Physiology (SFE) - Sports Training Center (CTE) of Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

³ Exercise Physiology Laboratory (LAFISE), School of Physical Education, Physiotherapy and Occupational Therapy, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

⁴ Department of Physiology, Ribeirão Preto Medical School, Universidade de São Paulo, SP, Brazil.

⁵ Department of Physical Education, Universidade Estadual de Minas Gerais, Divinópolis, MG, Brazil.

⁶ Universidade Federal de Uberlândia, Uberlândia, MG, Brazil.

Keywords: Female. High-fat Diet. Obesity. Physical Performance.

Abstract:

Obesity is a global epidemic, more prevalent in women. It can impair insulin sensitivity, alter metabolism, and disrupt protein metabolism. This dysfunction may cause muscle atrophy and decreased physical performance. Our study aimed to evaluate the effects of a high-fat diet (HFD) on body mass (BM), glucose responses, and physical performance in female mice tested at different times of the day. The sample consisted of 80 female CD1 mice, divided into three groups: 20 mice fed a HFD control diet (C), 20 mice fed a standard commercial diet (CM), and 40 mice fed a HFD. The animals were kept on a 12-hour light/dark cycle, with the temperature controlled at $30 \pm 1^\circ\text{C}$. Before and after 12 weeks of diet consumption, they performed an incremental load test (ILT) on a treadmill, grip strength test (GST), and glucose tolerance test (GTT). Approval in Animal Research Ethics Committee/UFGM: 204/2024. HFD group showed an increase in BM compared to C and CM groups ($p = 0.0155$ vs CM, $p = 0.0035$ vs C). HFD animals evaluated at night (ZT14) had higher retroperitoneal white adipose (RETRO) mass relative to BM compared to C and CM groups ($p = 0.0082$ vs CM, $p = 0.0113$ vs C). In the morning (ZT2), HFD animals had a higher RETRO-to-BM ratio than the CM group ($p = 0.0406$). In GTT, HFD group showed higher peak glycemia and elevated blood glucose levels at 15, 30, and 60 minutes ($p < 0.0001$ vs CM and C at 15 and 30 min, $p = 0.0044$ vs CM, $p = 0.0242$ vs C at 60 min). Regarding physical performance, no differences were observed in ILT. However, in the GST, the HFD and CM groups performed worse at ZT2 compared to C ($p = 0.0047$ vs CM, $p = 0.0035$ vs HFD). No differences were found at ZT14. In HFD group, performance was



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

lower at ZT2 compared to ZT14 ($p = 0.0067$). The 12-week HFD increased BM and RETRO, impaired GTT, and had no effect on aerobic performance, but GST was better in the obesity-induced animals when tested at night.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Home-based inspiratory muscle training improves cardiac autonomic response to postural stress in Parkinson's disease: a preliminary study

Authors:

Felipe Castro Ferreira^{1,2}
Michelle Cristina Salabert Vaz Padilha^{1,2}
Pedro Paulo da Silva Soares^{1,2}
Marco Antonio Araujo Leite^{1,2}
Gabriel Dias Rodrigues^{1,2,3}

Affiliations:

¹ Laboratory of Experimental and Applied Exercise Physiology, Department of Physiology and Pharmacology, Fluminense Federal University – UFF, Niterói/RJ – Brazil.

² Post-graduate Program in Neurology, Fluminense Federal University, Niteroi, Rio de Janeiro, Brazil.

³ Department of Clinical Sciences and Community Health, University of Milan, 20122, Milan, Italy.

Keywords: Parkinson's disease. Heart rate variability. Motor disorders. Respiratory muscles. Orthostatic stress.

Abstract:

The weakness of the inspiratory muscles and blunted cardiac autonomic response to orthostatic stress (ORT) are characteristics present in Parkinson's disease (PD), whereas inspiratory muscle training (IMT) has been shown to enhance inspiratory muscle strength and cardiac autonomic control in older adults. However, the potential benefits of IMT on cardiac autonomic response to ORT in individuals with PD remain unclear. The study aimed to evaluate the effects of IMT on cardiac autonomic modulation at rest and during ORT in patients with PD and healthy controls (HC). Methods: In this pilot study, eight PD (67 ± 8 years old) and eight age-matched (64 ± 3 years old) HC participated in a home-based IMT protocol for five weeks (CAAE: 58755522.5.0000.5243). The maximal inspiratory pressure (MIP), a proxy of inspiratory muscle strength, and the active standing test were performed before and post-IMT. ECG was continuously recorded by 10 minutes at sitting position (SIT) and during 6 minutes at ORT position. Heart rate variability (HRV) analysis was conducted on samples of 300 beats in SIT and ORT. HRV spectral analysis identified two oscillatory components: low frequency (LFnu) and high frequency (HFnu). The sympatho-vagal balance was calculated as the LF/HF ratio. The cardiac autonomic response to ORT was calculated ($\Delta = \text{ORT} - \text{SIT}$). Paired t-test was used for comparison within same group. Results: MIP increased significantly post-IMT in both PD (46.5 ± 26.3 vs. 60.8 ± 31.6 cmH₂O; $p < 0.01$) and HC (79.6 ± 12.7 vs. 111.0 ± 14.9 cmH₂O; $p < 0.01$) groups. Also, cardiac vagal modulation (HFnu) increased in PD (22.1 ± 8.5 vs. 45.7 ± 15.9; $p < 0.01$) and HC (26.8 ± 12.4 vs. 37.8 ± 13.9; $p < 0.01$) groups in SIT post-IMT. However, only PD group improved $\Delta \text{LF/HF}$ (-1.7 ± 2.6 vs. 0.9 ± 0.8; $p = 0.03$) and ΔHFnu (11.8 ± 18.5 vs. -16.7 ± 16.7; $p < 0.01$) in response to ORT post-IMT. Conclusion: These findings suggest IMT may improve cardiac autonomic response to ORT in PD, highlighting the need for further investigation.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Effects of creatine supplementation in zebrafish

Authors:

Paloma C. Vieira¹
Murilo Spineli¹
Kayo M. Bagri¹
Manoel Luis Costa¹
Claudia Mermelstein¹

Affiliations:

¹ Laboratório de Diferenciação Muscular e Citoesqueleto, Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

Keywords: Creatine. Physical exercise. Supplementation. Zebrafish.

Abstract:

Maintaining muscle mass at all ages plays a crucial role in preserving body homeostasis and preventing diseases. Creatine supplementation has attracted attention for its potential to enhance muscle performance, however its mechanisms, especially in the elderly, are not fully understood. This study aims to investigate its effects using two well-established vertebrate models: primary chick muscle cell cultures and aged zebrafish. Chick myogenic cultures were treated with creatine (10–80 mM) and analyzed by phase-contrast microscopy, immunofluorescence, and MTT viability assay. Zebrafish underwent 8-week creatine supplementation (64 mg/L) in addition or not to swimming exercise. Morphometric parameters as body weight, volume and size were monitored every two weeks. The swimming performance was assessed by real-time imaging and tracking algorithms, 4 and 8 weeks after treatment. Animal procedures were approved by the Ethics Committee for Animal Use (CEUA - UFRJ) under protocol number 081/22 (chick) and 036/21 (zebrafish). *In vitro*, creatine treatment from 10 to 80 mM did not alter cell viability. Creatine supplementation increased myogenesis, resulting in the formation of larger, striated muscle fibers (20 mM, 48 h; $p < 0.001$). In zebrafish, creatine alone significantly enhanced swimming speed at both 4 (8.6 vs. 5.9 mm/s control; $p < 0.01$) and 8 weeks after treatment (9.6 vs. 5.7 cm/s control; $p < 0.0001$) and total distance after 8 weeks. Morphometric analysis revealed increased body density in creatine-treated fish, similar to the observed in exercised animals (6 weeks after treatment, $p < 0.05$). These findings provide evidence supporting creatine's role in promoting muscle differentiation and improving exercise performance in aged zebrafish.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: A Zero-Cost System to Evaluate Swimming Performance in Zebrafish Adults and Embryos

Authors:

Murilo Nespolo Spineli¹
Paloma C. Vieira¹
Claudia Mermelstein¹
Manoel Luis Costa¹

Affiliations:

¹ Laboratório de Diferenciação Muscular e Citoesqueleto, Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

Keywords: Exercise. ImageJ. Video tracking. Zebrafish.

Abstract:

Physical exercise has been recognized as a critical intervention for preventing numerous diseases, and enhancing systemic health. The development of accessible, cost-effective research methodologies in this field is desirable, especially when it comes to developing countries, such as Brazil. Here, we present a novel, zero-cost system for quantifying swimming performance in both adult and embryonic zebrafish (*Danio rerio*), using consumer-grade smartphones for image acquisition and open-source software (FFmpeg, ImageJ) for computational analysis. Adult zebrafish were recorded in a standardized arena using a home-built imaging apparatus, while embryonic specimens (larval stages) were recorded using trans-illumination through a 6-well plate (CEUA UFRJ 036/21). Video files were preprocessed using FFmpeg to optimize format compatibility and computational efficiency, enabling analysis on low-specification computer hardware. It was demonstrated that smartphone cameras have sufficient resolution (≥ 1080 , 30 fps) to capture locomotor dynamics. Once the files were imported to the computer, ImageJ was employed to derive quantitative kinematic parameters, including total swimming distance, mean velocity, bout frequency, trajectory patterns, and high-resolution positional tracking. We developed several graphical outputs of these results. Using ethanol treatment, whose biphasic effects are well described, it was possible to demonstrate the applicability of the system. We could measure the stimulant effects of ethanol in embryos at lower doses (0.5 - 2%) and the depressive effects, leading to sedation at higher doses (4%). In conclusion, this framework generates high-fidelity quantitative data without specialized instrumentation, offering an accessible tool for researchers - its adaptable approach holds great potential for limited resources laboratories and can become a reliable model for future studies in exercise, toxicological screening and behavioral analysis.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Strength training improves body composition, muscle metabolism and hypertrophy in high-fat-fed ovariectomized mice

Authors:

Jessica Denielle Matos dos Santos¹
Alessandra Gonçalves da Cruz¹
Maria Eduarda Ramos Cezine²
Ester Dos Santos Alves¹
Felipe Nunes de Camargo¹
Anne Raissa Melo Santos¹
Ian Martins Figueiredo¹
José Francisco Turino Silva¹
Bruna Maria Fantini Trinca¹
Sandro Leão Matos¹
Mariana Kiomy Osako²
João Paulo Camporez¹

Affiliations:

¹ Department of Physiology, Ribeirão Preto Medical School, University of São Paulo (FMRP/USP).

² Department of Cellular and Molecular Biology, Ribeirão Preto Medical School, University of São Paulo.

Keywords: Menopause. Muscle metabolism. Strength training.

Abstract:

Menopause leads to hormonal decline, resulting in metabolic changes such as atrophy and loss of muscle strength. Strength training (ST) promotes important muscle metabolic benefits. This study evaluated the effects of ST on body composition, physical performance, and muscle metabolism in ovariectomized C57BL/6J mice fed a high-fat diet (45% fat). The animals were divided into four groups (n=8): Sham Sedentary (S-SED), Sham Trained (S-EXE), Ovariectomized Sedentary (O-SED) and Ovariectomized Trained (O-EXE), CEUA nº 1096/2022. The training protocol was carried out 3x/week for 8 weeks. The data was analyzed by two-way ANOVA followed by Tukey's test ($p < 0.05$). Body weight was reduced in the O-EXE (21 ± 1 g) and S-EXE (21 ± 1 g) groups by ~30% and 6% compared to the O-SED (29 ± 1 g) and S-SED (22 ± 1 g) groups. Body fat decreased by 40% in O-EXE ($5 \pm 0\%$) and 22% in S-EXE ($2 \pm 0\%$) compared to the O-SED ($9 \pm 0\%$) and S-SED ($3 \pm 0\%$) groups. Strength increased by ~69% (O-EXE: 40 ± 2 g) and ~56% (S-EXE: 46 ± 7 g) compared to the O-SED (23 ± 1 g) and S-SED (29 ± 5 g) groups. Muscle endurance was higher in the trained groups (O-EXE: 22 ± 1 ; S-EXE: 29 ± 1) compared to the sedentary groups (O-SED: 5 ± 1 ; S-SED: 13 ± 2), $p < 0.0001$. Muscle triglycerides were reduced in the O-EXE and S-EXE groups compared to the sedentary groups ($p < 0.0001$; $p < 0.0012$). SDH activity increased in the trained groups (O-EXE: 0.48 ± 0.01 ; S-EXE: 0.43 ± 0.01) compared to the sedentary groups (O-SED: 0.38 ± 0.02 ; S-SED: 0.35 ± 0.04). The cross-sectional area was greater in the trained groups O-EXE and S-EXE ~20% compared to the sedentary groups, respectively. Atrogin-1 expression was lower in the O-EXE group (0.483 ± 0.009) compared to the O-SED group (0.928 ± 0.192). These data suggests that ST promotes important metabolic benefits in skeletal muscles independently of sexual hormones, being an interesting approach to mitigates the deleterious metabolic effects observed in menopause.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Aerobic Exercise Training and BACH1/HO-1 Regulation in Colon and Melanoma Cancer Cells: *in vitro* model

Authors:

Sabrina Meiriane da Silva Cruz¹
Gabriel Albanese Kafouri¹
Matheus Silveira Costa¹
Janaina da Silva Vieira¹
Patrícia Chakur Brum^{1,2}

Affiliations:

¹ School of Physical Education and Sports, University of Sao Paulo, Sao Paulo, Brazil.

² Department of Physiology & Biophysics in Institute of Biomedical Sciences, University of Sao Paulo, São Paulo, Brazil.

Keywords: Aerobic Exercise Training. BACH1. Cancer. HO-1.

Abstract:

Aerobic Exercise Training (AET) has been proved to be beneficial in cancer, but the pathways involved in its benefits need to be better elucidated. In this sense our group has been working in several intracellular targets altered by cancer, which can also be modulated by AET. Accordingly, some of these targets are the protein BTB Domain And CNC Homolog 1 (BACH1) and Heme oxygenase 1 (HO-1). BACH1 is known for regulating cancer cells proliferation in various types of cancer. HO-1 is an intracellular enzyme responsible for Heme (HM) degradation which is also related to pro-tumor processes, and is directly repressed by BACH1. Both pathways change tumor metabolism, proliferation and survival of tumor cells, and the AET can influence these pathways in normal conditions. Our study aims to investigate whether AET can mitigate the carcinogenic process by modulating these pathways within colon carcinoma (CT26) and melanoma (B16) cells using a AET mimetic drug, AICAR, and if treatment with Hemin (HE), a BACH1 inhibitor, can modulate cancer cells viability and proliferation. CT26 or B16 cells were cultured and treated with Hemin (HE) and incubated for 24h for the viability assay or for 6 days for clonogenic assay; CT26 cells were also treated with AICAR for viability assay. Statistical analysis: One-way ANOVA ($p < 0.05$). We observed that HE treatment increased viability of CT26 ($p < 0.0001$) and B16 cells ($p < 0.001$), but decreased CT26 ($p < 0.05$) and B16 ($p < 0.001$) cells proliferation. HE treatment decreased CT26 cells proliferation ($p < 0.05$), but increased B16 cells proliferation ($p < 0.05$). Also, CT26 cells' viability were also decreased by AICAR ($p < 0.0001$). Our findings indicate that HE treatment did not reduce cancer cells viability or proliferation, which might be related with HO-1 pathway, but not BACH1, since HE is also a HO-1 inducer. Despite that, AICAR treatment was able to reduce CT26 cells viability, reenforcing the potential of AET as a complementary therapy in cancer.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: In obese rats, physical training showed more effectiveness than carbohydrate-restricted and high-protein diet in antioxidant defenses in subcutaneous adipose tissue

Authors:

Karine Chaves Silva¹
Júlia de Oliveira Borges¹
Luciano Evangelista dos Santos Filho¹
Grazielle Prates Lourenço dos Santos Bittencourt¹
Thiago Macêdo Lopes Correia¹
Amanda Alves de Almeida¹
Maiara Raulina de Jesus Dias¹
Regiane Ribeiro Dias¹
Douglas Santos Batista¹
Rafael Pereira¹
Samuel Santos Bittencourt Pereira²
Amélia Cristina Mendes de Magalhães¹

Affiliations:

¹ Universidade Federal da Bahia.

² Universidade Estadual do Sudoeste da Bahia.

Keywords: High-protein diet. Obesity. Oxidative stresses. Physical training. Subcutaneous adipose tissue.

Abstract:

Obesity impacts on redox imbalance are known; therefore, non-pharmacological interventions, such as diets and physical exercise are associated with treating obesity. In this context, our study evaluated the effects of a carbohydrate-restricted and high-protein diet (HP), with or without physical training, on the redox balance in the subcutaneous adipose tissue of obese rats. 28 male Wistar rats with obesity induced by a hypercaloric diet (HC) were divided into four groups (n=7): HC diet and sedentary (HCS), HC diet and trained (HCT), HP diet and sedentary (HPS) and HP diet and trained (HPT). At the end of 12 experimental weeks, the subcutaneous adipose tissue (SAT) was weighed and stored. Antioxidant capacity on SAT was assessed through the activity of catalase (CAT), glutathione peroxidase (Gpx), and superoxide dismutase (SOD). Oxidative damage was evaluated by measuring nitrites, carbonylated proteins, and thiobarbituric acid reactive substances (TBARS). Nrf2 gene expression was analyzed using qPCR. This study was approved by CEUA of IMS/CAT-UFBA (No. 053/2017). Data were analyzed by two-way ANOVA followed by the Bonferroni post-test; the statistical significance was $p < 0.05$. Physical training increased Gpx activity (HCS: 0.16 ± 0.05 ; HCT: 0.3 ± 0.09 ; HPS: 0.20 ± 0.06 ; HPT: 0.32 ± 0.09 , $p = 0.0002$) and Nrf2 gene expression (HCS: 1.0 ± 0.2 ; HCT: 1.68 ± 0.4 ; HPS: 0.85 ± 0.3 ; HPT: 1.5 ± 0.7 , $p = 0.0022$) and reduced carbonylated proteins (HCS: 4 ± 1.6 ; HCT: 2.5 ± 1.2 ; HPS: 3.6 ± 1.7 ; HPT: 1.9 ± 1.2 , $p < 0.0001$) and nitrites (HCS: 10.2 ± 2.9 ; HCT: 5.2 ± 0.8 ; HPS: 8.5 ± 1.7 ; HPT: 4.8 ± 1.3 , $p < 0.0001$). SOD activity (HCS: 0.5 ± 0.1 ; HCT: 0.7 ± 0.2 ; HPS: 0.7 ± 0.1 ; HPT: 0.9 ± 0.1 , $p_{PT} = 0.0042$ and $p_{Diet} = 0.0006$) and TBARS content (HCS: 14 ± 4 ; HCT: 7.2 ± 2.5 ; HPS: 8.8 ± 3.2 ; HPT: 5.5 ± 3.3 , $p_{PT} = 0.0010$ and $p_{Diet} = 0.0114$) were influenced by both diet and training. CAT activity has not changed. Thus, training provides greater protection to SAT against oxidative damage and increases antioxidant defenses in the context of obesity.

Financial support: Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Resistance exercise for mice: a new approach to training rodents

Authors:

Raphael da Silva Lau¹
Breno Reis Corrêa Arigoni¹
Roberto Laureano-Melo¹
Victor Hugo de Maria Paura de Souza¹
Guilherme Ferreira Bueno¹
Fernando da Silveira Ribeiro Rocha²
Bruno Henrico Bertoni de Mendonça¹
Lívia Carvalho Dantas¹
Liliani Aparecida Sereno Fontes de Medeiros¹
Jonas Xavier Torres¹
Yasmin Cavalcante de Sousa¹
Brena Barcellos Luiz¹
Amanda da Silva Salgado¹
Wellington da Silva Côrtes¹

Affiliations:

¹ Universidade Federal Rural do Rio de Janeiro.

² Universidade Leonardo Da Vinci.

Keywords: resistance exercise; ladder climbing, mice,

Abstract:

Animal models have been used to understand the phenomena involved in the practice of physical activities on the various physiological systems and, normally, involve aerobic exercise and/or resistance exercise. Among the most widely used resistance training models is the inclined ladder for rodents recommended by Honrberger and Farrar. In this training model, external loads must be attached to the tail of the animal with which it must climb. Despite the interest in using smaller rodents, such as mice, its application is a minority. In our model, we inserted a fixed pulley system and developed a training outfit, which can be used to remove the stress of placing the load on the animal's tail. Therefore, the new methodology seeks to facilitate the "operant mode" of the method. For this, 21 male mice were divided into 2 experimental groups: control (C) and trained (T). The experimental protocol lasted 8 weeks with the following temporal organization: 1) acclimatization; 2) maximum load carrying test (MCWT) (pre); 3) ladder training; 4) MCWT (post). The work was registered with the CEUA under number 012282/2017. After the training protocol, no statistical difference in body mass was observed between groups C and T. Our results demonstrate that the acclimatization period was able to teach the animals to climb the stairs without noxious stimulus [$F_{(interaction)} (3, 51) = 0.49, p=0.69$; $F_{(time)} (1.297, 22.04) = 68.24, p<0.0001$; $F_{(treatment)} (1, 17) = 0.003, p = 0.96$; $F_{(learning)} (17, 51) = 2.41, p=0.008$], as well as increasing post-training strength verified by the TCCM ($t (17) = 4.170; p=0.0006$). Our adapted model of inclined ladder training for mice demonstrates that animals are capable of obtaining strength gains and, possibly, muscle hypertrophy after performing the training protocol.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Climb training modifies the effects of arterial insufficiency in the pelvic limb of elderly rats

Authors:

Walid Zayan Harati¹
Maria Eduarda de Almeida¹
Nuha A. Dsouki¹
Bárbara do Vale¹
Gisele Giannocco^{2,3}
Alexandre César Fioretti¹
Monica A. Sato¹

Affiliations:

¹ Dept. Morphology and Physiology, Faculdade de Medicina do ABC, Centro Universitário FMABC, Santo André, SP, Brazil.

² Dept. Medicine, Federal University of Sao Paulo, Sao Paulo, SP, Brazil.

³ Dept. Biological Sciences, Federal University of Sao Paulo, Diadema, SP, Brazil.

Keywords: Arterial insufficiency. Exercise training. Femoral artery.

Abstract:

Arterial insufficiency (AI) occurs when the arterial blood supply is impaired in comparison to the demand of a tissue. We investigated if the climb training (CT) without body weight (b.w.) load can modify the effects of AI in elderly rats. Male Wistar rats (10 months-old, CEUA-FMABC#07/2024) were divided into 4 groups: SHAM-SED (Sham surgery+sedentarism, n=6), SHAM-T (Sham surgery+training), n=6), LIG-SED (ligature surgery+sedentarism, n=7), LIG-T (ligature surgery+training, n=7). Ligature of the right femoral artery in isoflurane-anesthetized rats was performed with suture thread distal to the inguinal ligament, simulating blood flow limitations of AI. CT was performed on a 110 cm vertical ladder with an 80° inclination. Five wks after surgery, rats were adapted to climb the ladder for 2 wks. For 10 wks, on alternate days, 3 times/wk, the number of climbs was gradually increased weekly from 1 climb/bout until 10 climbs/bout with 1 min of interval between climbs without b.w. load. One day after the last exercise bout, rats were euthanized, and the heart, kidneys, and skin of the pelvic limb were harvested for later histological evaluation with HE and Masson's Trichrome stainings. The thickness of the left ventricle (LV) wall of the heart was evaluated using the Image J software. Data are as mean±SEM (One-way ANOVA, Tukey post-test, p<0.05). The LV wall of LIG-T was significantly thicker ($0.85\pm0.01\ \mu\text{m/g}$) than in SHAM-SED ($0.73\pm0.04\ \mu\text{m/g}$, p=0.023), and showed no difference compared to LIG-SED ($0.85\pm0.05\ \mu\text{m/g}$) or SHAM-T ($0.75\pm0.05\ \mu\text{m/g}$). Skin sections of LIG+SED was ischemic, dehydrated, with disorganized collagen fibers, loss of vascularization and multiple necrosis, which was not observed in LIG+T (with evident neovascularization) or SHAM-SED or SHAM-T groups. Kidneys sections of LIG-T and LIG-SED showed slightly hypertrophied glomeruli. Therefore, CT without b.w. load elicited LV hypertrophy and prevented skin damages in the pelvic limb in elderly rats with AI.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Physical exercise changes expression of mRNA- mitochondrial dynamics and biogenesis related in heart of overnourished juvenile male rats

Authors:

Jefferson Thadeu Arruda Silva^{1,2}
Thyago de Oliveira Rodrigues^{1,2}
Reginaldo Correia da Silva Filho^{1,2}
Elenilson Maximino Bernardo^{1,3}
Allifer Rosendo Pereira^{1,4}
Matheus Santos de Sousa Fernandes^{1,5}
Mariana Pinheiro Fernandes^{1,2,3}
Claudia Jacques Lagranha^{1,2,3}

Affiliations:

¹ Universidade Federal de Pernambuco (UFPE).
² Programa de Pós-Graduação em Nutrição, Atividade Física e Plasticidade Fenotípica (UFPE/CAV).
³ Programa de Pós-Graduação em Neuropsiquiatria e Ciências do Comportamento (UFPE).
⁴ Programa de Pós-Graduação em Nutrição (UFPE).
⁵ Instituto Keizo Asami (UFPE).

Keywords: Heart. Mitochondria. Overnutrition. Males. Physical Exercise.

Abstract:

INTRODUCTION: The classic literature points out that moderate aerobic training is one of the most effective strategies for promoting metabolic and mitochondrial health, and is a powerful tool for combating various conditions associated with modern lifestyles, such as overweight and obesity. **AIM:** Evaluate the effect of moderate aerobic training in the expression of mRNA of mitochondrial dynamics and biogenesis function (PGC1 α , TFAM, UCP2, OPA1, FIS1) in the male heart at 54 days of life, overnourished during the lactation. **METHODS:** This study was approved by the ethics committee (protocol nº 117/2022). Male offspring were divided according to the nutritional insult into normonourished-NS or overnourished-OS groups on the third day of life. At 54 days of life, rats were euthanized, and hearts were collected. Data were expressed as mean $\pm$ SD. The significance level was maintained at 5% ($p < 0.05$) for all analyses. Statistical analyses were performed using GraphPad Prism 8.0 software. **RESULTS:** Our data showed that the OS group's body weight was higher than NS ($p = 0.04$). Our data suggest that aerobic training in the OT group increased TFAM expression compared to the NT group (NT: 1.1 ± 0.2 ; OT: 1.2 ± 0.1 ; $p = 0.004$). Similar data were found for PGC1 α (NT: 1.6 ± 0.4 ; OT: 2.1 ± 0.3 ; $p = 0.0009$). Regarding genes related to mitochondrial dynamics, we found that FIS1 expression was higher in the OT rats compared to NT rats (NT: 0.9 ± 0.2 ; OT: 1.4 ± 0.4 ; $p = 0.005$). However, OPA1 expression did not differ between groups. Finally, we observed that UCP2 expression was decreased in the hearts of overnourished rats compared to normonourished rats (N: 1.0 ± 0.2 ; O: 0.3 ± 0.4 ; $p = 0.037$). Additionally, the expression of this gene was also lower in the NT group compared to N (N: 1.0 ± 0.2 ; NT: 0.2 ± 0.4 ; $p = 0.017$). **CONCLUSION:** In conclusion, our results highlight that aerobic training in the OT group led to increased expression of key mitochondrial genes, such as TFAM, PGC1 α , and FIS1, compared to the NT group. Additionally, overnourished rats exhibited reduced UCP2 expression in the heart, suggesting metabolic disruptions. These findings underscore the potential effects of exercise and overnutrition on mitochondrial dynamics and gene expression.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Effects of moderate physical exercise on the redox homeostasis of white adipose tissue of overnourished juvenile male rats

Authors:

Jefferson Thadeu Arruda Silva^{1,2}
Allifer Rosendo Pereira^{1,4}
Reginaldo Correia da Silva Filho^{1,2}
Elenilson Maximino Bernardo^{1,3}
Thyago de Oliveira Rodrigues^{1,2}
Matheus Santos de Sousa Fernandes^{1,5}
Mariana Pinheiro Fernandes^{1,2,4}
Claudia Jacques Lagranha^{1,2,3}

Affiliations:

¹ Universidade Federal de Pernambuco (UFPE).
² Programa de Pós-Graduação em Nutrição, Atividade Física e Plasticidade Fenotípica (UFPE/CAV).
³ Programa de Pós-Graduação em Neuropsiquiatria e Ciências do Comportamento (UFPE).
⁴ Programa de Pós-Graduação em Nutrição (UFPE).
⁵ Instituto Keizo Asami (UFPE).

Keywords: Adipose tissue. Obesity. Physical exercise. Oxidative stress.

Abstract:

The dysfunction of adipose tissue as a consequence of oxidative stress has proven to be a key factor in the development of obesity. On the other hand, physical exercise stands in the prevention of obesity. Our objective was to evaluate in overnourished male Wistar rats, during lactation, the effects of moderate physical training on the redox balance of white adipose tissue at 60 days of age. This study was approved by the ethics committee (protocol no. 117/2022). On the third day of life, the pups were randomly divided into four groups according to diet (normonutrient-N or supernutrient-S). At 30 days of life, the groups were further subdivided into untrained (NU and SU) and trained (NT and ST). The training consisted of 4 weeks of running at 50% of maximum capacity, for 5 days/week and 60 min/day. At 60 days, the animals were euthanized. The data were expressed as mean $\pm$ SD. The significance level was maintained at 5% ($p < 0.05$) for all analyses. Statistical analyses were performed using GraphPad Prism 8.0 software. We observed a 14% reduction in body mass in overnourished animals after training ($p < 0.01$). Regarding oxidative stress biomarkers, overnutrition increased lipid damage by 29% ($p < 0.0001$) and protein damage by 38% ($p < 0.0001$). In contrast, training attenuated lipid damage by 47% ($p < 0.0001$), and protein damage by 39% ($p < 0.0001$). When analyzing the enzymatic antioxidant defense, the overnourished animals had a reduction in SOD activity by 34% ($p < 0.001$), CAT by 65% ($p < 0.0001$) and GST by 55% ($p < 0.05$). On the other hand, physical training increased SOD activity by 39% ($p < 0.0001$), CAT by 57% ($p < 0.001$) and GST by 39% ($p < 0.01$). Finally, overnutrition reduced the redox state (GSH/GSSG) by 18% ($p < 0.0001$), while exercise seems to reverse this condition ($p < 0.0001$). Our results show that initial overnutrition impairs the redox balance in white adipose tissue, while a moderate 4-week exercise program seems to mitigate such conditions.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Assessment of the Effect of Acute Caffeine Supplementation on Affective Parameters and Cognition During a Resistance Training Session

Authors:

Kelly Monteiro de Barros Vargas¹
Lucas de Oliveira Siqueira¹
Rafaela dos Santos Veiga Dias¹
Rafael Arêas Vargas¹
Gustavo Vieira de Oliveira¹

Affiliations:

¹ Federal University of Rio de Janeiro.

Keywords: Caffeine. Cognition. Mental health. Physical exercise. Supplementation.

Abstract:

Physical activity offers numerous health benefits. Studies suggest that caffeine may improve mood, cognition, and exercise adherence, while reducing perceived exertion. This crossover, randomized, placebo-controlled study included three groups of 12 adults (aged 18–45), all regular resistance training practitioners. Four visits were conducted: screening (data collection, questionnaires, anthropometric assessment, and 12RM testing) and three sessions involving supplementation with CAF– (3 mg/kg), CAF+ (6 mg/kg), and PLA (placebo), administered 60 minutes prior to the training session. Affective and cognitive assessments were conducted before, 60 minutes after supplementation, and 30 minutes post-exercise. The study was approved by the Ethics Committee (CAAE: 80696924.0.0000.5699). CAF+ reduced negative affect after 60 minutes ($M = 12.17$; $SD = 3.24$), compared to baseline ($M = 14.42$; $SD = 5.25$; $p = 0.011$), and post-exercise ($M = 11.00$; $SD = 1.95$; $p = 0.028$). Affective valence increased in this group over time: pre ($M = 3.25$; $SD = 1.42$), post-supplementation ($M = 3.75$; $SD = 0.97$), and 30 minutes post-exercise ($M = 4.17$; $SD = 0.80$). The CAF– group showed reduced negative affect only after exercise: from 12.67 ($SD = 5.07$) to 10.83 ($SD = 1.19$; $p = 0.036$). Affective valence increased from 3.58 ($SD = 1.16$) to 4.00 ($SD = 0.80$) at the same time point. In the cognitive test (DSST), only the CAF+ group showed improvements at all three time points: pre ($M = 76.75$; $SD = 18.48$), post-supplementation ($M = 81.00$; $SD = 17.95$), and 30 minutes post-exercise ($M = 85.25$; $SD = 20.21$). CAF– and PLA groups improved only after exercise: CAF– from 76.33 ($SD = 18.55$) to 87.92 ($SD = 19.43$), and PLA from 75.50 ($SD = 17.99$) to 83.17 ($SD = 21.66$), suggesting a positive effect of exercise on cognition. It is concluded that caffeine (6 mg/kg) promoted affective and cognitive benefits enhanced by exercise, which alone also contributed to cognitive improvement.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Analysis of the Quality of Life of Elderly People Included in a University Extension Program of the Undergraduate Course in Physical Education

Authors:

Tainá Aparecida Haubert¹

Kênia Natália Corrêa²

Patrick da Silveira Gonçalves²

Affiliations:

¹ Universidade Federal do Pampa - Campus Uruguai - RS. ² Universidade La Salle - Campus Canoas - RS.

Keywords: Aging. Quality of Life. University Extension.

Abstract:

The world's population is aging rapidly, bringing social and economic challenges, in addition to the need for public policies adapted to the elderly. Sports and leisure are essential for the health, well-being and quality of life of the elderly, promoting physical, psychological and social benefits. Therefore, this study aimed to analyze the quality of life of elderly participants in a university extension program of an undergraduate course in Physical Education. The program included 12 participants aged 60 years or older, assessed through sociodemographic, health and quality of life questionnaires, using the WHOQOL-OLD. Data collection occurred after physical exercise, for 4 weeks, and the data were analyzed by descriptive statistics and scoring methods to assess quality of life. The study followed the ethical guidelines established by the National Health Council and was approved by the Research Ethics Committee of La Salle University (CAAE: 78893224.70000.5307). Among the participants, 58.3% have a monthly income of one minimum wage and are out of the job market. High blood pressure was the most common condition (n=7), followed by osteoarthritis (n=6), arthritis, and depression (n=4). Regarding exercise, 41.7% do not exercise, 25% do individual aerobic exercises, 25% participate in group activities, and 8.3% do strength exercises. The total average of the quality of life questionnaire was 61.37. In the instrument's details, sensory functioning obtained the lowest average, with 32.29. Autonomy had an average of 73.44, and satisfaction with past, present, and future activities was 76.56. Social participation had an average of 75.52, while the domain related to death and dying also had an average of 32.29. The intimacy domain had the highest average, with 78.13. It is concluded that university programs play a central role in promoting the integral health of the elderly, integrating teaching, research and extension.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Gestational resistance training affects fetal development differently depending on fetal sex with minimal impact on maternal health in rats submitted to an obesogenic diet

Authors:

Raíssa Andrade de Araújo Silva¹
Débora Kathuly da Silva Oliveira¹
Rayanne Maria da Silva Lima¹
Paulo Gabriel Santos de Oliveira¹
Maria Laís de Barros Silva¹
Débora Priscila Lima De Oliveira²
Thaynan Raquel dos Prazeres Oliveira¹
José Antonio-Santos¹
Raquel da Silva Aragão¹

Affiliations:

¹ Universidade Federal de Pernambuco; ² Faculdade Pernambucana de Saúde.

Keywords: Fetal development. Maternal obesity. Placenta. Pregnancy. Strength exercise.

Abstract:

Maternal consumption of a high-fat/high-caloric diet can impair pregnancy development, impairing maternal health, placental function, and fetal development. Gestational physical exercise could be a non-pharmacological action to reverse or minimize these impairments. Female Wistar rats received a control diet (CD, 3.6 kcal/g, 14% fat, n=10) or an obesogenic diet (OD, 4.6 kcal/g, 49% fat, n=14) and condensed milk (3.28 kcal/g, 69% carbohydrate, 22% fat) supplemented with mineral and vitamin mix for six weeks before pregnancy and during pregnancy. During pregnancy, half of the rats performed daily resistance training (RT) on stairs (5 days/week, 50% of maximum carrying capacity at the first and third gestational weeks, and 80% at the second week). Euthanasia occurred on the 20th gestational day. CEUA-UFPE approval 009/2024. OD increased maternal body weight (OD 248.2±14.1; CD 230.8±13.0, g, p=0.02), weight gain (OD 46.5±8.1; CD 33.9±7.8, %, p<0.001) and blood glucose at 15 min in the glucose tolerance test (OD 488.1±69.6; CD 365.9±130, mg/dl, p=0.02) before pregnancy and maternal body weight (OD-NT 285.5±23.4; CD-NT 254.4±14.1, g, p=0.04) at G7 and fasting blood glucose (OD-NT 79±9.6; CD-NT 65.5±7.0, mg/dl, p=0.03) and the percentage of body fat (OD-NT 3.85±0.8; CD-NT 2.42±0.4, %, p=0.02) at G20. In the OD groups, resistance training increased placental efficiency in female fetuses (OD-RT 9.01±0.9; OD-NT 6.98±1.4, p=0.04) and reduced relative liver weight in male fetuses (OD-RT 5.26±1.2; OD-NT 7.09±1.2, %, p=0.04). There was no influence on placental weight and fetal murinometric parameters. Maternal diet was able to induce obesity and glycemic alterations in mothers, and gestational resistance training was unable to reverse these effects. However, gestational training had an impact on placental efficiency and organ development depending on fetal sex.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Relationship Between Mood State and Sleep in Rugby 7's Athletes During a Regional Championship

Authors:

Lucas Schimmack Redondo¹
Heloisa Bergamasco Vettor¹
Nayara Germano¹
Wanderson Rocha Oliveira¹
Sidney Barnabe Peres¹
Solange Marta Franzói de Moraes¹

Affiliations:

¹ Universidade Estadual de Maringá.

Keywords: BRUMS. Physical exercise. Performance. Sleep duration.

Abstract:

Rugby Sevens is an intermittent-intensity sport where performance can be influenced by mood state and pre-competition rest. The present work evaluated the relationship between mood state and sleep duration in Rugby Sevens athletes during a regional championship. All the procedures were approved by the Ethical Committee in Human Research of the State University of Maringá (Protocol No. 6.736.44). Twenty-seven male athletes from two teams (Team 1: n=12; Team 2: n=15) with similar anthropometric profiles were recruited (T1: age 24.2 ± 7.15 years, BMI 28.7 ± 5.20 kg/m²; T2: age 27.2 ± 7.84 years, BMI 27.8 ± 4.59 kg/m²). An anamnesis questionnaire was applied upon arrival at the competition venue. Mood state was assessed using the Brunel Mood Scale (BRUMS), which is composed of six dimensions (tension, depression, anger, vigor, fatigue, and confusion) 30 minutes before and immediately after each match. Current Emotional State Index (CESI) was calculated as a result of BRUMS scale. Sleep duration was categorized into three moments: less than 6 hours, 6 to 8 hours, and more than 8 hours of rest. Data were analyzed using SPSS statistical software. Results showed that Team 1 maintained normative BRUMS profiles, with high vigor (and lower scores in other dimensions), while Team 2 exhibited elevated tension across all matches and pronounced fatigue before the third game. CESI values and sleep duration correlated with team performance: Team 1 showed CESI positive values near 0 with longer sleep duration, whereas 33% of Team 2 athletes slept fewer than 6 hours before competition, with significantly negative CESI values. Vigor demonstrated a direct relationship with positive mood states. More stable and positive CESI levels were associated with better performance, suggesting that extended sleep duration may help maintain optimal mood levels for enhanced athletic output.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Anti-adiposity effects of swimming training are accented by vagus nerve and spleen ablation on male Wistar rats without alter iron or hepcidin circulating levels

Authors:

Caroline de Maman Oldra¹
Giovana Fanhani Tessaro¹
Ellen Carolina Zawoski Gomes¹²
Eveline Cristiane Batista Helene¹
Marianela Andrea Díaz Urrutia¹²
Amanda Rocha Fujita¹
Beatriz Machado Daudt¹
Bruna Schumaker Siqueira¹
Elizângela Vanessa da Cruz Hoffmann³
Matheus Dias Martins³
Sandra Lucinei Balbo¹
Sabrina Grassioli¹

Affiliations:

¹ Endocrine Physiology and Metabolism Laboratory, Center for Biological and Health Sciences, Stricto Sensu Postgraduate Program in Biosciences and Health, Western Paraná State University, Cascavel, PR, Brazil.

² Medical Sciences, Assis Gurgacz Foundation University Center (FAG), Cascavel, PR, Brazil.

³ Physiotherapy Undergraduates, Univel University Center, Cascavel, PR, Brazil.

Keywords: Adipocytes. Exercise. Iron. Spleen. Vagus nerve.

Abstract:

Introduction: Vagus nerve (VN) and spleen participate in the anti-inflammatory vagal reflex, modulating immune and metabolic responses, which impact white adipose tissue (WAT). WAT adipogenesis can be influenced by iron metabolism. Exercise alters both WAT and iron homeostasis, a response that may involve the anti-inflammatory vagal reflex. **Objective:** Here, we evaluated how VN and spleen ablation influence WAT mass and histology in lean rats subjected to swimming training. **Methods:** Ethical approval (Nº 16- 22). At 60 postnatal day (PND), rats underwent one of the following surgeries: subdiaphragmatic vagotomy (sv), splenectomy (spl), sv+spl, or sham. At 80 PND, the groups were randomly subdivided into exercised (Ex; swimming for 4 weeks) or sedentary (Sd). At PND 120, rats were euthanized and retroperitoneal (R) and inguinal (I) WAT depots were weighed and collected for histological analysis. Plasma was used for biochemical assays of glucose and triglycerides, from which the TyG index, a marker of insulin resistance (IR) was calculated. Circulating levels of hepcidin and iron were also measured. **Results:** Neither exercise nor surgeries (sv and spl) significantly altered fasting glucose levels. In contrast, Ex led to a reduction in triglycerides ($p=0.0007$) compared to Sd, with a more pronounced effect in the sv+spl groups. Thus, Ex improved IR ($p=0.0033$) in the sv+spl groups compared to Sd-sham rats. Spl alone resulted in a reduction in iron levels compared to other groups ($p=0.0288$). Furthermore, plasma hepcidin levels were higher in Ex-sv rats ($p=0.0001$) compared to other groups. WAT content (I-WAT: $p<0.0001$; R-WAT: $p=0.0072$) and adipocyte size (I-WAT: $p=0.0044$; R-WAT: $p<0.0001$) were significantly reduced in the Ex-sv+spl group compared to Sd-sham rats, an effect independent of circulating iron or hepcidin levels. **Conclusion:** The absence of the VN and spleen favors a reduction in adiposity during exercise, independent of changes in iron or hepcidin levels.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Impact of whey protein and mild aerobic training on renal function in rats

Authors:

Arthur Fornazari Iost¹
Júlia Gurgel Bernardo¹
Anne Tiemi Santos Watanabe¹
Maria Laura Limeres¹
Diogo de Barros Peruchetti^{1,2}
Maria Aparecida Ribeiro Vieira¹

Affiliations:

¹ Dept. of Physiology and Biophysics, Institute of Biological Sciences, Universidade Federal de Minas Gerais.

² INCT-Nanobiofar.

Keywords: Aerobic exercise. Chow intake. Whey protein.

Abstract:

Whey Protein (WP), a major milk protein, is used as a dietary supplement for non-athletes and mild-trained individuals, though data on its effects in mild-trained subjects are limited. This study evaluated the effects of a WP-supplemented diet in rats undergoing mild exercise training. Male Wistar rats (320–450 g) were divided into non-trained (NT) and trained (T) groups, further split into subgroups: NT with regular chow (NT RC, n=4), NT with WP chow (NT WP, n=5), T with regular chow (T RC, n=5), and T with WP chow (T WP, n=5). WP increased dietary protein to 30%. Rats adapted to treadmills (5 min/day, 3 days/week) for one week, followed by 8 weeks of training (30 min/day, 3 days/week). Metabolic parameters (water/chow intake, urine flow, proteinuria) were measured in the 9th week. Data were analyzed via two-way ANOVA and Tukey test. Trained groups outperformed NT in maximal effort tests (T RC: 27.7 ± 1.3 m/min; T WP: 24.0 ± 2.7 m/min vs. NT RC: 15.1 ± 0.8 m/min). WP and training did not significantly affect body weight or urine flow. Plasma creatinine rose only in T RC (2.14 ± 2.2 mg/dl vs. NT RC: 1.25 ± 0.05 mg/dl). It is noteworthy that chow intake decreased in WP groups (NT WP: 23.2 ± 1.3 g/24h vs. NT RC: 30.4 ± 1.3 g/24h; T WP: 21.3 ± 1.6 g/24h vs. T RC: 29.2 ± 1.6 g/24h). WP reduced chow intake without harming renal function, even in non-trained rats. These findings support further research on WP's health and performance effects.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Influence of Maternal Lifestyle Habits on the Determinants of Cardiovascular and Metabolic Health in Children

Authors:

Cheid Manuel Chongo^{1,2}
Jéssica Gonzaga Pereira^{1,2}
Maria Brenda Ellen dos Santos Pereira Nascimento^{1,2}
Anna Bheatriz Gomes Ferreira Marinho^{1,2}
Reginaldo Correia da Silva Filho³
Mariana Pinheiro Fernandes³
Aiany Cibelle Simões-Alves^{1,2}
João Henrique da Costa-Silva^{1,2}

Affiliations:

¹ Laboratory of Physical Assessment and Signal Processing - LAPS, CAV, Federal University of Pernambuco.
² Laboratory in Nutrition, Physical Activity and Phenotypic Plasticity - NAFPF, CAV, Federal University of Pernambuco.
³ Laboratory of General, Molecular, and Exercise Biochemistry - LABMEX, CAV, Federal University of Pernambuco.

Keywords: Maternal Lifestyle. Hypertension. Nutrition. Obesity. Serum Markers.

Abstract:

Maternal lifestyle directly impacts the health of both mothers and their children. Factors such as physical inactivity, alcohol consumption, and overweight are associated with metabolic and cardiovascular alterations, compromising the family's oxidative and metabolic balance. Thus, this study aimed to investigate the association between maternal lifestyle habits and cardiovascular and metabolic health markers in their children. A total of 26 mothers and 29 children (aged 7–11 years) from public schools in Vitória de Santo Antão (2024) were evaluated. Maternal lifestyle habits were classified based on physical activity, smoking, alcohol consumption, and nutritional status. Nutritional status, body composition, blood pressure, cardiac electrical activity, blood glycemia, lipid profile, and redox status were also assessed in both mothers and children. Ethics Committee approval: CAAE 80956824.3.0000.9430. Among the mothers, 27.6% presented healthy habits, while 72.4% had unhealthy habits. Regarding nutritional status, 20.7% were eutrophic, 34.5% overweight, and 44.8% obese. Among children, 75.9% were eutrophic, 10.3% overweight, and 13.8% obese. Based on maternal lifestyle, children of mothers with unhealthy habits had a shorter QT interval (ventricular repolarization time) (0.32 ± 0.02 ms) compared to those whose mothers had healthy habits (0.34 ± 0.01 ms, $p=0.034$). When considering maternal physical activity, children of sedentary mothers had lower high-density lipoprotein (HDL) levels (61.2 ± 19.4 mg/dL) compared to those of physically active mothers (71.4 ± 13.9 mg/dL, $p=0.02$). In mothers, healthy habits were associated with a higher standard deviation of RR intervals (SDRR, 46.0 ± 25.5 ms) compared to unhealthy habits (29.8 ± 10.6 ms, $p=0.02$), and with greater antioxidant activity of the enzyme superoxide dismutase ($p=0.02$). Healthy maternal habits are associated with better cardiometabolic indicators in children and enhanced antioxidant defense in mothers.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Effect of Endurance Running Training in a Hot Environment on Phenotype and Heat Shock Protein in the Skeletal Muscles of Mice

Authors:

Gustavo de Oliveira Zanetti^{1,2,3}
Pedro William Martins-Pessoa^{1,2,3}
Isis do Carmo Kettelhut⁴
Luiz Carlos Carvalho Navegantes⁵
Dawit Albieiro Pinheiro Gonçalves^{1,2,3,6,7}

Affiliations:

¹ ERGOgenic and Systemic and Molecular Physiology Research Group (ErgoFis), School of Physical Education, Physiotherapy and Occupational Therapy, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

² Exercise Physiology Laboratory (LAFISE), School of Physical Education, Physiotherapy and Occupational Therapy, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

³ School of Physical Education, Physiotherapy and Occupational Therapy, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

⁴ Biochemistry & Immunology.

⁵ Physiology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, SP, Brazil

⁶ Section of Sports Physiology (SFE), Sports Training Center (CTE), Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

⁷ Sports Training Center (CTE), Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

Keywords: Endurance training. Intracellular signaling pathways. Skeletal muscle. Oxidative metabolism. Heat shock proteins.

Abstract:

This study aimed to evaluate the morphological, metabolic, and molecular adaptations in skeletal muscle induced by long-term endurance-trained (ET) in hot (H). Two-month-old male Swiss mice (40g) were divided into 4 groups: 1) sedentary (SED) in a temperate (T) environment (22°C; SED/T), 2) SED in H (32°C; SED/H), 3) ET in T (ET/T), and 4) ET in H (ET/H). ET groups performed treadmill running (1h/day, 5 days/week, 8 weeks) at 60% of maximum speed (S_{max}). Incremental load tests (ILT) in T and H assessed performance before and after ET. Muscles were evaluated through hematoxylin-eosin (HE) morphology, succinate dehydrogenase (SDH) activity, myosin-heavy chain (MyHC) isoforms (immunofluorescence), and western blot analysis of proteins involved in heat response and muscle, energy, and mitochondrial metabolism. Ethics Committee on the Use of Animals (CEUA 220/2019). Pre-ET results showed that H impaired performance by reducing S_{max} (~30%), while VO_{2peak} remained similar between environments. After 8 weeks, despite ET/H trained at a lower absolute intensity (~30%) than ET/T, both ET groups showed similar increases in S_{max} (~22%) and VO_{2peak} . After 2 days of rest, HE indicated that ET/T increased the number of centrally nucleated fibers, an indicative of muscle injury, whereas ET/H ablated it. SDH activity (9%) and Type 2A fiber proportion (16%) increased in both ET groups. Heat shock protein (HSP70) increased (275,5%) in ET groups. No differences were observed in mitochondrial proteins (OxPhos, TOM20) or intracellular regulators (AMPK, p38, PGC1 α , TFEB). These findings suggest that ET/H induces heat shock response and may serve as an alternative to higher ET intensity in T, offering similar benefits in VO_{2peak} , muscle metabolism, and performance. This approach could be useful for individuals unable to sustain high mechanical loads (e.g., obese individuals) and for recovery strategies in sports training.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Resistance training and secondhand smoke negatively modulate FIS1 protein expression in mitochondrial dynamics in glycolytic muscle

Authors:

Gabriel Evangelista dos Santos¹ Priscila Arisa Sasaki^{1,2}

Ana Caroline Rippi Moreno¹

Marcos Fernando de Souza Teixeira³ Patricia Monteiro Seraphim^{1,2}

Affiliations:

¹ Department of Physical Therapy – School of Sciences and Technology – UNESP – Presidente Prudente, Brazil.

² Multicenter Graduate Program in Physiological Sciences, FOA – Araçatuba, UNESP / SBFis.

³ Department of Chemistry and Biochemistry - School of Sciences and Technology – UNESP – Presidente Prudente, Brazil.

Keywords: Mitochondrial Biogenesis. Skeletal Muscle. Resistance Exercise. Mitochondrial Fission. Secondhand Smoke.

Abstract:

Secondhand smoke represents an important risk factor for the development of mitochondrial dysfunction, especially in metabolically active tissues such as skeletal muscle. Resistance training has been proposed as a therapeutic strategy due to its ability to promote cellular adaptations and stimulate mitochondrial biogenesis. This study aimed to investigate the effects of resistance training (TR) on the expression of mitochondrial fission proteins (FIS1 and DRP1) in the glycolytic extensor digitorum longus (EDL) muscle of Wistar rats chronically exposed to secondhand smoke (SHS). Male rats were divided into four groups: Control (C), Smoker (F), Exercised (E), and Smoker + Exercised (FE). Groups F and FE were exposed to smoke from four cigarettes for 30 minutes, twice a day, five days a week, for 16 weeks. Groups E and FE underwent resistance training by climbing with progressive overload over the same period. Euthanasia was performed under anesthesia, and the glycolytic EDL muscle was collected. The study was approved by the local ethics committee (CEUA nº 02/2017). Expression of mitochondrial fission proteins (FIS1 and DRP1) was evaluated by Western Blotting. Data were analyzed using two-way ANOVA, with significance set at $p < 0.05$. From the 4th week of intervention, groups F and FE showed lower body mass compared to groups C and E ($p < 0.001$), and this difference persisted until the final week. A significant reduction in periepididymal white adipose tissue mass was also observed in groups F and FE ($p < 0.001$), along with reduced food intake in group FE compared to C and E. The maximum load supported in the strength test was lower in group FE ($p = 0.0197$ vs CE). FIS1 expression was reduced in groups F, E, and FE compared to group C ($p < 0.0001$). DRP1 protein content remained similar among the groups. In conclusion, secondhand smoke impaired food intake, which resulted in lower body mass due to reduced fat gain, and decreased strength development. Both TR and SHS impaired FIS1 expression in glycolytic muscle, although they did not affect DRP1 protein expression, suggesting a disturbance in mitochondrial dynamics that may lead to the formation of more elongated and interconnected mitochondria.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Impact of resistance training on mitochondrial fission of glycolytic fibers in obese rats

Authors:

Anna Júlia Leal Rodrigues¹
André Hideaki Quaresma Ueda¹
Orlando Camilo Mendes Neto¹
Priscila Arisa Sasaki¹
Murillo Silva Cardoso¹
Marcos Fernando de Souza Teixeira¹
Patrícia Monteiro Seraphim¹

Affiliations:

¹ Universidade Estadual Paulista "Júlio de Mesquita Filho".

Keywords: High-calorie diet. Mitochondrial dynamic. Obesity. Strenght training.

Abstract:

Considering the consequences of obesity associated with a "junk food" diet, this study aimed to analyze the impact of resistance exercise and a high-calorie diet on the expression of mitochondrial fission proteins in glycolytic fibers of obese rats. A study was conducted using 24 Wistar rats, which were randomly divided into 4 groups (n = 6/group): Sedentary Control (SC), Sedentary Obese (SO), Exercised Control (EC), and Exercised Obese (EO). The SC and EC groups were fed standard chow and water ad libitum, while the SO and EO groups received a cafeteria diet, standard chow, soda and water ad libitum. The resistance training (RT) protocol for EC and EO groups consisted of climbing an inclined vertical ladder with progressively increasing loads over 12 weeks. All interventions were previously approved by the Ethics Committee on Animal Use (CEUA 01/2017). Blood sample and glycolytic muscle fiber (EDL) were removed under anesthesia after the intervention period. The high-calorie diet induced a significant increase in body weight ($P < 0.0001$), adipose tissue ($P < 0.0001$), glycemia ($P < 0.005$) and triglyceridemia ($P < 0.005$) compared with SC, EC, and EO. RT caused reduction on body fat by 30% ($P < 0.0001$) and glucose levels by 27% ($P < 0.005$) in EO compared with SO. In addition, DRP1 protein expression was 3x-higher in SO compared with SC, and 3.5x-higher in EO and EC compared with SC. FIS1 protein expression was similar among the groups. The RT protocol was effective in reducing adipose tissue, glucose, and triglycerides, thus attenuating the negative effects of obesity. Both interventions, high-calorie diet or RT, increased the expression of DRP1 protein in glycolytic muscle fibers, suggesting an increased mitochondrial fission. Although mitochondrial fission helps maintain cellular health, an excessive increase without a balance with mitochondrial fusion can impair mitochondrial efficiency and compromise ATP production, potentially leading to dysfunctions in energy metabolism.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Effects of Immobilization and Exercise Training on Muscle Carnosine and Anserine content in Rats

Authors:

Ana Carolina Ferreira da Silva¹
Guilherme G Artioli¹

Affiliations:

¹ Department of Anatomy, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil.

Keywords: Anserine. Carnosine. Muscle disuse. Physical exercise.

Abstract:

Carnosine (L-histidyl- β -alanine) is a histidine-containing dipeptide (HCD) abundantly expressed in skeletal muscle, where it exerts several functions, including intracellular pH regulation, neutralization of reactive species, detoxification of cytotoxic aldehydes, and regulation of Ca^{2+} dynamics and excitation-contraction coupling. The multifunctional nature of carnosine has attracted considerable attention due to its potential use as an ergogenic aid and therapeutic adjuvant in conditions involving oxidative stress. Therefore, understanding interventions that increase intracellular carnosine and the mechanisms controlling carnosine content is crucial. We investigated muscle carnosine content at the two extremes of muscle activity-inactivity spectrum. After obtaining ethical approval (EEFE-USUP #2020/02), 45 30-day-old male Wistar rats were divided into 3 groups: denervated (DEN, n=16), SHAM control (SHAM, n=14) and denervated+trained (DEN+Ex, n=15). DEN rats underwent sciatic nerve transection in one hind limb, with the contralateral side undergoing SHAM surgery. DEN+Ex rats underwent the same surgeries plus 10 weeks of swimming training. SHAM rats underwent SHAM surgery only. Muscle carnosine, anserine, and total HDCs were quantified using HPLC-MS/MS. In the tibialis anterior muscle, carnosine content was significantly lower in denervated conditions (DEN: 15.1 ± 1.9 mmol/kg dry muscle; DEN+Ex: 14.9 ± 2.1 mmol/kg dry muscle) compared to non-denervated conditions (SHAM: 21.2 ± 2.3 mmol/kg dry muscle; SHAM+Ex: 21.0 ± 2.0 mmol/kg dry muscle) ($p < 0.05$ for all comparisons). Similar results were observed for anserine and total HDCs. No differences were observed between DEN vs. DEN+Ex or SHAM vs. SHAM+Ex for any of the variables. In conclusion, immobilization and physical inactivity appear to diminish carnosine, anserine and total HDCs levels in rat skeletal muscle, while exercise training did not modify these parameters.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Effect of pharmacological and non-pharmacological therapy on spatial learning and memory in an experimental model of ADHD in ovariectomized females

Authors:

Roberto Victor Figueiredo de Oliveira Gonçalves^{1,2}
Ana Késsia do Nascimento Gomes^{1,2}
Luana Basilio Ferreira^{1,3}
Marilene da Mata Gomes Patrício^{1,3}
Antonia Frez^{1,2}
Emerson Lopes Olivares^{1,3}
Anderson Luiz Bezerra da Silveira^{1,2}

Affiliations:

- ¹ Federal Rural University of Rio de Janeiro.
² Laboratory of physiology and human performance.
³ Laboratory of Cardiovascular Physiology and Pharmacology.

Keywords: ADHD. Methylphenidate. Ovariectomy. Physical Exercise. Spatial learning.

Abstract:

Attention Deficit Hyperactivity Disorder is described as a neurodevelopmental disorder that is noticeable in childhood and may also have consequences in adolescence and adult years. Scientific literature indicates a higher prevalence in males, with more studies in this population. However, in-depth researches in both sexes are necessary to understand factors promoting differences in ADHD development due to sexual dimorphism and to optimize treatments. Psychopharmacological interventions are common, but long-term adverse effects motivate the search for non-pharmacological approaches. Evidence suggests resistance training can mimic the effects of psychostimulants, attenuating ADHD symptoms. This study investigates the effect of high-intensity resistance training, alone and combined with pharmacological therapy, on spatial learning and memory in an experimental model of ADHD in ovariectomized females. Approved by CEUA-UFRRJ 10/2023, the study separated forty SHR females, aged 12-week-old, into four groups: control, trained, pharmacotherapy, and trained+pharmacotherapy. Bilateral ovariectomy preceded protocols to avoid estrous cycle interference. Pharmacological treatment: methylphenidate (2 mg/kg, 0.2 mg/mL, orogastrically). Non-pharmacological therapy: 8 weeks of high-intensity resistance training. Behavioral assessment: Barnes Maze test. Results show that training, pharmacological therapy, and the combination reduced latency to find the escape box, differing from control. There was a significant interaction between time and treatment. Trained and combined groups had less time to find the escape box compared to control (CTRL: 75.0 ± 7.6 vs. TR: 32.1 ± 8.7 , $p = 0.01$; CTRL: 75.0 ± 7.6 vs. TR+MPH: 18.2 ± 4.8 , $p = 0.0007$). Other comparisons were not significant. Resistance training proved to be a low-cost and efficient non-pharmacological therapy in improving spatial learning and memory in the experimental model of ADHD, with benefits similar to pharmacological treatment.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: The influence of physical exercise in mice with severe generalized lipoatrophy

Authors:

Rhaissa Klaumann de Souza¹
Loreana S. Silveira¹
Bianca F. Leonardi¹
Natália M. Pessoa¹
Erika V. M. Pessoa¹
Luciano Pedro S. Júnior¹
Érique Castro¹
Álbert S. Peixoto¹
Marina Honda¹
Carolina A. Lino¹
William T. L. Festuccia¹

Affiliations:

¹ Institute of Biomedical Sciences, University of São Paulo (ICB - USP), São Paulo, SP.

Keywords: Lipoatrophy. Metabolic syndrome. Physical exercise.

Abstract:

Adipose tissue, beyond serving as an energy reserve, plays a key role in regulating metabolism by secreting adipokines—hormones essential for maintaining metabolic homeostasis. Dysfunctions in this tissue may lead to diseases such as obesity and lipoatrophy. Lipoatrophy is characterized by partial or total loss of fat deposits leading to ectopic lipid accumulation in organs and metabolic dysregulation, causing insulin resistance, cardiac hypertrophy, and hepatic steatosis. Treatment in humans includes adiponectin and leptin replacement, whereas omega-3 supplementation has shown benefits in mice models. Although physical exercise is a promising non-pharmacological intervention, evidence regarding its efficacy in lipoatrophy remains limited. This study (CEUA, ICB/USP: 6160250820) aimed to evaluate the effects of aerobic physical training (APT) on hepatic steatosis, insulin resistance, and cardiac hypertrophy in mice with severe generalized lipoatrophy (A-PPAR γ KO). A-PPAR γ WT and KO mice underwent APT (3x/week, 1h, 55–65% max speed). Two groups were assessed: Protocol 1 (6 months old, 4 weeks APT – WT sedentary: n=5; WT trained: n=7; KO sedentary: n=4; KO trained: n=4) and Protocol 2 (13 weeks old, 11 weeks APT – WT sedentary: n=6; WT trained: n=5; KO sedentary: n=6; KO trained: n=7). Analyses included body weight, food intake, glucose tolerance, serum cholesterol, glucose, triacylglycerol (TAG), aspartate aminotransferase, alanine aminotransferase, liver/heart/pancreas mass and hepatic cholesterol, TAG and glycogen. Statistical analysis was performed using GraphPad Prism 9.1, with two-way ANOVA and Tukey's post-test ($p < 0.05$). In Protocol 1, APT improved glucose tolerance and reduced cardiac hypertrophy in KO mice. In Protocol 2, APT had limited effects on glycemia, serum TAG, and cholesterol. Still, APT improved metabolic and cardiovascular markers in older animals, even after short-term training, supporting its potential as a protective intervention.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Effect of resistance training on behavioral responses in the experimental model of female ADHD

Authors:

Ana Késsia do Nascimento Gomes^{1,2}
Roberto Victor Figueiredo de Oliveira Gonçalves^{1,2}
Marilene da Mata Gomes Patrício^{1,3}
Luana Basilio Ferreira^{1,3}
Emerson Lopes Olivares^{1,2,3}
Anderson Luiz Bezerra da Silveira^{1,2,3}

Affiliations:

¹ Federal Rural University of Rio de Janeiro.
² Laboratory of Physiology and Human Performance.
³ Laboratory of Cardiovascular Physiology and Pharmacology.

Keywords: ADHD. Behavior. Ovariectomy. Resistance Training.

Abstract:

There are few studies investigating attention deficit hyperactivity disorder (ADHD) in females, making them susceptible to erroneous reports and treatments. Thus, the objective of the study was to investigate the effect of pharmacological and non-pharmacological therapy on ADHD-like behaviors. The study was approved by CEUA-UFRRJ 10/2023. 40 ovariectomized female SHR, 12 weeks of age, were allocated into groups: control (CTRL), trained (TR), combined (TR+MPH) and drug (MPH). The trained group consisted of high-intensity resistance training for 8 weeks; the drug group consisted of the orogastric administration of methylphenidate at a dose of 2 mg/kg; and the control group received 0.9% saline administration. Subsequently, the animals were subjected to the Elevated Plus Maze (EPM) and Open Field (OF) tests. The following results were evidenced through from the EPM, Stretched Attend Posture (SAP): (CTRL: 13.0 ± 1.4 vs. TR: 6.1 ± 0.77 , $p = 0.00$), (CTRL: 13.0 ± 1.4 vs. TR+MPH: 5.6 ± 1.2 , $p = 0.00$), (MPH: 11.1 ± 0.4 vs. TR: 6.1 ± 0.77 , $p = 0.01$) and (MPH: 11.1 ± 0.4 vs. TR+MPH: 5.6 ± 1.2 , $p = 0.00$), entries into the open arms: (CTRL: 1.16 ± 0.1 vs. TR: 4.8 ± 0.79 , $p = 0.00$), (CTRL: 1.16 ± 0.1 vs. TR+MPH: 4.5 ± 0.6 , $p = 0.00$), (MPH: 1.16 ± 0.1 vs. TR: 4.8 ± 0.79 , $p = 0.00$) and (MPH: 1.16 ± 0.1 vs. TR+MPH: 4.5 ± 0.6 , $p = 0.00$). Number of transitions in the open and closed arms: (CTRL: 5.0 ± 0.9 vs. TR: 13.5 ± 1.6 , $p = 0.000$), (CTRL: 5.0 ± 0.9 vs. TR+MPH: 10 ± 0.8 , $p = 0.04$). The results found from the OF: Central quadrant (CTRL: 21.20 ± 3.5 vs. TR: 39.50 ± 2.3 , $p < 0.0001$), (CTRL: 21.20 ± 3.5 vs. TR+MPH: 31.2 ± 1.6 , $p = 0.02$), (TR: 39.50 ± 2.3 vs. MPH: 19.4 ± 1.2 , $p < 0.0001$) and (TR+MPH: 31.2 ± 1.6 vs. MPH: 19.4 ± 1.2 , $p = 0.006$). Signs analogous to anxiety were therefore reduced when resistance training was incorporated into the animals' routine.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Effects of maca (*Lepidium meyenii*) supplementation on sports performance in amateur runners: a randomized, double-blind, cross-over, clinical trial

Authors:

Bruno Gomes Paschoal¹
Carolina Kei Furusato¹
Luiz Eduardo Dias Diniz¹
Juliano Guarnieri²
Giuseppe Potrick Stefani^{1,3}

Affiliations:

¹ Olympic Studies Research Group (GPEO). School of Health and Life Sciences. Pontifical Catholic University of Rio Grande do Sul (PUCRS), Porto Alegre – RS, Brazil.

² Iron Legion Sports Consulting, Novo Hamburgo – RS, Brazil.

³ Postgraduate Program in Nutrition Sciences (PPGNUT). Federal University of Health Sciences of Porto Alegre (UFCSPA). Porto Alegre, RS, Brazil.

Keywords: Maca. Peruvian Maca. Sports Performance. Running. Supplementation.

Abstract:

Dietary supplementation has been investigated as a possible strategy to improve physical performance in athletes. Preclinical studies suggest that acute supplementation with Peruvian maca can be an ergogenic resource in different sports. This study aimed to evaluate the acute effects of Peruvian maca supplementation on physical performance, body composition, and food intake in amateur runners. This is a randomized, double-blind, crossover clinical trial with seven runners (5 men, 2 women). The Research Ethics Committee approved the project under number 6.057.800. Each participant underwent a submaximal test with prior supplementation of Peruvian maca or placebo, with at least 72 hours of washout for each test. Performance was assessed in different variables, such as test time, distance traveled and estimated VO_{2max} during a running protocol. In addition, food consumption, energy availability and urinary gravimetry were performed to assess variations between placebo and Maca. In all conditions, no differences were observed in energy availability (placebo 23.69 ± 11.58 kcal/kg/day versus Maca 22.01 ± 13.08 kcal/kg/day; P-value > 0.05), which indicates similar testing conditions. For the exercise test (Maca condition presented $+ \Delta 15.71 \pm 366.00$ meters) and test time (Maca condition presented $- \Delta 2.57 \pm 86.20$ sec) with no significant changes compared to placebo (P-value > 0.05). Additionally, VO_{2max} (Maca condition presented $- \Delta 10.31 \pm 19.92$ mL/kg/min) did not demonstrate significant changes between the supplementation and placebo condition (P-value > 0.05). Also, no difference was observed regarding urine gravimetry for placebo 1.022 ± 0.01 mg/dL versus for Maca 1.018 ± 0.01 mg/dL (P-value > 0.05). The results indicate that acute supplementation with Peruvian maca did not exert an ergogenic effect on physical performance or influence the hydration status of amateurs runners, indicating ineffectiveness as a short-term ergogenic strategy in amateurs runners.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Semaglutide combined with physical training: impact on diabetic kidney disease morbidity

Authors:

Eloiza de Oliveira Silva¹
Jessica Garcia¹
Carla Djamila de Pina Victoria¹
Juliana Veloso Gusmão¹
Mykelly Gomes Alves¹
Fernanda Borges²
Maria de Fatima Fernandes Vattimo¹

Affiliations:

¹ School of nursing the university of Sao Paulo.

² Southern Cross University Thematic axis: endocrinology, nephrology, exercise physiology.

Keywords: Type 2 diabetes mellitus. Diabetic kidney disease. Physical training. GLP-1. Semaglutide. HK-2 cells.

Abstract:

Introduction: Diabetic kidney disease (DKD) is one of the most common complications of type 2 diabetes mellitus (T2DM) and a leading cause of renal replacement therapy. This study investigated the renoprotective effects of a glucagon-like peptide-1 receptor agonist (GLP-1RA), semaglutide, combined with moderate physical training (PT), using both in vivo and in vitro approaches. **Methods:** Male wistar rats were allocated into six groups: controls, GLP-1RA, T2DM, T2DM+PT, T2DM+GLP-1RA and T2DM+PT+GLP-1RA, or both. Interventions lasted 28 days. Renal function (inulin clearance, serum creatinine), microalbuminuria, renal hemodynamics, oxidative biomarkers (urinary peroxides, lipid peroxidation, nitrates, tissue thiols, catalase), and mitochondrial enzyme activity (citrate synthase) were evaluated. In parallel, HK-2 cells were cultured under normoglycemic (5.5 mM) or hyperglycemic (30 mM) conditions and treated with semaglutide (400 nM) for up to 4 days. Cell viability was assessed using MTT assay. **Results:** The combination of semaglutide and PT in diabetic rats significantly improved glomerular filtration rate, reduced serum creatinine, microalbuminuria, renal oxidative stress, and vascular resistance, while enhancing renal blood flow and citrate synthase activity. In vitro, semaglutide attenuated high-glucose-induced cytotoxicity in HK-2 cells, preserving cellular viability. **Conclusion:** Combined treatment with semaglutide and moderate physical training attenuated the progression of DKD induced by T2DM. These findings reinforce the superior renoprotective effects of the combined therapy compared to isolated interventions, particularly through improved renal hemodynamics and reduced oxidative stress. Overall, the data highlight the therapeutic potential of this strategy in mitigating DKD progression



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: De IGF-1 em fibras musculares do tipo II e a adaptação óssea e muscular à natação em camundongos fêmeas

Authors:

Gisele Cristina Lopes Couto¹
Danielly Xavier Marino¹
Sabelle Gottsfritz Fernandes Queiroz Gomes¹
Jefferson Alves Costa¹
Nickollas Guilera Gonçalves Nascimento Moreira¹
Cecília Helena de Azevedo Gouveia¹

Affiliations:

¹ Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, SP, Brazil.

Keywords: Hiperexpressão. Hipertrofia. IGF-1. Interação. Ossos.

Abstract:

Fêmeas transgênicas que superexpressam uma isoforma muscular de IGF-1 (mIgF-1) em fibras do tipo II (TgIGF1) apresentam hipertrofia muscular e melhora da microarquitetura óssea femoral. Em estudo anterior, mostramos que a suspensão dos membros posteriores por 14 dias causou atrofia muscular semelhante em TgIGF1 e WT, mas piorou a microarquitetura óssea apenas nos TgIGF1. Isso sugere que o mIgF-1 depende de carga mecânica para efeitos anabólicos ósseos. Nosso objetivo foi investigar se a natação, que impõe carga pela contração muscular sem impacto, afeta osso e músculo de forma distinta em TgIGF1 e WT. Animais com três meses foram submetidos a oito semanas de natação moderada com carga progressiva. Como esperado, TgIGF1 tinham peso 12% maior que WT ($p < 0,01$), sem alterações com o treino. No início, TgIGF1 tinham maior massa muscular no tibial anterior (17%), EDL (41,3%) e gastrocnêmio (11,6%). A natação aumentou a massa de EDL nos WT (21%, $p < 0,001$), mas reduziu nos TgIGF1 (14%, $p < 0,01$), indicando adaptação distinta. TgIGF1 também apresentaram maior força de preensão nos membros anteriores (9,4%) e posteriores (12,4%). A natação teve pouco efeito no WT, mas atenuou o ganho de força nos posteriores dos TgIGF1. Apesar da maior massa muscular, TgIGF1 não apresentaram maior força óssea no início. A natação prejudicou parâmetros biomecânicos em WT, reduzindo carga máxima (12%), tenacidade (7,7%) e rigidez (11%), efeito não observado em TgIGF1. Concluímos que a superexpressão de IGF-1 altera a adaptação músculo-esquelética ao exercício, protegendo o osso, mas limitando a resposta muscular.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Gestational Amplification of Metabolic Disruptions Induced by Excessive Supplementation of Polyunsaturated Fatty Acids

Authors:

Djenifer de Toledo Maciel¹
Eduarda Martinowski
Henriques Guia¹
Bárbara Pedrozo Coelho¹
Lucas Mendes Galvão¹
Mariana Inocêncio Manzano¹
Mônica Maciel²
Katya Naliwaiko²
Matheus Felipe Zazula^{1,2}

Affiliations:

¹ Grupo de Pesquisa em Gestação e Doenças Metabólicas, Universidade Cesumar – Curitiba, Paraná, Brasil.

² Laboratório de Plasticidade Morfofuncional, Departamento de Biologia Celular, Universidade Federal do Paraná – Curitiba, Paraná, Brasil.

Keywords: Hepatic Function. Metabolic Parameters. Lipid supplementation. Pregnant Rats.

Abstract:

Pregnant rats, analyzing biochemical, metabolic, and morphometric parameters. Forty-eight Wistar rats were used, receiving supplementation with polyunsaturated fatty acids n-3 and n-6 for 30 days before mating. After this period, the rats were mated and divided into two groups: half were euthanized 24 hours after mating (GD0), and the other half on the 18th day of pregnancy (GD18). Body, biochemical, and hepatic metabolic parameters were evaluated (CEUA 1452/2022). A significant interaction between group and age was observed for several variables. On GD0, there were no differences in body weight between groups, but on GD18, the control group had the highest weight, while the supplemented groups showed progressively lower values. The Lee Index followed this trend, with the highest values in the control group. Adiposity was highest in the SPL3 group on GD0, while on GD18, the SPL9 group had the highest values. Liver weight was higher in the control group at both time points. Supplementation with n-3 and n-6 fatty acids positively modulated glucose and insulin levels, as well as impacting TAG and cholesterol levels, especially on GD18. Hepatic enzymes, such as AST and ALT, also showed variations, with lower ALT levels in the supplemented groups at the end of pregnancy. These results highlight the impact of supplementation with polyunsaturated fatty acids on liver metabolism and other metabolic parameters during pregnancy, emphasizing the importance of balanced intake of these nutrients to prevent metabolic and inflammatory complications.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Time course of hepatic bioconversion of alpha-linolenic acid into long- chain polyunsaturated fatty acids

Authors:

Thaiane da Silva Rios¹
Talitha Silva Meneguelli¹
Amanda Rocha Fujita¹
Beatriz Moreira Nascimento¹
Camila Venturini Ayres Cunha¹
Leandro Kansuke Oharomari¹
Adelino Sanchez Ramos Da Silva³
Eduardo Rochete Ropelle²
José Rodrigo Pauli²
Dennys Esper Cintra¹

Affiliations:

¹Nutritional Genomics Laboratory – Faculty of Applied Sciences – State University of Campinas (UNICAMP).

²Exercise Molecular Biology Laboratory – Faculty of Applied Sciences – State University of Campinas (UNICAMP).

³School of Physical Education and Sport of Ribeirão Preto, University of São Paulo (USP).

Keywords: Fatty acid metabolism. Fatty acid desaturase 2. Nutrigenomics. Omega-3. Omega-6.

Abstract:

Linoleic (LA) and alpha-linolenic (ALA) acids are polyunsaturated fatty acids (PUFA) that are bioconverted mainly in the liver, through desaturation and elongation, into arachidonic acid (AA) or eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), respectively. The study aimed to evaluate the time course of hepatic bioconversion of these long-chain PUFA. The study was approved by CEUA (#6398-1/2024) from UNICAMP. Male C57BL/6 mice (6wk old; n=7/group) received 100 µL of saline or flaxseed oil (~50% ALA) at times 0, 4, 8, 16, and 24 hours. After euthanized, the liver was removed for *Fads2* gene expression and fatty acids from the liver and serum evaluated by gas mass spectrometry. Enzymatic activity was assessed by C18:4(ω3)/C18:3(ω3) and C18:3 (ω6)/C18:2(ω6) ratios. An increase in the *Fads2* was observed after 16h of ALA administration, with normalization after 24h. Regarding enzymatic activity, a reduction was observed at 4h and 8h for the C18:4 (ω3)/C18:3(ω3) and at 8h for the C18:3 (ω6)/C18:2(ω6) in the liver. While after 16h, an increase in enzymatic activity was observed compared to the saline and 0h groups. In the evaluation of hepatic fatty acids, an increase in the incorporation of ALA was observed after 4h and a reduction after 24h. An increase in EPA was observed at 4h, 8h, and 16h, while a decrease in the ω6/ω3 was seen at 4h and 8h. No statistical differences were observed for DHA, the sum of EPA+DHA, LA, AA, and PUFA. In the serum, no difference was observed in the C18:3 (ω6)/C18:2 (ω6) or the LA and AA content. An increase in ALA was also observed after 4h, with a reduction after 8h. There was an increase in EPA at 4h, 8h, and 24h, and in DHA and EPA+DHA after 24h. The ALA peak kinetics coincided with serum and liver concentration after 4 hours, in parallel with bioconversion time into EPA; meanwhile, in serum, there was an increase in DHA after 24h. Thus, the fatty acids elongation and desaturation obey specific times, faster than expected.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: The short- or long-term exposition to a high-fat diet does not change the behavioral profile of adolescents C57BL/6 mice

Authors:

Gabriela Pandini Silote¹
Aline Franco de Campos¹
Nilton José dos Santos²
Ísis De Cássia Alves Martins¹
Priscilla Karla Fernandes Lopes³ Adriana Souza Torsoni^{1,4}
Marcio Alberto Torsoni^{1,4}

Affiliations:

¹ Laboratory of Metabolic Disorders (Labdime), School of Applied Sciences, University of Campinas (UNICAMP), Limeira, SP, Brazil.
² Department of Genetics, Medical School of Ribeirão Preto (FMRP), University of São Paulo – USP, Ribeirão Preto, SP, Brazil.
³ Federal University of Lavras (UFLA).
⁴ Obesity and Comorbidities Research Center, University of Campinas (UNICAMP), Campinas, SP, Brazil.

Keywords: Adolescent C57BL/6 mice; anxiety; depression; obesity.

Abstract:

Background: Adolescent obesity has increased worldwide during the pandemic, due to lifestyle changes and exposure to stressful events. Adolescence is a vulnerable period for consumption of palatable high-fat diets (HFD), which can consequently lead to metabolic dysfunction and have important psychological impacts, including anxiety and depression. Here, we investigated the effect of a short-term (3 days) or long-term (30 days) high-fat diet on depressive and anxiety-related behaviors in adolescent C57BL/6 mice undergoing behavioral testing. Methods: Adolescent male C57BL mice (PN35-PN70; Weighing: 18-22.5 g) from the animal facility of the University of Campinas (CEUA Number: 6184-1/2023). The animals received standard diet (Ctrl) or HFD (45% kJ fat) for 4 (short-term) or 30 days (long-term). On test day, we submitted the mice to novel object recognition (NOR), open field test (OFT), elevated plus-maze (EPM) and forced swim test (FST). The mice were subjected to fasting for 12h to determine the fasting blood glucose. Results: The exposition to short- and long-term HFD in adolescent C57BL/6 mice increased in body weight (3 days: Bonferroni: $p=0.0109$; 30 days: Bonferroni: $p<0.0001$) compared to Ctrl diet group, without changing the cumulative weight gain (3 days: $t(18)=0.5089$; $p=0.617$; 30 days: $t(18)=1.093$; $p=0.2886$) and fasting blood glucose (3 days: $t(18)=1.339$; $p=0.1973$; 30 days: $t(18)=0.9272$; $p=0.3661$). Otherwise, no changes were found in the parameters evaluated in the NOR, OFT, EPM and FST test. Conclusion: The results indicate that short- and long-term HFD produces changes in murinometric parameters without altering the behavioral profile of male adolescent C57BL/6 mice exposed to anxiety- and depression-related tests. Further investigations in neuroinflammatory markers are needed to better understand the molecular mechanism involved in our results.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Cardiometabolic responses to Vitamin D supplementation in elderly ribeirinhos

Authors:

Elca Silvania da Silva Abreu¹
Caroline Oliveira dos Anjos¹
Renata Miyabara¹
Ovidiu C. Baltatu^{1,2,3}
Luciana A Campos³

Affiliations:

1 Center of Innovation, Technology, and Education (CITE) at Anhembi Morumbi University, Anima Institute, Sao Jose dos Campos Technology Park, São José dos Campos, Brazil.
2 Alfaisal University College of Medicine, Riyadh, Saudi Arabia.
3 UNINOVAFAPI University Center, Teresina, Piauí, Brazil.

Keywords: Diabetes. Elderly ribeirinhos. Femur fracture. Hypertension. Vitamin D supplementation.

Abstract:

Vitamin D's role in calcium homeostasis and glucose metabolism suggests complex physiological interactions affecting post-surgical recovery. This study investigated the physiological responses to vitamin D supplementation in elderly Ribeirinhos following femur fracture surgery. Sixty participants (mean age 77.8 ± 1.2 years) were evaluated based on pre-existing adherence to weekly oral vitamin D supplementation (5,000 IU; n=22 supplemented, n=38 non-supplemented). Physiological parameters assessed included serum 25-hydroxyvitamin D, glucose homeostasis, calcium regulation, and recovery indicators. Supplemented patients exhibited enhanced vitamin D status (50.26 ± 2.55 vs. 21.74 ± 0.84 ng/mL; $p < 0.0001$) and calcium homeostasis (ionized calcium: 1.29 ± 0.01 vs. 1.08 ± 0.02 mg/dL; $p < 0.001$). Glucose regulation was significantly improved in the supplemented group (83.8 ± 1.4 vs. 152.2 ± 9.6 mg/dL; $p < 0.0001$), suggesting enhanced metabolic control. These physiological improvements corresponded with accelerated recovery, evidenced by reduced hospitalization duration (18.2 ± 2.73 vs. 23.1 ± 0.91 days; $p = 0.0383$). Notably, cardiometabolic assessment revealed high prevalence of hypertension (73.3%) and diabetes (31.7%), with 25% presenting both conditions. These findings demonstrate the integrative physiological effects of vitamin D supplementation on mineral homeostasis, glucose regulation, and recovery processes, while highlighting the need for comprehensive cardiometabolic screening in this population. Ethics Committee Approval: CAAE n. 69275023.8.0000.5492

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Preventive effect of *Citrus bergamia* on pathophysiological processes involved in metabolic hepatic steatosis in animals

Authors:

Núbia Alves Grandini¹
Maria Júlia Ribeiro²
Taynara Aparecida Vieira¹
Murilo Dalarme Tanganini²
Fabiane Valentini Francisqueti-Ferron³
Fernando Moreto¹
Guilherme Ribeiro Romualdo¹
Camila Renata Correa¹

Affiliations:

¹ São Paulo State University (UNESP), Medical School, Botucatu.

² São Paulo State University (UNESP), Institute of Biosciences, Botucatu.

³ Integrated Colleges of Bauru (FIB), Bauru.

Keywords: Bioactive compounds. Inflammation. Metabolic hepatic steatosis. Obesity. Oxidative Stress.

Abstract:

Oxidative stress and inflammation resulting from high consumption of diets rich in sugar and fat is related to the pathophysiology of Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD). *Citrus bergamia* (CB) is rich in flavonoids with antioxidant and anti-inflammatory properties. The aim was to evaluate the preventive effect of CB on pathophysiological processes involved in MASLD. Male *Wistar* rats were allocated into 3 groups: control diet (C, n=6), high sugar and fat diet (HSF, n=6), and HSF diet+CB (HSF+CB, n=6), for 20 weeks. CB and vehicle were administered by gavage at 250 mg/kg/day. Then, adiposity index (AI, %), systolic blood pressure (SBP, mmHg), triglycerides (TG, mg/dl), insulin resistance (TyG), tumor necrosis factor alpha (TNF- α , pg/mg of protein), Interleukin-6 (IL-6, pg/mg of protein), Malondialdehyde (MDA, nmol/mg of protein), Carbonyl Proteins (CBO, nmol/mg of protein), and steatosis in liver tissue were evaluated. Data were compared by one-way ANOVA or Kruskal-Wallis with *post-hoc* Tukey's or Dunn's tests, respectively. CEUA: 1337/2019. The HSF group presented higher AI (HSF:8.20 $\pm$ 1.67 vs C:2.18 $\pm$ 0.409, p <0.001), SBP (HSF:148 $\pm$ 11.1 vs C:122 $\pm$ 5.09, p <0.001), TG (HSF: 123 $\pm$ 21.8 vs C:18.2 $\pm$ 2.64, p <0.001), TyG (HSF:8.60 $\pm$ 0.223 vs C:6.66 $\pm$ 0.226, p <0.001), TNF- α (HSF:54.5 $\pm$ 9.26 vs C:31.2 $\pm$ 7.66, p =0.001), IL-6 (HSF:223 $\pm$ 38.8 vs C:157 $\pm$ 41.4, p =0.018), MDA (HSF:34.1 $\pm$ 4.78 vs C:23.3 $\pm$ 3.04, p <0.001), CBO (HSF:3.22 $\pm$ 0.511 vs C:2.07 $\pm$ 0.282, p <0.001) and hepatic steatosis compared to the C group. CB improved the AI (HSF+CB:5.23 $\pm$ 1.14 vs HSF:8.20 $\pm$ 1.67, p =0.002), TG (HSF+CB:52.5 $\pm$ 12.2 vs HSF: 123 $\pm$ 21.8, p <0.001), TyG (HSF+CB:7.74 $\pm$ 0.310 vs HSF:8.60 $\pm$ 0.223, p <0.001), TNF- α (HSF+CB:39.5 $\pm$ 9.45 vs HSF:54.5 $\pm$ 9.26, p =0.026), MDA (HSF+CB:21.7 $\pm$ 3.29 vs HSF:34.1 $\pm$ 4.78, p <0.001) and CBO (HSF+CB:2.03 $\pm$ 0.295 vs HSF:3.22 $\pm$ 0.511, p <0.001) compared to the HSF group. CB acted on important parameters involved in the pathophysiological processes of MASLD and may be applied as a preventive agent.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: The effect of bergamot on adipose tissue modulates changes in the redox and inflammatory state in animals fed a high sugar-fat diet

Authors:

Taynara Aparecida Vieira¹ Jordanna Cruzeiro¹ Matheus Antônio Filiol Belin¹
Marina de Paula Salomé dos Santos¹ Núbia Alves Grandini¹ Erika Tiemi Nakandakare Maia¹
Júlia Camargo de Almeida² Luís Eduardo Sormani¹
Murilo Dalarme Tanganini²
Fabiane Valentini Francisqueti Ferron³ Artur Junio Togneri Ferron³ Camila Renata Correa¹

Affiliations:

¹ São Paulo State University (UNESP), Medical School, Botucatu, Brazil.

² São Paulo State University (UNESP), Institute of Biosciences, Botucatu, Brazil.

³ Integrated Colleges of Bauru (FIB), Bauru, Brazil.

Keywords: Adipose tissue. Bioactive compounds. Inflammation. Obesity. Oxidative stress.

Abstract:

Obesity results from excessive adipose tissue accumulation, promoting inflammation and oxidative stress. Bergamot (*Citrus bergamia*) is a fruit rich in flavonoids, which can act to combat the changes caused by this condition. Thus, this study aimed to evaluate the effect of bergamot (BG) on adipose tissue as a modulator of changes in the redox and inflammatory state in animals fed a high sugar-fat diet. Male Wistar rats ($n=28 \pm 150g$) were allocated into two groups: a control diet (C, $n=14$) and a high sugar-fat diet (HSF, $n=14$) plus 25% sucrose in drinking water for 20 weeks. After the 20th week, the groups were reallocated to receive, via gavage, BG (250 mg/kg/day) or water for 10 weeks: C ($n=7$), C+BG ($n=7$), HSF ($n=7$) and HSF+BG ($n=7$). This study was approved by the Animal Ethics Committee (1337/2019). In the end, the adiposity index (AI, %) and, in adipose tissue, the levels of inflammatory cytokines were evaluated: tumor necrosis factor alpha (TNF- α , pg/mg protein) and interleukin-6 (IL-6, pg/mg protein), and markers of oxidative stress: malondialdehyde (MDA, nmol/mg protein) and protein carbonylation (PC, nmol/mg protein). Data were compared by two-way ANOVA with Tukey's post-hoc test; ($p<0.05$). The results showed that the HSF group presented obesity (HSF 11.2 ± 1.9 vs C 5.0 ± 0.9 , $p<0.001$) and alterations in the redox and inflammatory state compared to the C group. In contrast, the HSF+BG group presented a reduction in AI (HSF+BG 8.16 ± 1.41 vs HSF 11.2 ± 1.9 , $p<0.001$) and improvement in the levels of MDA (HSF+BG 592 ± 167 vs HSF 1037 ± 276 , $p<0.001$), PC (HSF+BG 166 ± 40 vs HSF 352 ± 32 , $p<0.001$), TNF- α (HSF+BG 468 ± 112 vs HSF 798 ± 100 , $p<0.001$) and IL-6 (HSF+BG 20665 ± 29 vs HSF 2736 ± 641 , $p=0.012$) when compared to the HSF group. Therefore, BG was able to act on adipose tissue, reducing the adiposity index and modulating changes in the redox and inflammatory state in animals fed an HSF diet.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Effect of Ora-Pro-Nóbis supplementation in tilapia diets: Amino Acid Metabolism and Mitochondrial Energy Metabolism

Authors:

Pedro Trábulo Junqueira Franco¹ Charles Henrique dos Santos¹ Milena Cia Retcheski¹
Sílvia Romão¹
Luisa Helena Cazarolli¹

Affiliations:

¹ Universidade Federal da Fronteira Sul.

Keywords: *Pereskia aculeata*. Tilapia. Amino acid metabolism. Mitochondrial energy metabolism.

Abstract:

The ora-pro-nóbis (OPN, *Pereskia aculeata*) is a plant with a high content of proteins, minerals, vitamins and bioactive compounds, as well as low toxicity, and has a high potential as a substitute for traditional protein sources in aquatic organism diets, such as fishmeal and soy. This study evaluated the influence of supplementing tilapia diets with OPN on amino acid metabolism and mitochondrial energy metabolism. The experiment was conducted in a recirculating system where tilapia was fed diets with different concentrations of OPN meal (0, 5, 10 and 15%) for 57 days (CEUA protocol number 2545170619). At the end of the experimental period, the livers (n = 10 for each treatment) were collected, homogenized and the mitochondria were isolated. The activities of the enzymes citrate synthase (CS), malate dehydrogenase (MDH) and NADH dehydrogenase (NADH-DES) were assessed from the isolated mitochondria. The activities of the enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST) and glutamate dehydrogenase (GLDH) were determined in liver homogenates. Data were expressed as the mean $\pm$ standard deviation and to the statistical analysis, Shapiro-Wilk test and Bartlett's test were used. Also, data were analyzed by analysis of variance, and the means were compared using the Tukey test at the 5% level of significance. No significant changes were observed in the activities of CS, MDH and NADH-DES enzymes. On the other hand, significant increases in the activity of amino acid metabolizing enzymes were observed in the tilapia liver, with AST and ALT showing increases in the 10 and 15% groups compared to the control (AST - 58 and 73%; ALT - 22 and 25%), while GLDH was significantly higher in the 10% group compared to the control (67%). The lack of effect of OPN on the enzymes of mitochondrial energy metabolism may indicate that it was a good quality dietary source that did not alter the necessary energy requirements of tilapia and probably directed the amino acids towards protein synthesis, reinforcing its potential as a growth inducer in fish.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Evaluation of safety and effects of *Pereskia aculeata* on cellular carbohydrate metabolism in Nile tilapia liver

Authors:

Pedro Trabulsi Junqueira Franco¹
Charles Henrique dos Santos¹
Milena Cia Retcheski¹
Silvia Romão¹
Luisa Helena Cazarolli¹

Affiliations:

¹ Universidade Federal da Fronteira Sul.

Keywords: Cellular metabolism. *Oreochromis niloticus*. Ora-pro-nóbis. PANC.

Abstract:

The maintenance of homeostasis and cellular energy production is extremely important for the proper development of aquatic organisms. In this study, we evaluated ora-pro-nóbis (OPN; *Pereskia aculeata*), a plant rich in proteins, vitamins and minerals, and its influence on the energy metabolism and biochemical profile of tilapia. The experiment was conducted in a recirculating system where tilapia were fed diets with different concentrations of OPN meal (0, 5, 10 and 15%) for 57 days (CEUA protocol number 2545170619). At the end of the experiment, blood samples were collected to determine the activity of alkaline phosphatase (AP), lactate dehydrogenase (LDH), alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and liver samples were collected to determine the activity of hexokinase, phosphofructokinase and glucose-6-phosphate dehydrogenase. The absence of changes in the activity of ALT, AST, LDH and FA enzymes in the blood indicates that the concentrations used in the test are safe and do not cause toxicity to the various organs. On the other hand, significant reductions in HK activity were observed in the 15% group compared to the control group (29%) and the 10 and 15% groups (35 and 26%, respectively). PFK activity was decreased in the 10 and 15% groups compared to the control (53 and 62%, respectively). In addition, G6PDH activity was significantly reduced in the 15% group compared to the other experimental groups (24% on average). The reduction in the activity of these enzymes at the higher concentrations of OPN in the tilapia diet indicates that there may be a decrease in energy availability, since a reduction in the activity of these enzymes leads to a reduction in the energy readily available for cellular processes. Thus, OPN supplementation at low concentrations may be indicated without compromising the biochemical and physiological functions of the fish.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: The modulatory effect of Ora-pro-nóbis on the lipid metabolism of the Nile tilapia

Authors:

Charles Henrique dos Santos¹
Luciano Tormen¹
Milena Cia Retcheski¹
Pedro Trabulsi Junqueira Franco¹
Silvia Romão¹
Luisa Helena Cazarolli¹

Affiliations:

¹ Universidade Federal da Fronteira Sul.

Keywords: Digestion. lipid metabolism. *Oreochromis niloticus*. supplementation.

Abstract:

The activity of enzymes involved in digestion and cellular lipid metabolism is extremely important in determining the profile of fatty acids and reserves in aquatic organisms. Therefore, we evaluated the effect of supplementing ora-pro-nóbis flour (OPN; *Pereskia aculeata*) on lipid metabolism and fatty acid profile of tilapia flesh. The experiment was carried out in a recirculating system where tilapia were fed diets with different concentrations of OPN meal (0, 5, 10 and 15%) for 57 days (CEUA protocol number 2545170619). The activities of intestinal lipase, fatty acid synthase (FAS) and β -hydroacyl CoA dehydrogenase (HOAD) in the tilapia liver were evaluated. The fatty acid profile of the meat was determined by GC coupled to a Shimadzu mass spectrometer, model GCMS-QP2010 Ultra. There was an increase in lipase activity compared to the control and 5% groups (89 and 80%, respectively) and in AGS in the 10 and 15% groups compared to the control group (100% and 81%, respectively). On the other hand, HOAD activity was not affected by OPN treatments. In addition, there was a predominance of polyunsaturated fatty acids (PUFA) (65%) and less saturated fatty acids (SFA) (34%) in the fatty acid profile of the meat. In addition, a significant and dose-dependent increase in PUFA was observed in all experimental groups (Σ PUFA control, 5, 10 and 15%: 30.91 ± 0.15 ; 31.91 ± 0.13 ; 33.43 ± 0.14 ; 34.07 ± 0.25) and for very long chain fatty acids (LC-PUFAs) (Σ LC-PUFAs control, 5, 10 and 15%: 9.98 ± 0.53 ; 10.45 ± 0.59 ; 12.5 ± 0.56 ; 11.13 ± 0.50) the significant increases were in the 5 and 10% groups compared to the control group. The metabolic changes observed in this study indicate that OPN has the potential to positively modulate the lipid metabolism of tilapia by improving the fatty acid profile, especially at higher concentrations.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Effect of Ora-pro-nobis on growth and body composition of Nile tilapia

Authors:

Charles Henrique dos Santos¹ Milena Cia Retcheski¹ Pedro Trabulsi Junqueira Franco¹ Silvia Romão¹ Luisa Helena Cazarolli¹

Affiliations:

¹ Universidade Federal da Fronteira Sul.

Keywords: Diet. Growth. Ora-pro-nóbis. Tilapia.

Abstract:

The main challenges in aquaculture are related to the cost and quality of the feed and the nutrition of the animals, with protein being the most expensive component of the feed. The ora-pro-nóbis (OPN; *Pereskia aculeata*), a non-conventional food plant (NCFP), has a high concentration of proteins, minerals and bioactive compounds and has a high potential for partial substitution of traditional protein sources. This study evaluated the potential use of OPN in Tilapia diets. The experiment was conducted in a recirculating system where tilapias were fed diets with different concentrations of OPN meal (0, 5, 10 and 15%) for 57 days (CEUA protocol number 2545170619). Zootechnical parameters were evaluated based on measurements of weight, total and standard length, height, width and feed consumption (weight gain; width gain; height gain; standard length gain; total length gain; condition factor; specific growth rate; relative feed consumption; apparent feed conversion) and centesimal composition of tilapia meat (total protein and lipid content, ash and fillet moisture). The results indicate that the inclusion of 5% OPN meal in the diet resulted in significant improvements in weight gain, feed efficiency and protein quality of the fillets compared to the control group. In addition, the specific growth rate showed no significant changes in any of the experimental groups. However, higher concentrations (15%) resulted in a decrease in condition factor and overall performance, possibly due to the presence of antinutritional factors. In terms of centesimal composition, there were no significant differences in moisture and ash in any of the treatments, and the 5% group had a higher total lipid content compared to all the experimental groups. These results suggest that ora- pro-nóbis meal has great potential as an additive in tilapia diets at concentrations below 10%.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Gut microbiota composition of non-obese type 2 diabetic rats (Goto-Kakizaki)

Authors:

Gabriela Mandú Gimenes¹ Vinícius de Rezende Rodovalho² Tiago Bertola Lobato¹

Thaís Martins Rodrigues³

Joice Naiara Bertaglia Pereira⁴ João Carlos de Oliveira Borges¹ Amara Cassandra dos Anjos

Alves¹ Renata Gorjão¹

Marco Aurelio Ramirez Vinolo² Rui Curi^{1,4}

Affiliations:

¹ Cruzeiro do Sul University (São Paulo, SP).

² Laboratory of Immunoinflammation, Institute of Biology, University of Campinas (São Paulo, SP).

³ Laboratory of Endocrine Physiology, Institute of Biomedical Sciences, University of São Paulo (São Paulo, SP).

⁴ Butantan Institute (São Paulo, SP).

Keywords: Gut microbiota. Type 2 diabetes. Intestinal transit. Inflammation.

Abstract:

Gut microbiota dysbiosis is associated with the onset of type 2 diabetes mellitus (T2DM) by affecting intestinal permeability, immune function, inflammation, insulin-signaling pathways, and glucose metabolism. The Goto-Kakizaki (GK) rat is an experimental model that develops T2DM spontaneously without obesity and exhibits chronic inflammation, insulin resistance (IR), glucose intolerance, and gastrointestinal features such as constipation, intestinal remodeling, decrease of excitatory neurons in the myenteric plexus, and altered gut microbiota composition. We studied 21, 60, and 120-day-old GK and Wistar (WT) rats to investigate how the diabetic phenotype (without obesity), intestinal transit, and gut microbiota composition change over time. This study was approved by the Cruzeiro do Sul University Animal Ethics Committee (001/2024). At 21 days old, GK rats already exhibit IR, glucose intolerance, and slow intestinal transit compared to age-matched WT. The beta diversity (Bray-Curtis) is different longitudinally (same group at the three ages) and between the age-matched diabetic and control groups at the studied time points. The differential abundance analysis of gut microbiota shows a lower abundance of *Faecalibaculum* and *Enterococcus* at the genus level in 21-day-old GK compared with WT rats. At 60 days, the GK rats have decreased *Faecalibaculum* and *Dubosiella* and increased *Peptoclostridium* abundance compared to the control group. The gut microbiota of GK at 120 days has high *Pseudomonas*, *Escherichia-Shigella*, and *Alistipes* and low *Shuttleworthia* and *Lachnospira* than WT rats. In conclusion, altered gut microbiota composition (decrease in short-chain fatty acids-producing bacteria and increase in inflammation-associated genus) become more notable with age and is probably associated with the slow intestinal transit, IR and glucose intolerance in GK rats.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title:

Gestational protein restriction accelerates male bone loss during aging

Authors:

Bruno Calsa¹

José Antônio Rocha Gontijo¹ Milton Santamaria-Jr² Patrícia Aline Boer¹

Affiliations:

¹ Fetal Programming and Hydroelectrolyte Metabolism Laboratory, Department of Internal Medicine, Faculty of Medical Sciences at State University of Campinas - UNICAMP, Campinas, SP, Brazil.

² Department of Social and Pediatric Dentistry, Institute of Science and Technology - College of Dentistry, UNESP - São Paulo State University, São José Dos Campos, SP, Brazil.

Keywords: DOHaD, Femur, Osteoporosis.

Abstract:

Gestational protein restriction is associated with metabolic disruptions, including hypertension, cardiac hypertrophy, and diabetes during adulthood. However, no studies have evaluated bone physiology in low-protein animals during aging, leaving a gap in understanding how gestational nutritional stress affects bone health in later life. This study was approved by ethic committee (5923-1/2021). C57BL/6J female mouse were mated and randomized into two groups during gestation: normal protein (NP, 17% casein, n=5) or low protein (LP, 6% casein, n=5). After birth, dams and pups received standard diet. Male pups' femurs were collected with 12- and 18-months age. Microtomography computerized was employ to evaluate trabecular parameters in femoral metaphysis. The gestational protein restriction induces a significant decrease in percentage of bone (NP: 7.46 ± 1.0 ; LP: 3.75 ± 0.7 ; $p=0.0009$) and bone mineral density (NP: 19.4 ± 1.8 ; LP: 12.6 ± 1.9 ; $p=0.002$) at 18 months. Trabecular number was reduced in 18M-LP animals (LP: 0.00053 ± 0.0003 ; $p=0.008$) compared to their age-control group (NP: 0.0012 ± 0.0001). Trabecular spacing was reduced in 12M-LP animals (LP: 252.3 ± 10.4 ; $p=0.006$) compared to NP group (313.0 ± 27.2). Trabecular Pattern Factor was increased in 18M-LP animals (LP: 0.038 ± 0.004 ; NP: 0.024 ± 0.002 ; $p=0.002$). Also, Structural Model Index was increased in 18M-LP animals (LP: 2.86 ± 0.15 ; $p=0.006$) compared to age-control (NP: 2.46 ± 0.11). Maternal malnutrition significantly impairs bone histoarchitecture, leading to lower bone mass and mineral density, along with a disorganized trabecular structure characterized by rod-like trabeculae. We have previously demonstrated that protein restriction during pregnancy accelerates aging and now we show early occurrence of bone loss and onset of osteoporosis.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Effects of lycopene supplementation on oxidative stress and collagen deposition in the liver of diabetic and normal rats.

Authors:

Marcus Vinicius de Souza ^{1,2}

Silas Ouma Akalla¹ Bruno Teixeira Martins ^{2,3}

Raianne dos Santos Baleeiro ^{2,3} Wanderson Geraldo de Lima^{2,3} Carla Speroni Ceron⁴

Silvia Paula-Gomes^{1,2}

Affiliations:

¹ Laboratory of Biochemistry and Molecular Biology, Department of Biological Sciences, Research Center in Biological Sciences, Federal University of Ouro Preto, Ouro Preto, Minas Gerais, Brazil.

² Postgraduate Program in Biological Sciences, Research Center in Biological Sciences, Federal University of Ouro Preto, Ouro Preto, Minas Gerais, Brazil.

³ Laboratory of Morphopathology, Department of Biological Sciences, Research Center in Biological Sciences Federal University of Ouro Preto, Ouro Preto, Minas Gerais, Brazil.

⁴ Laboratory of Endocrine Physiology, Department of Biological Sciences, Research Center in Biological Sciences, Federal University of Ouro Preto, Ouro Preto, Minas Gerais, Brazil.

Keywords: Liver. Diabetes. Oxidative stress. lycopene.

Abstract:

Diabetes mellitus (DM) can cause serious liver complications, such as hepatic steatosis, inflammation, and even cirrhosis. This study evaluated the effects of lycopene on the liver of normal and diabetic rats. 80 male Wistar rats were divided into 5 groups: control (CON), lycopene control (LIC), diabetic (DM), diabetic insulin (DINS), and diabetic lycopene (DLIC) (Ethical No. 7807301122). DM was induced with alloxan (60 mg/kg) and lycopene supplementation (45 mg/kg) administered by gavage for 30 days. DM showed reduced body mass (39%) and liver mass (20%) compared to CON, while DLIC improved body mass (51%) and liver (16%) compared to DM. DM increased food intake (40%) compared to CON, and DLIC reduced it (30%) when compared to DM. Blood glucose (119%), triglycerides (163%) and total cholesterol (50%) increased in DM compared to CON and DLIC reduced glycemia (53%), triglycerides (60%) and total cholesterol (50%) compared to DM. DM demonstrated a reduction (87%) in catalase (CAT) activity and reduced glutathione (GSH) levels (45%) compared to CON. DLIC showed an increase (~5x) in CAT activity and GSH levels (108%) compared to DM. DM demonstrated an increase in protein carbonyl (PCO) levels (~2x), TBARS levels (~4x) and a reduction (92%) in sulfhydryl groups (NPSH) compared CON. DLIC showed a decrease in PCO (75%) and TBARS (79%) and an increase in NPSH (~10x) compared to DM. DM increased inflammatory infiltrates (27%) and type III/IV collagen deposition (3.6x) compared to CON and DLIC showed a decrease in inflammatory infiltrates (15%) and type III/IV collagen deposition (76%). Lycopene improves body and liver masses, reduces food intake. Furthermore, it improves biochemical parameters, oxidative stress/inflammation and reduces collagen deposition in the liver of diabetic rats. These findings suggest that lycopene is an interesting strategy for controlling diabetic complications in the liver. Supported by FAPEMIG (APQ02029-21) and UFOP.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Metformin controls oxidative stress, inflammation, and collagen deposition in the liver of normal and diabetic rats.

Authors:

Silas Ouma Akalla¹

Marcus Vinicius de Souza^{1,2} Bruno Teixeira Martins^{2,3} Raianne dos Santos Baleeiro^{2,3}

Wanderson Geraldo de Lima^{2,3} Carla Speroni Ceron⁴

Silvia Paula-Gomes^{1,2}

Affiliations:

¹ Laboratory of Biochemistry and Molecular Biology, Department of Biological Sciences, Research Center in Biological Sciences, Federal University of Ouro Preto, Ouro Preto, Minas Gerais, Brazil.

² Postgraduate Program in Biological Sciences, Research Center in Biological Sciences, Federal University of Ouro Preto, Ouro Preto, Minas Gerais, Brazil.

³ Laboratory of Morphopathology, Department of Biological Sciences, Research Center in Biological Sciences Federal University of Ouro Preto, Ouro Preto, Minas Gerais, Brazil.

⁴ Laboratory of Endocrine Physiology, Department of Biological Sciences, Research Center in Biological Sciences, Federal University of Ouro Preto, Ouro Preto, Minas Gerais, Brazil.

Keywords: Diabetes, Liver, Metformin, Oxidative Stress.

Abstract:

Diabetes mellitus is a metabolic condition characterized by impaired cellular metabolism due to inadequate insulin production and/or insulin resistance and it is connected to hepatic complications such as loss of liver mass, steatosis, fibrosis and cirrhosis. This study investigated the effects of metformin in the control of oxidative stress/inflammation and collagen deposition in the liver of normal and diabetic rats. 80 male Wistar rats were divided into 5 groups: control (CON), metformin control (MET), diabetic (DM), diabetic insulin (DINS), and diabetic metformin (DMET) (Ethical No. 7807301122). DM was induced with alloxan (60 mg/kg) and metformin treatment (250 mg/kg) administered by gavage for 30 days. DM showed reduced body (39%) and liver (20%) masses in relation to CON, while DMET improved body (51%) and liver (26%) masses relative to DM. DM increased food intake (40%) in comparison to CON, and DMET reduced it (31%) relative to DM. Glycemia (120%), triglycerides (119%), and total cholesterol (163%) were elevated in DM compared to CON, but DMET reduced glycemia (55%), triglycerides (53%), and total cholesterol (28%) relative to DM. DM showed increased TBARS levels (~4x) and reduced sulfhydryl groups (NPSH) (92%) compared to CON, while DMET decreased TBARS (85%) and increased NPSH (~10x) relative to DM. DM also increased inflammatory infiltrates (27%) and type III/IV collagen deposition (79%) compared to CON, whereas DMET reduced inflammatory infiltrates (39%) and collagen deposition (44%). Metformin improved body and liver masses, reduced food intake and biochemical parameters, alleviated oxidative stress/inflammation, and decreased collagen deposition in diabetic liver. These findings suggest that in addition to classic effects of metformin on glycemic control, it is also an effective therapeutic strategy for blocking liver complications as fibrosis.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Impact of Different Dietary Patterns on Inflammatory and Immune Response Following Exercise-Induced Muscle Damage in Trained Men

Authors:

Beatriz Rugila Salvalággio¹ Andressa Roehrig Volpe-Fix¹ Bijar Ghafouri² Bruno Moreira Silva¹
Denisse Marginean²
Ronaldo Vagner Thomatieli-Santos¹

Affiliations:

¹ Universidade Federal de São Paulo (UNIFESP). ² Linköping University.

Keywords: Exercise. Inflammation. Neutrophils. Vegetarianism.

Abstract:

Exercise-induced muscle damage (EIMD) triggers inflammatory responses. Nutritional factors as vegetarian diets can modulate exercise responses and muscle recovery. The aim of this study was to evaluate the impact of different dietary patterns on the concentration of plasmatic cytokines and neutrophil function following EIMD. This study was approved by the Research Ethics Committee of UNIFESP/Hospital São Paulo, No. 0084/2023. An EIMD protocol was applied in healthy, trained men aged 20-45 years. Participants were assigned to groups: omnivore (OMN), ovolactovegetarian (OLV), and strict vegetarian (SV). A maximal voluntary contraction (MVC) test was conducted, and blood samples were collected at three time points: (1) pre (PRE), (2) immediately post (POST), and (3) 24 hours post-exercise (24H POST). Twenty-two participants (OMN = 7; OLV = 7; SV = 8) were evaluated. A significant age difference was observed between groups ($F(2, 19) = 6.93$; $p = 0.005$; $\eta^2 = 0.422$), specifically between the OMN and SV groups (-10.57 years; $p_{\text{Tukey}} = 0.004$). Therefore, age was considered a covariate for the following statistical analyses. MVC tests revealed a significant difference in peak torque over time ($F(2, 21) = 3.397$; $p = 0.045$; $\eta^2 p = 0.159$) between the PRE (262 ± 69.3 Nm) and POST (211 ± 63.8 N·m) (50.03 N·m; $p_{\text{Tukey}} < 0.001$), and PRE and 24H POST (218 ± 68.4 Nm) (43.32 Nm; $p_{\text{Tukey}} < 0.001$). No significant differences were observed between the groups or in the group-time interaction. No significant differences were found in IL-6, IL-8, and TNF- α levels over time, between groups, or in the group-time interaction. The same was observed for neutrophil phagocytic activity, both stimulated by *E. coli* and unstimulated. The results indicate that following a vegetarian diet does not provide any additional benefits or drawbacks compared to an omnivorous diet following EIMD. More intense or prolonged exercise sessions may be necessary to highlight potential differences in these parameters.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Beta-hydroxy-beta-methyl-butyrate (HMB) supplementation enhances mitochondrial capacity in hepatic stress induced by partial hepatectomy (PHx) in mice

Authors:

Ana Laura Vieira da Silva¹ Marcos Vinícius Esteca¹ Franciely Alves da Silva² Isabela Aparecida Divino¹ Fernanda Silva Carneiro³ Eduardo Rochete Ropelle³ Adriana Souza Torsoni²
Igor Luchini Baptista¹

Affiliations:

¹ Laboratory of Cell and Tissue Biology (LaBCeT), School of Applied Sciences, University of Campinas, Limeira, Brazil.

² Laboratory of Metabolic Disorders (LabDiMe), School of Applied Sciences, University of Campinas, Limeira, Brazil.

³ Laboratory of Molecular Biology of Exercise (LaBMEx), School of Applied Sciences, University of Campinas, Limeira, Brazil.

Thematic axis: Digestive System, Nutrition and Metabolism

Keywords: Liver metabolism. Liver regeneration. Mitochondrial network.

Abstract:

The hepatic regeneration process depends on the energetic state of the remaining tissue. Mitochondrial dysfunction can be associated with mortality risk after partial hepatectomy (PHx), highlighting the importance of preserving mitochondrial integrity for effective regeneration and postoperative survival. Dietary modulation of mitochondrial population or dynamics is a potential strategy to enhance liver repair. This study investigates the effects of beta-hydroxy-beta-methyl-butyrate (HMB) supplementation on mitochondrial quality pathways post-PHx. To this end, C57BL/6J mice received 600 mg/kg HMB via gavage for 10 days. On the 10th day, supplementation was interrupted, and the mice underwent a PHx followed by 7 days of liver regeneration. To induce a second injury, acetaminophen (APAP) overdose was administered at the end of the 7th day of regeneration (CEUA, protocol no. 5712-1/2021). The HMB supplementation did not significantly impact mitochondrial biogenesis markers levels in intact liver. However, post-PHx, the supplementation increases the content of PGC-1 α (Sham 1.0 vs. HMB+PHx 1.68 a.u.) while decreasing the levels of TFAM (Sham 1.0 vs. PHx+HMB 0.63 a.u.) and Parkin (PHx 2.52 vs. HMB+PHx 0.92 a.u.) without altering mtDNA copy number. The supplemented group showed a lower content of MFN2 (PHx 1.33 vs. HMB+PHx 0.89 a.u) and DRP1 (Sham 1.0 vs. HMB+PHx 0.67 a.u.), both mitochondrial dynamics markers. This group exhibits higher levels of mitochondrial markers VDAC2 (PHx 1.13 vs. HMB+PHx 2.31 a.u) and Tom20 (PHx 1.11 vs. HMB+PHx 1.37 a.u). These changes were associated with enhanced basal tissue respiration (PHx 27.24 vs. HMB+HPx 36.89 a.u.). Following an APAP overdose, the supplemented group exhibited an increased mtDNA content (PHx 1.08 vs. PHx+HMB 2.28 a.u.) and both basal (PHx 11.87 vs. 21.77 a.u.) and maximal (PHx 43.48 vs. PHx+HMB 112.39 a.u.) tissue respiration. These findings suggest that HMB supplementation enhances mitochondrial capacity post-PHx.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Reversal of metabolic dysfunction-associated steatohepatitis by galectin-3 inhibition

Authors:

Anne Raissa Melo Santos¹ Alessandra Gonçalves da Cruz¹ Jéssica Denielle Matos dos Santos¹
Helena Gaspar Monteiro¹
Felipe Nunes de Camargo¹ Layanne Cabral da Cunha Araujo² Sandro Leão Matos¹
João Paulo Gabriel Camporez¹

Affiliations:

¹ Department of Physiology at the Ribeirão Preto Medical School.

² Superior Institute of Biomedical Sciences, State University of Ceará

Keywords: Insulin Resistance. MAFLD. MASH.

Abstract:

Metabolic dysfunction-associated fatty liver disease (MAFLD) is characterized by triglyceride accumulation (TAG) in the liver and is frequently associated with hepatic insulin resistance and type 2 diabetes. It can progress to metabolic dysfunction-associated steatohepatitis (MASH), which is characterized by inflammation and fibrosis. Galectin-3 (Gal-3) plays a crucial role in inflammatory and fibrotic diseases. Therefore, this study aims to evaluate the effects of modified citrus pectin (MCP), a Gal-3 inhibitor, on the pathological features of MASH in an experimental model. **Methods:** Male ApoE KO mice (n=15) were divided into standard diet (CTL), Western diet (WD), and WD treated with MCP (WD+MCP) groups (CEUA approval 1023/2021). After 11 weeks, glucose tolerance, body composition, hepatic steatosis, collagen deposition, and liver damage were assessed. Statistical analysis used one-way ANOVA, with $p < 0.05$ considered significant. The data were represented as mean $\pm$ SEM. **Results:** The WD group showed a 77% fat increase compared to CTL. Both the WD and WD+MCP groups had a 22% lean mass increase versus CTL. Glucose intolerance increased by 58% in WD compared to CTL but was reduced by 22% in WD+MCP. Hepatic lipid droplets and TAG concentrations were higher in WD by 187% and 121%, respectively, compared to CTL, but were reduced in WD+MCP by 40% and 26%. Plasma ALT and AST enzyme levels increased by 294% and 267%, respectively, in WD compared to CTL, but were reduced in WD+MCP by 53% and 48%, respectively. Collagen deposition increased by 153% in WD compared to CTL, but by only 88% in WD+MCP. **Conclusion:** Overall, our data suggest that Gal-3 inhibition shows promise as a potential therapeutic approach to partially reverse MASH.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Gamma-oryzanol Modulates Hepatic Metabolism and Prevents MAFLD: An Integrative Omics and Molecular Approach

Authors:

Juliana Silva Siqueira^{1,2} Thiago Luiz Novaga Palacio¹ Jéssica Leite Garcia¹ Jordanna Cruzeiro¹
Marina de Paula Salomé dos Santos¹ Gilda Aiello^{2,3}
Fabiane Valentini Francisqueti-Ferron^{1,4} Alfonsina D'amato² Camila Renata Correa¹

Affiliations:

¹ Botucatu Medical School, São Paulo State University (Unesp), 18618687, Botucatu, Brazil.

² Department of Pharmaceutical Sciences, University of Milan, 20133, Milan.

³ Department for the Promotion of Human Science and Quality of Life San Raffaele Open University, Rome.

⁴ Integrated Colleges of Bauru (FIB), 17056100, Bauru, Brazil.

Keywords: Integratomics. Nutraceutical. Phytosterol.

Abstract:

Metabolic dysfunction-associated fatty liver disease (MAFLD) is closely linked to obesity and insulin resistance. Gamma-oryzanol (ORY), a bioactive compound with antioxidant and anti-inflammatory properties, has shown promise in modulating metabolic pathways. This study employed an integrative omics approach to elucidate ORY's hepatoprotective mechanisms in a diet-induced MAFLD model. Male Wistar rats were fed a control (CTRL), high sugar-fat (HSF), or HSF+ORY (0.5% w/w) diet for 30 weeks (CEUA 1451/2024). Nutritional-metabolic parameters, redox-inflammatory markers, and miR-122 expression were assessed. Proteomic and lipidomic analyses were performed to identify ORY-modulated molecular pathways. Statistical differences were determined by one-way ANOVA/Tukey's post-hoc test ($\alpha = 5\%$). Omics data were analyzed using MaxQuant (FDR = 1%), MetaboAnalyst 6.0, and Ingenuity Pathway Analysis (IPA). HSF diet induced obesity, insulin resistance, hepatic steatosis, and dyslipidemia. ORY supplementation prevented these alterations, improving lipid metabolism and reducing intrahepatic triglyceride accumulation. Proteomic analysis identified 2194 hepatic proteins, revealing key changes in sirtuin signaling, xenobiotic metabolism, and oxidative phosphorylation. Lipidomic analysis detected 438 differentially expressed lipids, predominantly diacylglycerols, phosphatidylcholines, and triacylglycerols. ORY modulated 84 lipids, restoring lipid profiles toward control levels. Functional network analysis associated ORY with reduced hepatocyte death, improved lipid metabolism, and enhanced cellular viability. ORY also upregulated miR-122, a key regulator of lipid homeostasis, linking its hepatoprotective effects to improved lipid handling and redox-inflammatory parameters mitigation. These findings underscore ORY's therapeutic potential in MAFLD by modulating metabolic and inflammatory pathways, offering new insights into its role as a dietary intervention for liver disease.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Cholesterol exacerbates the pathophysiology of metabolic dysfunction associated steatotic liver disease through upregulation of hypoxia-inducible factor 1 and modulation of microcirculatory dysfunction

Authors:

Evelyn Nunes Goulart da Silva Pereira¹ Beatriz Peres de Araujo¹
Carolina Souza Machado Marins¹ Karine Lino Rodrigues¹
Raquel Rangel Silveiras¹ Fernanda Verdini Guimarães² Edgar Eduardo Ilaquita Flores¹
Patrícia Machado Rodrigues e Silva² Anissa Daliry¹

Affiliations:

¹ Laboratory of Clinical and Experimental Physiopathology, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil.

² Laboratory of Inflammation, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil.

Keywords: Lipotoxicity. Metabolic dysfunction-associated steatotic liver disease. Microcirculation.

Abstract:

Cholesterol is a lipotoxic molecule associated with the progression of metabolic dysfunction-associated steatotic liver disease (MASLD). In this study, the role of cholesterol in MASLD-induced microcirculatory dysfunction was investigated. The MASLD model was induced in 20 male C57BL/6 mice by feeding a high-fat high-carbohydrate diet (HFC) for 39 weeks, supplemented with 2% cholesterol (HFC+Chol) at weeks 31-39 (License L-012/2018 A1). Microcirculation was assessed by leukocyte recruitment, hepatic stellate cell activation and in vivo perfusion analysis. Results were expressed as mean±SEM and analyzed by one-way ANOVA with Tukey's post hoc test ($p < 0.05$). At 39 weeks, the HFC+Chol group showed hepatomegaly (0.15 ± 0.009 vs CTL: 0.06 ± 0.003 g; $p < 0.001$), pale liver discoloration, and histologic evidence of severe liver injury, including steatosis, inflammation and fibrosis. Increased expression of alpha-smooth muscle actin and COL1A1 transcription (HFC+Chol: 40.8 ± 6.4 vs CTL: 1.0 ± 0.1 relative expression/ β -actin; $p < 0.001$) was observed. Stellate cell activation was increased (HFC+Chol: 28.5 ± 3.9 vs CTL: 124.3 ± 3.0 number of vitamin A+ cells; $p < 0.001$). Flow cytometry indicated TH1 polarization (IFN- γ /IL-4 ratio, $p = 0.022$). Hepatic microvascular blood flow was reduced by 56% and 71% in the HFC and HFC+Chol groups, respectively ($p < 0.001$), with greater impairment in the HFC+Chol group ($p = 0.045$). Cholesterol supplementation increased leukocyte rolling (13.3 ± 1.4 vs CTL: 2.2 ± 0.3 number of cells/30s; $p < 0.001$) and adhesion (13.0 ± 1.3 vs CTL: 2.0 ± 0.4 number of cells/30s; $p < 0.001$) and increased HIF1A (1.8 ± 0.14 vs CTL: 1.0 ± 0.12 relative expression/ β -actin; $p < 0.001$) and ICAM-1 (4.2 ± 0.1 vs CTL: 1.3 ± 0.4 relative expression/ β -actin; $p < 0.001$) mRNA levels. These results suggest that cholesterol exacerbates MASLD by inducing microcirculatory dysfunction and HIF1A upregulation via hypoxia and inflammation. This emphasizes lipotoxicity and microcirculation as important therapeutic targets in MASLD.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Mutation in the GPNMB protein induces negative regulation of energy metabolism and of rhythmic profile in mice fed a high-fat diet supplemented with fructose

Authors:

Eliz Maria de Oliveira Furtado^{1,2}

Juliano Jefferson da Silva³ Ana Flávia Tostes³ Pietra Souza Barsanele^{1,2} José Cipolla-Neto³

Luciana Chagas Caperuto⁴ Danusa Soares Dias⁵ Maristela Oliveira Poletini⁶ Leonardo Vinícius

Monteiro de Assis⁷ Maria Nathália Moraes^{1,2}

Affiliations:

¹ Graduate Program in General Physiology, Institute of Biosciences, USP, São Paulo - SP, Brazil.

² Molecular Chronobiology Laboratory, Institute of Environmental, Chemical and Pharmaceutical Sciences, UNIFESP, Diadema - SP, Brazil.

³ Neurobiology Laboratory, Institute of Biomedical Sciences, USP, São Paulo - SP, Brazil.

⁴ Metabolic Physiology Laboratory, Institute of Environmental, Chemical and Pharmaceutical Sciences, UNIFESP, Diadema - SP, Brazil.

⁵ Exercise Physiology Laboratory, School of Physical Education, Physiotherapy and Occupational Therapy, UFMG, Belo Horizonte - MG, Brazil.

⁶ Endocrinology and Metabolism Laboratory, Institute of Biomedical Sciences, UFMG, Belo Horizonte - MG, Brazil.

⁷ Department of Chemistry and Molecular Biology and the Wallenberg Centre for Molecular and Translational Medicine. University of Gothenburg, Gothenburg, Sweden.

Keywords: Energy substrates. GPNMB. High-fat diet. Metabolic disorder.

Abstract:

Sedentarism and high-fat diets contribute to metabolic disorders, such as insulin resistance and type 2 diabetes (T2D), potentially leading to liver damage. In T2D and non-alcoholic fatty liver disease, GPNMB is highly expressed and associated with a higher BMI, making it a potential metabolic marker and risk factor. This study investigates GPNMB's role in metabolic alterations in control and mutant mice subjected to a high-fat diet (HFD) with 30% fructose in water (FRUT). Male DBA/2J mice (2-3 months old), expressing the mutant form of GPNMB (GP-) and control mice (GP+), were fed an HFD (48.4% fat, 30.1% carbohydrates, 21.5% proteins) with FRUT for 12 weeks (CEUA 7699101023). We demonstrated that GP+ animals, after 12 weeks of HFD+FRUT, showed a strong increase in hepatic *Gpnmb* expression, whereas GP- animals had no detectable hepatic *Gpnmb* expression. Both groups increased body mass in response to HFD+FRUT compared to the normolipid diet, with no statistical differences between genotypes. However, GP- mice showed increased fat mass percentage and higher plasma triglyceride and cholesterol levels compared to GP+ mice. In the GTT, GP- exhibited better glucose response. After 12 weeks, indirect calorimetry at 30°C revealed a loss of metabolic rhythm in GP-. At 10°C, GP- showed lower carbohydrate oxidation, higher lipid oxidation, and loss of the oscillatory profile for most parameters analyzed. Additionally, GP- exhibited reduced AKT phosphorylation, leading to high GSK3- α/β activity, reducing hepatic glycogen stores in the dark phase. GP- mice maintained the anti-phase relationship of *Bmal1* with *Per1*, *Per2*, and *Nr1d1*, despite increased mesor in the liver during the light phase. In GP- livers, *Tnf- α* lacked rhythmicity, while *IL-6* maintained rhythmicity with increased mesor at the end of the light phase. These findings indicate that GP absence negatively affects metabolic processes, impacting energy homeostasis and carbohydrate-lipid metabolism.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Effects of alpha-linolenic fatty acid and physical exercise on long-chain polyunsaturated fatty acids metabolism

Authors:

Talitha Silva Meneguelli¹ Thaiane da Silva Rios¹ Amanda Rocha Fujita¹
Camila Venturini Ayres Cunha¹ Leandro Kansuke Oharomari¹ Eduardo Rochete Ropelle²
Adelino Sanchez Ramos da Silva³ José Rodrigo Pauli²
Dennys Esper Cintra¹

Affiliations:

¹ Laboratório de Genômica Nutricional (LabGeN) - Faculdade de Ciências Aplicadas - Universidade Estadual de Campinas.

² Laboratório de Biologia Molecular do Exercício (LaBMEx) - Faculdade de Ciências Aplicadas - Universidade Estadual de Campinas.

³ Escola de Educação Física e Esporte - Universidade de São Paulo - Ribeirão Preto.

Keywords: Alpha-linolenic fatty acid. Delta-6-desaturase. Fatty acid desaturase 2. Long-chain polyunsaturated fatty acid metabolism. Physical exercise.

Abstract:

The biosynthesis of long-chain polyunsaturated fatty acids (LC-PUFA) from α -linolenic acid (ALA) relies on desaturases and elongases in the liver. The conversion of linoleic acid (18:2 ω 6, LA) into ω 6 LC-PUFA follows the same enzymatic pathway, creating competition with ALA metabolism, particularly at the Δ 6-desaturation level, which is considered the rate-limiting stage. This issue is of epidemiological relevance, as dietary LA intake has significantly increased in recent decades leading to an elevated dietary LA/ALA ratio. This study aimed to assess the impact of ALA and physical exercise on enzymes involved in PUFA bioconversion. Male C57BL/6J mice (6-week-old; n=6/group) were gavaged (100 μ L of saline or flaxseed oil). Animals were distributed into four groups: exercised saline (ES)/ALA (EA); and sedentary saline (SS)/ALA (SA). The study lasted 6 weeks, and the exercised mice performed treadmill training. The animals were euthanized, liver and blood samples were collected for fatty acid analysis by gas chromatography-mass spectrometry and RT-qPCR was performed on the liver. All the procedures were approved by the Ethics Committee on the Use of Laboratory Animals

(CEUA:6411-1/2024). Data are expressed as mean $\pm$ SD, and statistical significance was determined using one-way ANOVA and Tukey *posthoc* test. The group that consumed ALA had a lower ω 6/ ω 3 ratio in serum (SA= 3.44 $\pm$ 0.86; EA= 2.76 $\pm$ 0.33 vs. SS= 10.34 $\pm$ 1.74; ES= 8.38 $\pm$ 0.65); and a lower 18:3/18:2 ω 6 ratio in serum (SA= 0.004 $\pm$ 0.0005; EA= 0.004 $\pm$ 0.0009 vs. SS= 0.009 $\pm$ 0.002; ES= 0.011 $\pm$ 0.0004) and liver (SA= 0.006 $\pm$ 0.0009; EA= 0.006 $\pm$ 0.0009 vs. SS= 0.011 $\pm$ 0.002; ES= 0.012 $\pm$ 0.002). The EA group showed lower expression of *Fads2* compared to the SA group (0.415 $\pm$ 0.259 vs. 1.079 $\pm$ 0.589 *Fads2*/ β -*actin*). ALA intake was able to alter Δ 6-desaturase index by modulating the synthesis of ω 6, suppressing the conversion of 18:2 to 18:3 ω 6. Also, ALA associated with physical exercise modulated *Fads2*.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: The Impact of Air Pollution in Combination with Ultra-Processed Foods on Glucose Metabolism in C57BL/6J Mice

Authors:

Fernanda Garofalo Xavier¹
Bianca Camilo Schimenes²
Clara Machado Campolim³
Kênia Moreno de Oliveira¹
Mariana Matera Veras⁴
Patrícia de Oliveira Prada^{1,2,3}

Affiliations:

- ¹Laboratório de pâncreas endócrino e metabolismo, LaPEM, OCRC, Universidade Estadual de Campinas, Campinas, SP, Brasil
² Faculdade de Ciências Aplicadas, Universidade Estadual de Campinas, Limeira, SP, Brasil
³ Departamento de Clínica Médica, Faculdade de Ciências Médicas, Universidade Estadual de Campinas, Campinas, SP, Brasil
⁴ Laboratório de Patologia Ambiental e Experimental LIM05, Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo, São Paulo, SP, Brasil

Keywords: Air pollution. Glucose homeostasis. Obesity. Particulate Matter 2.5 (PM2.5). Ultra-processed foods.

Abstract:

Lifestyle, genetic background, a high-fat and sugar diet, and sedentary behavior are classic risk factors for developing metabolic diseases. Even considering these factors together, they fail to fully explain the magnitude of the incidence of these diseases, suggesting additional environmental factors are involved. Air pollution exposure, represented by fine particulate matter with a diameter equal to or less than 2.5 μm (PM2.5) levels, is associated with obesity and insulin resistance. Considering the current food system, which includes several ultra-processed foods (UPF), we aimed to investigate whether PM2.5 exposure combined with a UPF during 4 weeks would impair energy homeostasis. Six-week-old male C57BL/6J mice were assigned to four experimental groups exposed for 4 weeks: one group was exposed to filtered air (FA) and a chow diet (CD); the second group was exposed to FA and a UPF (FA/UPF); the third group exposed to CD and PM2.5 (CD/PM2.5); last group was exposed to UPF and PM2.5 (UPF/PM2.5). We followed the body weight (BW) and food intake (FI), performed an intolerance test (GTT), and determined adiposity (CEUA/UNICAMP-5916-1). Throughout the experiment, their body weights remained comparable to those of the animals in the other three groups. However, after exposure, we observed increased gonadal fat mass from UPF/PM2.5 mice compared to FA and PM2.5 fed with a chow diet. Food intake was higher in kilocalories in the groups consuming ultra-processed foods, independent of exposure conditions (FA or PM2.5), and partially explained the higher adiposity after UPF and PM2.5 exposures. In addition, we observed that UPF alone was deleterious to the glucose homeostasis; however, when we added PM2.5 together, we observed a marked glucose intolerance at 60 minutes from GTT ($p=0.0200$). Our findings suggest that UPF alone damages the energy balance, but if we combine it with air pollution exposures, we add a significant glucose intolerance profile.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Morphometric changes in colon fragments exposed to dexamethasone in utero and subjected to excess fructose intake in adulthood

Authors:

Lorena de Souza ALMEIDA¹

Kênia Moreno de OLIVEIRA³

Carolina Vieira CAMPOS¹

Julia Modesto VICENTE¹

Caio Jordão TEIXEIRA¹

Gabriel Forato ANHÊ¹ Silvana BORDIN²

Affiliations:

¹ Department of Pharmacology, Faculty of Medical Sciences, State University of Campinas, São Paulo 13083-887, Brazil.

² Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, São Paulo 05508-000, Brazil.

³ Obesity and Comorbidities Research Center (OCRC), Department of Structural and Functional Biology, State University of Campinas, São Paulo 13083-864, Brazil.

Keywords: Colon. Dexamethasone. Fructose. Morphometrie.

Abstract:

Intrauterine glucocorticoid exposure combined with fructose intake during adulthood may exacerbate metabolic and absorption disorders, increasing the risk of cell proliferation. Colon cancer, the third most common cancer worldwide, is a growing concern in developing regions due to lifestyle changes. In the present study, we investigated morphometric changes in the distal colon of rats exposed to intrauterine dexamethasone (DEX) and excessive fructose intake in adulthood. Pregnant Wistar rats were treated or not with DEX (0.1 mg/kg/day in drinking water) during the 3rd gestational period. Adult pups (CTL and DEX) were assigned to receive 10% fructose in drinking water (fructose and DEX-fructose) for eight weeks. Colon fragments were collected, and histological images were captured and quantified (8 fields/sample) using ImageJ software. Results were expressed as mean $\pm$ SEM and analyzed by two-way ANOVA followed by Tukey's post-test (CEUA/UNICAMP 5530-1/2020). Offspring exposed to DEX-fructose showed a reduction in goblet cells per crypt ($p < 0.05$), increased crypt distance ($p < 0.005$), and reduced submucosal thickness ($p < 0.05$) compared to the CTL group. Reduced goblet cells suggest increased proliferation and altered mucus properties, affecting the protective barrier. Increased crypt distance indicates hyperplasia or hypertrophy, possibly as an adaptive response to increased absorption. Submucosal thinning, associated with possible reduced vascularization, may impair tissue regeneration and response to damage. These alterations suggest that DEX-fructose exposure induces significant morphometric changes, potentially contributing to colorectal carcinogenesis. Further studies are needed to clarify the functional impact and long-term consequences of these findings, particularly about epithelial proliferation, inflammatory responses, and colon cancer progression.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Effects of periodontal disease during gestation and lactation on salivary flow, biochemical composition, and redox status in rats

Authors:

Larissa Victorino Sampaio¹ Rayara Nogueira de Freitas¹ Gladiston William Lobo Rodrigues²
Isabel Dourado de Oliveira¹ Maria Clara Pacce Bispo¹
Renan José Barzotti¹
José Vitor Furuya de Lima¹
Rafaela Yumi Gregório Fuzishima¹ Leonardo dos Anjos Teruel¹ Gabriela Borba Crispim de Carvalho¹ Antonio Hernandez Chaves-Neto¹

Affiliations:

¹ Department of Basic Sciences, São Paulo State University (UNESP), School of Dentistry, Araçatuba, São Paulo.

² Department of Preventive and Restorative Dentistry, São Paulo State University (UNESP), School of Dentistry, Araçatuba, São Paulo.

Keywords: Oxidative stress. Periodontitis. Saliva.

Abstract:

Gestation and lactation are unique periods in a woman's life, marked by hormonal and immunological changes that can affect oral health. Periodontitis, an inflammation of the supporting tissues of the teeth, is common during this period and may influence various physiological and systemic parameters. Given this, the study evaluated the effects of ligature-induced periodontitis, established before conception and maintained during gestation and lactation, on salivary flow, biochemical composition, and redox status. For this purpose, sixteen 3-month-old Wistar rats were randomly divided into two groups ($n=8$): Control and Periodontal Disease (PD). Periodontal disease was induced by applying a 4-0 silk ligature around the cervical region of the first lower molars. Seven days later, both groups were mated with healthy male rats. After pregnancy was confirmed, the rats were housed individually during gestation and lactation. After weaning, the rats were sedated, and pilocarpine-induced saliva was collected for biochemical parameter analysis (CEUA FOA/UNESP No. 1054-2023). Salivary flow, pH, and buffering capacity were determined immediately after collection. Spectrophotometric methods were used to analyze salivary total protein, amylase, calcium, and phosphate, as well as total oxidant capacity and total antioxidant capacity. The results were analyzed using the unpaired Student's t-test ($p < 0.05$). PD did not alter salivary flow, pH, buffering capacity, or phosphate. However, it increased total protein ($p < 0.05$) and reduced amylase activity ($p < 0.05$) and calcium concentration ($p < 0.05$). PD did not affect total oxidant capacity but reduced total antioxidant capacity ($p < 0.05$). Thus, we conclude that experimental periodontitis during gestation and lactation induces disturbances in biochemical composition and impairs the redox status of rat saliva, which may increase susceptibility to oral diseases. (FAPESP process nº 2023/15915-6; PIBIC- UNESP nº 13938).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Vagus nerve neural circuit regulates bile acid secretion in the liver

Authors:

João Sérgio da Fonseca Guimarães¹ Camila de Fátima Carvalho Brito¹ Roberta Cristelli Fonseca¹
Lucas Rodrigues-Ribeiro¹
Izadora Zhong Liang Ferreira Feng¹ Maisa Antunes² Gustavo Braga Menezes²
Thiago Verano-Braga¹ Maria De Fátima Leite¹ André Gustavo de Oliveira¹

Affiliations:

¹ Departamento de Fisiologia e Biofísica, ICB, UFMG.

² Departamento de Morfologia, ICB, UFMG.

Keywords: Bile secretion. Gut-brain axis. Liver. Vagus nerve.

Abstract:

INTRODUCTION: Bile is a complex secretion produced by hepatocytes, essential for fat digestion and absorption. Albeit an essential secretion, bile secretion regulation remains incompletely understood. Humoral and neuronal factors are known modulators of this process. Here, we investigated the role of vagal signaling in bile secretion. **METHODS:** Eight-week-old C57Bl/6 mice were assigned to sham or left cervical vagotomy (VNX) groups (n=5/group). We employed liver proteomics and confocal intravital microscopy to study *in vivo* bile secretion. All procedures were approved by the local ethics committee (CEUA 280/2018). Raw mass spectra were analyzed using MaxQuant software, with protein identification against the mouse UniProt FASTA database. Differential expression was evaluated via one-way ANOVA ($p < 0.05$), and bile secretion dynamics were assessed using Linear Mixed Effects Models ($p < 0.05$). **RESULTS:** VNX mice displayed alterations in the hepatic proteomic landscape when compared to sham animals. We identified 453 differentially expressed proteins out of the 2121 proteins. KEGG pathway enrichment identified eight proteins involved in bile acid secretion (Abcb4, Abcc3, Atp1a1, Atp1b1, Ephx1, Prkacb, Slc22a1 and Slc10a1). To assess functional impact, we developed a novel *in vivo* real-time imaging technique using indocyanine green and liver intravital microscopy. This enabled visualization of all phases of intrahepatic bile secretion: sinusoidal uptake (phase 0), metabolic conversion (phases I and II), and apical release (phase III). VNX mice exhibited reduced fluorescence signal intensity when compared to sham group, indicating impaired bile secretion (Sham: mean = 0.5331433; sd = 0.07309349; VNX: mean = 0.4854003; sd = 0.04076579; $p = 0.0138$). **CONCLUSIONS:** These findings support a regulatory role for vagal input in hepatic bile secretion.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Mice with experimental Ulcerative Colitis treated with Sodium Butyrate had reduced distal colon collagen fiber damage

Authors:

Caroline Bures de Paulo¹

Marcos Antônio Ferreira Caetano²

Patricia Castelucci²

Affiliations:

¹ Department of Anatomy, Faculty of Veterinary Medicine and Animal Science, University of São Paulo (FMVZ-USP). São Paulo - SP.

² Department of Anatomy, Institute of Biomedical Sciences - USP (ICB-USP). São Paulo - SP.

Keywords: Butyrate. Collagen fibre. Ulcerative colitis.

Abstract:

Ulcerative colitis results in inflammation of the gastrointestinal tract and changes in the extracellular matrix. This project aims to analyse changes in collagen fibers in experimental ulcerative colitis (EUC). Male C57BL/6 (8-week-old) mice (CEUA 5643021024) received an intrarectal injection of 100µL of 2,4,6- trinitrobenzenesulfonic-acid (TNBS group). The Sham group received vehicle (ethanol 35%). After EUC induction, some animals were treated for 7 days with Sodium Butyrate (100mg/kg, Butyrate group) via gavage. Sham and TNBS groups received saline. Animals were euthanized 7 days after TNBS injection. Distal colon was collected for histological analysis. Picrosirius red was used to visualize collagen fibers. The slides were analyzed with a Nikon 80i microscope. The collagen areas of the mucosa, submucosa and ganglion were measured using Image J software. Collagen fibers were thickened in all three regions in the colitis group, and these fibers were reduced in the butyrate group. In polarized light, type I and III fibers were deposited in the three regions of the colitis group and there was a reduction in the butyrate group. The Sham group showed mucosa (0.27 ± 0.06), submucosa (0.26 ± 0.07) and ganglion (0.32 ± 0.11). The Colitis group showed increases in mucosa by 19.47% (0.32 ± 0.07), in submucosa by 32.34% (0.35 ± 0.08) and in ganglion by 34.32% (0.43 ± 0.09) compared to Sham group, respectively, $p < 0.05$. The butyrate group reduced the mean mucosa by 28.2% (0.25 ± 0.03), submucosa by 29.9% (0.27 ± 0.06) and ganglion by 17.74% (0.37 ± 0.04) compared to Colitis group, respectively, $p < 0.05$. Ulcerative colitis causes an increase in collagen fibers and treatment with butyrate recovered the collagen damage caused by EUC.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Reduction of advanced glycation end products formation by Pyridoxamine improves adipose tissue inflammation and microvascular dysfunction in C57BL/6 obese mice

Authors:

Juliana Florencio da Silva¹ Raquel Rangel Silveiras¹
Beatriz Peres de Araujo¹
Karine Lino Rodrigues¹
Evelyn Nunes Goulart da Silva Pereira¹
Vivian Vieira Dias da Silva¹
Anissa Daliry¹

Affiliations:

¹ Laboratory of Clinical and Experimental Pathophysiology, Oswaldo Cruz Institute, Oswaldo Cruz Foundation

Keywords: Adipose tissue. Advanced glycation end products. Inflammation. Obesity. Pyridoxamine.

Abstract:

Obesity is a chronic disease associated to metabolic and inflammatory disorders, in which advanced glycation end products (AGEs) contribute to its progression. The binding between AGEs and their receptor (RAGE) drives inflammatory processes that affect adipose tissue. Pyridoxamine (PYR) has been investigated as a therapeutic agent for inhibiting AGE formation by interfering with post-Amadori products and for its antioxidant properties. This study investigates PYR's effects on adipose tissue inflammation and microcirculation in obese mice. Animal experimental procedures were approved by L-012/2018A2. Male C57BL/6 mice (n=40) were divided into control (CTL) and hypercaloric diet with 2% cholesterol (HCOL2%) groups for 12 weeks. The HCOL2% group received vehicle (VEI) or PYR (200 mg/kg/day) for the last 6 weeks. Analyses included body weight, fat depots, glycemia, blood pressure, microcirculation, and inflammation. Data are mean $\pm$ SD, analyzed by two-way ANOVA ($p < 0.05$ significant). HCOL2%+VEI showed increased body weight (33.9 ± 5.4 vs. CTL 28.5 ± 3.8 , $p < 0.001$), which was reduced by PYR (29.6 ± 0.5 , $p < 0.001$). Fat depots weight followed the same trend. Glycemia was higher in HCOL2%+VEI (164.0 ± 25.5 vs. CTL: 149.1 ± 17.9 , $p < 0.001$) but lower in PYR group (152.1 ± 4.37 , $p < 0.001$). Blood pressure was elevated in HCOL2%+VEI (139.3 ± 13.7 vs. CTL: 126.7 ± 6.5 , $p < 0.001$), but PYR attenuated this effect (129.1 ± 2.7 , $p < 0.001$). PYR partially restored microvascular flow (HCOL2%+VEI 84.1 ± 21.6 vs. HCOL2%+PYR 104.7 ± 9.05 , $p < 0.001$) and reduced leukocyte rolling (HCOL2%+VEI 1.24 ± 1.09 vs. HCOL2%+PYR 0.53 ± 0.25 , $p < 0.001$) and adhesion (HCOL2%+VEI 0.45 ± 0.45 vs. HCOL2%+PYR 0.15 ± 0.11 , $p < 0.01$). TNF- α , IL-6, and IL-2 levels, elevated in HCOL2%+VEI ($p < 0.001$), were lower with PYR (TNF- α : $p < 0.05$; IL-6: $p < 0.01$; IL-2: $p < 0.05$). Therefore, we hypothesize that PYR improves adipose tissue health in obesity by reducing inflammation, enhancing microcirculation, and mitigating metabolic complications aggravated by AGEs.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: The role of cholesterol on renal microcirculatory dysfunction in diet-induced obesity (DIO) model

Authors:

Felipe Raed Xavier¹
Evelyn Nunes Goulart da Silva Pereira¹ Daniel Castro¹
Beatriz Peres de Araujo¹ Karine Lino Rodrigues¹ Raquel Rangel Silveiras¹
Carolina Souza Machado Martins¹ Edgar Eduardo Ilaquita Flores¹ Anissa Daliry¹

Affiliations:

¹ Laboratory of Clinical and Experimental Physiopathology, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil.

Keywords: Lipotoxicity. Microcirculation. Obesity.

Abstract:

The continuing global rise in obesity prevalence parallels the rise in chronic kidney disease (CKD). Although the molecular mechanisms linking the two diseases are not well understood, the role of lipotoxicity in obesity-induced kidney damage is becoming increasingly important. Renal lipotoxicity is known to have deleterious effects on the kidney through various mechanisms of action such as inflammation, development of oxidative and endoplasmic reticulum stress, insulin resistance, deregulation of lipid metabolism or overactivation of the renin-angiotensin-aldosterone system. Cholesterol is an important lipotoxic molecule that contributes to the progression of CKD. In addition, microcirculatory changes are a critical component of CKD pathogenesis. The aim of this study was to investigate the role of cholesterol as an insult modulating microcirculatory damage in CKD. To this end, an obesity model was established with 20 male C57BL/6 mice fed a high-fat, high-carbohydrate (HFHC) diet for 39 weeks. 2% cholesterol (HFHC+Chol) was added to the diet between weeks 31–39 (license L-012/2018 A1). Microcirculation was assessed by in vivo perfusion analysis. Statistical analyzes were performed using one-way ANOVA with Tukey's post hoc test ($p < 0.05$). After 39 weeks, the HFHC and HFHC+Chol groups showed higher body weight (HFHC: 52.41 ± 3.75 g vs. HFHC+Chol: 48.15 ± 8.12 g vs. CTL: 29.71 ± 2.51 g; $p < 0.001$) and a lower kidney weight (HFHC: 0.006 ± 0.001 g vs. HFHC+Chol: 0.007 ± 0.001 g vs. CTL: 0.011 ± 0.001 g; $p < 0.001$) compared to CTL. When analyzing microcirculation, both HFHC and HFHC+Chol mice exhibited renal endothelial dysfunction, evidenced by a reduced response to acetylcholine (HFHC: -8.805 ± 10.33 vs. HFHC+Chol: -3.256 ± 4.093 vs. CTL: 2.477 ± 3.385 ; $p < 0.001$). However, a significant reduction in basal renal microvascular blood flow was observed only in HFHC+Chol mice (CTL: 330.539 ± 33.672 vs. HFHC: 334.436 ± 62.328 vs. HFHC+Chol: 238.596 ± 86.738 ; $p < 0.001$). This study highlights the importance of cholesterol-induced lipotoxicity causing microcirculatory dysfunction associated with CKD pathology, reinforcing the potential of lipotoxicity and microcirculation as therapeutic targets for CKD.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Feeding pattern of rats submitted to the maternal neglect model

Authors:

Wilkson Rafael de Miranda Santos¹ Fernando Antônio Bezerra Filho¹ Maria Eduarda Santana do Nascimento¹ Swane Miranda Alves²
Aline Ananias de Lima³ Sandra Lopes de Souza^{2,3}

Affiliations:

¹ Department of Nutrition.

² Graduate Program in Nutrition.

³ Graduate Program in Neuropsychiatry and Behavioral Sciences.

Keywords: Food Preference. Maternal neglect. Stress.

Abstract:

Child adversity, in the form of abuse and neglect, is prevalent worldwide and represents a public health problem. The animal model of maternal neglect is the combination of maternal separation followed by early weaning, interrupting breastfeeding and maternal care. It is considered neonatal stress and has negative consequences throughout life. The research was approved by the ethics committee (protocol no. 0036/2024), with the hypothesis that maternal neglect would promote greater consumption of standard food and preference for palatable foods. Male (n=13) and female (n=12) Wistar rats were used. The neglect group (NG) was subjected to maternal separation from postnatal day (PND) 2^o to 14^o, followed by early weaning on PND-15. The control group (C) was weaned on PND-30. Body weight in the neonatal phase was assessed (DPN-2 to 20); food preference at PND-45 to 50 and PND-140 to 145, offered with 30g of palatable food (Cookie Chocolate Bauducco®) and standard feed (Presence®). The amount ingested was quantified by the difference between offer and rejection over 24h/5 days. Circadian food consumption (12h-12h) was assessed at PND-56 and PND-156, in the inverted light-dark cycle. In this, 30g of standard feed was offered during the dark (06:00-18:00h) and light cycle (18:00-06:00h). It was observed that body weight was lower ($p<0.001$) in the neglect group compared to controls males at PND-5 to PND-20 and females at PND-11 to 20. Regarding food preference, both groups of males and females consumed more ($p<0.001$) palatable food. In circadian consumption, there was no difference between groups for both sexes. It was concluded that maternal neglect did not affect the consumption of standard food or promote a preference for palatable food. However, these animals showed a reduction in body weight.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Effects of gestrinone administration on the gastrointestinal tract of rats fed on a hyperlipidic diet

Authors:

Filipe Augusto Baier de Andrade¹ Isabela de Oliveira Kapp²
Elisângela Pinotti Conci Costa Rosa³ Dionízia Xavier Scomparin⁴
Rosane Aparecida Ribeiro⁵

Affiliations:

¹ Universidade Estadual de Ponta Grossa – Doutorado do programa de Pós-Graduação em Ciências da Saúde.

² Universidade Estadual de Ponta Grossa – Curso de Ciências Farmacêuticas.

³ Universidade Estadual de Ponta Grossa-Mestranda do programa de Pós-Graduação em Ciências Farmacêuticas.

⁴ Universidade Estadual de Ponta Grossa-Programa de Pós-Graduação em Ciências da Saúde.

⁵ Universidade Estadual de Ponta Grossa-Programa de Pós-Graduação em Ciências Farmacêuticas.

Keywords: Gestrinone. Obese. Gastrointestinal Tract. High fat diet.

Abstract:

Gestrinone is a synthetic steroid mostly used for myoma treatments. In recent years, physicians used it empirically to improve body composition in women. This study aimed to investigate the effects of gestrinone administration on morphofunction of the gastrointestinal tract of female rats submitted to different diets. Adult female *Wistar* rats were fed on a normo- or a high fat diet and received 3 times/ week, a gavage of soybean oil containing 0,16% DMSO (CTL and HFD groups), without or with 0,26 (CGES 0,26 and HGES 0,26 groups), or 0,52 mg gestrinone/kg body weight (BW; CGES 0,52 and HGES 0,52 groups), for 12 weeks. All procedures were approved by CEUA/UEPG. HFD rats displayed increased BW ($271,80 \pm 18,35\text{g}$), abdominal adiposity ($81,37 \pm 18,70\text{mg/g BW}$), mesenteric fat weight ($14,45\text{mg/g BW}$), triglycerides (TG; $1,856 \pm 0,71 \text{ ug/mg feces}$) and cholesterol (CHOL; $1,794 \pm 0,48 \text{ ug/mg feces}$), but not small intestine weight ($20,30\text{mg/g BW}$), colon weight ($4,50\text{mg/g BW}$) and fat in fecal content ($70,375$

$\pm 15,79 \text{ ug/mg feces}$) in comparison with CTL group ($255 \pm 11,54\text{g}$; $46,60 \pm 3,58 \text{ mg/g BW}$; $9,80 \pm 1,79\text{mg/g BW}$; $1,77 \pm 0,83\text{ug/mg feces}$; $1,64 \pm$

$0,33\text{ug/mg feces}$; $25,82 \pm 2,74\text{mg/g BW}$; $8,58 \pm 3,30\text{mg/g BW}$; $76,62 \pm 7,62 \text{ ug/mg feces}$, respectively). Gestrinone administration prevented HFD effects, since HGES 0,26 and HGES 0,52 groups showed lower BW ($268,14 \pm 19,58$ and $249 \pm 25,28\text{g}$), visceral adiposity ($69,504$

$\pm 13,30$ and $41,90 \pm 18,06\text{mg/g BW}$), mesenteric fat ($8,35 \pm 1,8$ and $8,40 \pm 0,7\text{mg/g BW}$), TG ($1,50 \pm 0,83$ and $1,74 \pm 0,83\text{mg/g feces}$) and CHOL in CGES 0,26 group ($1,81 \pm 0,45\text{ug/mg feces}$), when compared to HFD groups. However, TG and CHOL values on CGES 0,52 group were higher than HGES 0,52 group ($1,76 \pm 0,8\text{ug/mg feces}$; $1,97 \pm 0,77\text{ug/mg feces}$, respectively). Finally, the treatment did not influence gut and fecal weight, but did on fecal fat. Gestrinone administration reduced BW, visceral fat, metabolic variables and fat on fecal content, but had no influence on gut and fecal weight.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Cannabinoid Receptor 1 (*CNR1*) expression in the gastric antrum and fundus of patients with obesity or overweight

Authors:

Marina Costa Reis¹

Camilla Pereira Dias-Rocha¹ Mariana Macedo Almeida² Isis Hara Trevenzoli¹

Affiliations:

¹Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, RJ.

²Instituto de Ciências Biológicas, Universidade Federal de Juiz de Fora, MG.

Keywords: Endocannabinoid system. Energy metabolism. Human obesity. Stomach.

Abstract:

Obesity is associated with hyperactivation of the endocannabinoid system. Activation of the cannabinoid receptor 1 (CB1; *CNR1* gene) promotes positive energy balance and increases the release of the orexigenic hormone ghrelin in the gastric fundus of rodents. We hypothesize that *CNR1* expression would be upregulated in gastric antrum and fundus of humans with obesity. This study evaluated *CNR1* mRNA expression in the stomach of eutrophic, overweight, or obese individuals of both sexes (CEP 5.525.787/UFRJ and 7.021.975/UFGF). Volunteers aged 18–65 years completed health questionnaires and anthropometric assessments. Gastric biopsies of the fundus and antrum were collected during endoscopy. Twenty-seven participants were divided into control (C), overweight (OW), or obese (O) groups, classified by body mass index (BMI) and sex (female-F or male-M); n=3–5 per group. Total RNA was extracted from gastric samples, and *CNR1* and *HPRT1* expression (housekeeping gene) were analyzed by qPCR. Results are expressed as percentages relative to the C-F group and analyzed using one-way ANOVA ($p < 0.05$). No significant differences in *CNR1* expression were found in the fundus ($p = 0.5328$) or antrum ($p = 0.6215$), possibly due to the small sample size. In the fundus, higher averages were observed in OW-F (117 ± 29.37) and OW-M (96.96 ± 18.27) versus C-F (100 ± 25.04) and C-M (93.24 ± 33.45) respectively. In the antrum, the highest values were in O-F (136.4 ± 81.05) and O-M (125.1 ± 84.17), compared to C-F (100 ± 27.92) and C-M (112 ± 34.5) respectively. We speculate that increasing the sample size may reveal significant associations between BMI and gastric *CNR1* expression (in progress). Furthermore, we intend to analyze the ghrelin expression to investigate possible interactions between *CNR1* upregulation and ghrelin expression (perspective).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Effects of the systemic inflammation on the intestinal tone of endotoxemic mice: functional evidence of disordered contractile regulation involving calcium- sensitization

Authors:

Stherfanni Almeida Lima-Moura¹ José Eduardo da Silva-Santos¹

Affiliations:

¹ Laboratory of Cardiovascular and Smooth Muscle Biology, Department of Pharmacology, Federal University of Santa Catarina – SC, Brazil.

Keywords: Contraction. Intestinal function. Peristalsis. Relaxation. Systemic inflammation.

Abstract:

Lipopolysaccharide (LPS) injection is used to study systemic inflammation (SI) and sepsis in rodents. We used this model to explore the hypothesis that intestinal smooth muscle regulation is disordered during SI. Male (M-) and female (F-) mice (3-4 months) received saline (1 mL/kg) or 10 mg/kg LPS given by ip route and were assigned (4-12/group) into control (CT), LPS6h or LPS24h groups. The mice were euthanized under anesthesia, and their ileum and colon (~1.5 cm segments) were mounted in organ baths. We evaluated the contractile or relaxation responses. The differences described come from statistical analysis ($p < .05$; ANOVA followed by Tukey's test). The peristalsis of the ileum and colon segments did not differ between CT and LPS groups. However, the spontaneous force developed was enhanced by ~100% in the colon of the F-LPS24h group and reduced by ~50% in the ileum from M- and F-LPS24h. We found no significant differences in contractile responses in LPS6h groups. Reduced responses to 30 μ M acetylcholine (ACh) were found in the colon (phasic and tonic contractions) from the female (but not male) LPS24h group. For instance, ACh-induced tonic contraction was 47.4 ± 2.9 and 31.5 ± 2.2 g/g of tissue in the colon of CT and LPS24h groups. On the other hand, contractile responses elicited by electrical field stimulation (EFS; 20 V, 10 ms, 1-128 Hz) were enhanced by over 300% in the colon from M- and F-LPS24h groups. Although we found only slight differences for norepinephrine-induced relaxation, the relaxation induced by Y-27632 (a Rho-kinase inhibitor) was enhanced in the colon of LPS24h groups. These results confirm our hypothesis, suggesting that, at least in the colon, SI results in an augmented release of contractile mediators, which is partially counterbalanced by intracellular mechanisms involving calcium sensitization. The role of these findings for early and late gastrointestinal conditions after a systemic inflammatory insult deserves further investigation.

Financial support and ethical approval: CAPES, 001; CNPq 405538/2021-9 e 403615/2023-2. Approved by CEUA/UFSC (5366250520).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Sex-related differences in the gut function: evidence of increased contractile activity in the colon of females

Authors:

Stherfanni Almeida Lima-Moura¹ José Eduardo da Silva-Santos¹

Affiliations:

¹ Laboratory of Cardiovascular and Smooth Muscle Biology, Department of Pharmacology, Federal University of Santa Catarina – SC, Brazil.

Keywords: Contraction. Gut function. Peristalsis. Sexual dimorphism.

Abstract:

Epidemiological data indicate that women are more likely to develop intestinal diseases. However, the potential role of sex-associated differences in intestinal contractility remains unknown. We explored the hypothesis that the female colon is more responsive to contractile mediators. Female and male Swiss mice (3-4 months) were euthanized under anesthesia to remove their colon. The colon segments (~ 1.5 cm length) were mounted in organ baths under a basal tone of 0.5 g ($n \geq 6$ -12/group). The spontaneous activity, responses to 120 mM KCl, and reactivity for 1-300 μ M acetylcholine (ACh), carbachol (CCh), and angiotensin I and II (AI and AII) were recorded. We also assessed the contractile responses elicited by electric field stimulation (EFS, 20 V, 10 ms, 1-128 Hz, interval 10 s) in colon segments without drug incubation. The differences described come from statistical analysis ($p < .05$; ANOVA followed by Tukey's test). Peristalsis did not differ between male and female groups. However, the spontaneous force developed was ~70% greater in the colon of the female group. We found no significant differences in the 120 mM KCl and CCh contractile responses. Nevertheless, responses were increased in the colon of females for 30 μ M ACh (phasic and tonic contractions). We also found higher contractile responses in the female colon for cumulative concentration-response curves of ACh (~68%), AI (~43%), and AII (~80%). In addition, EFS (2-16 Hz)-induced contraction was 80-30% more intense in the female colon than in male mice samples. We confirmed the hypothesis that the colon of female mice is more sensitive to exogenous (drugs added) and endogenous (released by EFS) contractile mediators. Importantly, our results suggest that cholinergic and angiotensin systems are more functional in the colon of female mice. The role of the myenteric plexus and the importance of these differences in developing intestinal conditions deserve further investigation.

Financial support and ethical approval: CAPES, 001; CNPq 405538/2021-9 e 403615/2023-2. Approved by CEUA/UFSC (5366250520).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Effects of chronic central ghrelin infusion on the glycemic profile of offspring of mild hyperglycemic rats.

Authors:

Milena Silva Agostini^{1,2}

Karla Martinucho Godeguez^{1,2} Caroline Martins Sierro^{1,2} Carla de Moraes Machado¹

Ana Carolina Inhasz Kiss^{1,2}

Affiliations:

¹ Department of Structural and Functional Biology, Institute of Biosciences of Botucatu, São Paulo State University 'Júlio de Mesquita Filho' (UNESP).

² Graduate Program in General Physiology, Institute of Biosciences, University of São Paulo (USP).

Keywords: Diabetes. Glycemia. Orexigenic factors. Pregnancy. Rodents.

Abstract:

Ghrelin plays a role in maintaining glycemic homeostasis; however, the effects on the offspring of hyperglycemic mothers have not been investigated. The aim was to evaluate the effect of chronic central ghrelin infusion on the glycemic profile of male offspring of rats with mild hyperglycemia. Our hypothesis was that maternal hyperglycemia would reduce offspring glucose tolerance and insulin sensitivity, and those would be exacerbated by ghrelin infusion. Adult offspring from normoglycemic (Control) and hyperglycemic rats (streptozotocin (STZ) 35 mg/kg i.p. day 7 of pregnancy) underwent stereotaxic surgery and intracerebroventricular infusion of saline or ghrelin (1 µg/day) for 7 days. The oral glucose tolerance test (OGTT) and insulin tolerance test (ITT) were conducted before and during ghrelin infusion, and the area under the glycemic curve (AUC) was calculated (mean ± SEM mg/dL/120min) (CEUA 3041270422). The AUC analysis of basal ITT (Control-Saline (CS), n=4: 11853.7 ± 576.4; Control-Ghrelin (CG), n=5: 11704.5 ± 604.8; STZ-Saline (SS), n=5: 11202.1 ± 862.8; STZ-Ghrelin (SG), n=4: 11741.2 ± 513.4; maternal metabolism: F(1,14)=0.117, p=0.74; treatment: F(1,14)=0.256, p=0.62) and during treatment (CS: 8553.7 ± 400.0; CG:10591.5 ± 934.7; SS:8909.5 ± 497.9; SG:10601.2 ± 925.6; maternal metabolism:F(1,14)=2.253, p=0.16; treatment: F(1,14)=1.546, p=0.23) revealed no significant differences. The AUC analysis of basal OGTT (CS:15718.1 ± 219.8; CG:14961.0 ± 133.7; SS: 15108.0 ± 393.5; SG:15408.7 ± 440.0; maternal metabolism: F (1,14) = 0.064, p=0.80; treatment: F (1,14) = 0.509, p=0.49) and during treatment (CS:16253.0 ± 457.6; CG:16057.5 ± 185.6; SS:16336.5 ± 337.4; SG:16516.8 ± 660.1; maternal metabolism: F (1,14) = 0.429, p=0.52; treatment: F (1,14) = 3.107×10⁻⁴, p=0.99) also showed no significant differences. In conclusion, ghrelin did not modify glucose tolerance or insulin sensitivity, and the offspring of hyperglycemic mothers did not differ from those of normoglycemic ones.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Differential effects of maternal obesity and metformin on male and female Wistar rats

Authors:

Aline Milena Dantas Rodrigues¹ Mateus Silva Campos¹
Ana Caroline Schoenberger Kipper¹ Júlio Cezar de Oliveira¹

Affiliations:

¹ Grupo de Pesquisa em Programação Perinatal de Doenças Metabólicas: conceito DOHaD, Laboratório de Doenças Metabólicas e Cardiovasculares, Núcleo de Pesquisa e Apoio Didático em Saúde (NUPADS), Instituto de Ciência da Saúde (ICS), Universidade Federal de Mato Grosso, Campus Universitário de Sinop. Sinop, MT, Brazil.

Keywords: Metformin. Obesity. Metabolic Programming.

Abstract:

Introduction: Obesity is a condition that may be associated with the metabolic state of the mother. Metformin, a medication used to treat diabetes, is known also by reducing body weight. Objective: To evaluate the effect of obesogenic-diet consumption by lactating mother on breast-milk energy composition and the long-term efficacy of metformin used during adolescence on body weight and fasting glucose levels of the rat-offspring. Methodology: Wistar lactating rats were fed a hypercaloric diet, while control mothers consumed a standard diet. Milk was collected on the 12th day. At 30 days-old, offspring from control (C-Met) and obese (Ob-Met) mothers treated with metformin (250 mg/kg body weight in drinking water from 30 to 60 days-old) were analyzed, along with those from control (C) and obese (Ob) mothers that were not treated with metformin. The protocols were approved by the animal ethics committee (protocol: 23108.017073/2019-56). Results: We observed a 50.9% increase in the energy content of milk from obese mothers compared to controls ($P < 0.01$). At weaning, when compared to C group, body weight was 14% higher in male Ob, and 16% in female Ob ($P < 0.01$). During metformin treatment, body weight was reduced by 10% in male Ob-Met, while in females it was reduced by 8.5% ($P < 0.05$). In the post-treatment period, the body weight of male Ob-Met was 7% smaller than the Ob group ($P < 0.05$) than the Ob group, with no differences between females. Glycemia increased by 86% in Ob group compared to C ones ($P < 0.01$). On the other hand, Ob-Met male group was normoglycemic. There was no statistical difference in glucose levels among females. Conclusion: Maternal high-caloric intake, increased milk energy content influencing fast body weight gain in their offspring. Metformin treatment only during the adolescence was effective on ameliorating glucose homeostasis in male sex, as short and long-term consequence, which was not observed in females.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Butyrate attenuates enteric neuron loss and reduces caspase-3 cleavage in experimental ulcerative colitis

Authors:

Marcos Antônio Ferreira Caetano¹ Roberta Figueiroa de Souza¹ Caroline Bures de Paulo²
Patricia Castelucci¹

Affiliations:

¹ Department of Anatomy, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil.

² Department of Surgery, School of Veterinary Medicine and Animal Sciences, University of São Paulo, São Paulo, Brazil.

Keywords: Enteric nervous system. Gut microbiota. Inflammatory bowel diseases. Short chain fatty acids.

Abstract:

The enteric nervous system (ENS) is affected by inflammatory bowel diseases (IBDs). Butyrate, a short-chain fatty acid (SCFAs) produced by gut microbiota, binds to receptors such as GPR43 and GPR109A, improving gut health, reducing cytokines like tumor necrosis factor alpha (TNF- α), and preventing cell death by inhibiting cell death. The hypothesis is that Butyrate, via SCFAs receptors, would protect neurons, reduce inflammation, and inhibit apoptosis. This work aimed to investigate the effects of Butyrate and GPR43/GPR109A on myenteric neurons in experimental ulcerative colitis (EUC). Male C57BL/6 (8 weeks) mice received an intrarectal injection of 100 μ L of 2,4,6-trinitrobenzenesulfonic-acid (TNBS group) while Sham group received 35% ethanol. After EUC, some mice were treated for 7 days with Sodium Butyrate (100mg/kg, Butyrate group) via gavage. Sham and TNBS groups received saline (CEUA ICB/USP-5482071122). Mice were euthanized 7 days after EUC, and distal colon was collected for immunofluorescence. Double labeling included: a) GPR43 + neuronal nitric oxide synthase (nNOS), Calretinin (Calr), Protein Gene Product 9.5 (PGP9.5), and Glial Fibrillary Acidic Protein (GFAP); b) GPR109A + PGP9.5; c) Total Caspase-3 and cleaved Caspase-3 + PGP9.5; d) TNF- α + nNOS, Calr, PGP9.5 and GFAP. The number of neurons/glia cells per ganglion and corrected total fluorescence (CTCF) were analyzed. TNBS reduced GPR43-ir (20.3%), GPR109A-ir (18.9%), Calr-ir (38.5%), nNOS-ir (40.1%), and PGP9.5-ir neurons (16.9%), and increased GFAP-ir glia (35.4%) vs. Sham ($p < 0.05$). Butyrate restored these neurons by 20.9%, 15.0%, 35.4%, 37.6%, 15.5%, respectively, and reduced GFAP-ir glia by 30.8% vs. TNBS ($p < 0.05$). TNF- α , total Caspase-3, and cleaved Caspase-3 CTCF increased (49.0%, 36.6%, 28.3%) in TNBS and decreased (35.7%, 44.1%, 22.7%) in Butyrate group ($p < 0.05$). Butyrate prevented neuronal loss and inflammation, likely via GPR43/GPR109A signaling, supporting its therapeutic potential for IBDs.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Probiotic in periostropause: impact on cognition and behavior Wistar rats

Authors

Júlia Zanuto Douradinho^{1,2}

Thainá Daguane Esperança^{1,2} Débora Prazias Cavalcante^{1,2} Luana Galante Douradinho^{1,2}

Isabella Ribeiro Pradela²

Beatriz Procópio STRINGHETTA-Villar² Antônio Hernandes CHAVES-Neto^{1,2} Rita Cássia

Menegati Dornelles^{1,2}

Affiliations

¹ Multicentric Graduate Program in Physiological Sciences– SBFis/UNESP, São Paulo State University, Araçatuba, São Paulo, Brazil.

² Department of Basic Sciences, School of Dentistry. São Paulo State University, São Paulo/SP.

Keywords: Brain-Gut Axis. Oxidative stress. Senescence.

Abstract:

We investigated the action of *VSL#3* on cognition, anxiety, intestinal inflammation and redox status in periostropause Wistar rats. Our central hypothesis is that *VSL#3* exerts a neuroprotective effect in aging rats. Female rats in persistent diestrus (21 months) were given NaCl (0.1 mL/kg/day) or *VSL#3* (12.86 billion live bacteria/kg; in 0.1 mL/kg/day/NaCl) via gavage for 42 days (CEUA no. 1028-2023). Behavioral analyses [Open Field (AC) and Object Recognition (OR)] performed before (21 months) and after (23 months) the intervention. Redox status (blood and hippocampus) and evaluation of mesenteric mast cells (MCs) (toluidine blue 1%), at 23 months. ANOVA analysed showed a decrease in the number of MCs ($p=0.0011$) and activated MCs ($p=0.0077$) after *VSL#3*. However, 23Me/NaCl and 23Me/*VSL#3* revealed a greater number ($p<0.0001$) of deactivated MCs. In the AC, the 23Me/*VSL#3* group spent less time in the periphery ($p=0.125$), more time in the center ($p=0.0219$) and more vertical exploration ($p=0.002$) than the 23Me/NaCl group. Both at 23Me showed a higher tigmotaxis index. In the RO test, the interaction ($p=0.0208$) and the *post hoc* showed greater memory acquisition in the 23Me/*VSL#3* group compared to 21Me ($p=0.0003$) and 23Me/NaCl ($p=0.0008$). The 23Me/NaCl group showed a decrease in short-term memory (STM) compared to their 21Me counterparts ($p=0.0025$). The interaction ($p=0.0384$) in the long-term memory test led to better performance in the 23/*VSL#3* animals compared to 23Me/NaCl ($p=0.0466$). *Student t* showed improved hippocampal redox balance, with increased TAC ($p=0.0194$) and lower TOC ($p=0.0334$) after *VSL#3*. In plasma, decreased TOC ($p=0.0075$), decreased GSH ($p=0.0328$) and TBARs ($p=0.0026$). STM decline reveals brain biochemical alterations in senescent rats. *VSL#3* shows anxiolytic potential, cognitive improvement and reduced intestinal inflammation. These findings, together with the redox balance of the hippocampus, suggest a possible neuroprotective effect in periostropause.



XXXIX REUNIÃO ANUAL DA FeSBE
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XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Influence of nicotinic cholinergic receptor alpha-7 deletion in hypothalamic POMC neurons in brown adipose tissue's activity of mice.

Authors:

Ana Luisa Gomes Fernandes¹ Wenicios Ferreira Chaves¹ Caroline Lobo Costa¹ Marcio Alberto Torsoni¹

Affiliations:

¹ Faculdade de Ciências Aplicadas – UNICAMP.

Keywords: Brown adipose tissue. Nicotinic cholinergic receptor alpha-7. Thermogenesis.

Abstract:

Nicotinic cholinergic receptor alpha-7 ($\alpha 7$ nAChR) has a main role in the body's cholinergic anti-inflammatory response and energy balance. $\alpha 7$ nAChR is expressed in POMC neurons in the hypothalamus. It can signal through the JAK2-STAT3 pathway, similar to leptin. Previous results showed that POMC $\alpha 7$ nAChR deletion delays the anorexigenic leptin response. Thereby, $\alpha 7$ nAChR might affect peripheral tissues' activity, like brown adipose tissue (BAT)'s thermogenesis. This project aimed to study this interaction. Methodology: It was used C57BL/6L mice (CEUA: 6214-1/2023) with specific $\alpha 7$ nAChR gene deletion in hypothalamic POMC through Cre-Lox system. Therefore, $\alpha 7^{fl/fl}$ POMC^{Cre+} mice are the ones with gene deletion and $\alpha 7^{fl/fl}$ mice were used as control animals. Before BAT extraction, the animals were kept fasting for 12 hours and stimulated with leptin (IP) (2,5 μ g/g of weight), PNU-282987 (1mg/kg of weight), an $\alpha 7$ nAChR agonist, and saline solution. There was no difference in Cidea, Ucp1, Sirt1, Prdm16, Ucp1, Tfam and Pparg expression between saline groups, but in $\alpha 7^{fl/fl}$ POMC^{Cre+} animals there was increase in Chrna7 expression and a tendency to reduction in Adrb3 expression. Comparing all groups, it was observed higher Ucp1 and Adrb3 expressions on $\alpha 7^{fl/fl}$ POMC^{Cre+} with PNU than $\alpha 7^{fl/fl}$. Furthermore, $\alpha 7^{fl/fl}$ POMC^{Cre+} mice stimulated with leptin exhibited lower expressions of Ucp1 and Cidea compared to those treated with PNU or saline and showed reduced Pparg expression in the PNU and saline $\alpha 7^{fl/fl}$ groups compared to the control group. It was used $p \leq 0,05$ for significance. Thus, deleting hypothalamic POMC $\alpha 7$ nAChR may lead to a compensatory response characterized by increased peripheral $\alpha 7$ nAChR levels and decreased Adrb3 expression in BAT. PNU $\alpha 7^{fl/fl}$ POMC^{Cre+} thermogenesis activity might have been affected by these peripheral $\alpha 7$ nAChR. Results suggest POMC $\alpha 7$ nAChR deletion impairs the nervous system signaling to BAT and modulates its thermogenesis ability, decreasing leptin action in the tissue.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Aging and inflammatory bowel disease: The role of P2X7 receptor in enteric neuronal loss after experimental ulcerative colitis

Authors:

Marcos Antônio Ferreira Caetano¹ Henrique Inhauser Riceti Magalhães² Patricia Castelucci¹

Affiliations:

¹ Department of Anatomy, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil.

² Department of Surgery, School of Veterinary Medicine and Animal Sciences, University of São Paulo, São Paulo, Brazil.

Keywords: Inflammatory bowel disease. Enteric nervous system. P2X7 receptor. Aging. ATP.

Abstract:

The enteric nervous system (ENS) is affected by inflammatory bowel diseases (IBDs). Aging also causes changes in the ENS, and IBDs in the elderly can lead to serious consequences. The P2X7 receptor is activated by ATP, which is released in greater quantities during IBDs and mediates the inflammatory response. This work aimed to explore the effects of aging on myenteric neurons in experimental ulcerative colitis (EUC), comparing 18 months wild-type (WT) and P2X7 knockout (KO) (P2X7R -/-) male C57BL/6 mice. Animals from WT/Colitis and KO/Colitis groups received an intrarectal injection of 100µL of 2,4,6-Trinitrobenzenesulfonic acid (TNBS). WT/Sham and KO/Sham received TNBS vehicle (ethanol 35%). Animals were euthanized 4 days after TNBS (CEUA-ICB/USP-2372300921). Large intestines were collected for immunofluorescence double labeling for the P2X7 receptor with neuronal nitric oxide synthase (nNOS), and choline acetyltransferase (ChAT). The number of neurons/ganglia immunoreactive(-ir) for P2X7, nNOS, and ChAT were analyzed. Colocalization of P2X7 receptor with nNOS-ir and ChAT-ir neurons was observed in WT groups. The number of nNOS-ir neurons/ganglion reduced by 40.1% in WT/Colitis and 27.7% in KO/Colitis ($p < 0.0001$) compared to WT/Sham and KO/Sham, respectively. The number of ChAT-ir neurons reduced by 31.5% and 12.7% ($p < 0.0001$) in WT/Colitis and KO/Colitis compared to the WT/Sham and KO/Sham, respectively. Regarding the number of P2X7-ir neurons, WT/Colitis showed a reduction of 17.1% compared to the WT/Sham ($p < 0.0001$). Histological analysis showed mucosal architecture loss, submucosal edema, and inflammatory infiltrate in WT/Colitis, which were attenuated in KO/Colitis. WT/Sham and KO/Sham had normal distal colon morphology. EUC in elderly mice led to neuronal loss, and morphological alterations. However, KO mice lost fewer neurons, and had better morphology, suggesting a protective effect of P2X7 receptor absence, indicating potential therapeutic tool for IBDs.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Antagonism of RAGE ameliorates cholesterol-induced exacerbation of metabolic dysfunction- associated steatotic liver disease.

Authors:

Beatriz Peres de Araujo¹ Raquel Rangel Silveiras¹
Evelyn Nunes Goulart da Silva Pereira¹ Karine Lino Rodrigues¹
Anissa Daliry¹

Affiliations:

¹ Laboratory of Clinical and Experimental Physiopathology, Oswaldo Cruz Institution, Oswaldo Cruz Foundation.

Keywords: Advanced glycation end products. Advanced lipoxidation end products. Cholesterol. Lipotoxicity. Metabolic dysfunction-associated steatohepatitis.

Abstract:

Metabolic dysfunction associated steatotic liver disease ranges from steatosis to metabolic dysfunction-associated steatohepatitis (MASH). Lipotoxicity and advanced glycation/lipoxidation end products (AGEs/ALEs) bind to liver receptors, triggering oxidative stress, inflammation, and metabolic dysfunction. However, the molecular mechanisms remain unclear. We hypothesized that lipids modulate the AGE-RAGE pathway, aggravating MASH. MASH was induced in C57BL/6 male mice (n=26) using a high-fat diet (55%) and high-carbohydrate drink (25% fructose) for 43 weeks. In the final 12 weeks, a subgroup received 2% cholesterol. To assess RAGE inhibition, FPS-ZM1 was administered in a MASH model using a 60% fat diet

+ 2% cholesterol and a high-carbohydrate drink (55% fructose, 45% sucrose) for 12 weeks (n=20). After 6 weeks, animals received FPS-ZM1 (1 mg/kg/day) or vehicle intraperitoneally. Controls received a standard diet for 43 weeks (n=18) or 12 weeks (n=18). Animal experiments were approved (L-012/2018A2). Data were analyzed by one-/two-way ANOVA with Tukey's test. Cholesterol worsened MASH, increasing LDL (p<0.001), liver cholesterol (p<0.05), steatosis (p<0.01), inflammation (p<0.001), fibrosis (p<0.05), AST (p<0.05), reactive oxidative species (p<0.001), superoxide dismutase (p<0.05), and CML protein expression (p<0.01). RAGE protein (p<0.01), RAGE gene (p<0.001), and DIAPH1 gene expression (p<0.05) also increased. FPS-ZM1 normalized hepatic cholesterol (p<0.001), triglycerides (p<0.05), ALT (p<0.01), reactive oxidative species (p<0.05) and catalase (p<0.05), and serum/liver AGEs/ALEs (p<0.01; p<0.05). Findings suggest cholesterol exacerbates MASH via AGE-ALE/RAGE, while RAGE inhibition mitigates liver injury.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Targeting AGE/RAGE Pathway with Pyridoxamine Attenuates Inflammatory and Microcirculatory Abnormalities in Metabolic dysfunction-associated Steatohepatitis

Authors:

Raquel Rangel Silveiras¹ Beatriz Peres de Araujo¹ Juliana Florêncio da Silva¹ Karine Lino Rodrigues¹

Evelyn Nunes Goulart da Silva Pereira¹ Juliana Magalhães Chaves Barbosa² Vivian Vieira Dias da Silva¹

Marjo P. H. Van de Waarenburg³ Jean L. J. M. Scheijen³

Kristiaan Wouters³ Casper Schalkwijk³ Anissa Daliry¹

Affiliations:

¹ Laboratory of Clinical and Experimental Pathophysiology, Oswaldo Cruz Institute, Oswaldo Cruz Foundation.

² Cell Biology Laboratory, Oswaldo Cruz Institute, Oswaldo Cruz Foundation.

³ Department of Internal Medicine, Maastricht University, Maastricht, the Netherlands.

Keywords: Advanced glycation end products. Metabolic dysfunction-associated steatohepatitis. Pyridoxamine

Abstract:

Metabolic dysfunction-associated steatotic liver disease (MASLD) results from excessive fat accumulation in the liver, associated with obesity, type 2 diabetes, and dyslipidemia. Its progression to metabolic dysfunction-associated steatohepatitis (MASH) involves inflammation and microcirculatory dysfunction, potentially leading to fibrosis, cirrhosis, and hepatocellular carcinoma, as well as increasing cardiovascular and renal risks. Advanced glycation end-products (AGEs) and the receptor RAGE play a central role in this progression, making them promising therapeutic targets. Pyridoxamine, an inhibitor of AGE formation, emerges as a potential strategy to mitigate MASH progression. To evaluate its effects, we used a MASH model in C57BL/6 mice fed a high-fat, high-carbohydrate diet with 2% cholesterol (HFHC+COL2%) for 16 weeks. During the last six weeks, the animals were treated with pyridoxamine (200 mg/kg/day). Microcirculation was assessed in vivo, and metabolic, inflammatory, and AGE/RAGE markers in the tissue ex vivo. Animal experimentation was approved by license L-012/2018A2. HFHC+COL2% mice exhibited increased body weight, visceral and subcutaneous fat ($p < 0.001$), glucose ($p < 0.05$), serum cholesterol ($p < 0.001$), ALT levels, and hepatic lipid accumulation ($p < 0.05$). Treatment prevented these alterations and reduced both serum ($p < 0.05$) and hepatic ($p < 0.01$) AGE levels. Hepatic microcirculation was compromised in the MASH group, with reduced blood flow and increased leukocyte adhesion ($p < 0.001$), whereas pyridoxamine demonstrated vasoprotective effects ($p < 0.001$). Additionally, hepatic inflammation was attenuated by treatment, with reduced levels of TNF- α , IFN- γ , IL-6, NF- κ B, and IL-2 ($p < 0.05$). Thus, pyridoxamine effectively modulated key mechanisms of MASH, including the AGE-RAGE pathway, reducing inflammation and microcirculatory dysfunction while preserving hepatic metabolic homeostasis. These findings support its therapeutic potential in preventing progression.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: PNPLA3 polymorphism as a possible determinant of bile acid dysregulation in MASLD progression

Authors:

Julia Helena Oliveira de Barros¹ Yasmin Pestana^{1,2}
Raiana Andrade Quintanilha Barbosa¹ Gustavo José de Souza Kreispaine¹ Débora Alves Comune¹
Alexandre Cerqueira Silva Filho¹ Guilherme Ferreira da Motta Rezende³ Nathalia Ferrari Fonseca de Sales¹ Marina Amaral Alves^{1,2}
Marlon Lemos Dias¹
Tais Hanae Kasai-Brunswick^{1,4} Regina Coeli dos Santos Goldenberg¹

Affiliations:

¹ Precision Medicine Research Center, IBCCF-UFRJ, Rio de Janeiro-Brazil.
² Technological Development Support Laboratory, LADETEC-UFRJ, Rio de Janeiro-Brazil.
³ Clementino Fraga Filho University Hospital, UFRJ, Rio de Janeiro-Brazil.
⁴ National Center for Structural Biology and Bioimaging, UFRJ, Rio de Janeiro- Brazil.

Keywords: Bile acid. MASLD. PNPLA3 polymorphism. Precision medicine.

Abstract:

Metabolic dysfunction-associated steatotic liver disease (MASLD) includes a spectrum of conditions that can progress in severity. PNPLA3 gene polymorphism (SNP) is associated with its progression. Despite being invasive, liver biopsy remains the gold standard for MASLD assessment. This study aims to address these limitations by integrating omics- based techniques and induced pluripotent stem cells (iPSC) as an in vitro model to elucidate PNPLA3 SNP underlying MASLD severity. Patients were recruited from the Clementino Fraga Filho University Hospital (UFRJ) (CAAE: 16079319.0.0000.5257). Fibrosis severity was determined via liver biopsy. The PNPLA3 SNP was analyzed by Sanger sequencing. Biochemical and bile acid profiling of blood samples were performed. The cohort consisted of liver disease patients and healthy individuals (control group) aged 22–77 years. Histological analysis showed that four patients had absent or mild fibrosis, and four had advanced fibrosis. Genetic analysis revealed three heterozygous and one homozygous carriers of the *PNPLA3* SNP. Advanced fibrosis was observed in one heterozygous and one homozygous patient. Biochemical profiles showed no significant differences across fibrosis stages and controls, except for higher ALT levels in the advanced fibrosis group (mean: 28.75) versus controls (mean: 12.00) ($p < 0.05$). Bile acid profiling by bileomic showed altered bile acid profiles in SNP carriers, with reduced levels of CDCA and its taurine conjugates, including taurine-conjugated CA. These results suggest *PNPLA3* SNP as a potential marker of bile acid dysregulation and MASLD severity. Future work includes developing iPSC-derived hepatocyte models to explore the impact of genetic and clinical backgrounds. This research advances precision medicine by providing insights into MASLD pathophysiology and highlighting the utility of omics and iPSC for patient-specific approaches as disease model.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Chronic intracerebroventricular treatment with irisin increases lipid metabolism in adipose tissues in diet-induced obesity mice.

Authors:

Tassiana Cristina Talpo¹ Kelly Cristina Pereira Bem¹
Guilherme Augusto da Silva Nogueira¹ Helena Cristina de Lima Barbosa¹

Affiliations:

¹ Department of Structural and Functional Biology, State University of Campinas, São Paulo.

Keywords: Adipose tissue. Irisin. Obesity. Thermogenesis.

Abstract:

Obesity is characterized by lipid excess in adipose tissue, impairing energy homeostasis. The hypothalamus controls adipose tissue metabolism via the autonomic nervous system (ANS). Irisin, an adipomyokine released into the circulation after exercise, crosses the blood-brain barrier, is expressed in the hypothalamus, and could mediate the effects of exercise on adipose tissue modulation via the ANS, contributing to the control of energy homeostasis. This study aimed to evaluate the effects of chronic intracerebroventricular (ICV) treatment with irisin on lipid metabolism in adipose tissues in diet-induced obese mice. CEUA-UNICAMP:5995-1/2022. C57BL/6J male mice, 4w, were divided into 3 groups: CTL fed a chow diet 10% fat, treated ICV with vehicle (saline 0,9%); HFD fed a high-fat diet 45% fat, treated ICV with vehicle; HFD- Iri fed a high-fat diet, treated ICV with irisin 300 ng/day. After 13w in diets, right lateral ventricle cannulation surgery was performed, and animals were submitted to ICV treatment for 7d. Plasma and tissues were collected after euthanasia. Sample size: 5-7 animals. Results were analyzed by One-way ANOVA ($P < 0,05$), followed by Tukey post-test. HFD showed increased plasma cholesterol (119.7 ± 7.9 mg/dL), larger adipocyte area in iWAT (5477 ± 566.5 μm^2), and higher percentage of lipid content in BAT ($53.9 \pm 2.6\%$), with decreased protein expression of PRDM16, with no changes in UCP1 (0.19 ± 0.1 and 1.09 ± 0.1 , fold change of CTL), compared with CTL (77.3 ± 2.7 mg/dL; 878.5 ± 179.6 μm^2 ; $45.6 \pm 1.8\%$). HFD-Iri showed decreased plasma cholesterol (99.6 ± 3.8 mg/dL), smaller adipocyte area in iWAT (3334 ± 427.8 μm^2), and normalization of a percentage of lipid content in BAT ($48.7 \pm 1.2\%$), with increased protein expression of PRDM16 and UCP1 (0.85 ± 0.1 and 1.6 ± 0.1 , fold change of CTL), compared with HFD. Chronic ICV treatment with irisin decreased plasma cholesterol, reduced iWAT deposits, improved BAT cytoarchitecture by decreasing whitening, and increased the expression of thermogenesis markers in this tissue, protecting mice against obesity challenges.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Lipidomic Signature of Somatic Muscles in Diet-Induced Metabolic Syndrome Animal Model Supplemented with Gamma-Oryzanol

Authors:

Thiago Luiz Novaga Palacio¹ Juliana Silva Siqueira^{1,2} Giovanna Baron²
Francesca Galasso² Gilda Aiello^{2,3} Giancarlo Aldini² Alfonsina D'Amato² Camila Renata Correa¹

Affiliations:

¹ Botucatu Medical School, São Paulo State University (UNESP), 18618687, Botucatu, Brazil.

² Department of Pharmaceutical Sciences, University of Milan, 20133, Milan.

³ Department for the Promotion of Human Science and Quality of Life San Raffaele Open University, Rome.

Keywords: Chromatography. Mass spectrometry. Obesity. Skeletal muscle.

Abstract:

The somatic musculature (SM) contributes to whole-body homeostasis. Metabolic syndrome (METS) can cause SM dysfunction. Gamma-Oryzanol (ORY) is a cost-effective, non-drug approach to managing METS and its comorbidities. Mechanistic data on ORY in SM treatment are scarce. This study investigated key lipid metabolism changes in oxidative and glycolytic muscles in a high sugar-fat (HSF) diet-induced METS model supplemented with ORY. Male Wistar rats were fed with a control (n=6) or HSF (n=6) diet for 20 weeks. Then, HSF animals were split and fed with an HSF+ORY (0.5% w/w) diet for 10 weeks (1458/2024- CEUA). METS risk factors were assessed. Lipidomic analysis identified key lipid classes modulated by ORY. Statistical significance by ANOVA/Kruskal-Wallis with Tukey's/Dunn's post-hoc test $p < 0.05$. Lipidomic data were analysed using MaxQuant (FDR = 1%), MetaboAnalyst 6.0, and Ingenuity Pathway Analysis (IPA). Compared to the control, the HSF group showed higher caloric intake (2082.02 ± 1193.43 vs 18309.09 ± 1382.83), body weight (586.75 ± 34.45 vs 472.18 ± 43.61), adiposity index (8.33 ± 0.44 vs 4.49 ± 0.25), triglycerides ($119.32(122.32-117.09)$ vs $60.04(63.42-55.55)$), glucose ($5.60(6.03-5.02)$ vs $4.31(4.51-4.22)$), insulin levels ($132.00(159.36-95.95)$ vs $31.15(35.17-27.98)$), and insulin resistance (34.02 ± 7.71 vs 6.07 ± 1.37). PLS-DA showed clear lipid signature differences in soleus and EDL muscles of the HSF group versus control. 10 weeks of treatment with ORY improved the triglycerides ($88.71(98.00-75.47)$ vs $119.32(122.32-117.09)$), insulin ($94.23(116.03-76.90)$ vs $132.00(159.36-95.95)$), and insulin resistance (20.86 ± 6.08 vs 34.02 ± 7.71) and modulated three main classes of SM lipids: diacylglycerol, phosphatidylcholine, and phosphatidylethanolamine (fold change > 1.3) despite any changes in body weight (587.03 ± 68.63 vs 586.75 ± 34.45) and adiposity index (8.50 ± 1.31 vs 8.33 ± 0.44). ORY improved METS markers and altered lipid profiles in both SM types, suggesting new therapeutic targets.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Glycolytic Muscle Metabolomic Signature of Obese Animals Submitted to Western Diet Supplemented with Gamma-Oryzanol

Authors:

Thiago Luiz Novaga Palacio¹ Juliana Silva Siqueira^{1,2} Giovanna Baron²
Giancarlo Aldini² Alfonsina D'Amato² Camila Renata Correa¹

Affiliations:

¹ Botucatu Medical School, São Paulo State University (UNESP), 18618687, Botucatu, Brazil.

² Department of Pharmaceutical Sciences, University of Milan, 20133, Milan.

Keywords: Chromatography. Mass Spectrometry. Metabolites. Phytosterol. Somatic muscle.

Abstract:

Obesity (Ob) is an epidemiologic global problem. In Brazil, about 1.5 billion reais are spent annually on this disease, and it is estimated that by 2044, 75% of adult Brazilians will be obese. The glycolytic musculature (GM) is the main responsible for the contraction, force generation, and functional capacity of the skeletal muscles, negatively affected by the Western diet-related Ob. Gamma-Oryzanol (GOZ) is a cost-effective phytosteroidal strategy for the treatment of Ob and its comorbidities. However, no information about the therapeutic mechanistic effects of GOZ on GM metabolites of diet-induced obesity was reported. This study aimed to investigate metabolomic alterations in the glycolytic extensor digitorum longus (EDL) muscle of obese animals fed with a Western diet (WD) supplemented with GOZ. Rats received either a control (n=6) or WD (n=6) diets for 20 weeks. Then, WD animals were split and fed with WD+GOZ (0.5% w/w) for 10 weeks (1458/2024-CEUA). Total caloric intake, body weight, and adiposity index were assessed. Metabolomic analyses were performed to identify the GOZ-modulated metabolites. Statistical significance by one-way ANOVA/Kruskal-Wallis test with Tukey's/Dunn's post-hoc test $p < 0.05$. Metabolomic data were analyzed using MaxQuant (FDR = 1%), MetaboAnalyst 6.0, and Ingenuity Pathway Analysis (IPA). The WD group presented higher caloric intake, body weight, and adiposity index in comparison to the control group. Also, in the GM of the WD group, a clear distinction between the identified metabolites in comparison to the control group through partial least squares-discriminant analysis (PLS-DA) can be observed. 10 weeks of treatment with GOZ could modulate the following metabolites: 1-Methyladenosine, Cytidine, Cytosine, Guanine, Guanosine, Inosine and Proline, independently of effects on obesity. GOZ modulated the metabolomic signature in EDL muscle, providing first insights about the compound action in the glycolytic muscle metabolites.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Effects of chronic central ghrelin infusion on food preference of male offspring of mild hyperglycemic rats.

Authors:

Karla Martinucho Godeguez^{1,2} Milena Silva Agostini^{1,2} Caroline Martins Sierro^{1,2} Carla de Moraes Machado¹ Ana Carolina Inhasz Kiss^{1,2}

Affiliations:

¹ Department of Structural and Functional Biology, Institute of Biosciences of Botucatu, São Paulo State

University 'Júlio de Mesquita Filho' (UNESP).

² Graduate Program in General Physiology, Institute of Biosciences, University of São Paulo (USP).

Keywords: Diabetes. Food choice. Orexigenic factors. Pregnancy. Rodents.

Abstract:

Ghrelin is a hormone that modulates food intake and taste perception; however, there are no studies on its effects in offspring of hyperglycemic mothers. The aim of the present study was to evaluate the effect of chronic central ghrelin infusion on the food preference of male offspring of rats with mild hyperglycemia. Adult offspring from normoglycemic (control) and hyperglycemic (streptozotocin (STZ) 35 mg/kg i.p. on day 7 of pregnancy) mothers underwent stereotaxic surgery and intracerebroventricular infusion of saline or ghrelin (1 µg/day) for 7 consecutive days. The food preference test was conducted before and during the ghrelin infusion, and the index was calculated (mean ± SEM) (Animal ethics committee protocol: 3041270422). Ghrelin treatment reduced the offspring preference for high-fat diet (Control-Saline (CS), n = 3: 50.7 ± 10.5%; Control-Ghrelin (CG), n = 4: 21.0 ± 5.4%; STZ-Saline (SS), n = 4: 33.0 ± 5.6%; STZ-Ghrelin (SG), n = 5: 24.9 ± 5.7%; time: $F(2,24) = 7.424$, $p = 0.003$; time and treatment: $F(2,24) = 3.623$, $p = 0.042$; treatment: $F(1,12) = 5.530$, $p = 0.037$) and increased the preference for high-protein diet (CS: 7.34 ± 2.03%; CG: 27.58 ± 8.67%; SS: 5.42 ± 1.58%; SG: 20.43 ± 6.95%; treatment: $F(1,12) = 7.025$, $p = 0.021$). Regarding the preference for the high-sugar diet, there was no change related to ghrelin treatment. Additionally, there was no significant effect of maternal metabolism on the preference index for any of the diets analyzed. In conclusion, ghrelin modified the food preference of male offspring for high-fat and high-protein diets. However, there was no difference in the preference for the high-sugar diet. Finally, offspring of hyperglycemic mothers did not show any difference in food preference compared to offspring of normoglycemic mothers. Further studies will be conducted to increase the size of the experimental groups and include analysis of female offspring.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Role of cholesterol in triggering the AGE-RAGE pathway in an in vitro steatosis model

Authors:

Mariana Bonno MATTOS¹

Beatriz Peres de ARAUJO¹

Marjo P. H. Van de Waarenburg² Jean L. J. M. Scheijen²

Kristiaan Wouters² Casper G. Schalkwijk² Anissa DALIRY¹

Affiliations:

¹ Laboratory of Clinical and Experimental Physiopathology, Oswaldo Cruz Institution, Oswaldo Cruz Foundation.

² Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Maastricht, The Netherlands.

Keywords: AGEs. ALEs. Cholesterol. Lipotoxicity. MASLD.

Abstract:

Metabolic dysfunction-associated steatotic liver disease (MASLD) is characterized by excessive accumulation of triglycerides in the liver in the presence of at least one cardiometabolic risk factor. The term MASLD encompasses a spectrum that begins with simple steatosis and can progress to metabolic dysfunction-associated steatohepatitis (MASH). The development and progression of MASLD is due to the synergic interaction of several factors that can cause metabolic and molecular changes. These factors include the imbalance in lipid metabolism leading to toxic lipids causing lipotoxicity and the presence of advanced glycation and lipoxidation end products (AGEs/ALEs). It is still unclear whether lipoproteins (LDL) and oxidized lipoproteins (oxLDL), which transport cholesterol, can modulate the AGE-ALE/RAGE signaling pathway. To determine whether cholesterol is able to modulate this pathway, HepG2 cells have been incubated with LDL or oxLDL 50 µg/mL and the intracellular triglycerides levels were assessed using kit. The AGEs intermediates (methylglyoxal and glyoxal) were analyzed by ultra performance liquid chromatography/ mass spectrometry and the content of fluorescent AGEs/ALEs and carboxymethyl lysine will be measured in the cell lysate. In addition, gene and protein expression of the receptor for AGEs (RAGE) will be evaluated. We found that LDL and oxLDL (50 µg/mL) were unable to increase triglyceride levels ($p < 0.05$). Regarding AGE intermediates, incubation with LDL did not modulate MGO and GO levels, while oxLDL increased MGO levels, with no changes in GO levels. These findings suggest that LDL and oxLDL do not modulate intracellular triglyceride levels and that LDL oxidation may be involved in the modulation of glycoxidative stress, which could have implications for related metabolic disorders.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Effects of estradiol and fish oil on white and brown adipose tissue of ovariectomized mice

Authors:

Anna Beatriz Agio Secco¹ Vanessa Morales Torres¹ Anna Luiza Curty Cipriano¹ Deborah Biscacio Cardoso¹ Caroline Fernandes-Santos¹ Thereza Cristina Lonzetti Bargut¹

Affiliations:

¹ Núcleo de Estudos em Metabolismo, Nutrição e Histopatologia (NEMENUTH) - Instituto de Saúde de Nova Friburgo (ISNF) - Universidade Federal Fluminense (UFF).

Keywords:

Adipose tissue. Hormone replacement. Menopause. Polyunsaturated fatty acids.

Abstract:

Menopause is defined as the cessation of menstruation, resulting from the decline in ovarian hormones production. This process is accompanied by changes in body composition and metabolism that impact women's quality of life. We evaluated whether hormone replacement with estradiol (E) and/or fish oil (FO) was able to attenuate the changes promoted by menopause in female C57Bl/6 mice. At 4 months of age, the animals were divided into 2 groups: Control (SHAM) and Menopause (OVX). After 8 weeks, the OVX group was randomly subdivided into 4 groups according to the treatment received, totaling 5 groups: SHAM (n=7), OVX (n=7), OVX+E (n=8), OVX+FO (n=6), and OVX+E+FO (n=6). CEUA no 8714100123. Menopause was induced through bilateral ovariectomy. Estradiol was administered as 10 µg of 17-beta-estradiol 3x/week via subcutaneous injections, and FO was provided at 1% of the total energy of the diet, both for 6 weeks. The uterus, white genital adipose tissue (gWAT) and brown adipose tissue (BAT) were weighed and also stored for histological analysis. The uterine weight of the OVX group showed a decrease compared to the SHAM group (-51.5%). OVX+E and OVX+E+FO were higher than the OVX group (+289.5% and +244%). In gWAT, OVX was higher than the SHAM group (+37%), while OVX+E and OVX+E+FO were lower than the OVX group (-35.5% and -42%). The adipocyte diameter of the OVX+E and OVX+E+FO groups were smaller than the OVX group (-12.4% and -16.4%). The BAT weight, as well as the area of the lipid vesicles, were similar between the groups. In conclusion, estradiol was able to alter uterine mass and attenuate adiposity, regardless of the presence of fish oil. Therefore, although more studies are necessary, it could be an interesting adjuvant tool in the changes promoted by menopause.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Effects of Gestrinone Administration on Body Composition and food intake in Female Rats Fed a High-Fat Diet

Authors:

Dionizia Xavier Scomparin¹ Clara Wacelkoski⁴
Filipe Augusto Baier de Andrade¹ Isabela Kapp³
Elisângela Pinotti Conci Costa Rosa² Rosane Aparecida Ribeiro⁴

Affiliations:

- ¹ Universidade Estadual de Ponta Grossa-Programa de pós graduação em Ciências da saúde.
² Universidade Estadual de Ponta Grossa- Programa de pós graduação em Ciências Farmacêuticas.
³ Universidade Estadual de Ponta Grossa-Curso de Farmácia.
⁴ Universidade Estadual de Ponta Grossa Curso de Pós Graduação em Ciências Biomédicas

Keywords: Food intake. Gestrinone. High fat diet. Obese.

Abstract:

In recent years, the internet has brought attention to the potential role of the gestrinone implant in fat mass regulation. Gestrinone is a synthetic steroid with androgenic, antiprogesterin, and anti-estrogenic properties, primarily used to treat endometriosis and myomas. This study aimed to investigate the effects of gestrinone administration on the body composition of female rats fed a high-fat diet (HFD). Adult female Wistar rats were maintained on an HFD and received oral gavage three times per week with soybean oil containing 0.16% DMSO, with or without gestrinone at doses of 0.13 mg/kg (HGES0.13), 0.26 mg/kg (HGES0.26), or 0.52 mg/kg body weight (BW; HGES0.52). Approved by the CEUA/protocol 23.000077157-5). Gestrinone treatment did not significantly affect final body weight: HFD (313 ± 47.24 g), HGES0.13 (299.83 ± 34.28 g), HGES0.26 (283.4 ± 32.4 g), and HGES0.52 (294.5 ± 43.45 g). However, it significantly reduced fat depots, including periuterine fat: HFD (43.09 ± 8.4 mg/BW), HGES0.13 (27.95 ± 8.9 mg/BW), HGES0.26 (25.9 ± 9.49 mg/BW), and HGES0.52 (27.6 ± 8.33 mg/BW); retroperitoneal fat: HFD (30.31 ± 8.52 mg/BW), HGES0.13 (18.5 ± 5.4 mg/BW), HGES0.26 (14.96 ± 3.59 mg/BW), and HGES0.52 (16.26 ± 2.4 mg/BW); and total abdominal adiposity: HFD (125.3 ± 34.14 mg/BW), HGES0.13 (78.7 ± 27.10 mg/BW), HGES0.26 (71.2 ± 23.27 mg/BW), and HGES0.52 (76.03 ± 24.53 mg/BW), compared to the HFD group. Muscle mass, assessed in the soleus, gastrocnemius, and femoral muscles, remained unchanged in gestrinone-treated rats. Additionally, gestrinone administration did not affect daily food intake: HGES0.13 (23.15 ± 14.31 Kcal/rat), HGES0.26 (25.16 ± 5.81 Kcal/rat), and HGES0.52 (22.03 ± 7.74 Kcal/rat), compared to HFD (32.85 ± 8.10 Kcal/rat). No significant differences were observed in nocturnal food consumption either. In conclusion, gestrinone administration effectively reduces HFD-induced obesity by decreasing fat depots without altering overall food intake or muscle mass.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Supplementation preserves muscle functionality and morphology in aging wistar rats

Authors:

Yasmin Leal¹
Beatriz Borges Matthes¹ Thomas Horlem²
Diego Francis Saraiva Rodrigues¹ Matheus Felipe Zazula¹
Luiz Claudio Fernandes² Katya Naliwaiko¹

Affiliations:

¹ Laboratório de Plasticidade Morfofuncional, Departamento de Biologia Celular, Universidade Federal do Paraná – Curitiba, Paraná, Brasil.

² Laboratório de Metabolismo Celular, Departamento de Fisiologia, Universidade Federal do Paraná – Curitiba, Paraná, Brasil.

Keywords: Fiber types. Fish oil. Skeletal muscle.

Abstract:

Aging is characterized by a decline in functionality, changes in fiber composition, and reductions in muscle fiber size. Omega-3 have been shown to exert a protective effect on muscle tissue and functional performance. This study investigated the effects of Omega-3 on muscle functionality and morphology in aging Wistar rats. Animals were divided into a control group (CTL) and a supplemented group (SPL), which received 1 g/kg of fish oil orally for eight weeks. At 15 and 18 months, functional tests assessed muscle strength, joint mobility, and locomotor activity. After euthanasia, the plantar muscle was dissected for histological analysis (CEUA 1497/2022). A two-way ANOVA was conducted to examine the effects of age and fish oil supplementation on muscle functionality and morphology followed by Tukey's post hoc test. SPL animals exhibited milder histopathological alterations than CTL, with reduced inflammatory infiltration, fibrosis, and centrally nucleated fibers, indicating a protective effect against aging-related muscle degeneration ($p < 0.0001$, in all). Supplementation significantly altered fiber type composition, increasing the proportion of type IIa and IIb fibers, while aging itself led to reductions in type I and IIb fibers ($p < 0.001$, in all). Cross-sectional area revealed that supplementation reduced fiber size at 15 months, but values became comparable to CTL by 18 months, suggesting an adaptive response over time ($p < 0.01$, in all). The diameter ratio indicated that omega-3 preserved the structural integrity of fast-twitch fibers, preventing the typical rounding and atrophy associated with aging ($p < 0.01$, in all). Functionally, SPL animals exhibited superior muscle strength, maintaining higher peak force in grip strength tests at both time points. Joint mobility was also improved in SPL, with greater range of motion observed in flexion and extension tests. Locomotor activity analysis revealed a significant reduction in the number of falls in the SPL group, indicating better neuromuscular control and coordination ($p < 0.01$, in all). These findings suggest that omega-3 supplementation exerts a protective effect by modulating fiber composition, preserving muscle architecture, and enhancing functional performance.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Effects of maternal curcumin supplementation on offspring in an experimental model of protein restriction during the gestational period

Authors:

Ana Carolina Lourenço Ferreira¹ Ana Beatriz Farias de Souza^{1,2} Pedro Alves Machado-Junior¹
Samuel dos Santos Valença² Frank Silva Bezerra¹

Affiliations:

¹ Laboratory of Experimental Pathophysiology (LAFEx), Department of Biological Sciences (DECBI), Institute of Exact and Biological Sciences (ICEB), Federal University of Ouro Preto, Ouro Preto, MG, Brazil.

² Laboratory of Redox Biology, Institute of Biomedical Sciences (ICB), Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

Keywords: Antioxidant defense. Curcumin. Inflammation. Lung. Protein restriction.

Abstract:

Maternal nutrition influences the health of offspring. Curcumin has antioxidant and anti-inflammatory properties. This study evaluated the effects of curcumin administration on the offspring of females subjected to protein restriction during pregnancy. All procedures were approved by Ethics Committee (n°6251150822). C57BL/6 mice aged 8 to 10 weeks were used for breeding. The pregnant mice were divided into 5 groups: normoprotein diet (NP); low protein diet (LP) 8% protein; protein restriction + curcumin 50 mg/kg (LPC50); 100 mg/kg (LPC100) and 200 mg/kg (LPC200). After birth, the male offspring were euthanized 31 days postnatal. Lung mass (g) in the LPC100 (0.288 ± 0.006) was lower compared to NP (0.344 ± 0.01) ($p < 0.05$). Lung density (g/cm³) in the LP [0.40 (0.40; 0.43)] and LPC50 [0.17 (0.14; 0.46)] was lower compared to NP [0.76 (0.72; 0.80)] ($p < 0.05$). The influx of cells into the airways was greater in the LP (8.50 ± 0.58) compared to NP (3.58 ± 0.49). The animals that received curcumin had lower leukocytes compared to LP ($p < 0.0001$). There was a decrease in superoxide dismutase (SOD) activity (U/g tissue) in the RP (262.8 ± 9.48), LPC50 (201.5 ± 22.20), LPC100 (179.4 ± 23.0) and LPC200 (183.1 ± 10.56) compared to NP (320.2 ± 17.92) ($p < 0.001$). The Catalase activity (U/g tissue) decreased in LP (27.01 ± 3.65) compared to NP (68.46 ± 8.57), the LPC50 (88.74 ± 24.8) showed higher activity compared to LP ($p < 0.05$). There was a decrease in GSH/GSSG ratio in LPC50 [2.24 (1.07; 5.15)] and LPC100 [2.98 (2.34; 6.01)] compared to NP [8.86 (8.12; 11.33)] ($p < 0.05$). Carbonylated protein (nmol/g tissue) was higher in the LP (518.1 ± 68.53) compared to the NP group (241.0 ± 72.47); LPC50 (84.01 ± 2.98), LPC100 (188.5 ± 128.1) and LPC200 (153.8 ± 40.98), but showed lower levels when compared to LP ($p < 0.0001$). The results show that maternal supplementation with curcumin can exert protective effects on the 31-day-old offspring, minimizing inflammation and oxidative damage.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Supplementing with n-3 fatty acids to balance the n-6:n-3 ratio leads to metabolic dysfunction, oxidative stress, and liver structural changes in rat offspring, caused by a pro-inflammatory response

Authors:

Matheus Felipe Zazula¹ Mônica Maciel¹

Heloisa Deola Confortim² Luiz Claudio Fernandes³ Katya Naliwaiko¹

Affiliations:

¹ Laboratório de Plasticidade Morfofuncional, Departamento de Biologia Celular, Universidade Federal do Paraná – Curitiba, Paraná, Brasil.

² Laboratório de Plasticidade Morfofuncional, Universidade Federal do Paraná – Toledo, Paraná, Brasil.

³ Laboratório de Metabolismo Celular, Departamento de Fisiologia, Universidade Federal do Paraná – Curitiba, Paraná, Brasil.

Keywords: Maternal health. Unsaturated fatty acids. Metabolic programming. Dyslipidemia. DOHaD.

Abstract:

Due to its beneficial effects during pregnancy, pregnant women are advised to include n-3 fatty acids in their diets. This increased fatty acid intake, combined with Western dietary patterns, enhances lipid bioavailability during development. However, the consequences of excessive fatty acid intake on developmental traits remain underexplored. This study investigates the effects of higher-than-recommended doses of n-3 and n-6 fatty acids supplementation during pregnancy and lactation on liver and lipid homeostasis in offspring rats. Wistar rats were divided into three groups: control (C), supplemented with 4 g/kg of fish oil (FO; n-3) or soybean oil (SO; n-6). Supplementation started before mating and continued throughout pregnancy and lactation, and was interrupted at weaning. At 60 days of age, the rats were euthanized, and tissues were collected, weighed, and analyzed histomorphometrically (liver) and biochemically (liver, adipose tissue, plasma) (CEUA 1303/2019). Supplementation with FO or SO at these supply schemes led to a reduction in body size, increased markers of adiposity, and dyslipidemia. Alterations in energy metabolism, oxidative stress onset, and liver histological damage were observed, alongside the accumulation of damage and increased secretion of pro-inflammatory cytokines by adipose tissues. These results suggest that increased lipid availability during development induces liver damage through energy imbalance and oxidative stress, contributing to the development of metabolic syndrome. Additionally, elevated adiposity markers were observed, indicating excessive fat accumulation. This stress led to histological liver lesions, including inflammatory infiltration and cellular degeneration. These results indicate that excessive supplementation with unsaturated fatty acids during pregnancy and lactation induces liver damage, lipid metabolism imbalances, and increased oxidative stress in the offspring, with potential consequences for the development of metabolic syndrome.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Chronic supplementation with polyunsaturated fatty acids induces histopathological liver alterations in wistar rats

Authors:

Eduarda Martinowski Henriques Guia¹ Douglas Moritz Moreira¹
Otávio Sales¹
Mariana Inocêncio Manzano¹ Mônica Maciel²
Luiz Claudio Fernandes² Katya Naliwaiko² Matheus Felipe Zazula^{1,2}

Affiliations:

¹ Grupo de Pesquisa em Gestaç o e Doenas Metab licas, Universidade Cesumar – Curitiba, Paran , Brasil.

² Laborat rio de Plasticidade Morfofuncional, Departamento de Biologia Celular, Universidade Federal do Paran  – Curitiba, Paran , Brasil.

Keywords: Chronic inflammation. Dyslipidemia. Lipid supplementation. Visceral adiposity.

Abstract:

Fatty acids play essential roles in the body, acting as energy reserves and structural components of cell membranes. They are classified into saturated and unsaturated, including monounsaturated and polyunsaturated fatty acids, such as omega-3 and omega-6. Although adequate consumption of these fatty acids is associated with reduction in cardiovascular diseases and inflammatory processes, the human body cannot synthesize them, making their intake through diet essential. Despite the benefits of fatty acids, excessive and prolonged intake may be associated with an increased risk of obesity, diabetes, metabolic syndrome, and hepatic steatosis. In this study, 120-day-old Wistar rats were divided into three experimental groups: control, omega-3 supplementation (SPL3), and omega-6 supplementation (SPL6). After euthanasia, blood and hepatic tissue samples were collected (CEUA 1452/2022). The results indicated increase in abdominal adiposity in the supplemented groups, as well as elevated blood glucose levels in both experimental groups. In the SPL6 group, triglyceride levels increased, while total cholesterol and LDL levels were elevated in both groups, with a reduction in HDL levels in the SPL6 group. Furthermore, albumin and uric acid levels decreased in the supplemented rats. Morphologically, the supplemented groups exhibited reduction in hepatocyte density and an increase in sinusoids. Histopathological analysis revealed hepatic inflammation, macrophage infiltration, steatosis, and necrosis, suggesting chronic inflammation and a predisposition to metabolic syndrome. Thus, it is concluded that chronic supplementation with polyunsaturated fatty acids increased visceral adiposity, blood glucose, dyslipidemia, and hepatic inflammation, contributing to a metabolic syndrome-like condition. These findings reinforce the importance of balancing the n-6/n-3 ratio, highlighting the need for increased omega-3 consumption alongside dietary re-education to minimize adverse impacts.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Parkin Deficiency Alters Ubiquitination Levels in Liver Regeneration in Mice

Authors:

Felipe Oliveira Gomes da Cruz¹ Ana Laura Vieira da Silva¹ Rafael Paschini Tonon¹
Isabela Aparecida Divino¹ Igor Luchini Baptista¹

Affiliations:

¹ Laboratory of Cell and Tissue Biology (LaBCeT), School of Applied Sciences, University of Campinas, Limeira, Brazil

Keywords: Ubiquitination. Liver regeneration. Parkin.

Abstract:

The liver plays a crucial role in maintaining body homeostasis and exhibits remarkable regenerative capacity, capable of restoring its functions even after significant damage, such as the removal of $\frac{2}{3}$ of its total mass. However, the repair process involves intense cellular stress, caused by the high metabolic demand necessary to promote tissue regeneration. Parkin is a E3 ligase able to ubiquitinate lysine residues at position 48 (K48) — associated with degradation by the 26S proteasome — and at position 63 (K63) — associated with alterations in target protein activity. Under stress, Parkin can translocate from the cytosol to the nucleus which has already been demonstrated in other cell models. However, it is unclear whether this translocation also occurs during liver repair. Therefore, this study investigated the levels of ubiquitination in the nucleus and cytosol, and effects if Parkin loss at these levels. In nuclear and cytoplasmic fractions in both intact tissue and during liver regeneration in mice subjected to partial hepatectomy (PHx). The animals for this were provided by CEMIB, CEUA 6443-1/2024. For this purpose, male C57BL/6J Wild-type (WT) and Parkin^{-/-} (Parkintm1Shn) mice were subjected to surgical removal of $\frac{2}{3}$ of the liver and analyzed at two distinct time points of regeneration: 4 hours (preparation phase) and 7 days (termination phase). Our results show that, in the absence of Parkin, there is a decrease in total ubiquitination in the cytosolic fraction at the 4-hour regeneration time point (WT 1.94 vs KO 0.55). We also observed a trend of increased ubiquitin K63 in the nuclear fraction in Parkin-deficient animals (WT 0.97 vs KO 1.67), while in the cytosolic fraction, a decrease in ubiquitination levels was observed in Parkin-deficient animals (WT 1.86 vs KO 1.28). The results suggest that Parkin may be involved in physiological responses during liver repair in mice. However, it is important to emphasize that these are preliminary results, which require further analysis.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Association between inflammatory foods, excess weight and quality of life of patients with autoimmune disease

Authors:

Geovana Gonçalves Figueiredo¹ Geovana da Silva Abreu¹ Mariana Inêz Gregório da Silva¹
Milena Ribeiro da Costa¹
Beatriz de Mezas Tarocco¹ Edna de Souza Batista¹ Karianne Delalibera Hinokuma¹
Marcela de Andrade Bernal Fagiani¹ Ana Paula Marques Andrade²
Ana Paula Desiree de Oliveira² Sandra Cristina Genaro¹

Affiliations:

¹ Universidade do Oeste Paulista.

² Universidade Estadual de Maringá.

Keywords: Autoimmune disease. Diet. Inflammation. Obesity. Symptoms.

Abstract:

The number of people with autoimmune diseases (AD) such as rheumatoid arthritis, lupus, psoriasis, vitiligo, multiple sclerosis among others, has been increasing in recent years, especially in countries where industrialization has been growing rapidly. In this sense, there was a need to evaluate the relationship between the impact of consuming inflammatory foods and the worsening of symptoms experienced by patients, since anti-inflammatory foods can contribute to the process of remission of symptoms presented by most patients. This study aimed to identify the Inflammatory Index of the Diet, BMI (body mass index) and quality of life of 78 patients with autoimmune disease over 18 years old, admitted to a hospital in Presidente Prudente. The research was approved by the Research Ethics Committee and followed the standards of Resolution CNS 466/2012 of CONEP, by the Universidade do Oeste Paulista (UNOESTE), receiving a protocol by CAEE 78769624.6.0000.5515 and by CAPI 8670. The mean age was 55.6 ± 18.09 years, female gender (60.26%) prevailed. Hashimoto's thyroiditis presented a prevalence of 42.30%, followed by Rheumatoid Arthritis (15.38%) and Type 1 Diabetes Mellitus (8.97%). Overweight was common in 43.59% for usual weight BMI and 58.97% for current weight BMI. Physical health was compromised in the domains of physical aspects (30.51 ± 42.57), pain (37.02 ± 33.58) and mental health in the domain of emotional aspects (31.6 ± 44.59). The intake of a pro-inflammatory diet was the majority (94.87%). It is concluded that dietary and behavioral interventions are necessary to improve quality of life and control inflammation, since these aggravate chronic pain, affect mental health and worsen quality of life.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Abbreviation of preoperative fasting in patients with heart disease

Authors:

Mariana Inêz Gregório da Silva¹ Gabriel Ricordi Gerbasi Cardoso¹ Kateleen de Lima Alcântara¹
Kimberlyn Reis da Silva¹
Ana Paula Marques Andrade² Karianne Delalibera Hinokuma¹ Marcela de Andrade Bernal
Fagiani¹ Sandra Cristina Genaro¹

Affiliations:

¹ Universidade do Oeste Paulista.

² Universidade Estadual de Maringá.

Keywords: Disorders. Elective surgery. Food. Heart. Recovery.

Abstract:

The research began after the approval of the Research Ethics Committee and followed the norms of Resolution CNS 466/2012 of CONEP, by the Universidade do Oeste Paulista (UNOESTE), receiving a protocol by CAEE 81435324.0.0000.5515 and CAPI 8929. The aim of this study was to analyze gastrointestinal symptoms, anxiety, glycemic variation, and length of hospital stay of patients undergoing cardiac surgery with or without abbreviation of preoperative fasting, including oral nutritional supplementation with carbohydrates. The research was a Clinical Case Study carried out in the Cardiology Clinic and Coronary Unit of a Public Hospital in the city of Presidente Prudente, SP, from September to December 2024. Patients > 18 years old were randomly divided into a Control Group (CG) n = 9 and an Intervention Group (IG) n = 7, with the use of 23,25 g of carbohydrate, diluted in 200 mL of water to abbreviation of fasting. There was a reduction in the days of hospitalization and ICU stay among the IG (22,83±3,19 and 4,29±1,8 respectively). The CG had a higher incidence of nausea and anxiety (0,89 and 6,67 respectively, scale of 0 to 10). Capillary blood glucose showed no difference. It was concluded that offering fasting abbreviation provided greater comfort and better outcome to patients.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Impact of Chemotherapy Treatment on the Nutritional and Behavioral Status of Oncology Patients

Authors:

Leticia Manfio Cavalheiro¹

Maria Elaine Ferreira dos Santos¹ Thaís Mara Aguiar Rampazzo¹ Emily Storto Dela Bandeira¹

Gabrielly Caroliny de Souza Gomes¹ Kimberlyn Reis da Silva¹

Ana Paula Marques Andrade² Karianne Delalibera Hinokuma¹ Marcela de Andrade Bernal

Fagiani¹ Sandra Cristina Genaro¹

Affiliations:

¹ Universidade do Oeste Paulista.

² Universidade Estadual de Maringá.

Keywords: Chemotherapy. Health. Malignant neoplasm. Nutrition. Symptoms.

Abstract:

This study was initiated following approval by the Research Ethics Committee and conducted in accordance with Resolution CNS 466/2012 of the National Research Ethics Commission (CONEP), through the Universidade do Oeste Paulista (UNOESTE), under protocol CAEE 78777324.8.0000.5515 and CAPI 8671. Cancer remains a major public health concern, and chemotherapy, one of its primary treatments, often leads to adverse side effects. This study aimed to observe the symptoms associated with chemotherapy in cancer patients, as well as assess their nutritional status and quality of life. A total of 113 patients were evaluated at the Oncology Clinic of a hospital in Presidente Prudente, SP, from April to December 2024. The mean age was $59,8 \pm 13$ years, with 56,64% being female. Breast cancer was the most prevalent diagnosis (25,66%). Excess weight was observed in 42,25% of patients based on usual BMI and in 51,33% based on current BMI. The most reported side effects were nausea (57,14%) and xerostomia (44,90%). Quality of life was notably affected in the domains of physical functioning ($34,07 \pm 38,08$) and emotional well-being ($48,81 \pm 44,05$). It is concluded that chemotherapy can significantly impact both the nutritional status and quality of life of oncology patients. A multidisciplinary healthcare team is essential to provide comprehensive care and minimize the adverse effects of both the disease and its treatment.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Maternal administration of curcumin protects the liver of offspring at 31 days postnatal age of females subjected to protein restriction.

Authors:

Maria Eduarda Campos Lage VIEIRA¹ Ana Beatriz Farias de SOUZA^{1,2} Samuel dos Santos VALENÇA²
Frank Silva BEZERRA¹

Affiliations:

¹ Laboratory of Experimental Pathophysiology (LAFEx), Department of Biological Sciences (DECBI), Institute of Exact and Biological Sciences (ICEB), Federal University of Ouro Preto, Ouro Preto, MG, Brazil.

² Laboratory of Redox Biology, Institute of Biomedical Sciences (ICB), Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

Keywords: Curcumin. Protein restriction. Offspring. Oxidative stress. Liver.

Abstract:

This study investigated the effects of curcumin on the liver of the offspring of females subjected to protein restriction during pregnancy. All procedures were approved by the ethics committee (n°6251150822). C57BL/6 mice (8-10 weeks old) were used for mating. Pregnant females were divided into five groups: normoprotein (NP), low protein (LP, 8%), and LP + curcumin at 50 (LPC50), 100 (LPC100), or 200 mg/kg (LPC200). After birth, the male offspring remained with the females until day 31 (n = 5/group) when they were euthanised. In the peripheral blood, the monocyte Count (x103/mm³) was higher in the LP (0.07 ± 0.04) compared to the NP (0.52 ± 0.08) (p < 0.0001), and a higher monocyte count was observed at all doses of curcumin compared to the LP (p < 0.0001). ALT levels (U/L) in LP (59.13±7.01), LPC50 (71.59±8.13), LPC100 (61.84±2.53), LPC200 (58.67±8.32) were lower than in NP (90.79±5.67) (p < 0.05). Total cholesterol (mg/dL) levels were higher in LP (208.8±65.32) than NP (112.0±12.0), and triglyceride (mg/dL) levels were higher in LP (121.8±13.89) compared to NP (55.87±5.75), LPC200 (61.11±24.07) showed lower levels compared to LP (p < 0.05). The levels of carbonylated protein (nmol/g tissue) were higher in LP (518.1±68.53) compared to NP (241.0±72.47), all doses of curcumin resulted in lower levels of carbonylated protein compared to LP (p < 0.05). The levels of sulfhydryl groups were higher in LPC50 (13944± 3050), LPC100 (8916±1768) and LPC200 (12730±840.8) compared to NP (5685± 371.8) and LP (5789±333.8). There was a decrease in SOD activity (U/g tissue) in the LP group (262.8±9.48) compared to NP (320.2±17.92), and CAT activity was lower in LP (27.01±3.65) compared to NP (68.46±8.57), but there was an increase in RPC50 enzyme activity (88.74±24.82) compared to LP (p < 0.05). The data obtained so far show that maternal supplementation with curcumin can exert a protective effect on the offspring at 31 days postnatal, minimising inflammation and oxidative stress.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Impact of fish oil supplementation on obesity and metabolic syndrome in a rat model of litter size reduction: effects on oxidative stress and adiposity

Authors:

Tayná Nery Banckes¹ Matheus Felipe Zazula¹
Stephanie Rubianne da Silva Carvalhal² Luiz Claudio Fernandes²
Katya Naliwaiko¹

Affiliations:

¹ Laboratório de Plasticidade Morfofuncional, Departamento de Biologia Celular, Universidade Federal do Paraná – Curitiba, Paraná, Brasil.

² Laboratório de Metabolismo Celular, Departamento de Fisiologia, Universidade Federal do Paraná – Curitiba, Paraná, Brasil.

Keywords: Metabolic programming. Adiposity. Antioxidant Potential. Omega-3. DOHaD.

Abstract:

Obesity is a multifactorial disease caused by genetic and environmental factors, with its prevalence increasing due to the industrialization of food, which has altered the lipid profile of the human diet. Lactation is a critical period for the development of feeding behavior, and overnutrition during this stage may predispose individuals to comorbidities such as obesity and diabetes. This study investigated the effects of metabolic programming induced by litter size reduction on morphological changes and obesity-related parameters, as well as the impact of supplementation with fish oil, rich in n-3 polyunsaturated fatty acids. Male Wistar rats (n=10 per group) were used, whose litters were reduced on postnatal day 3 to three pups (obese group), while control litters were maintained with ten. After weaning, the animals were distributed into four experimental groups: control (CON), supplemented control (CONS), obese (OB), and obese supplemented (OBS), receiving daily supplementation of 1 g/kg/day of fish oil, administered orally via micropipette for 60 days. Serum parameters such as glucose, lipidemia, and lipid profile were evaluated, and adipose tissue was analyzed using enzymatic and non-enzymatic oxidative stress markers, including SOD, CAT, GPx, GR, GST, LPO, NP-SH, GSSG/GSH ratio, DCF-DA, and ChE activity (CEUA 1274/2018). Data were analyzed using two-way ANOVA followed by Tukey's post hoc test ($p \leq 0.05$). The results showed that the metabolic programming model induced obesity in the reduced-litter animals, triggering the production of reactive oxygen species and hyperactivation of the antioxidant system. The OB group exhibited increased oxidative stress, lipid peroxidation, and antioxidant enzyme activity, along with signs of adipose tissue dysfunction. Fish oil supplementation significantly attenuated these alterations in the OBS group, reducing ROS production and improving redox balance. Moreover, adipose tissue parameters such as lipid accumulation and oxidative damage were reversed. Supplementation not only reduced oxidative damage but also effectively prevented the development of metabolic syndrome, with a significant reduction in serum markers of obesity, such as glucose and lipids. Additionally, supplementation reversed adipose tissue parameters, indicating that n-3 polyunsaturated fatty acids may modulate obesity and metabolic syndrome.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Effects of Amazon açai (*Euterpe precatoria Martius*) supplementation on biochemical parameters and oxidative stress in the serum and liver of healthy Wistar rats

Authors:

Carolina de Oliveira Ramos Petra de Almeida¹ Milla Christie Rodrigues de LIRA²
Karen Pereira COUTINHO³ Julyana Oliveira ALBINO³
Nara Michi de Faria ISHIKIRIYAMA³ Klenicy Kazumy de Lima YAMAGUCHI⁴ Valdir Florêncio da Veiga JUNIOR⁵ Mariana Sarto FIGUEIREDO⁶
Anderson Junger TEODORO⁶

Affiliations:

¹ Pos-graduated in Food and Nutrition, Faculty of Nutrition, Federal University of the State of Rio de Janeiro, Rio de Janeiro, Brazil.

² Pos-graduated in Medical Science, Faculty of Nutrition, Fluminense Federal University, Niteroi, Brazil.

³ Nutrition Graduate, Emília de Jesus Ferreiro College of Nutrition, Fluminense Federal University, Niteroi, Brazil.

⁴ Institute of Health and Biotechnology, Federal University of Amazonas, Amazonas, Brazil.

⁵ Department of Chemical Engineering, Military Institute of Engineering, Rio de Janeiro, Brazil.

⁶ Departament of Nutrition and Dietetic, Faculty of Nutrition, Fluminense Federal University, Niteroi, Brazil.

Keywords: Amazon biome. Biochemical parameters. *Euterpe precatoria*. Oxidative stress.

Abstract:

Euterpe precatoria is an underexplored açai species from the Amazon biome, rich in phytochemicals and antioxidants. This study evaluated the effects of Amazon açai pulp supplementation on biochemical parameters and oxidative balance in Wistar rats. 40 rats (90 days old) were divided into: Control (C, n=10), *Euterpe precatoria* 50 mg/kg/day (50PRE, n=10), 100 mg/kg/day (100PRE, n=10) and 200 mg/kg/day (200PRE, n=10). The animals received commercial feed, water *ad libitum* and gavage for 30 days. Our protocol was approved by the Ethical Committee (CEUA 1645110921). Biochemical parameters (BioClin Analyzer), serum and hepatic antioxidant capacity and oxidative stress were assessed by colorimetric methods. 200PRE showed a 17% reduction in ALT compared to C. No significant differences were observed in AST or alkaline phosphatase levels ($p>0.05$). 100PRE exhibited lower total serum and hepatic antioxidant capacity compared to C (-46% and -22%) and serum compared to 200PRE (-48%) by DPPH assay. In serum, 200PRE showed a 49% increase in FRAP compared to 50PRE. No significant differences were observed in serum thiol and FOX, or in hepatic thiol, TBARS, FOX or protein carbonyl ($p>0.05$). However, 200PRE exhibited higher serum protein carbonyl compared to C, 50PRE and 100PRE (+48%, +41% and +99%). 100PRE showed increased TBARS serum levels compared to C and 50PRE (+178% and +141%). Despite the recognized antioxidant effects of polyphenols, high doses may act as pro-oxidants, especially in low oxidative stress conditions. The reduction in hepatic antioxidant capacity without evident liver damage suggests an adaptive response involving redox homeostasis, possibly mediated by the Nrf2-ARE pathway, favoring hepatoprotection. In contrast, in serum, higher doses indicate that systemic antioxidant defenses might have been overwhelmed, leading to redox imbalance. These findings highlight the complexity of antioxidant action that Amazon açai and the need for caution, especially in healthy conditions.

Committee number: 1645110921



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Diet-Induced Insulin Resistance Reduces Brain BDNF Levels in Female but Not Male Mice Without Affecting Memory

Authors:

Júlia Sousa Ramos¹ Camila Berbert Gomes¹
Daniel Massote de Melo Leite¹ César Augusto da Silva Caetano^{1,2} Maristela de Oliveira Poletini³ Bruno Pereira Melo^{1,4}
Danusa Dias Soares¹

Affiliations:

¹ Exercise Physiology Laboratory, School of Physical Education, Physiotherapy and Occupational Therapy, Federal University of Minas Gerais, UFMG, Belo Horizonte, Brazil.
² School of Pharmacy – Federal University of Minas Gerais, UFMG, Belo Horizonte, Brazil.
³ Endocrinology and Metabolism Laboratory, Institute of Biological Sciences, Federal University of Minas Gerais, UFMG, Belo Horizonte, Brazil.
⁴ University Center, UNITOP - PALMAS/Tocantins, Brazil.

Keywords: Diabetes. Cognition. Sexual dimorphism. High fat diet. Fructose.

Abstract:

Type 2 Diabetes Mellitus (T2DM) accounts for 90-95% of diabetes cases globally and is primarily characterized by insulin resistance (IR), often associated with sedentary behavior and poor diet habits. Although IR is known to impair glucose metabolism and reduce neurotrophins such as brain-derived neurotrophic factor (BDNF), potentially leading to cognitive decline, the influence of biological sex on these effects remains poorly understood. This study investigated sex-specific effects of IR - induced by a highfat-diet (HFD) combined with a 30% fructose solution - on BDNF levels and memory performance (short-term memory: STM and long-term memory: LTM) in male and female mice. The project was approved by the Ethics Committee for Animal Use (CEUA) of the Federal University of Minas Gerais (UFMG) (protocol numbers: 119/2024 and 120/2024). Sixteen 10-week-old mice (8 male, 8 female), were fed HFD and fructose for 8 weeks to induce IR confirmed via oral glucose tolerance testing. Cognitive function was assessed using the Novel Object Recognition (NOR) test, analyzing short-term memory (STM) and long-term memory (LTM) through the recognition index. After behavioral testing, animals were euthanized for tissue collection, and BDNF levels in the hippocampus and hypothalamus were quantified via ELISA. No significant differences between sexes were observed for STM ($p = 0,41$; males: $0,55 \pm 0,12$, $n=8$; females: $0,58 \pm 0,16$, $n=8$) or LTM ($p = 0,86$; males: $0,52 \pm 0,21$, $n=8$; females: $0,59 \pm 0,20$, $n=8$). However, female mice exhibited significantly lower BDNF concentrations than males in both the hippocampus ($p < 0,001$; male: 7; female: 8) and hypothalamus ($p < 0,001$; male: 6; female: 6). These findings suggest that IR induced by 8 weeks of HFD and fructose consumption selectively reduces BDNF levels in females, without impairing memory performance in either sex.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Influence of Yerba Mate Extract (*Ilex paraguariensis*) on Weight Gain and Metabolism in Wistar Rats

Authors:

Ana Paula Marques Andrade¹ Karianne Delalibera Hinokuma² Karolinnny Cristiny de Oliveira Vieira² Marcela de Andrade Bernal Fagiani² João Vítor Jordão Maia Silva² Sabrina Alves Lenquiste²
Sandra Cristina Genaro²

Affiliations:

¹ Universidade Estadual de Maringá.

² Universidade do Oeste Paulista.

Keywords: Yerba mate. *Ilex paraguariensis*. Lipid profile. Body weight.

Abstract:

Yerba mate (*Ilex paraguariensis*) is a plant native to South America, traditionally consumed as a hot infusion (chimarrão) or a cold beverage (tereré), and is known for its antioxidant, diuretic, and metabolic effects. This study evaluated the effects of tereré consumption on body weight, lipid profile, biochemical parameters, and food and liquid intake in male Wistar rats. Twenty-four young adult rats were divided into two groups: a control group (CG), which received a standard diet and filtered water, and a tereré group (TG), which received the same diet and an aqueous extract of *Ilex paraguariensis* as their only source of hydration. The extract was prepared daily at a concentration of 6 g per 100 mL of water, steeped for 15 minutes, filtered, and offered ad libitum in drinking bottles (300 mL per bottle), replacing water. The intervention lasted 30 days. The animals were monitored for weight gain, Lee index, food and liquid consumption, lipid parameters, serum calcium and iron, red blood cell counts, and renal markers (urea and creatinine). The TG showed significantly reduced weight gain compared to the CG ($p = 0.0328$), with no significant differences in food or fluid intake. Although not statistically significant, lipid profile markers such as total cholesterol, HDL, and triglycerides tended to be lower in the TG. No significant differences were found in serum calcium, iron, urea, creatinine, or hematological parameters. These findings suggest that tereré may aid in moderating weight gain without negatively affecting renal function or the metabolism of essential minerals. The study protocol was approved by the Ethics Committee on Animal Use (CEUA/UNOESTE, protocol no. 5300). Further studies are needed to explore the physiological mechanisms associated with these effects.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Impact of physical training on diabetes renal dysfunction: role of advanced glycation end products

Authors:

Daniel Olindo de Castro Linhares¹ Karine Lino Rodrigues¹
Mariá Lobosco Pinto Mendes¹ Vivian Vieira Dias da Silva¹
Evelyn Nunes Goulart da Silva Pereira¹ Raquel Rangel Silveiras¹
Beatriz Peres de Araujo¹
Caroline Fernandes dos Santos Bottino² Anissa Daliry¹

Affiliations:

¹ Laboratory of Clinical and Experimental Pathophysiology, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil.

² Center for Studies in Metabolism, Nutrition and Histopathology, Rio de Janeiro, Brazil.

Keywords: Advanced glycation end products. Type 2 diabetes. Physical training.

Abstract:

Diabetic nephropathy (DN) is one of the most frequent microvascular complications of type 2 diabetes (T2D). Oxidative stress is believed to be responsible for inducing the formation of advanced glycation end products (AGEs), which could contribute to renal alterations. In the present study, we aimed to study the effects of aerobic training and its underlying mechanisms, including the modulation of the advanced glycation end products pathway and oxidative stress in mice with T2D. T2D was induced by feeding C57BL/6 mice a high-fat, high-carbohydrate diet for 24 weeks. In the following 12 weeks, a subgroup of T2D mice underwent aerobic exercise training (T2D EX). At the end of the protocol, the parameters of oxidative stress, glomerular morphology and the AGE-RAGE pathway were evaluated. Results were expressed as mean $\pm$ SEM and analyzed by one-way ANOVA with Tukey's post hoc test ($p < 0.05$). Animal experimentation was approved by license L-012/2018A2. Physical training in T2D was able to decrease malondialdehyde levels in the kidney ($p > 0.001$), but had no significant effects on the quantification of the activity of the antioxidant enzymes SOD and catalase when compared to sedentary T2D. Regarding the morphological parameters of the kidney, we observed in the EX T2D group of a decrease in the glomerular area and mean glomerular diameter ($p > 0.01$), and in the area of the Bowman space ($p > 0.001$) compared to T2D. Regarding the AGE-RAGE pathway, T2D EX demonstrated decreased serum CML levels, fluorescent AGEs and RAGE protein expression in the kidney ($p > 0.05$) compared to T2D. However, when we evaluated the gene expression of GLO1, DIAPH1, RAGE, AGER1 and galectin, we did not observe significant differences between the T2D and T2D EX groups. We conclude that physical training may be a potential non-pharmacological treatment for renal dysfunction associated with T2D, via decreased renal deposition of AGEs and oxidative stress.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Fetoplacental microcirculatory and morphometric abnormalities in mice with preeclampsia

Authors:

Daniella Leal Lamarca BOMFIM¹

Fábio das Índias dos Santos CARVALHO¹ Evelyn Nunes G. S. PEREIRA¹

Raquel Rangel SILVA¹ Karine Lino RODRIGUES¹ Beatriz Peres de ARAÚJO¹ Caroline FERNANDES-SANTOS^{1,2}

Elen Mello de SOUZA³ Anissa DALIRY¹

Affiliations:

¹ Laboratory of Clinical and Experimental Pathophysiology.

² Oswaldo Cruz Institute, Oswaldo Cruz Foundation.

Keywords: Fetoplacental microcirculation. Hypertension. L-NAME. Preeclampsia.

Abstract:

Preeclampsia (PE) is a hypertensive disorder that occurs after the 20th week of gestation, marked by complications such as proteinuria or organ dysfunction, probably due to a dysfunctional placenta, which, by releasing factors into the maternal bloodstream, leads to systemic inflammation and endothelial dysfunction. This study aims to analyze fetoplacental microcirculation using laser speckle fluxometry in a PE model induced by L-NAME. Main assessments will include systolic blood pressure, proteinuria, microcirculation and fetal morphometry. The experiment was approved (L-016/2021) and results expressed as mean $\pm$ SEM and analyzed using Student's t-test ($p < 0.05$), with $n = 10$ /group. Compared to the control group, the PE group showed an increase in SBP (227.7 ± 10.5 vs. CTL: 150.4 ± 3.4 mmHg; $p < 0.0001$), proteinuria (88.3 ± 11.4 vs. CTL: 15.11 ± 6.5 mg/dL; $p = 0.0001$), and number of resorption sites (1.7 ± 0.2 vs. CTL: 0.9 ± 0.21 ; $p = 0.024$). There was a decrease in the number of fetuses per female (6.67 ± 0.41 vs. CTL: 8.2 ± 0.29 ; $p = 0.011$) and the flowmetry parameters of the placenta (54.28 ± 5.4 vs. CTL: 78.64 ± 3.9 APU; $p = 0.0109$), umbilical cord (43.6 ± 5.2 vs. CTL: 75.21 ± 7.1 APU; $p = 0.0011$), thorax (92.0 ± 7.0 vs. CTL: 126.9 ± 7.9 APU; $p = 0.0027$), kidney (79.4 ± 4.4 vs. CTL: 105.2 ± 9.6 APU; $p = 0.0093$), paw (40.0 ± 2.2 vs. CTL: 55.9 ± 3.9 APU; $p = 0.0007$), transverse sinus (96.2 ± 4.8 vs. CTL: 124.3 ± 8.2 APU; $p = 0.0080$), anterior facial vein (101.0 ± 7.2 vs. CTL: 129.8 ± 8.4 APU; $p = 0.0236$), and cephalic vein (82.5 ± 6.3 vs. CTL: 108.1 ± 6.5 APU; $p = 0.0083$). In fetal morphometry we observed a reduction of the elbow-paw length (0.36 ± 0.009 vs. CTL: 0.39 ± 0.007 centimeters; $p = 0.04$) and paw length (0.26 ± 0.005 vs. CTL: 0.29 ± 0.006 centimeters; $p = 0.004$). The results demonstrate that L-NAME led to the manifestation of clinical signs of PE in mice through increased SBP and proteinuria, as well as impaired fetal development, evidenced by morphometric abnormalities and reduced fetoplacental microcirculation parameters. These findings deepen the understanding of preeclampsia and are essential for the search for new treatments, with a significant impact on maternal and child public health.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Intermittent Cafeteria Diet in Adolescent Rats: Metabolic Effects in Adolescence and Adulthood

Authors:

Cleusa Whichoski¹

Fabiano Takeo Komay Tsutsui² Lucas Leonardo França de Oliveira² Polyana Keiko Shishido²

Maria Heloisa Bernardes² Nicole Kemi Ida Miya² Rhauany Pelisson Guergolette² Larissa Rugila Stopa²

Ernane Torres Uchoa²

Cássia Thaís Bussamra Vieira Zaia²

Affiliations:

¹ Centro Universitário Filadélfia.

² Universidade Estadual de Londrina.

Keywords: Adipose tissue. Body weight. Energy metabolism. Hypercaloric diet. Ultra-processed foods.

Abstract:

The cafeteria diet (CD), rich in hyperpalatable, high-calorie foods, promotes excess weight and fat. This study assessed its effects on energy metabolism in adolescence and adulthood. Wistar rats (36 males, 36 females) were divided into two groups: one received only balanced chow (SD), while the other received a CD on alternate days alongside balanced chow (CD) from post-natal day (PND) 28 to 50. Half were euthanized on PND 51 (adolescents), the rest on PND 100 (adults), with both groups receiving only chow from PND 51 to 100. Data were analyzed via Student's t-test or Mann-Whitney ($p < 0.05$) and expressed as mean $\pm$ standard deviation. The Ethics Committee of the State University of Londrina approved the study (058/2021, 12/2022). During adolescence, food intake was higher in CD males (SD: 358.5 $\pm$ 17.4g; CD: 575.5 $\pm$ 48.9g; $p < 0.001$) and females (SD: 334.9 $\pm$ 18.4g; CD: 448.8 $\pm$ 30.8g; $p < 0.001$), as was caloric intake in males (SD: 1058.9 $\pm$ 56.4 kcal; CD: 2890.1 $\pm$ 281.7 kcal; $p < 0.001$) and females (SD: 985.1 $\pm$ 62.8 kcal; CD: 2514.6 $\pm$ 198.8 kcal; $p < 0.001$). Body weight remained unchanged. The Lee index was higher in CD males (SD: 0.285 $\pm$ 0.005g^{1/3}/cm; CD: 0.294 $\pm$ 0.007g^{1/3}/cm; $p < 0.001$) and females (SD: 0.290 $\pm$ 0.006g^{1/3}/cm; CD: 0.300 $\pm$ 0.006g^{1/3}/cm; $p < 0.001$). CD adolescents had more adipose tissue in males (SD: 0.94 $\pm$ 0.18g; CD: 1.40 $\pm$ 0.31g; $p = 0.001$) and females (SD: 1.25 $\pm$ 0.31g; CD: 1.99 $\pm$ 0.80g; $p = 0.018$). CD females had higher triacylglycerol levels (SD: 40.4 $\pm$ 16.01mg/dL; CD: 57.4 $\pm$ 15.3mg/dL; $p = 0.038$), while glucose and cholesterol remained unchanged. After CD ended, food intake normalized. In adulthood, only CD males showed greater weight gain (SD: 297.4 $\pm$ 19.1g; CD: 333.6 $\pm$ 29.9g; $p = 0.012$), while only CD females had a higher Lee index (SD: 0.289 $\pm$ 0.004g^{1/3}/cm; CD: 0.300 $\pm$ 0.004g^{1/3}/cm; $p < 0.001$). Other parameters showed no differences. Intermittent CD exposure in adolescence altered food intake, caloric intake, and adipose mass. Most metabolic effects reversed post-diet, but increased adiposity suggests lasting metabolic risks.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Effect of physical training on renal microcirculatory changes and metabolic parameters in preclinical models of diabetes

Authors:

Mariá Lobosco Pinto Mendes¹ Karine Lino Rodrigues¹
Daniel Olindo de Castro Linhares¹ Vivian Vieira Dias da Silva¹
Evelyn Nunes Goulart da Silva Pereira¹ Raquel Rangel Silveiras¹
Beatriz Peres de Araujo¹
Juliana Magalhães Chavez Barboça¹ Anissa Daliry¹

Affiliations:

¹ Laboratory of Clinical and Experimental Physiopathology, Oswaldo Cruz Institution, Oswaldo Cruz Foundation.

Keywords: Physical training. Renal microcirculation and T2D.

Abstract:

Type 2 diabetes (T2D) represents a serious global health problem, affecting millions of people and being associated with microvascular and macrovascular complications, particularly in the kidney. Exercise training has emerged as an essential strategy to prevent and delay T2D complications by improving insulin sensitivity and reducing cardiovascular risks. However, the specific effects of exercise on renal microcirculation and the underlying molecular mechanisms remain unclear. Therefore, this study investigates the impact of aerobic training on renal microcirculation in T2D mice. C57BL/6 mice (6-8 weeks of age) were divided into three groups: control (CTL), diabetic (T2D) and diabetic subjected to aerobic training (T2D EX). At the end of the 36-week protocol, the animals underwent histopathological (hematoxylin and eosin and Masson's trichrome), biochemical, and renal microvascular flow analyses through laser speckle (LSCI) flowmetry technique. Results were expressed as mean $\pm$ SEM and analyzed by one-way ANOVA with Tukey's post hoc test ($p < 0.05$). Animal experimentation was approved by license L-012/2018A2. Physical training in T2D decreased body weight ($p > 0.01$), systolic blood pressure ($p > 0.001$) and subcutaneous fat ($p > 0.05$) compared to sedentary T2D mice. Regarding glucose parameters, physical training did not present a significant effect. Regarding biochemical parameters, we observed in the T2D EX group an increase in HDL ($p > 0.05$), a decrease in total cholesterol ($p > 0.05$) and hepatic cholesterol ($p > 0.01$). Regarding microcirculation and histopathology, the T2D EX group showed an increase in renal flow ($p > 0.01$) and a decrease in fibrosis and inflammatory infiltrate ($p > 0.05$ and $p > 0.001$, respectively). Physical training improved metabolic, microcirculatory and histopathological parameters. Thus, we conclude that physical training is a possible non-pharmacological treatment for T2D and its complications.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Lack of Sexual Dimorphism in Hepatic Enzyme Alterations Induce by Diet-Induced Insulin Resistance in Mice

Authors:

César Augusto da Silva Caetano¹ Júlia Sousa Ramos²

Bruno Pereira Melo^{2,3} Camila Berbert Gomes²

Daniel Massote de Melo Leite² Gleide Fernandes de Avelar⁴ Danusa Dias Soares²

Affiliations:

¹ School of Pharmacy – Universidade Federal de Minas Gerais, UFMG, Belo Horizonte.

² Exercise Physiology Laboratory, School of Physical Education, Physiotherapy and Occupational Therapy, Universidade Federal de Minas Gerais, UFMG, Belo Horizonte, Brazil.

³ University Center, UNITOP - PALMAS/Tocantins, Brazil.

⁴ Cell Biology Laboratory, Department of Morphology, Institute of Biological Sciences, Universidade Federal de Minas Gerais, UFMG, Belo Horizonte, Brazil.

Keywords: Type 2 Diabetes Mellitus. High-fat diet. Lipid profile. Insulin resistance.

Abstract:

Diabetes Mellitus (DM) is a chronic metabolic disorder marked by persistent hyperglycemia, often associated with physical inactivity and hypercaloric diets. Insulin resistance (IR) a key component of DM pathophysiology, can promote metabolic disturbances, including alterations in liver function. However, the role of sex differences in this context remains unclear. This study, approved by UFMG's Ethics Committee (161/2020), investigated whether IR induces sex-specific changes in hepatic enzyme levels in mice. Male and Female C57BL/6J mice were divided into two groups: Control (CON; n = 16), which received a standard diet, and Insulin Resistance (IR; n = 16), fed a high-lipid diet (22% saturated fat) with unrestricted access to a 30% fructose solution for 8 weeks. Blood levels of hepatic enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assessed via colorimetric analysis. IR significantly increased AST and ALT in both sexes: AST in males (CON: 35.90 ± 3.29 vs. IR: 88.6 ± 4.00) and females (CON: 45.53 ± 3.27 vs. IR: 83.22 ± 7.55); ALT in males (CON: 35.44 ± 2.37 vs. IR: 67.36 ± 4.26) and females (CON: 45.89 ± 2.34 vs. IR: 59.18 ± 6.52) (all $p < 0.01$). The AST/ALT ratio also increased significantly in both sexes. In addition, these alterations were accompanied by signs consistent with hepatic steatosis, indicating early-stage liver injury. Despite these hepatic alterations no significant sex differences were observed in the lipid-related outcomes. These findings suggest that diet-induced IR leads to liver enzyme elevation in both male and female mice, but without evidence of sexual dimorphism, pointing to a shared vulnerability to hepatic dysfunction under metabolic stress.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Characterization of a Model of Steatohepatitis Associated with Metabolic Dysfunction for Use in Post-Transplant Evaluation Studies of Acellular Hepatic Scaffolds

Authors:

Giulia Roldão Barbosa Freire¹ Alexandre Cerqueira da Silva Filho¹ João Vitor Santana da Silva¹ Marlon Lemos Dias¹
Regina Coeli dos Santos Goldenberg¹

Affiliations:

¹ Carlos Chagas Filho Institute of Biophysics - Federal University of Rio de Janeiro (UFRJ), Brazil.

Keywords: Acellular Scaffold. Fibrosis. Liver Transplantation. Metabolic Dysfunction. Steatohepatitis.

Abstract:

Liver transplantation presents complex challenges, and the development of acellular liver scaffolds (ALS) for transplants aims to mitigate incompatibility issues, reduce waiting lists, and increase patient survival. This study aimed to characterize a model of steatohepatitis associated with metabolic dysfunction (MASH) for use in post-transplant evaluation studies of ALS. Female Wistar rats (2 months old) were initially subjected to a hyperlipidic diet containing 45% of calories from fats, 20% from fructose, and 2% from cholesterol for 16 weeks, associated with carbon tetrachloride (CCl₄, 1 mL/Kg) administration during the last week. Histological analysis was performed on weeks 12 and 16 to confirm MASH development. In addition, 6% sucrose was added to the animals' drinking water throughout the entire protocol (CEUA/CCS-UFRJ protocol no. 069/24). After protocol induction, biochemical and histological analysis (H&E and Picrosirius) were performed. Biochemical results showed a significant increase in triglyceride levels, LDL, HDL, ALB, ALT, AST, total cholesterol, and body weight in the MASH group. However, no significant differences in blood glucose were observed between MASH and control groups. H&E staining showed micro- and macrosteatosis, characterized by hepatocellular ballooning with nuclear displacement to the periphery due to lipid accumulation in the cytoplasm, distinguishing them from the control group. In Picrosirius-stained MASH tissue slides, collagen fiber deposition was observed. To obtain ALS, healthy rat livers were perfused through the portal vein at 4 ml/min with water for 2 h followed by 1% Triton X-100 for 2 hours and SDS 1% for 18h. After that, livers were washed with distilled water for 1 day and then submitted to DNA quantification and histology analysis. ALS macroscopy, H&E staining, and DNA quantification analysis showed that the decellularization process removed more than 99.5% of the DNA content. Currently, studies with ALS transplantation are being conducted. In conclusion, the results confirm that the MASH model was effective in inducing hepatic and metabolic pathological features associated with liver dysfunction.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Influence of Different Macronutrients on Skin Temperature in Para Athletes

Authors:

Carolina Batista Simões^{1,2,3,4} Lucas Siqueira-Moraes^{1,2,3,4} Marcelo Teixeira Andrades⁵
Gabriel Adler Gomes-Martins^{1,2,3,4} Wellington Douglas de Souza^{1,2,3,4} Marcelo Danillo Matos dos Santos⁴ Carlos Makleyton Caetano Schuchter⁴ Marcos Daniel Motta Drummond^{4,7} Marco Túlio de Mello^{4,6,7}
Andressa Silva^{4,6,7}
Eduardo Mendonça Pimenta⁷
Dawit Albieiro Pinheiro Gonçalves^{1,2,3,4}

Affiliations:

- ¹ Sports Physiology Section (SFE), Sports Training Center (CTE), Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.
- ² Ergogenic and Systemic and Molecular Physiology Research Group (ErgoFis), School of Physical Education, Physiotherapy and Occupational Therapy, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.
- ³ Exercise Physiology Laboratory (LAFISE), School of Physical Education, Physiotherapy and Occupational Therapy, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.
- ⁴ Sports Training Center (CTE), Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.
- ⁵ Psychology Program, Faculdade de Minas (FAMINAS), Belo Horizonte, MG, Brazil.
- ⁶ Center for Studies in Psychobiology and Exercise (CEPE), School of Physical Education, Physiotherapy and Occupational Therapy, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.
- ⁷ Department of Sports, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

Keywords: Infrared thermography; Metabolism; Thermogenesis

Abstract:

Introduction: Infrared thermography (IT) is a non-invasive, contactless technique that records body thermal patterns through skin temperature (Tsk). It is widely used to detect sports injuries and assess physiological responses to exercise and nutrition. While Tsk reflects metabolic heat production and is influenced by environmental, individual, and technical factors, the dietary impact on Tsk remains underexplored. **Objective:** This study investigated the acute effects of meals with different macronutrient compositions on the thermographic response of para-athletes. **Methods:** Ten athletes (8 men, 2 women), including 5 para-powerlifters (4 with spinal cord injury, 1 with achondroplasia) and 5 para-athletic throwers (4 with physical disabilities, 1 visually impaired), participated in a crossover study. They underwent four meal interventions: carbohydrate-rich (CHO), protein-rich (PTN), and fat-rich (FAT) meals, and overnight fasting control (OVF). IT was used to measure Tsk at rest and at seven postprandial time points (0, 10, 20, 30, 60, 90, and 120 minutes) across ten regions of interest. A mixed linear model analyzed the effects of meal type, time, and their interactions. The b value indicates each variable's estimated effect on Tsk. Ethics Committee (CEP 68427923.8.0000.5149). **Results:** Time had a significant negative effect on Tsk ($b = -0.002$, $p < 0.001$), indicating a gradual decrease over time. Meal-time interactions showed that CHO and PTN meals significantly increased Tsk compared to OVF (CHO: $b = 0.004$, $p < 0.001$; PTN: $b = 0.003$, $p < 0.001$), whereas FAT intake had no significant effect. **Conclusion:** CHO and PTN meals significantly elevate Tsk in para-athletes, suggesting a thermogenic effect of these macronutrients. These findings highlight IT as a valuable tool for monitoring metabolic responses and advancing the understanding of nutritional influences on thermoregulation in para-athletes.

Funding: Pro-Reitoria de Pesquisa - Federal University of Minas Gerais, Conselho Nacional de Desenvolvimento Científico e Tecnológico (N.444769/2023- 4 and N. 405140/2021-5),



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Secretaria Especial do Esporte do Ministério da Cidadania (Governo Federal, Brazil - N.58000.008978/2018-37 and N. 71000.056251/2020-49), Comitê Paralímpico Brasileiro, Centro de Treinamento Esportivo e Fundação de Apoio ao Ensino, Pesquisa e Extensão.

Title: High-Protein Diet Modulates Hepatic Gluconeogenesis: From CREB to FoxO1

Authors:

João Batista Camargo Neto¹ Henrique Jorge Novaes Morgan² Matheus Leonardo Moro²
Ana Paula Assis¹ Leticia Cirelli Ruiz²
Aline Zanatta Schavinski² Natalia Lautherbach E. S.¹
Luiz Carlos Carvalho Navegantes² Isis do Carmo Kettelhut¹

Affiliations:

¹ Department of Biochemistry and Immunology, Ribeirao Preto Medical School, University of São Paulo

² Department of Physiology, Ribeirao Preto Medical School, University of São Paulo.

Keywords: CREB. FoxO1. Hepatic gluconeogenesis. High protein diet.

Abstract:

Introduction: Although it is well known that animals adapted to a high-protein, carbohydrate-free diet (HP) maintain glycemia through enhanced hepatic gluconeogenesis, the molecular mechanisms underlying this adaptation remain to be elucidated. **Methods:** We evaluated, in liver and in primary hepatocytes of C57BL6J mice fed a control (20% protein; 66% carbohydrate; 8% lipids) or a HP (86% protein; 0% carbohydrate; 8% lipids) diet, the gluconeogenesis capacity by pyruvate tolerance test and protein content (western blotting) and gene expression (RT-qPCR) of hepatic PEPCK and G6Pase. Insulin and glucagon serum levels were evaluated by ELISA, and its hepatic signaling pathways components by western blotting. CREB transcriptional activity was assessed using CRE-Luc transgenic mice. Hepatic FoxO1 function was investigated using a pharmacological inhibitor (AS1862458). Statistical analyses were performed using two-way ANOVA and Student's t-test. $P \leq 0.05$ was defined as significant. CEUA: 1167/2023. **Results:** HP-adapted mice exhibited a twofold increase in hepatic gluconeogenesis, which was not associated with elevated hepatic cAMP and phospho-PKA-substrates content and CREB activation (~50% reduced), despite increased glucagon serum levels (~20%). Meanwhile, we reported that hepatic cAMP and phospho-PKA-substrates content and CREB activity were initially elevated (~50%), which became attenuated in prolonged HP feeding, leading to resistance to glucagon-induced gluconeogenesis. Conversely, HP-adapted mice exhibited lower insulin serum levels, coupled with its reduced hepatic phospho-AKT content (~50%) and decreased hepatic FoxO1 phosphorylation and acetylation (~50%). Notably, FoxO1 inhibition reduced hepatic gluconeogenesis (~50%) and glycemia in these mice (~20%). **Conclusion:** Our findings reveal a shift in the control of hepatic gluconeogenesis in HP-fed animals, transitioning from CREB activation in the early periods to sustained activation by FoxO1 during later periods.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Influence of high-Fat diet (HFD) consumption combined with colon inflammation on hepatic miR-122 expression in mice

Authors:

Luíze Naomi Machado UTINO¹ Caroline Lobo COSTA¹
Márcio Alberto TORSONI¹ Adriana Souza TORSONI¹ Carolina PANZARIN¹

Affiliations:

¹ Universidade Estadual de Campinas (FCA).

Keywords: miR-122. Ulcerative Colitis. High-Fat Diet. Metabolic Dysfunction-Associated Steatotic Liver Disease.

Abstract:

Inflammatory bowel diseases (IBD) have high prevalence in the population and are associated with genetic and environmental factors. High-fat diets (HFD) can increase intestinal permeability, the presence of pro-inflammatory cytokines in the bloodstream, to inhibit Hepatocyte Nuclear Factor 4 Alpha (HNF4- α), and stimulates the expression of miR-122 in the liver. In this context, we aim to investigate the effects of UC combined with HFD on miR-122 expression in the liver. Male C57BL/6 mice, aged six weeks, were used in the study. Experimental colitis was induced with 2.5% DSS in drinking water for seven days. The animals were divided into four groups: control diet, HFD, control diet + DSS, and HFD + DSS. After treatment the liver was extracted for qPCR analysis. Regardless of diet, weight loss was observed in the colitis groups. However, the CT+DSS group showed greater weight loss compared to the HFD+DSS group. In the Disease Activity Index score, the CT+DSS group had a higher score than the HFD+DSS group. Both HFD and the disease significantly reduced colon length compared to the control group. The relative liver weight was lower in the HFD group than in the control group, while the CT+DSS group had a significantly higher liver weight. However, when combining HFD and the disease, liver weight decreased compared to the CT+DSS group but increased relative to the HFD group. The hepatic expression of miR-122 was lower in the DSS groups. However, the HFD group appeared to have increased miR-122 levels compared to the control group. The results suggest that decreased miR-122 levels in the DSS groups may be linked to the inflammatory process of colitis, where IBD inhibits HNF4- α in hepatocytes—a transcription factor for miR-122. In contrast, the increased miR-122 in the HFD group indicates that saturated fat consumption may trigger an early stage of Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Effects of Amazonaçaí (*Euterpe precatoria Martius*) pulp supplementation on body composition and bone parameters in Wistar rats

Authors:

Milla Christie Rodrigues de Lira¹

Carolina De Oliveira Ramos Petra de Almeida² Bruna Almeida Nascimento¹

Thais Silva Santos³

Karla de Araujo Coutinho³ Yasmin Cunha Silva⁴

Valdir Florêncio Veiga Junior⁵ Anderson Junger Teodoro⁶ Mariana Sarto Figueiredo⁶

Affiliations:

¹ Pos-graduated in Medical Science, Fluminense Federal University, RJ, Brazil.

² Pos-graduated in Food and Nutrition, Federal University of State of Rio de Janeiro, RJ, Brazil.

³ Integrated Food and Nutrition Center, Fluminense Federal University, Niteroi, Brazil.

⁴ Universidade Federal do Amazonas, Manaus, Brazil.

⁵ Instituto Militar de Engenharia – RJ, Brazil.

⁶ Departament of Nutrition and Dietetic, Faculty of Nutrition, Fluminense Federal University, RJ, Brazil.

Keywords: Bone health. Bioactive compounds. Biodiversity.

Abstract:

Euterpe precatoria is a species of açai found in Brazil, rich in nutrients and phytochemicals, which can contribute to diseases prevention, such as bone diseases. We aimed to investigate supplementation effects of different doses of lyophilized precatoria açai pulp on bone parameters in rats. Our protocol was approved by the Ethical Committee (CEUA1645110921). Forty rats (90-day-old) were subdivided into: Control (C, n=10), Açai precatoria 50mg/kg/day (50PRE, n=10), Açai precatoria 100mg/kg/day (100PRE, n=10) and Açai precatoria 200mg/kg/day (200PRE, n=10), gavage for 30 days. At 120 days of age, biochemical parameters (BioClin Analyzer), body composition, and left femur were examined to evaluate bone parameters (DXA), radiodensity (computed tomography), and biomechanics (3-point flexion test). 100PRE dose showed higher femoral weight (+10%, $p<0.05$), length (+4%, $p<0.05$), distance from greater trochanter (+7%, $p<0.05$) and distance from epicondyle (+5%, $p<0.05$) compared to C. 200PRE group showed higher femoral weight and epicondyle distance (+11% and +4%, respectively, $p<0.05$) compared to C group and 200PRE group presented higher femoral length and distance from greater trochanter (+4.2% and +9.2%, respectively, $p<0.05$) compared to 50PRE. No changes were observed in body composition. 50PRE, 100PRE and 200PRE showed higher femoral radiodensity (+31%, +32% and +40%, respectively, $p<0.05$) compared to C. 50PRE increased creatinine (+11%, $p<0.05$), 100PRE increased creatinine (+11.3%, +7% and +13%, respectively, $p<0.05$) and 200PRE increased calcium and phosphorus (+6% and +15%, respectively, $p<0.05$) compared to C. No significant changes were observed in other serum biochemical parameters. Supplementation with açai pulp has shown promising effects on bone health, mainly attributed to the anthocyanins present in the fruit. Açai precatoria has demonstrated benefits for bone health, influencing biochemical, anatomical parameters of femur and radiodensity, suggesting that its inclusion in the diet can strengthen bone health. **Committee Number:** 1645110921



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Effects Baru oil (*Dipteryx alata* Vog.) supplementation on body composition and biochemical parameters in Wistar rats

Authors:

Mariana Sarto Figueiredo^{1,2} Pamela Navarro Brito²
Letícia de Almeida Sant'anna Trindade³ Pamela Braga Louback²
Julia Scotelaro de Araujo Barros² Gabriel de Alcantara Noblat³
Beatriz Alem Nascimento de Araujo³ Alana Louzada Millions Monteiro³ Thais Silva Santos²
Anderson Junger Teodoro^{1,2}

Affiliations:

¹ Departament of Nutrition and Dietetic, Faculty of Nutrition, Fluminense Federal University, RJ, Brazil.

² Integrated Food and Nutrition Center, Fluminense Federal University, Niteroi, Brazil.

³ Pos-graduated in Medical Science, Fluminense Federal University, RJ, Brazil.

Keywords: Baru oil. *Dipteryx alata* Vog. Body composition. Biochemical parameters.

Abstract:

Baru oil is obtained from baru almonds (*Dipteryx alata* Vog.), which is a source of monounsaturated and polyunsaturated fatty acids, such as oleic, linoleic, palmitic, lignoceric, stearic, gadenic and arachidic acids, that can modulate lipotoxicity, decrease oxidative stress, improve lipid profile and antioxidant potential, especially due to the presence of phenolic compounds, catechin, ferulic acid and vitamin E. We aimed to evaluate effects of baru oil supplementation in body composition and biochemical parameters in male rats. Thirty male rats (90 days old, CEAU 9204110520) were divided: Control group, gavage with saline (CS, n=10), Soy oil group, gavage with soybean oil (SO, n=10) and Baru oil group, gavage with baru oil (BARU, n=10). All animals received commercial diet and water ad libitum. Gavage was performed for 30 consecutive (0.5 ml of oil/100 g of body mass). At 120 days old, we evaluated body composition (Dual-energy X-ray absorptiometry) and biochemical parameters (Colorimetric assays). Results were analyzed by GraphPad Prism and One-way ANOVA analysis of variance and Tukey post-test were used, considered statistically significant when $p < 0.05$. BARU presented lower triglycerides, HDL-cholesterol, Glutamic Pyruvic Transaminase, iron, magnesium, uric acid and alkaline phosphatase in serum (-22%, -11%, -23%, -31%, -14%, -32% and -30%, respectively, $p < 0.05$) when compared to CS. OS group presented lower triglycerides, calcium, iron, magnesium, phosphorus and alkaline phosphatase in serum (-32%, -8%, -22%, -21%, -16% and -27%, respectively, $p < 0.05$) when compared to CS. No changes were observed in body mass, food intake and body composition. Thus, our results demonstrated that Baru oil did not change body composition, improve lipid profile and liver biomarkers. However, Baru and soybean oil supplementation presented negative effects on serum concentration of key metabolic minerals. More studies are necessary to elucidate the molecular mechanism involved.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Bisphenol-A alters the expression of genes involved in lipogenesis, inflammation, and oxidative stress in the liver of adult zebrafish.

Authors:

Eronides A. de Heberle Salau¹ Giuliano Rizzotto²

Daniela Diglio³

Orlando V. Furtado-Filho³ Marilene Porawski¹

Affiliations:

¹ Department of Hepatology and Basic Health Sciences, Federal University of Health Sciences of Porto Alegre, Porto Alegre 90050-170, Brazil.

² Pathology Laboratory, Federal University of Health Sciences of Porto Alegre, Porto Alegre 90050-170, Brazil.

³ Department of Basic Health Sciences, Federal University of Health Sciences of Porto Alegre, Porto Alegre 90050-170, Brazil.

Keywords: Steatosis. Liver disease. Bisphenol-A. Zebrafish. Oxidative stress.

Abstract:

Bisphenol A (BPA) is a synthetic organic compound widely used in the manufacture of plastic products and commonly found in the environment. Recognized as an endocrine disruptor, BPA has been associated with various diseases, including metabolic dysfunction-associated steatotic liver disease (MASLD). This study aimed to investigate the hepatic effects of BPA exposure in adult zebrafish (*Danio rerio*), an established model for liver disease research. Two experimental groups were analyzed: control (n=30) and exposed to 20 µg/L BPA (n=30) for seven days. Following euthanasia with eugenol, livers were collected for histological assessment or frozen in liquid nitrogen for gene expression analysis via RT-qPCR. Quantitative data were analyzed using Student's t-test ($p < 0.05$). Histological evaluation and lipid quantification using Nile Red staining revealed significant fat accumulation in BPA-exposed livers, while hematoxylin and eosin (H&E) staining showed hepatic parenchyma alterations consistent with steatosis. Gene expression analysis demonstrated significant upregulation of *fasn*, *acc1*, *srebp-1c*, *nfkb*, and *il-6*, along with downregulation of *adipor-2*. Additionally, BPA exposure led to increased expression of *gpx1*, *sod*, *cyp1a*, and *cyp2ad2*, and decreased expression of *gpx4*. These findings suggest that BPA promotes hepatic steatosis through lipogenesis, activation of pro-inflammatory cytokines, xenobiotic metabolism dysregulation, and impairment of antioxidant defenses. Together, these results underscore the hepatotoxic potential of BPA at environmentally relevant concentrations and its role in the pathogenesis of MASLD. All procedures were conducted following current legislation that regulates the use of animals in research. The Federal University of Health Sciences of Porto Alegre Ethics Committee approved the project under protocol 713/20.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Acute Effect of Nutrition on Physical Performance in Para Athletes

Authors:

Thais Fernanda Pires Barbosa^{1,2,3,4} Carolina Batista Simões^{1,2,3,4} Lucas Siqueira Moraes^{1,2,3,4}
Gabriel Adler Gomes-Martins^{1,2,3,4} Wellington Douglas de Souza^{1,2,3,4} Jessé Borges de
Oliveira^{1,2,3,4} Beatriz Lopes da Silva^{1,2,3,4}
Carlos Makleyton Caetano Schuchter⁴ Marcelo Danillo Matos dos Santos⁴ Marcos Daniel Motta
Drummond^{4,6} Eduardo Mendonça Pimenta⁶ Andressa da Silva de Mello^{4,5,6}
Marco Túlio de Mello^{4,5,6}
Dawit Albieiro Pinheiro Gonçalves^{1,2,3,4}

ERGOnic and Systemic and Molecular Physiology Research Group.

Affiliations:

¹ Section of Sports Physiology (SFE), Sports Training Center (CTE), Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

² Ergogenic and Systemic and Molecular Physiology Research Group (ErgoFis), School of Physical Education, Physiotherapy and Occupational Therapy, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

³ Exercise Physiology Laboratory (LAFISE), School of Physical Education, Physiotherapy and Occupational Therapy, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

⁴ Sports Training Center (CTE), Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

⁵ Center for Studies in Psychobiology and Exercise (CEPE), School of Physical Education, Physiotherapy and Occupational Therapy, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

⁶ Department of Sports, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

Keywords: Macronutrients. Maximal isometric voluntary contraction. Nutrition. Para athletes. Physical performance.

Abstract:

Introduction: Maximal Isometric Voluntary Contraction (MVIC) is very useful in athletes to assess muscle strength. Carbohydrate-rich (CHO) meals enhance performance in MVIC, but the acute effects of other macronutrients, such as protein (PRO) and fat (FAT), remain underexplored. Objective: This study aimed to evaluate the acute effect of meals with different macronutrient compositions on MVIC performance in para-athletes before and after preparatory activities (PA). Methods: Ten para-powerlifters and para-throwers athletes participated in a crossover study with 3 dietary (CHO, PRO, FAT) and 14-h fasting conditions. After meal ingestion, athletes remained at rest for 2-h in a controlled environment (25°C). Athletes performed MVIC test before (pre) and after (post) standardized PA (stretching and progressive load in the bench press). MVIC test was performed unilaterally in the supine position, with three attempts of 5-s and a 30-s interval. The highest value obtained in the dominant arm was analyzed, determining the variation between pre- and post-PA periods. Statistical analysis was conducted using two-way ANOVA. The study was approved by the Research Ethics Committee of UFMG (68427923.8.0000.5149). Results: No significant differences in MVIC (Kg) were observed between macronutrient conditions in either pre-PA or post-PA assessments. In the pre-PA condition, MVIC values were similar across meals, ranging from 31.18 ± 16.07 kg (CHO) to 35.08 ± 16.85 kg (FAT). A similar pattern was found post-PA, with values between 28.92 ± 15.63 kg (PRO) and 30.88 ± 17.87 kg (Fasting). Although a trend toward performance decline post-PA was noted ($p = 0.054$), neither time nor macronutrient intake significantly influenced MVIC ($p > 0.05$). Conclusion: The analysis indicated that meals rich in different macronutrients did not influence MVIC performance in the dominant arm before and after PA in para-athletes.

Funding: Pro-Reitoria de Pesquisa - Federal University of Minas Gerais, Conselho Nacional de Desenvolvimento Científico e Tecnológico (N.444769/2023- 4 and N. 405140/2021-5), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Coordenação de



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Secretaria Especial do Esporte do Ministério da Cidadania (Governo Federal, Brazil - N.58000.008978/2018-37 and N. 71000.056251/2020-49), Comitê Paralímpico Brasileiro, Centro de Treinamento Esportivo e Fundação de Apoio ao Ensino, Pesquisa e Extensão.

Title: The effect of early weaning on eating and emotional behavior in adult Wistar rats

Authors:

Swane Miranda-Alves¹ Aline Ananias de Lima²
Maria Eduarda Santana do Nascimento³ Wilkson Rafael de Miranda Santos³ Fernando Antônio Bezerra Filho³ Sandra Lope de Souza^{1,2}

Affiliations:

¹ Graduate Program in Nutrition - UFPE, Recife-PE, Brazil.

² Graduate Program in Neuropsychiatry and Behavioral Sciences - UFPE, Recife-PE, Brazil.

³ Department of Nutrition - UFPE, Recife-PE, Brazil.

Keywords: Early weaning. Food preference. Satiety. Serotonin. Stress.

Abstract:

Breastfeeding is essential for the healthy growth and development of an individual because it provides complete nutrition and allows for mother-child contact, which contributes to emotional development. However, the abrupt interruption of breastfeeding through early weaning has negative consequences throughout life, in modulating both eating and emotional behavior. The research was approved by the ethics committee (protocol nº0099/2023). The adult male (M,n=16) and female (F,n=16) *Wistar* rats were used, early weaned (EW) on the 15th postnatal day (PND) and the control group (C) weaned on PND-30. Males and females were assessed for: body weight (PND-15 to 210) individually; experimental anxiety (PND-160) using the elevated cross maze; behavioral sequence of satiety (PND-170), the rats were fasted for 4 hours, some were given fluoxetine (Flu.) or saline (S) 30 minutes before the end of the fast, then they were offered 30g of standard chow (Presence®) and their behaviors (consumption, cleaning and resting) were observed for 1 hour; food preference (PND-200), 30g of palatable food (Cookie Chocolate Bauducco®) and standard chow were offered at the same time, and the quota ingested (g) was quantified by the difference between the offer and the rejection over 24h/5 consecutive days. A lower body weight ($p<0.001$) was observed in the EW group compared to the C group, at adulthood (PND-210) in males and females; delay in the satiety point in the EW group for males (C:3.8 vs PD:4.0) and females (C:2.5 vs PD:3.3), which was anticipated by fluoxetine for males (MEWS:4.0 vs MEWFlu:2.8) and females (FEWS:3.2 vs FEWFlu:2.6); higher consumption of cookies for both EW and C groups; anxiety-like behavior ($P<0.01$) for MEW and FEW. Thus, early weaning negatively alters emotional behavior as well as the modulation of eating behavior in relation to the point of satiety, but does not promote a greater preference for palatable foods.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Brazil nut oil (*Bertholletia excelsa*) supplementation on biochemical parameters and bone composition of healthy adult male rats and those programmed by early weaning

Authors:

Beatriz Alem Nascimento de Araújo¹ Alana Louzada Millions Monteiro¹ Gabriel de Alcantara Noblat¹

Paulo Roberto Nuyens Leme² Deysla Sabino Guarda Figueira² Aline D'ávila Pereira³

Anderson Junger Teodoro^{2,4} Mariana Sarto Figueiredo^{2,4}

Affiliations:

¹ Pos-graduated in Medical Science, Fluminense Federal University, RJ, Brazil.

² Integrated Food and Nutrition Center, Fluminense Federal University, Niteroi, Brazil.

³ Departament of Geography, Rio de Janeiro State University, Cabo Frio, Brazil.

⁴ Departament of Nutrition and Dietetic, Faculty of Nutrition, Fluminense Federal University, RJ, Brazil.

Keywords: *Bertholletia excelsa*. Bioactive compounds. Bone metabolism. Biochemical parameters.

Abstract:

Brazil nut oil presented high levels of bioactive compounds, which influence bone metabolism, reducing oxidative stress and inflammation. This study aimed to evaluate the effects of Brazil nut oil supplementation on biochemical parameters related to bone metabolism in adult male rats programmed for early weaning (EW). Sixty Wistar rats, offspring of healthy animals (Control) and programmed for EW (CEUA no.9204110520) were used. At PN150, for gavage, control group was divided into: 1) Control, saline solution (C, n=10); 2) Control, soybean oil (CSO, n=10); 3) Control, Brazil nut oil (CBNO, n=10); and EW group was subdivided into: 1) EW, saline solution (EW, n=10); 2) EW, soybean oil (EWSO, n=10); 3) EW, Brazil nut oil (EWBNO, n=10). At PN150, gavage was performed for 30 consecutive days (0.5ml oil/100g body mass). All animals received water and commercial diet ad libitum. In PN180, body bone and femur parameters were evaluated (dual-energy X-ray absorptiometry), biochemical parameters (colorimetric tests), femur anatomical parameters (digital universal caliper), femur head radiodensity (tomography scanner) and femur biomechanical characteristics (three-point flexion test). Data were analyzed by statistical software RStudio (version 2021.09.0), using a significance level of 5%. Results showed that Brazil nut oil supplementation reduced alkaline phosphatase levels (28.7% and 23.1%, $p < 0.05$) and there was a tendency for animal weight reduction (4.1% and 3.8%) when compared to groups that received soybean oil and saline solution, respectively. A reduction in femoral head radiodensity (11.3%, $p < 0.05$) was observed in the group subjected to metabolic programming, when compared to the control group. No changes were observed in other parameters. In conclusion, Brazil nut oil supplementation had a moderate effect on bone metabolism and health, indicating that further studies on dosage and intervention duration is necessary to elucidate mechanism involved.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: The role of the MrgD receptor and Angiotensin-(1-7) in adipose tissue remodeling in obese mice.

Authors:

Ana Beatriz Proença^{1,2} Nícia Pedreira Soares³
Ana Livia Ladislau de Souza Vieira^{1,2} Luiza Da Franca Losito^{1,2}
Yasmin Kolz Brozeghini³ Gabriela de Castro Magalhães³
Matheus Luan dos Santos Oliveira¹ Guilherme dos Santos Reis^{1,2}
Maria Jose Campagnole Santos³ D'angelo Carlo Magliano²
Antonio Claudio Lucas da Nobrega¹ Robson Augusto Souza Santos³ Eliete Dalla Corte Frantz^{1,2}

Affiliations:

¹ Exercise Science Laboratory and Morphology and Metabolism Research Center, Fluminense Federal University, Niteroi, Rio de Janeiro.

² Morphology and Metabolism Research Center, Fluminense Federal University, Niteroi, Brazil

³ INCT Nanobiofar - Hypertension Laboratory, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais.

Keywords: Obesity. Renin-angiotensin system. Adipose tissue.

Abstract:

Obesity overactivates the classical renin-angiotensin system (RAS) axis and damages adipose tissue. Conversely, the counterregulatory RAS axis, Angiotensin (Ang)-(1-7)/MAS receptor and alamandine/MrgD receptor, modulates white adipose tissue (WAT) function and morphology, exhibiting anti-obesity effects. This study investigates the role of MrgD and Ang-(1-7) in WAT in obese animals. Male C57BL/6J wild-type (WT) and MrgD receptor knockout (MrgD-ko) mice received a control (SC) or high-fat (HF) diet for eight weeks. The animals were randomly divided into six experimental groups (n=12/group): WT (SC, HF, and HF+Ang-(1-7)) and MrgD-ko (SC, HF, and HF+Ang-(1-7)). Ang-(1-7) (30 µg/kg/day) was administered by gavage for the last 14 days. Data were analyzed using one and two-way ANOVA and Holm-Sidak post-test. HF-fed animals increased body mass, subcutaneous WAT mass, adiposity index and adipocyte hypertrophy compared to controls, WT (+31.57%, p=0.0001; +177.35%, p=0.083; +159.91%, p=0.0002; +175%, p=0.002) and MrgD-ko (+12.68%, p=0.0002; +102.45%, p=0.074; +78.72%, p=0.0038; +294%, p=0.003). Ang-(1-7) improved these parameters only in WT animals (-14.44%, p=0.0004; -50.47%, p=0.0438; -40.25%, p=0.156; -170%, p=0.002).

MrgD-ko induced adipocyte hyperplasia in SC animals (+87.11%, p=0.0067), while HF reduced adipocyte density (-46.52%, p=0.032), and Ang-(1-7) failed to reverse this effect. HF increased cholesterol and glucose intolerance compared to controls: WT (+21.42%, p=0.0034; +36.84%, p=0.0001) and MrgD-ko (+15.71%, p=0.0254; +26.52%, p=0.0027). Ang-(1-7) improved glucose intolerance in both WT (-15.79%, p=0.0152) and MrgD-ko (-15.63%, p=0.031), suggesting this effect is independent of MrgD. Our findings suggest that MrgD plays an important role in adipocyte plasticity. While Ang-(1-7) mitigates metabolic dysfunction in WT animals, its beneficial effects are significantly blunted in MrgD-ko, underscoring the receptor's importance in WAT homeostasis.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Effects of tauroursodeoxycholic acid (TUDCA) on adiposity and brown adipose tissue activation in malnourished mice fed with high-fat diet

Authors:

Lívia Terumi Odo Lopes^{1,2} Joel Alves da Silva Junior¹ Kênia Moreno de Oliveira¹ Israel Netto Freitas¹ Tassiana Cristina Talpo¹
Everardo Magalhães Carneiro¹

Affiliations:

¹ Universidade Estadual de Campinas.

² Pontifícia Universidade Católica de Campinas.

Keywords: Adiposity. TUDCA. Brown adipose tissue.

Abstract:

Intrauterine/prenatal malnutrition disrupts energy homeostasis, predisposing individuals to obesity in adulthood. Brown adipose tissue (BAT) plays a key role in energy balance, undergoing morphofunctional alterations during obesity. Tauroursodeoxycholic acid (TUDCA) is investigated as a potential therapeutic against obesity and its complications. This study evaluated the effects of TUDCA's effects on BAT activation in malnourished mice fed a high-fat diet (HFD). Male C57Bl/6J mice (30–75 days old) were divided into control (C) and protein-restricted (R) groups. Half of each group received HFD for 12 weeks (CH, RH). In the last 15 days, mice received TUDCA (CHT, RHT) or PBS. Data were analyzed via two-way ANOVA ($P < 0.05$) (CEUA UNICAMP: 5768-1/2021). CH and RH groups showed higher body weight (37.4 ± 1.6 g and 34 ± 0.6 g), food intake (1.8 ± 0.04 and 1.9 ± 0.05 Kcal), and feed efficiency (0.05 ± 0.002 g and 0.05 ± 0.002 g) compared to the C (25.4 ± 0.7 g; 1.3 ± 0.04 Kcal; 0.029 ± 0.001 g) and R (22.4 ± 0.4 g; 1.5 ± 0.04 Kcal; 0.024 ± 0.0009 g). TUDCA reduced body weight and feed efficiency in CHT (29.5 ± 1.1 g; 0.03 ± 0.001 g) and RHT (29.5 ± 0.8 g; 0.03 ± 0.002 g) but did not alter food intake. CH and RH mice exhibited increased retroperitoneal (1.02 ± 0.08 g and 0.74 ± 0.1 g), inguinal (1.64 ± 0.2 g and 1.21 ± 0.2 g) depots, and BAT (0.18 ± 0.02 g for RH) compared to C (0.23 ± 0.01 g; 0.34 ± 0.03 g; 0.11 ± 0.005 g) and R mice (0.19 ± 0.01 g; 0.30 ± 0.02 g; 0.10 ± 0.007 g). TUDCA reduced fat mass in CHT (0.48 ± 0.07 g; 0.65 ± 0.05 g; 0.11 ± 0.01 g) and RHT mice (0.33 ± 0.08 g; 0.49 ± 0.1 g; 0.12 ± 0.001 g). During TUDCA treatment, body temperature (BT) was measured, and no differences were observed. BT also remained unchanged at room temperature and after 3 hours of cold exposure. Histological analysis of BAT showed that HFD intake in RH mice increased the percentage of white adipose area ($53.5 \pm 3.6\%$) compared to R mice ($39 \pm 2.2\%$). TUDCA treatment did not attenuate this phenomenon ($45.4 \pm 3.1\%$). TUDCA reduced weight gain and adiposity in malnourished and obese mice but did not affect body temperature or BAT activation.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Maternal Vit.D status plays a crucial role in determining bone and fat mass in male offspring but not in females

Authors:

Bianca Bozzi¹

Matheus Leonardo Moro¹ João Batista Camargo Neto¹ Leticia Cirelli Ruiz¹ Andressa Pereira¹

Neusa Maria Zanon¹

Iana Mizumukai de Araújo¹

Francisco José Albuquerque de Paula¹ Isis Do Carmo Kettelhut¹

Luiz Carlos Carvalho Navegantes¹

Affiliations:

¹ University of São Paulo, Ribeirão Preto, SP, Brazil.

Keywords: Vitamin D. Maternal deficiency. Body composition. Bone mass. Ovariectomy.

Abstract:

Vitamin D (Vit.D) deficiency during pregnancy and lactation is associated with long-term, sex-dependent effects on offspring. However, its impact on body composition in adult offspring of both sexes remains poorly understood. To address this, female Wistar rats were subjected to either a Vit.D-deficient (VDD) or sufficient (CT) diet during gestation and lactation. After weaning, male (M) and female (F) offspring were maintained on a CT diet until 20 weeks of age. To explore the potential role of sex hormones in the dimorphic effects of maternal VDD, female offspring underwent bilateral ovariectomy (OVX) or sham surgery (SHAM) at six weeks of age. At the end of the experiment, body composition was assessed using Dual-Energy X-ray Absorptiometry. Data are expressed as mean $\pm$ SEM (n=6), and statistical analysis was performed using two-way ANOVA ($p < 0.05$) (CEUA 1241/2023). The dietary intervention successfully induced VDD in dams, as evidenced by serum calcidiol concentrations (6.5 ± 0.5 ng/mL vs. 31.9 ± 1.3 ng/mL in CT). Ovariectomy efficacy in both CT and VDD female offspring was confirmed by uterine atrophy. Moreover, OVX promoted gain of body weight, fat mass and retroperitoneal white adipose tissue (rWAT) mass equally in CT and VDD offspring. In male offspring, VDD significantly reduced bone mass (9.9 ± 0.2 mm vs. 11.7 ± 0.2 mm in CT) and rWAT (142 ± 0.2 mg/mm vs. 190.3 ± 0.2 mg/mm in CT) but did not affect body weight, lean mass or total fat mass. Interestingly, the catabolic effect of VDD on bone and rWAT were not observed in female offspring, regardless of OVX status, indicating a sex-specific response independent of sex hormones. As expected, as compared to male CT, female CT offspring exhibited lower lean mass (7.3 ± 0.2 mg vs. 4.6 ± 0.2 mg in CT-SHAM) and skeletal muscle mass (gastrocnemius, tibialis, soleus and extensor digitorum). However, neither VDD nor OVX influenced these parameters. In conclusion, these findings suggest that maternal Vit.D status during gestation and lactation plays a crucial role in determining bone and fat mass in male offspring but not in females. Furthermore, the observed sexual dimorphism appears to be independent of female sex hormones.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Liraglutide, a GLP-1 analog, enhances brown adipose tissue sympathetic activity of obese mice independently of food intake

Authors:

Bruna de Souza Moraes¹ Gabriel Hunzinker Skiba¹ Matheus Miranda Anjos E Silva¹ Matheus Leonardo Moro¹
João Batista Camargo Neto¹ Leticia Cirelli Ruiz¹
Lilian do Carmo Heck¹ Isis do Carmo Kettelhut¹
Luiz Carlos Carvalho Navegantes¹

Affiliations:

¹ *Ribeirão Preto Medical School, University of São Paulo.*

Keywords: Brown adipose tissue. Food intake. Liraglutide. Thermogenesis. Sympathetic nervous system.

Abstract:

Previous studies have demonstrated that intracerebroventricular injection of liraglutide, a GLP-1 analog, (LIRA) stimulates sympathetic nervous system (SNS) activity in brown adipose tissue (BAT), enhancing thermogenesis. However, the systemic effects of LIRA treatment on SNS activity in the BAT of obese mice remain poorly understood, as does whether these effects are dependent on food intake. To address this question, thirty seven-week-old male C57Bl/6 mice were fed either a standard diet (ND, N=15) or a high-fat diet (HFD, N=15) for twelve weeks. Subsequently, the animals were treated with LIRA (200 µg/kg, twice daily) (NDL; HFL), vehicle (ND; HFD), or subjected to a food-pairing regimen (PF-NDL; PF-HFL) for 10 days. On the seventh day of treatment, a glucose tolerance test (2 mg/kg) was performed. Three days later, the animals were euthanized for BAT collection, which was used for histological and molecular analyses as well as norepinephrine quantification. Data are expressed as mean ± SEM (n=5), and statistical analyses were performed using two-way ANOVA followed by Tukey's post-test, with a significance level of p<0.05 (CEUA: 192/2020). As expected, HFD increased body weight and impaired glucose tolerance (44.4±2.2 mg/dL in HFD vs. 29.4±0.7 mg/dL in ND). LIRA treatment induced greater weight loss in HFD-fed mice compared to the effect of food restriction alone (36±0.9 g in HFDL vs. 41±0.6 g in PF-HFDL) and improved glucose tolerance, an effect not observed in the PF group. These findings were associated with a reduction in BAT mass (117±8 mg in HFDL vs. 196±26 mg in HFD) and a decrease in lipid droplet area (-23%), suggesting intracellular fat mobilization and increased thermogenic activity. Supporting this notion, UCP-1 gene expression (1,81±0.5 in HFDL vs. 0,7±0.2 in HFD) and norepinephrine content (1344±45 ng/g in HFDL vs. 1163±38 ng/g in HFD) were elevated in the BAT of LIRA-treated HFD mice. These results suggest that LIRA treatment stimulates BAT thermogenic activity by enhancing SNS activity. The lack of similar effects in the PF group indicates that LIRA's systemic benefits on BAT are independent of food intake reduction.

Financial Support: CAPES, CNPQ, FAPESP



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Maternal obesity increases endocannabinoid signaling and triglyceride content in the liver of rat offspring at weaning

Authors:

Thamiris Chagas DIAS¹ Larissa de Brito Fassarella¹ Juliana Pena Gonçalves¹ Alexandre Torres²

Tatiana El Bacha² Carolina Ferreira²

Carmen Cabanelas Pazos-Moura¹ Isis Hara Trevenzoli¹.

Affiliations:

¹ Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, RJ.

² Instituto de Nutrição Josué de Castro, Universidade Federal do Rio de Janeiro, RJ.

Keywords: Endocannabinoid system. Fatty acids. Hepatic metabolism. Metabolic programming. Obesity

Abstract:

Nutritional insults during early development may predispose offspring to obesity and fatty liver disease, which are associated with increased endocannabinoid system (ECS) activity. The ECS comprises the endocannabinoids anandamide and 2-arachidonoylglycerol (2-AG), cannabinoid receptors, and several metabolizing enzymes, such as the monoacylglycerol lipase (MAGL). We hypothesized that maternal obesity would promote liver steatosis in weanling rat offspring associated with increased endocannabinoids and modulation of their metabolizing enzymes. Animal procedures were approved by the CEUA/CCS/UFRJ (protocol 059/19). Female rats (60 days old) were fed a control diet (CT; 9% kcal lipids) or an obesogenic diet (OD; 40% kcal lipids + 9.5% kcal sucrose) for 9 weeks before mating and during pregnancy and lactation. Offspring liver was collected at weaning for analysis of the endocannabinoid content (HPLC-MS), fatty acids (GC-FID) and enzymes (Western Blotting). Data was analyzed using two-way ANOVA with Sidak's post hoc test and Pearson correlations. Maternal obesity increased the content of triglycerides, 2-AG, and saturated, monounsaturated and polyunsaturated fatty acids (PUFA) in the offspring liver ($p < 0.05$), regardless of sex. Maternal obesity reduced n-3 PUFA content while increased n-6 PUFA in both sexes ($p < 0.05$), elevating the n-6/n-3 ratio at weaning ($p < 0.05$). Increased 2-AG was associated with reduced MAGL expression in both sexes ($p < 0.05$). Hepatic 2-AG showed a negative correlation with n-3 PUFA (males: $r = -0.5435$; $p < 0.05$; females: $r = -0.6494$; $p > 0.05$) and a positive correlation with the n-6/n-3 ratio (males: $r = 0.6288$; $p < 0.05$ 0.0091; females: $r = 0.8565$; $p < 0.05$), regardless of offspring sex. Maternal obesity induced early hepatic steatosis and reduced MAGL expression, possibly causing the increase of liver 2-AG. These findings suggest that maternal nutrition during critical periods of life may modulate the offspring's ECS and the development of metabolic disease.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Effect of Galactoglucomannan on the Protection of Intestinal Barrier Integrity in Sodium Dextran Sulfate-Induced Colitis

Authors

Thaise Caputo Silva¹ Fernando Vitor-Vieira¹ Amanda dos Santos Lima¹ Nathália Alves Bento¹
Julia Carvalho Cardoso Consentini¹ Gabriela Furlaneto¹
Giselly Karoline Paiva da Silva¹ Livya Maria Vidal Souza dos Santos¹ Laura da Silva Cruz¹
Marcell Crispim¹ Alanis Cristina Lima¹ Arthur Pereira¹ Daniel Granato² Petri Kilpeläinen³
Luciana Azevedo¹

Affiliations:

¹ Federal University of Alfenas – MG. ² University of Limerick – Ireland.

³ Natural Resources Institute Finland (Luke) – Finland.

Keywords: Dietary fiber; inflammation; hemicellulose; Colon;

Abstract:

Galactoglucomannan (GGM) is a hemicellulose found in plants and often discarded as a timber industry by-product. Previous studies have reported anti-inflammatory and anticancer effects of GGM-rich extracts. This study evaluated the potential of GGM to protect intestinal barrier integrity in a colitis model. Male C57BL/6 mice (6 weeks old) $n = 8$ per group, were pretreated by gavage for 21 days with GGM-rich extract obtained from Norway spruce (400 mg/kg/day). In the last 5 days, 3% dextran sulfate sodium (DSS) in water was offered ad libitum to induce colitis. One day after treatment, the mice were subjected to the open field test (OFT) and then euthanized for liver and spleen collection for molecular analyses. Procedures were approved by the Animal Ethics Committee #0024/2024. Data were analyzed using two-way ANOVA with $p < 0.05$ considered significant and expressed as standard error of the mean. The disease activity index (DAI) was used to assess the severity of colitis. On day 5th, mice receiving DSS or GGM + DSS had significantly higher DAI scores than those receiving water or GGM + water (3.71 ± 0.28 or 3.5 ± 0.33 vs 0 ± 0 or 0 ± 0 , $F(3,130) = 41.47$, $p < 0.0001$; Tukey's post hoc). Likewise, DSS or GGM + DSS groups traveled shorter distances (1068 ± 87.2 or 1030 ± 128.4 vs 1287 ± 75.7 or 1549 ± 132.8 , $F(1,26) = 10.7$, $p = 0.003$) and showed greater immobility (154.4 ± 9.73 or 160.5 ± 13.11 vs 137.3 ± 7.23 or 111.3 ± 6.44 , $F(1,26) = 11.78$, $p = 0.002$) in the OFT. Spleens were also larger in DSS or GGM + DSS groups compared to water or GGM + water (0.3 ± 0.02 or 0.31 ± 0.02 vs 0.27 ± 0.02 or 0.27 ± 0.01 , $F(1,26) = 15.85$, $p = 0.0005$), while liver size remained unchanged. Despite previous findings regarding its beneficial properties, the GGM-rich extract failed to protect against DSS-induced colitis in this model, suggesting that its use in inflammatory bowel conditions may be ineffective or potentially harmful.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Acute physical exercise decreases the P2X7 purinergic tone, facilitating cholinergic activity to delay solids gastric emptying

Authors:

Miguel Carvalho¹

Ana Isabel Guimarães²

Kalinne Kelly Lima Gadelha^{1,2,3} Cátia Vieira²

Paulo Correia-de-Sá²

Moisés Tolentino Bento da Silva¹

Affiliations:

¹ Lab. of Physiology.

² Lab. Pharmacology & Neurobiology, Center for Drug Discovery and Innovative Medicines (MedInUP/RISE-Health), School of Medicine and Biomedical Sciences – University of Porto, Portugal.

³ Department of Physiology and Pharmacology, Federal University of Ceará, Brazil.

Keywords: Acute high-intensity exercise. Cholinergic response. Enteric nervous system. Gastrointestinal motility. Myenteric plexus.

Abstract:

Acute physical exercise (AcEX) causes GI dysmotility by different mechanisms. Yet, how AcEX interferes with the enteric nervous system (ENS) activity remains uncertain. The purinergic tone via activation of the low-affinity ATP-sensitive P2X7 excitatory receptor is altered in many pathological conditions of the GI tract. This prompted us to investigate the impact of AcEX on the neurochemical coding and P2X7 receptor tone in the GI segments of rats using the selective P2X7 antagonist, A438079. The animals were divided into sedentary (SED), acute exercise (AcEX), SED+A438079, and AcEX+A438079 groups. AcEX consisted of (swimming with 5% b.w. load/15-min). We measured the blood concentration of lactate ([Lac]) and gastric emptying of solids (GE) in all groups. After sacrifice, we isolated the gastric fundus, the ileum, and the colon to measure nerve-evoked [3H] ACh release and for immunofluorescence confocal microscopy. AcEX increased ($P<0.05$) blood [Lac] (from 2.5 ± 0.1 to 5.6 ± 0.5 mM/L) and decreased ($P<0.05$) the GE of solids (from 81.2 ± 3.3 to $53.5\pm6.3\%$) vs. SED rats. AcEX decreased ($P<0.05$) nerve-evoked [3H] ACh release in the gastric fundus from an S2/S1 ratio of 1.17 ± 0.10 to 0.88 ± 0.06 in SED animals. Pretreatment with A438079 ($3\mu\text{M}$) decreased the release of [3H] ACh (S2/S1: 0.81 ± 0.07 vs 1.17 ± 0.10 ; $P<0.05$) in the gastric fundus of SED rats, but the P2X7 receptor antagonist failed to affect the neurotransmitter release in AcEX rats. In the colon, despite one could not perceive any difference in nerve-evoked [3H] ACh release among SED and AcEX groups, the P2X7 receptor blocker could still decrease ($P<0.05$) the neurotransmitter release in AcEX rats (S2/S1: 0.66 ± 0.02) vs. SED rats (S2/S1: 0.87 ± 0.03). No significant changes were observed in the ileum between groups. Data show that AcEX causes acidemia and delay in GE, most probably by decreasing the P2X7-mediated purinergic tone, facilitating cholinergic activity in the gastric fundus without repercussions downstream the GI tract. The study was approved by the ICBAS Animal Welfare and Ethics Committees (approval No.224/2017). Work was supported by FCT projects (FEDER funding, projects UIDB/04308/2020 and UIDP/04308/2020).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Consumption of high-fat diet impairs the cholinergic anti-inflammatory system and tight junctions proteins in dextran sulfate sodium (DSS)-induced colitis in mice

Authors:

Caroline Lobo Costa¹ Wenicios Ferreira Chaves¹ Isis de Cassis Alves Martins¹ Adriana Souza Torsoni¹ Marcio Alberto Torsoni¹

Affiliations:

¹ Graduate Program in Nutrition Sciences and Sports and Metabolism, PPGCNEM/UNICAMP.

Keywords: $\alpha 7$ nAChR. Colitis. High-fat diet. Inflammatory. Intestinal Permeability.

Abstract:

INTRODUCTION: $\alpha 7$ nicotinic cholinergic receptor ($\alpha 7$ nAChR) has fundamental role in the cholinergic anti-inflammatory pathway. Our previous studies have shown that HFD consumption reduces the expression of the $\alpha 7$ nicotinic cholinergic receptor ($\alpha 7$ nAChR). The present study aims to investigate the inflammatory process of colitis and the modulation of $\alpha 7$ nAChR expression in mice fed HFD. **METHODS:** C57BL/6J wild type and $\alpha 7$ knockout mice were divided into CT or HFD (45%) groups, offered for 4 weeks. For colitis induction, part of the animals in both groups were exposed to DSS (2.5%), added to the drinking water for 7 days. To evaluate intestinal permeability, FITC-dextran was administered by gavage and quantified in serum. Colon and isolated colonic epithelial cells (IEC) were collected for Western Blotting and RT-qPCR analyses. CEUA:5751-1/2021 and 6342-1/2023. **RESULTS:** CT+DSS showed a higher DAI score on days 4 and 7 and body weight loss compared to the HFD group. Regarding colon length, there was a reduction in the HFD and CT+DSS group, compared to the CT group, as well as in the HFD+DSS group compared to the HFD group. HFD colonic epithelial cells showed a reduction in the gene expression of Tjp1, Occludin, TNF α , and Chrna7 compared to CT. Furthermore, HFD consumption reduced the protein content of $\alpha 7$ nAChR, Claudin1, Claudin2 and Occludin compared to CT. HFD+DSS had a reduction in ZO1 protein content compared to CT+DSS. Intestinal permeability in the HFD+DSS group was higher than in the CT+DSS and HFD groups. HFD-fed $\alpha 7$ KO mice had short colon, reduced Claudin2 protein content, as well as Claudin4 and Tjp1 gene expression compared to CT. Furthermore, KO $\alpha 7$ animals had significantly higher TNF α and IL-1B gene expression compared to CT. **CONCLUSION:** Thus, HFD shortens the colon independent of colitis but exacerbates intestinal permeability in colitis animals. The reduction of $\alpha 7$ nAChR receptor may be potentiating an inflammatory response in both HFD-exposed groups.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: An interplay between the Sympathetic Nervous System and the Hypothalamic- Pituitary-Adrenal axis in the regulation of glycemia during acute cold stress

Authors:

Gustavo Fortunato Passos¹ Henrique Jorge Novaes Morgan Leticia Cirelli Ruiz¹
João Batista Camargo Neto² Matheus Leonardo Moro¹ Andressa Pereira Silva² Neusa Maria Zanon¹
Lilian do Carmo Heck¹ Isis do Carmo Kettelhut²
Luiz Carlos Carvalho Navegantes¹

Affiliations:

¹ Department of Physiology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, São Paulo, Brazil.

² Department of Biochemistry/Immunology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, São Paulo, Brazil.

Keywords: Adrenalectomy. Corticosteroid. Glycemia. Liver.

Abstract:

The interaction between the Sympathetic Nervous System (SNS) and the Hypothalamic-Pituitary-Adrenal (HPA) axis is a central component of the stress response, and its dysregulation can compromise metabolic health. It is well established that acute cold stress activates both the SNS and the HPA axis. In previous studies, we demonstrated that cold-induced SNS activation stimulates hepatic gluconeogenesis via CREB (cAMP response element-binding protein) signaling in rodents. Although we also observed ~80% upregulation of FoxO1, this effect was not modulated by sympathectomy, suggesting an alternative activation mechanism. We hypothesized that corticosterone contributes to gluconeogenesis during cold exposure by regulating FoxO1. To test this, male C57BL/6J mice (12 weeks old) were subjected to bilateral adrenalectomy (ADX) or sham surgery. One week later, mice were exposed to room temperature (28°C; RT) or cold (4°C) for 6 hours. Blood was collected for corticosterone, glucose, glycerol, and ketone body measurements, while rectal temperature was monitored. At the end, liver samples were collected for noradrenaline quantification by HPLC and further analysis. Two-way ANOVA followed by Holm-Sidak post hoc test was used ($p < 0.05$; $n = 5-7$ /group). Cold exposure increased corticosterone (~2-fold), hepatic noradrenaline, and glycemia in sham mice. ADX blocked the corticosterone rise (3.16 ± 1.01 vs. 17.9 ± 0.36 ng/dL in Sham) and tended to decrease glucose AUC (461.8 ± 9.79 vs. 502.7 ± 8.37 in Sham). Cold also elevated ketone bodies (1.84 ± 0.13 vs. 0.80 ± 0.08 mmol at RT), an effect attenuated in ADX mice. Cold increased plasma glycerol and decreased insulinemia and rectal temperature similarly in both groups. Interestingly, hepatic noradrenaline was significantly reduced in cold-exposed ADX mice vs. sham (18.5 ± 4.4 vs. 59.7 ± 19.7 ng/g). Our findings suggest interplay between the SNS and HPA axis in glycemia regulation during acute cold stress, possibly via FoxO1.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Therapeutic Potential of Angiotensin-(1-7) in Gut Dysbiosis-Induced Metabolic Disorders: Experimental Insights in Mice

Authors:

Nícia Pedreira Soares^{1,2} Gabriela de Castro Magalhães¹ Yasmin Kolz Brozeghini¹

Pedro Henrique Mayrink¹ Igor de Campos Fontes³

Robson Augusto Souza Santos² Thiago Verano-Braga¹

Affiliations:

¹ Núcleo de Proteômica Funcional (NPF), Departamento de Fisiologia, Universidade Federal de Minas Gerais (UFMG).

² Laboratório de Hipertensão, Departamento de Fisiologia, Universidade Federal de Minas Gerais (UFMG). ³ Laboratório de apoptose, Departamento de Patologia, Universidade Federal de Minas Gerais (UFMG).

Keywords: Angiotensin-(1-7). Antibiotic Use. High-Fat Diet. Intestinal Dysbiosis. Metabolic Disorders.

Abstract:

Gut dysbiosis is an imbalance in the gut microbiota caused by factors such as inadequate diet and antibiotic use leading to metabolic disorders. Angiotensin-(1-7) [Ang-(1-7)], in addition to its cardiovascular effects, has been investigated for its positive influence on various metabolic parameters, making it a promising target for new therapies. The aim of this study was to evaluate whether treatment with an oral formulation of Ang-(1-7) included in β -cyclodextrin (C) influences the metabolic parameters of mice with dysbiosis induced by a combination of high-fat diet and antibiotic therapy. Male mice (8 weeks old) were divided into two groups: Normobiosis (N): received standard AIN-93 diet and filtered water for 13 weeks. Dysbiosis (D): received high-fat diet for 13 weeks and treatment with antibiotics for the last two experimental weeks and a single dose of streptomycin (0.67g/kg) for the last 14 days. Additionally, from the 11th week, both groups were subdivided into control treatment [45 μ g/kg of C (C group)] or Ang-(1-7)-C treatment [30 μ g/kg of Ang-(1-7) + 45 μ g/kg of C (A group)] via gavage for 21 days. In the 14th week, oral glucose tolerance test, euthanasia, and tissue collection were performed (CEUA-UFMG: 144/2023). Gut dysbiosis increased body weight(g) (NC=24.3 $\pm$ 0.4; NA=25.3 $\pm$ 0.7; DC=28.1 $\pm$ 0.4; DA=27.2 $\pm$ 0.8; P <0.05), body adiposity index(%) (NC=2.8 $\pm$ 0.1; NA=2.8 $\pm$ 0.2; DC=4.2 $\pm$ 0.5; DA=3.6 $\pm$ 0.4; P < 0.05), adipocyte area(μ m) (NC=1862 $\pm$ 171.9; NA=2038 $\pm$ 96.6; DC=4557 $\pm$ 565.7; DA=3782 $\pm$ 490.6; P < 0.05), and induced glucose intolerance [AUC, (NC=664.4 $\pm$ 36.5; NA=679.8 $\pm$ 37.07; DC=893.5 $\pm$ 51.4; DA=717.8 $\pm$ 40.6; P < 0.05)] compared to controls, while Ang-(1-7)-C treatment reversed these changes. Additionally, animals subjected to gut dysbiosis exhibited a higher intestinal inflammatory histopathological score and a reduced number of goblet cells, both of which were attenuated by Ang-(1-7). Thus, Ang-(1-7) improves metabolic parameters linked to gut dysbiosis.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Effect of melatonin treatment on the intestine and liver of adult zebrafish exposed to alcohol

Authors:

Daniela Capelli Diglio^{1,3} Eronides Heberle Salau^{2,3} Sophia Costa de Almeida³ Nicole Bauermann Maffessoni³ Gabriela Kaminski³
Ana Cláudia Reis Schneider³ Orlando Vieira Furtado-Filho³ Themis Reverbel da Silveira³
Marilene Porawski^{1,2,3}

Affiliations:

¹ Programa de Pós-Graduação em Biociências, UFCSPA.

² Programa de Pós-Graduação Hepatologia, UFCSPA.

³ Laboratório de Fisiologia Comportamental e Metabólica, UFCSPA.

Keywords: Alcohol liver disease. Steatosis. Melatonin. Intestinal permeability. Zebrafish.

Abstract:

Introduction: Chronic alcohol consumption causes changes in the intestinal epithelium, promoting the rupture of tight junctions and increasing the translocation of endotoxins and inflammation. Melatonin is a hormone that regulates the circadian rhythm, enhances immune defense, and exhibits antioxidant and anti-inflammatory properties. **Objective:** To evaluate the action of melatonin in preventing changes in intestinal permeability, inflammation, and oxidative stress in zebrafish exposed to alcohol. **Methods:** Two hundred and fifty-six adult zebrafish of both sexes, kept under standard conditions, were used. They were divided into 4 groups: control, melatonin, and ethanol+melatonin. Animals exposed to ethanol received 0.5% ethanol (v/v) added to the aquarium water. Melatonin was dissolved in DMSO and added to the aquarium at a concentration of 100nM. Animals were euthanized after 6 weeks of exposure to ethanol and/or melatonin. The liver and intestine were dissected and fixed for histology with hematoxylin and eosin (HE) staining or frozen for evaluation of gene expression by qRT-PCR. Two-way ANOVA followed by Tukey's post-test was used with $p < 0.05$. Approval protocol nº 259/19. **Results:** We observed that animals exposed to ethanol presented moderate to severe steatosis, with increased expression of the *acc1* and *fasn* genes in the liver; these changes were prevented in the ethanol+melatonin group. In the intestine, the expression of the genes for *cldn-1* and *cld-3d* had a significant reduction in animals exposed to alcohol, which was prevented in the alcohol+melatonin group. Treatment with melatonin in animals exposed to alcohol increased the expression of *sirt-1* and reduced the expression of *il-6* and *nf-kb* in the liver and intestine. **Conclusion:** Melatonin was able to prevent the activation of the lipogenic and inflammatory pathway in the liver and preserved the intestinal barrier of animals with ALD.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Cold and restraint stress aggravates 5-fluorouracil-induced intestinal mucositis in mice

Authors:

Lorena Duarte da Silva¹ Ornildo Torres Fraga¹
Victória Martins Rodrigues dos Santos¹ Lucas Henrique Marques Rodrigues¹ João Lucas Silva Pinheiro¹
Cecília Lourena Olímpia Aragão da Cunha¹ Arthur Vinícios Tenório da Silva¹
Hana Hayashi Pinto¹
Jand Venes Rolim Medeiros²
Marcellus Henrique Loiola Ponte Souza³ Luiz Alberto Reis Mattos Junior⁴
Renan Oliveira Silva Damasceno¹

Affiliations:

¹ Department of Physiology and Pharmacology, Federal University of Pernambuco.
² Biotechnology and Biodiversity Center Research, Federal University of Parnaíba Delta.
³ Department of Physiology and Pharmacology, Federal University of Ceará.
⁴ Center for Medical Sciences, Federal University of Pernambuco.

Keywords: Stress. 5-Fluorouracil. Mucositis.

Abstract:

Intestinal mucositis is a common side effect of 5-fluorouracil (5-FU). Stress may aggravate gastrointestinal inflammation, though its role in chemotherapy-induced mucosal damage remains unclear. This study evaluated the effects of cold and restraint stress on 5-FU-induced intestinal mucositis in mice. Balb/c mice (Protocol 27/2024) were stressed daily in restraint tubes for 8 days (2 h at 4°C + 6 h at RT). On day 6, they received 5-FU (450 mg/kg, i.p.) and were euthanized on day 9. Body mass, intestinal length, leukocyte, villus morphometry, MPO, NP-SH, MDA, 4-HNE, intestinal transit, and fecal fluid were assessed. Body mass loss and leukopenia were greater in 5-FU (-15.2 ± 0.6 g and $2.3 \pm 0.2 \times 10^3/\text{mL}$) and S+5-FU (-18.5 ± 1.2 g and $2.4 \pm 0.19 \times 10^3/\text{mL}$) vs. Control ($+0.68 \pm 1.2$ g and $6.3 \pm 0.6 \times 10^3/\text{mL}$) and Stress ($+0.32 \pm 2.0$ g and $7.5 \pm 0.5 \times 10^3/\text{mL}$). Intestinal length was reduced in 5-FU (47.5 ± 1.4 cm) and S+5-FU (45.2 ± 0.5 cm) vs. Control (51.5 ± 1.5 cm). Villus height was reduced in S+5-FU (496.1 ± 40.7 μm) vs. 5-FU (959.9 ± 30.3 μm), Stress (1131 ± 27.0 μm), and Control (1288.0 ± 40.1 μm). Compared to Control 0.11 ± 0.05 U/mg, MPO activity was increased in 5-FU (1.41 ± 0.31 U/mg) and intensified ($p < 0.05$) in S+5-FU (2.41 ± 0.28 U/mg). NP-SH levels were reduced in 5-FU (124.5 ± 20.9 mg/g) and S+5-FU (162.7 ± 18.8 mg/g) vs. Control (209.3 ± 17.6 mg/g) and Stress (243.7 ± 47.7 mg/g), while lipid peroxidation markers (MDA and 4-HNE) were elevated in 5-FU (6.3 ± 0.5 mg/g and 0.150 ± 0.009 O.D.) and S+5-FU (6.5 ± 0.83 mg/g and 0.16 ± 0.01 O.D.) vs. Control (4.2 ± 0.4 mg/g and 0.108 ± 0.005 O.D.) and Stress (3.2 ± 0.4 mg/g and 0.11 ± 0.007 O.D.). The fecal fluid increased in Stress (63.7 ± 2.4 %) and S+5-FU (49.8 ± 6.9 %), but not in 5-FU alone (36.31 ± 10.33 %) vs. Control (19.8 ± 4.3 %). Intestinal transit was delayed in S+5-FU (1.90 ± 0.09 %) than in 5-FU (2.30 ± 0.09 %) compared to Control (2.83 ± 0.14 %). Stress exacerbates 5-FU-induced intestinal mucositis and may be a therapeutic strategy to reduce chemotherapy-related intestinal toxicity.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: A new murine model of chemically-induced ileitis: Insights into inflammation, antioxidant imbalance and gastrointestinal dysfunction

Authors:

Lucas Henrique Marques RODRIGUES¹ Victória Martins Rodrigues dos Santos¹ João Lucas Silva Pinheiro¹

Lorena Duarte da Silva¹ Ornildo Torres Fraga¹

Renan Oliveira Silva Damasceno¹

Affiliations:

¹ Department of Physiology and Pharmacology, Federal University of Pernambuco.

Keywords: Ileitis. DNBS. Inflammatory response. Oxidative stress. Gastrointestinal function.

Abstract:

Ileitis is a clinic inflammatory condition affecting the distal small intestine that remains poorly explored due to the scarcity of reliable experimental models. This study aimed to standardize a murine model of ileitis using 2,4-dinitrobenzenesulfonic acid (DNBS). Swiss mice (8 weeks old, Protocol 21/2024) received an intraluminal injection of saline or DNBS (2, 5, or 10 mg in 40% ethanol; 150 μ L) into the ileum and were euthanized on days 1, 3, or 7 post-induction. Ileal samples were analyzed for macroscopic and microscopic injury, oxidative stress, inflammation, and functional parameters. DNBS at 10 mg induced time- and dose-dependent ileal injury, with peak macroscopic scores on day 3 (median: 5 [4–8]), compared to saline (median: 0 [0–2]). No significant changes in villus length were observed on days 1 or 7, while on day 3, DNBS 10 promoted villus shortening (280.2 ± 23.0 μ m; $P < 0.05$) compared to DNBS 5 (440.6 ± 10.2 μ m), DNBS 2 (442.7 ± 16.2 μ m), and saline (384.6 ± 18.9 μ m). Compared to saline, DNBS 10 also increased ($P < 0.05$) ileal weight (488.4 ± 44.6 vs. 151.2 ± 8.0 mg), elevated myeloperoxidase (MPO) activity (13.4 ± 3.6 vs. 2.7 ± 0.7 U-MPO/mg), indicating intense inflammatory response, and decreased non-protein sulfhydryl (NP-SH) (48.9 ± 17.9 vs. 139.3 ± 20.4 mg/g) and glutathione (GSH) levels (7.9 ± 3.6 vs. 30.8 ± 2.8 mg/g), suggesting antioxidant imbalance. Additionally, DNBS 10 reduced ($P < 0.05$) mucus-producing goblet cells number (6.3 ± 0.3 vs. 8.9 ± 0.6 cells/villus), along with increased gastric retention (45.7 ± 3.5 vs. $31.9 \pm 2.4\%$) and delayed intestinal transit (1.60 ± 0.04 vs. 2.04 ± 0.14), compared to saline. In conclusion, our findings demonstrate that DNBS induces severe inflammatory, oxidative, and functional changes in the ileum, supporting its use as a valuable preclinical model for studying ileitis pathophysiology and evaluating therapeutic strategies.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Sleep deprivation prevents ethanol-induced gastric injury in mice: A dynamic and reversible effect modulated by rebound sleep

Authors:

Victória Martins Rodrigues dos Santos¹ Lucas Henrique Marques Rodrigues¹ Lorena Duarte da Silva¹

João Lucas Silva Pinheiro¹ Arthur Vinícios Tenório da Silva¹

Renan Oliveira Silva Damasceno¹

Affiliations:

¹ Department of Physiology and Pharmacology, Federal University of Pernambuco.

Keywords: Sleep deprivation. Gastric injury. Ethanol.

Abstract:

Sleep deprivation (SD) is known for its deleterious effects on health. However, studies suggest that SD may trigger adaptive responses in the gastric mucosa. This study evaluated the effects of SD on ethanol-induced gastric injury. Swiss mice (8 weeks old, Protocol 22/2024) were submitted to 72 h of SD, followed by administration of 50% ethanol (ET) or saline. Other groups underwent 7 days of recovery sleep (Rebound sleep: REB). After 1 h, stomachs were collected for macroscopic, histological, oxidative stress, vascular permeability, and gastric function analyses. SD+ET reduced macroscopic damage ($32.0 \pm 4.6\%$) compared to ET ($54.2 \pm 5.2\%$), while saline or SD alone caused no injury. ET increased histopathological scores ($12 [8-13]$ vs. $2 [1-4]$), vascular permeability (32.2 ± 1.8 vs. 14.2 ± 1.2 $\mu\text{g}/\text{mg}$), decreased NP-SH (518.6 ± 88.6 vs. 1643.0 ± 153.5 mg/g), GSH (11.7 ± 2.0 vs. 37.3 ± 2.4 mg/g), and increased 4-HNE (0.35 ± 0.04 vs. 0.16 ± 0.02 O.D.) compared to saline. SD+ET reduced these alterations (histology: $4 [2-7]$; permeability: 21.8 ± 1.3 $\mu\text{g}/\text{mg}$; NP-SH: 882.9 ± 97.2 mg/g and GSH: 18.9 ± 1.2 mg/g , and 4-HNE: 0.24 ± 0.03 O.D.). Compared to saline, SD also reduced gastric secretion volume (107.7 ± 10.3 vs. 287.2 ± 29.8 μL), acidity (0.033 ± 0.004 vs. 0.043 ± 0.001 $\text{Eq}[\text{H}^+]/\text{mL}/4\text{h}$), and increased pH (1.48 ± 0.05 vs. 1.36 ± 0.01), similar to omeprazole (153.0 ± 26.9 μL , 0.023 ± 0.001 $\text{Eq}[\text{H}^+]/\text{mL}/4\text{h}$ and 1.62 ± 0.02 , respectively). Gastric retention was also reduced by SD (15.0 ± 1.6 vs. $22.0 \pm 1.6\%$). Similar to ethanol, REB+ET reversed the protective effects of SD, promoting injury (55.9 ± 8.8 vs. $65.6 \pm 10.7\%$) and decreasing antioxidant defenses (NP-SH: 342.4 ± 38.7 vs. 262.2 ± 22.2 mg/g and GSH: 7.3 ± 0.9 vs. 6.7 ± 1.8 mg/g), but not 4-HNE levels. Compared to saline, SD or REB alone did not induce injury or alter biochemical markers. SD attenuated ethanol-induced gastric injury by reducing oxidative stress and acid secretion, while were reversed after rebound sleep, reinforcing the dynamic and time-dependent nature of this response.



XXXIX REUNIÃO ANUAL DA FeSBE
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XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Splenectomy attenuates 5-fluorouracil-induced intestinal mucositis in mice: Evidence for a key role of gut-spleen axis

Authors:

João Lucas Silva Pinheiro¹

Lucas Henrique Marques Rodrigues¹ Victória Martins Rodrigues dos Santos¹ Lorena Duarte da Silva¹

Ana Luiza Costa Ferreira¹

Marcellus Henrique Loiola Ponte Souza² Luiz Alberto Reis Mattos Junior³

Renan Oliveira Silva Damasceno¹

Affiliations:

¹ Department of Physiology and Pharmacology, Federal University of Pernambuco.

² Department of Physiology and Pharmacology, Federal University of Ceará.

³ Center for Medical Sciences, Federal University of Pernambuco.

Keywords: Intestinal mucositis. 5-Fluorouracil. Splenectomy. Chemotherapy.

Abstract:

Intestinal mucositis is a severe side effect of chemotherapy with 5-fluorouracil (5-FU), attributed to its low selectivity for tumor cells, leading to significant morbidity and reduced quality of life in cancer patients. The spleen plays a crucial role in immune regulation, but its involvement in chemotherapy-induced mucositis remains poorly understood. This study aimed to investigate the impact of splenectomy (SP) on 5-FU-induced intestinal mucositis. Balb/c mice (7-8 weeks old, n=6, Protocol No. 20/2024) were submitted to SP or sham surgery, followed by 5-FU (450 mg/kg, i.p.) administration. The body mass, leukocytes count, oxidative stress and inflammation markers were assessed. 5-FU reduced ($P<0.05$) leukocytes count (1408.0 ± 98.1 cells/ μ L) and body mass ($-14.5\pm1.2\%$) compared to control (5517.0 ± 503.7 cells/ μ L and $+2.3\pm2.3\%$, respectively). Compared to 5-FU, SP+5-FU animals had no alteration in body mass loss ($-15.5\pm1.1\%$) nor leukopenia (2095.0 ± 207.1 cells/ μ L). The antioxidant defense was reduced ($P<0.05$) by 5-FU, as indicated by increased NP-SH consumption (108.3 ± 13.2 mg/g vs 227.5 ± 10.2 mg/g in control), and elevated lipid peroxidation markers, MDA and 4-HNE (6.2 ± 0.3 μ M/mg and 0.132 ± 0.004 O.D., respectively, vs 3.5 ± 0.2 μ M/mg and 0.069 ± 0.008 O.D. in control). Notably, SP mitigated these alterations, restoring NP-SH levels (165.8 ± 16.7 mg/g) and reducing MDA (5.1 ± 0.2 μ M/mg) and 4-HNE (0.110 ± 0.004 O.D.). Additionally, 5-FU increased ($P<0.05$) MPO activity (2.17 ± 0.31 UMPO/mg) compared to control (0.73 ± 0.16 UMPO/mg), which was reduced ($P<0.05$) in SP+5-FU animals (1.13 ± 0.17 UMPO/mg). SP alone had no effect on the assessed parameters. These findings suggest that SP alleviates 5-FU-induced intestinal mucositis by preventing excessive inflammation and oxidative stress. The gut-spleen axis may play a pivotal role in chemotherapy-induced intestinal mucositis, warranting further investigation into its mechanisms and possible therapeutic application.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Subdiaphragmatic vagotomy improves obesity, vagal activity and leads to browning in the iBAT

Authors:

Vanessa Cristina de Souza Melo¹ Rodrigo Mello Gomes^{1,2}
Sandra Lucinei Balbo³
José Antônio Rocha Gontijo⁴ Patrícia Aline Boer⁴
Antônio Carlos Boschiero¹

Affiliations:

- ¹ Departamento de Biologia Estrutural e Funcional, Universidade Estadual de Campinas.
² Departamento de Ciências Fisiológicas, Universidade Federal de Goiás.
³ Departamento de Fisiologia, Universidade Estadual do Oeste do Paraná.
⁴ Departamento de Clínica Médica, Universidade Estadual de Campinas.

Keywords: High-fat diet. Obesity. Metabolic syndrome. Autonomic nervous system. Vagus nerve.

Abstract:

The autonomic nervous system (ANS), through the vagus nerve, regulates insulin release by pancreatic beta cells. Considering that in obese rodents the electrical activity of the vagus nerve (AVN) is increased, the aim of this study was to evaluate the role of the vagus nerve and the effects of vagotomy on the metabolic parameters of obese mice. Experimental protocol was approved by CEUA/Unicamp (6454-1/2024). C57BL/6J mice (8 weeks old, n=36) were distributed into 3 groups: control group (SD) fed a standard rodent diet, obese group fed a high-fat diet (HF), and group fed a HF and submitted to vagotomy (VAG) surgery. All mice were fed their respective diets for 16 weeks, and surgery performed in the eighth week. As expected, HF mice had higher body weight (BW=42,42g±5,36), visceral adiposity (Fat=1,70g±0,68) and adipocyte size (eWAT=1,17x10⁷µm²±3,34x10⁶) compared to DP mice (BW=31,23g±3,61/Fat=0,27g±0,10/eWAT=2,33x10⁶µm²±7,11x10⁵). VAG group showed significant improvement in these parameters (BW=36,11g±5,84/Fat=0,96g±0,56/eWAT=4,97x10⁶µm²±2,14x10⁶). As expected, VAG group had a significant larger stomach than other groups (VAG=0,27g±0,04/ SD=0,19g±0,03/HF=0,17g±0,04). Glucose intolerance and pancreatic islets hypertrophy were observed in HF (islet area HF=1,98x10⁷µm²±1,62x10⁷) compared to SD group (islet area SD=1,20x10⁷µm²±8,19x10⁶). However, VAG reversed this condition (islet area VAG=1,27x10⁷µm²±8,36x10⁶). We also observed (qualitative analysis) brown adipose tissue (iBAT) remodeling, with multilocular brown adipocyte structure showed whitening in the HF group, which was reverted in VAG mice. HF group showed significant increase in AVN (3,93µA±0,50) compared to DP group (2,99µA±0,47). Whereas, VAG group showed similar AVN to that of the DP mice. Our findings indicate that VAG ameliorates energy homeostasis in HF mice by improving vagal activity and browning in the iBAT, highlighting ANS as a therapeutic target for metabolic disorders.

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XXXIX REUNIÃO ANUAL DA FeSBE
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XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Bile acids and the P2Y₁ purinoceptor play complementary roles to decrease esophageal motility in a gastroesophageal reflux disease (GERD) rat model

Authors:

Kalinne Kelly Lima Gadelha^{1,2,3} Pedro Jorge Caldas Magalhães³ Armênio Aguiar dos Santos³
Paulo Correia-de-Sá²
Moisés Tolentino Bento da Silva¹

Affiliations:

¹ Lab. of Physiology.

² Lab. Pharmacology & Neurobiology, Center for Drug Discovery and Innovative Medicines (MedInUP/RISE-Health), School of Medicine and Biomedical Sciences – University of Porto, Portugal.

³ Nubimed - Biomedicine Center, Department of Physiology and Pharmacology, Federal University of Ceará, Brazil

Keywords: Bile acids. Cholinergic neurotransmission. Esophageal motility. Gastroesophageal reflux. P2Y₁ purinoceptor

Abstract:

Gastroesophageal reflux disease (GERD) is associated with increased contact of the esophageal mucosa with gastric acid, bile acids and pepsin. Other molecules, like adenine nucleotides, influence GI transit, affecting the aggressiveness of damaging agents in GERD. Here, we evaluated the role of the P2Y₁ purinoceptor on cholinergic neurotransmission in the GERD model. The esophagus of rats (~200g) was isolated and submitted to intraluminal incubation with a pH 1 Tyrode's solution plus pepsin (1mg/mL) and deoxycholic acid (DCA; 2mM) for 30min; CTR rats received pH 7.4 Tyrode's solution. Then dissected esophageal strips were mounted into vertical organ baths to record myographic responses and to measure [³H]ACh release evoked by electrical field stimulation (5Hz, 200 pulses; S1 and S2). ATP (100μM) relaxed by 30.64% CCh (1μM)-induced esophageal contractions in CTR rats, but this effect was attenuated to 19.12% in the GERD model. ATP (100μM)-induced relaxations were prevented by MRS2179 (0.3μM), in both CTR and GERD. In vitro incubation of esophageal strips with DCA (100μM) alone for 30-45 min reduced the amplitude of CCh (3μM)-induced contractions (34.6±11.3mg vs. 78.0±8.0mg; P<0.05), but no effect was observed when DCA (100μM) was applied on top of cholinergic contractions. The relaxing effect of DCA (100μM) was mimicked by the ganglionic cholinergic blocker, hexamethonium (1mM), while blockage of muscarinic M3 receptors with J104129 (100nM) abolished cholinergic contractions under the same experimental conditions. Data suggests that DCA may reduce cholinergic-induced esophageal contractions by a mechanism related to blocking ganglionic transmission, which is corroborated by the significant decrease in nerve-evoked [³H]ACh release in GERD-treated esophageal strips vs CTR. Given the kinetics and the pattern of the P2Y₁-mediated esophageal relaxation, it seems that DCA and the purinoceptor play distinct, yet complementary, roles to delay the esophageal motility in GERD. *The study was approved by CEUA-UFC n-3742051018.*



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Proteolytic pathways in protein metabolism of skeletal muscles in diabetic mice

Authors:

Andressa Pereira-Silva¹ Leticia Cirelli Ruiz²
João Batista Camargo Neto¹ Matheus Leonardo Moro² Henrique Jorge Novaes Morgan² Neusa Maria Zanon²
Lilian do Carmo Heck²
Luiz Carlos Carvalho Navegantes² Natalia Lautherbach¹
Isis do Carmo Kettelhut¹

Affiliations:

¹ Department of Biochemistry & Immunology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, SP, Brazil.

² Department of Physiology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, SP, Brazil.

Keywords: Muscle atrophy. Protein metabolism. Proteolytic pathways. Type 1 diabetes *mellitus*.

Abstract:

It is well known that Diabetes *mellitus* causes muscle atrophy, but the participation of proteolytic pathways at different times in this phenotype has not been elucidated. Therefore, the aim of this study was to verify the proteolytic activity of different pathways in skeletal muscles of diabetic mice. For this, male mice (C57Bl/6, ~25g, 8-12 weeks) received a single intravenous injection of streptozotocin (STZ;130mg/kg). Animals were euthanized at different periods (days 1, 2, 3, 5, 10 and 14 post-STZ). *Soleus* and EDL muscles were incubated for 2h with tendons fixed on appropriate supports and the total proteolytic activity *in vitro* was determined by tyrosine release into the medium. Values were expressed as mean $\pm$ SEM (n=6) and Student's t-test or one-way and two-way ANOVA (*p<0,205) were used for statistical analyses (CEUA: 1430/2025). At the first day, glycemic levels were twice higher in the diabetic group when compared to control (373 $\pm$ 6 vs. 150 $\pm$ 5 mg/dL). In addition, a reduction (~15%) in total body mass from the 5th day was also observed in the diabetic group (19,0 $\pm$ 0,3 vs. 23,2 $\pm$ 0,7 g, control group). The relative mass of *tibialis* anterior (TA) reduced 10% on the 3rd day of diabetes (175 $\pm$ 3 vs. 194 $\pm$ 4 mg/g, control group). The total proteolytic activity in EDL muscles increased on days 2 (~65%) and 3 (~30%) (0,558 $\pm$ 0,035; 0,452 $\pm$ 0,049 vs. 0,314 $\pm$ 0,017 nmol tyr/mg.muscle.2h, control group). There was a reduction (~20%) in *soleus* total proteolysis only on the 14th day (0,290 $\pm$ 0,021 vs. 0,377 $\pm$ 0,018 nmol tyr/mg.muscle.2h, control group). Moreover, there was an increase in the protein content of atrogin-1 in TA on the 3rd day, possibly indicating an activation of the proteasomal pathway at the onset of diabetes. These results suggest that the proteolytic pathways are more activated in skeletal muscle of mice in early periods of diabetes, returning to control levels in chronic stages.



XXXIX REUNIÃO ANUAL DA FeSBE
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XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Effects of post-weaning protein restriction in the intestinal morphometry and motility of C57BL/6 male mice

Authors:

Bruna Pires de Oliveira¹ Kênia Moreno de Oliveira¹ Bruna Lourençoni Alves²
Everardo Magalhães Carneiro¹

Affiliations:

¹ Laboratório de Pâncreas Endócrino e Metabolismo - LaPEM, Centro de Pesquisa em Obesidade e Comorbidades - OCRC, Universidade Estadual de Campinas.

² Departamento de farmacologia, FCM, Universidade Estadual de Campinas.

Keywords: Duodenum. Protein undernutrition. Post-weaning.

Abstract:

Protein restriction may impair gut function and contribute to metabolic impairments, but there is a lack of information regarding this relation. We investigated the effects of prolonged post-weaning protein restriction on small intestine (SI) morphology and motility in mice. 30 days-old, mice were divided in control (C) fed a 14% protein diet, and restricted (R) fed a 6% protein diet for 90 days. Body weight (BW) was weekly evaluated. Duodenal segments of 2 cm were mounted in a 10 ml organ bath containing Krebs-Henseleit solution heated (37°C) and gassed (95%O₂:5%CO₂). Tissues were equilibrated for 45 min under resting tension of 1g. The isometric tension was registered using a PowerLab system (ADInstruments Sydney, Australia). Then, 80 mM KCl was added in to assess viability and maximal contraction of the tissues. The tissues were washed 3 times, after 45 min the agonist-induced contraction or relaxation of the tissue was evaluated. Cumulative concentration-response curves for carbachol (CCh; 1nM-100µM) or isoproterenol (1pM-10µM) were performed. Curves were analyzed using a sigmoidal concentration-response model to assess EC₅₀ and maximum response (E_{max}). Data were analyzed using the Shapiro-Wilk normality test, followed by Student's t-test or Mann-Whitney U test (P<0.05; CEUA UNICAMP nº5564-1/2020). As expected, R mice had a lower final BW (22.2±0.7g) when compared to C mice (26.5±0.5g), SI length (3.6±0.2cm/nose-anus length NAL) and weight (8.9±9.6mg/g BW) in R group were similar to C group (3.4±0.3cm/NAL and 48.2±7.3mg/g BW, respectively). Protein restriction did not alter carbachol-induced contractions between the groups. On the other hand, the isoproterenol-induced relaxations were three times higher in R group (31,8 ± 6,9) than C group (11,99 ± 2,74). Overall, post-weaning protein restriction did not alter SI morphometry and cholinergic contractile responses in the duodenum, however β-adrenergic-mediated relaxation responses were significantly enhanced.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Effect of maternal obesity and perinatal fish oil supplementation on endocannabinoid system in the small intestine of adolescent rat offspring

Authors:

Matheus Vasconcellos BORGES¹ Marina Costa REIS¹
Larissa de Brito FASSARELLA¹ Carmen Cabanelas PAZOS-MOURA¹ Mariana Macedo de ALMEIDA²
Isis Hara TREVENZOLI¹

Affiliations:

¹ Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, RJ.

² Instituto de Ciências Biológicas, Universidade Federal de Juiz de Fora, MG.

Keywords: Endocannabinoids. Gut. Obesity. Fish oil. Metabolic programming.

Abstract:

Maternal obesity programs early metabolic syndrome and anxiety-like behavior in adolescent rat offspring, with endocannabinoid system (ECS) changes in the gut-brain axis. Cannabinoid receptor 1 (CB1) influences behavior while CB2 has anti-inflammatory actions. Here, we investigated the effect of maternal obesity and fish oil (FO) supplementation, an important anti-inflammatory, on the expression of CB2 and the endocannabinoid metabolizing enzymes fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) in the gut of adolescent rat offspring. Female rats were fed an obesogenic diet (OD, n=9; 43% fat and 9.5% sucrose) or a control diet (C, n=8; 10.9% fat) for 8 weeks before mating and throughout pregnancy and lactation. An OD subgroup received supplementation with 3% FO (ODFO, n=8) during gestation and lactation. Body weight, adiposity, glycemia, and small gut length of the offspring were evaluated on postnatal day 45 (adolescence). CB2, FAAH and MAGL were quantified in the distal small gut by Western Blotting. Data were analyzed by one-way ANOVA, $p < 0.05$. The study was approved by CEUA/CCS/UFRJ (A32/24-135-22). Maternal OD increased offspring body weight and adiposity during lactation ($p < 0.05$), and maternal FO supplementation attenuated this effect ($p < 0.05$). At 45 days, we did not observe changes in body weight, gut length, serum cholesterol and triglycerides, glycemia, inguinal adiposity, or in the gut expression of CB2, FAAH and MAGL in the OD or ODFO offspring compared to controls. Only male OD offspring exhibited greater retroperitoneal adiposity compared to controls ($p < 0.05$), with no FO effects in adolescence. Maternal FO was able to prevent offspring obesity at weaning, but this effect did not last until adolescence. Neither maternal obesity nor FO affected CB2 and ECS metabolizing enzymes in the distal gut of adolescent offspring. Future experiments will address other components of the ECS in the offspring, as CB1 and the lipid endocannabinoids.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Serum Metabolomic Analyses in Colorectal Cancer Patients: Impact of Cachexia

Authors:

Bruno Sérgio M. Madeira*¹ Maria Carolina S. Mendes*² Carla de M. Salgado¹
Lais R. Viana¹
Leisa Lopes-Aguiar¹ Fernanda A. Marqueto¹ Gabriela F. Mequita¹ Ninon F. Barrios¹ Fabiana
Lascala² Sandra R. Branbilla² Renata Contriciani²
Fabiola Furtuoso Zarpelão² Leo Victor Kim²
Aglecio L. Souza³ Sarah Monte Alegre³ Felipe O. Costa²
Carlos A. Real Martinez⁴
Jose Barreto C. Carvalheira^{#2}
Maria Cristina C. Gomes-Marcondes^{#1}

*: sharing the first authorship; #: correspondent authors

Affiliations:

¹ Department of Structural and Functional Biology, Biology Institute, State University of Campinas, University of Campinas, Campinas, Brazil.

² Department of Anesthesiology, Oncology and Radiology, School of Medical Sciences, University of Campinas, Campinas, Brazil.

³ Department of Internal Medicine, School of Medical Sciences, University of Campinas, Campinas, Brazil.

⁴ Department of Surgery, School of Medical Sciences, University of Campinas, Campinas, Brazil.

Keywords: Cachexia. Metabolomic Analysis. Metabolism. Rectal Cancer.

Abstract:

Introduction: Colorectal cancer is the second most common cancer in world, which can lead to cachexia syndrome. 80% of those patients present this metabolic syndrome, with severe complications such as muscle mass loss and deterioration of nutritional status. Early detection of metabolic alterations is crucial for improving prognosis and more effective interventions. **Materials and Methods:** Serum samples from patients diagnosed with colon cancer, collected at baseline (T0) and after 120 minutes (T1), were analysed by NMR (500 MHz) assay. Spectra were processed using CHENOMX software, and altered metabolic pathways were identified using MetaboAnalyst 6.0. **Results:** Metabolic profiles of cachectic patients – C (n=5) and non-cachectic – NC (n=5) were analysed at T0 and T1 after glycaemic clamp (Ethics certificate number CAAE: 91217418.2.0000.5404). In C group, comparing T1 vs T0, there was a significant reduction in leucine, histidine, isoleucine, tyrosine, asparagine, mannose, hypoxanthine, phenylalanine, valine, threonine, serine, alanine, malonate, 3-hydroxybutyrate, glutamine, and ornithine. These reductions significantly modulated pathways related to BCAA biosynthesis and degradation, and the metabolism of glutamate, alanine, glycine, serine, and threonine. In the NC group, there were no changes in metabolites between T0 and T1. The comparison between both groups (after glycaemic clamp at T1), C group showed lower levels of creatinine and valine; and increased content of lactate, pyruvate and methylhistidine, with similar levels of glucose and succinate, reflecting activation of pathways associated with BCAA biosynthesis and degradation, taurine, beta-alanine, histidine, and purine metabolism. **Conclusion:** This preliminary study identified relevant metabolic differences between cachectic and non-cachectic patients with colon cancer, enhancing the understanding of cancer-associated cachexia, which could support the development of more effective therapeutic strategies. **Ethics certificate number:** 91217418.2.0000.5404 (CAAE – Brazil's Certificate of Ethical Approval).

Financial Support: FAPESP (2017/02739-4; 2019/20558-2; 2021/08188-5; 2021/08931-0; 2018/23428-0; 2021/08931-0; 2021/10265-8; 2022/06239-4; 2023/09611-4. 2023/00608-0), CNPq 302997/2022-9; FAEPEX; SAE-UNICAMP.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Resveratrol exerts molecular effects on insulin resistance induced by maternal obesogenic diet and fatty acids in c2c12 myotubes

Authors:

Carolina Ramos de Mendonça¹ Lígia Cristina Monteiro Galindo^{1,2}
Fernando Wesley Cavalcanti de Araújo¹ Gabriel Vinicius Silva Amadeu¹
Vitor Carlos de Araújo Bandeira² Brenda Hilary Avelino de Vasconcelos¹ Bianch Alexsandro da Silva¹
Ana Catherine Alves Neves do Nascimento¹ Diogo Antonio Alves de Vasconcelos^{1,2}

Affiliations:

¹ Programa de Pós-Graduação em Nutrição, Universidade Federal de Pernambuco, 55670-901, Recife, PE, Brazil.

² Programa de Pós-Graduação em Nutrição, Atividade Física e Plasticidade Fenotípica, Universidade federal de Pernambuco, 55608-680, Vitória de Santo Antão, PE, Brazil.

Keywords: Developmental plasticity. Insulin resistance. Obesogenic diet.

Abstract:

The increasing prevalence of maternal obesity negatively impacts fetal development through epigenetic mechanisms, predisposing offspring to obesity and insulin resistance. This study investigated the molecular effects of resveratrol on insulin signaling pathways in skeletal muscle using both in vivo models and C2C12 myotube cells. The research project, *In vivo*, was approved by the Ethics Committee on Animal Use of UFPE (nº 0020/2021). Pregnant rats were fed a control (AIN-93G) or obesogenic diet during gestation and lactation, receiving either vehicle (ethanol) or resveratrol (1.2–2.6 mg/kg/day) in water. After weaning, male offspring were fed a standard diet until day 90 and divided into three groups: Maternal Control Diet (MatCtrlD, n=8), Maternal Obesogenic Diet (MatObD, n=8), and Maternal Obesogenic Diet + Resveratrol (MatObD+R, n=8). In vitro study, C2C12 cells were treated with BSA or palmitate (400 µM) to induce insulin resistance, with or without resveratrol (50 or 100 µM). Therefore, the offspring body weight was significantly higher in MatObD from day 63 ($305 \pm 7.1\text{g}$ vs. $273.8 \pm 7.4\text{g}$) to day 90 ($387.9 \pm 13.2\text{g}$ vs. $338.1 \pm 6.6\text{g}$) compared to the MatCtrlD, with resveratrol attenuating weight gain. In soleus muscle, AKT mRNA expression was lower in MatObD than MatCtrlD (0.66 ± 0.07 vs. 1.30 ± 0.19), with no resveratrol effect. Whereas In vitro, palmitate reduced p-AKT (Ser473) levels (0.58 ± 0.16 vs. 2.60 ± 0.69), which 50 µM resveratrol restored (2.77 ± 0.66). We conclude that resveratrol exerts protective molecular effect against skeletal muscle insulin resistance induced by maternal obesity in vivo, and by fatty acid exposure in vitro.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Assessment of the Glycemic Response to Various Culinary Preparations of White Potato in Healthy Adults

Authors:

Marianela Andrea Díaz Urrutia¹ Eduardo de Paula Encina² Bianca Fernanda Haag Leite² Bruna Schumacher Siqueira¹ Sabrina Grassioli¹

Affiliations:

¹ Universidade Estadual do Oeste do Paraná.

² Centro Universitário da Fundação Assis Gurgacz.

Keywords: Glycemia. Potato. Cooking methods.

Abstract:

Preserving glucose homeostasis is essential to avoid diseases, such as diabetes. In this regard, carbohydrate digestion and absorption are influenced by meal composition and by cooking methods; modulating post-prandial glucose levels. Here were evaluated glycemic profile in health subjects consumed potatoes prepared using different cooking methods. The study was approved by the Research Ethics Committee (CAAE: 65417722.4.0000.5219). Twenty-four participants were randomly assigned to four groups: Control (CTL; white bread), Boiled Potato (BP), Fried Potato (FP), and Baked Potato (BA). Capillary blood glucose was measured after a 6-hour fast, followed by ingestion of a portion containing 25 g of available carbohydrates. Glycemia was recorded at 15, 30, 60, and 120 minutes (min) and calculated the area under the curve (AUC). In the 30 min, BP group showed higher glycemia than CTL ($\uparrow 6.5\%$; $p=0.0008$), FP ($\uparrow 21\%$; $p<0.0001$), and BA ($\uparrow 15\%$; $p=0.001$) subjects. At 60 min, CTL group presented higher glucose levels in relation to FP ($\uparrow 21.3\%$; $p=0.0261$) and BA ($\uparrow 27\%$; $p=0.0012$) subjects. Moreover, BP group had higher glycemia than BA ($\uparrow 28.3\%$; $p=0.0028$) subjects. At 120 min, glycemia remained high in the CTL group compared to BP and BA (both $\uparrow 24.9\%$; $p<0.01$) subjects. Thus, glucose AUC was significantly higher in the CTL than FP ($\uparrow 11.5\%$; $p=0.0118$) and BA ($\uparrow 14.4\%$; $p=0.0025$) groups. Similarly, BP group also had higher glucose AUC than FP ($\uparrow 11\%$; $p=0.0194$) and BA ($\uparrow 13.9\%$; $p=0.0042$) subjects. Peak glycemia of CTL groups was higher than FP ($\uparrow 19.5\%$; $p=0.0048$) and BA ($\uparrow 21.2\%$; $p=0.012$) subjects as well as observed in the BP versus FP ($\uparrow 19.5\%$; $p=0.0033$) and BA ($\uparrow 21.2\%$; $p=0.0008$). Fried and baked preparations cause smaller impact on post-prandial glucose, reinforcing the relevance of food preparation techniques in dietary planning for preserve glucose homeostasis and avoids diseases.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Pentyl-*p*-Coumarate Prevents Ethanol-Induced Gastric Injury by Modulating Oxidative Stress

Authors:

Ana Luiza Costa Ferreira¹
Cecília Lourena Olimpia Aragão da Cunha¹ João Lucas Silva Pinheiro¹
Lucas Henrique Marques Rodrigues¹ Lorena Duarte da Silva¹
Victória Martins Rodrigues dos Santos¹ Hana Hayashi Pinto¹
Jand Venes Rolim Medeiros² Mayara Castro de Moraes³ Damião Pergentino de Sousa³ Renan Oliveira Silva Damasceno¹

Affiliations:

¹ Department of Physiology and Pharmacology, Federal University of Pernambuco.

² Biotechnology and Biodiversity Center Research, Federal University of Parnaíba Delta.

³ Department of Pharmaceutical Sciences, Federal University of Paraíba.

Keywords: Ethanol. Gastric damage. Oxidative stress. Pentyl-*p*-coumarate.

Abstract:

Introduction: Alcoholic gastropathy is primarily mediated by oxidative stress, underscoring the need for bioactive compounds with antioxidant potential. Pentyl *p*-coumarate (PpC) has emerged as a promising candidate. This study evaluated the protective effects of PpC against ethanol-induced injury. **Methods:** Swiss mice (7-8 weeks, Protocol 28/2025) received PpC (50, 100, or 200 mg/kg, p.o.) 1h before 50% ethanol. Controls received saline or 50% ethanol. Gastric macroscopic injury, markers of antioxidant defense (glutathione [GSH] and non-protein sulfhydryl groups [NP-SH]), lipid peroxidation markers (malondialdehyde [MDA] and 4-hydroxynonenal [4-HNE]), vascular permeability, and gastric emptying were evaluated. **Results:** Ethanol induced severe hemorrhagic lesions (59.5±5.9%), which were attenuated ($P<0.05$) by PpC, with maximal protection at 100 mg/kg (21.8±7.4%). Ethanol also reduced ($P<0.05$) gastric GSH (1.7±0.5) and NP-SH (140.3±25.1 µg/g) levels compared to control (4.2±0.2 and 431.1±54.3 µg/g, respectively), whereas PpC prevented this decrease (GSH: 3.4±0.5 and NP-SH: 279.2±48.7 µg/g). Compared to control, lipid peroxidation was increased ($P<0.05$) by ethanol, as shown by elevated MDA (7.7±0.8 µg/g vs. 4.9±0.4 µg/g) and 4-HNE (0.49±0.06 O.D. vs. 0.34±0.04 O.D.), which were reduced ($P<0.05$) by PpC (6.1±0.4 µg/g and 0.28±0.02 O.D., respectively). Ethanol also increased gastric vascular permeability (21.2±2.3 µg/mg), compared to the control (9.0±0.5 µg/mg), which was prevented by PpC (16.0±1.4 µg/mg). PpC did not affect gastric acid secretion (volume, acidity or pH), nor gastric emptying (24.0±2.0%) compared to control (23.0±4.6%), while they were altered by omeprazole or atropine (51.7 ± 2.4%), respectively. **Conclusion:** PpC protects against ethanol-induced gastric injury by preserving antioxidant defenses, reducing oxidative stress, and limiting vascular impairment, supporting its potential as a therapeutic agent for alcoholic gastropathy.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Effects of guava (*Psidium guajava*) supplementation on biochemical, bone and oxidative stress parameters of healthy Wistar rats

Authors:

Thuane Passos Barbosa Lima¹ Deysla Sabino Guarda Figueira²
Carolina de Oliveira Ramos Petra de Almeida¹ Julliana Melengati de Mendonça¹
Larissa da Costa Moreira de Simas² Igor Vellasco Marcos²
Priscila Pierre de Souza Cardoso² Carlos Eduardo de Faria Cardoso¹ Mariana Sarto Figueiredo²
Anderson Junger Teodoro²

Affiliations:

¹ Postgraduate in Food and Nutrition, Faculty of Nutrition, Federal University of the State of Rio de Janeiro, Rio de Janeiro, Brazil.

² Integrated Food and Nutrition Center, Fluminense Federal University, Niteroi, Brazil.

Keywords: Animal model. Antioxidant. Bioactive compounds. Health. Tropical fruit.

Abstract:

Guava is rich in phytochemicals with potential health benefits. This study aimed to evaluate the effects of guava pulp powder supplementation on biochemical, bone, and oxidative stress parameters in rats. Adult *Wistar* rats were distributed into a control group (CG, n=11) and a guava group (GG, 100 mg/kg/day, n=10), which received gavage for 30 days. Biochemical parameters were analyzed using a BioClin analyzer, oxidative stress was assessed with SpectraMax, and bone parameters were evaluated by DXA. The research was approved by the Animal Use Ethics Committee (CEUA: 9892020623). Data were analyzed using GraphPad Prism with Student's t-test, considering $p < 0.05$ as statistically significant. GG exhibited lower levels of total cholesterol (45.09 ± 5.59 mg/dL) and LDL (12.27 ± 3.95 mg/dL) compared to CG (56.2 ± 7.69 mg/dL and 21.06 ± 5.94 mg/dL, respectively), indicating favorable modulation of lipid metabolism ($p < 0.05$). FOX assay revealed a reduction in lipid peroxidation in the liver (1.368 ± 0.076 μ Mol/L) in GG, compared to CG (1.574 ± 0.048 μ Mol/L). GG showed lower TGO levels (127.55 ± 61.01 U/L) and a lower TGO/TGP ratio (3.63 ± 0.60) than CG (165.50 ± 47.97 U/L and 4.83 ± 0.98 , respectively), suggesting positive modulation of liver function. Total protein levels (4.96 ± 0.27 g/dL), albumin (3.05 ± 0.18 g/dL), and urea (56.27 ± 5.46 mg/dL) were lower in GG compared to CG (5.61 ± 0.31 g/dL, 3.36 ± 0.11 g/dL, and 0.7 ± 3.23 mg/dL, respectively), while creatinine was higher (CG: 0.59 ± 0.11 mg/dL; GC: 0.46 ± 0.15 mg/dL), suggesting alterations in renal function ($p < 0.05$). Bone mineral density of the femur was lower in GG (0.162 ± 1.302 g/cm²) compared to CG (0.171 ± 0.006 g/cm²) ($p < 0.05$), with a trend toward reduced calcium and increased phosphorus levels ($p > 0.05$), which may be related to the observed renal parameters. Guava pulp reduced cardiovascular risk and oxidative stress, as well as improved liver function, although it caused changes in rats renal and bone parameters, requiring further investigation. **Committee number:** 9892020623.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Effect of non-alcoholic fatty liver disease and fibrosis on the hepatic endocannabinoid system of obese patients undergoing bariatric surgery

Authors:

Leticia ALABURDA de Araujo¹ Larissa de Brito Fassarella¹ Juliana Pena Gonçalves¹
Fernando de Barros²
João Régis Ivar Carneiro³ Isis Hara Trevenzoli¹

Affiliations:

¹ Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro (UFRJ), RJ.

² Departamento de Cirurgia Geral, Universidade Federal Fluminense (UFF), RJ.

³ Hospital Universitário Clementino Fraga Filho, Universidade Federal do Rio de Janeiro (UFRJ), RJ.

Keywords: Endocannabinoid system. Hepatic metabolism. Non-alcoholic fatty liver disease. Obesity.

Abstract:

Obesity is an important risk factor for the development of non-alcoholic fatty liver disease (NAFLD). NAFLD encompasses a spectrum of hepatic changes, including steatosis, fibrosis, and even hepatocellular carcinoma. Both obesity and NAFLD have been associated with hyperactivation of the endocannabinoid system (ECS) in animal models. ECS is composed of type 1 (CB1) and type 2 (CB2) cannabinoid receptors, lipid ligands as anandamide (AEA) and 2-arachidonoylglycerol (2-AG), and various metabolizing enzymes, including monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH). In the liver, CB1 activation induces lipogenesis and reduces fatty acid oxidation, contributing to fat accumulation, while CB2 has anti-inflammatory actions. We tested the hypothesis that obese patients with liver steatosis and/or fibrosis have increased expression of hepatic ECS. The study was approved by the research ethics committee of the UFRJ (CAAE: 76686923.9.0000.5257). Forty-four women with obesity (body mass index (BMI) > 35kg/m²) undergoing bariatric surgery had liver biopsy collected during the surgical intervention. Liver samples were characterized by histopathology for steatosis (ST) and/or fibrosis (FB), and the obese patients were divided into three groups: control (without ST or FB, n=10), ST (n=17) and STFB (n=17). ECS components were analyzed by Western Blotting. Data were analyzed by Kruskal-Wallis and Spearman correlation tests (p<0.05). Steatosis and/or fibrosis did not change significantly the hepatic expression of CB1, but there was a trend to increase in the ST group, compared with control and FB groups (p=0.1582). MAGL liver content was similar across the three groups. No correlation was found between CB1 and BMI, serum triglyceride or LDL cholesterol levels. Future experiments will include other patients to increase sample size, and the analysis of CB2 and the endocannabinoids AEA and 2-AG to elucidate the role of the ECS in the NAFLD pathogenesis.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Time without endogenous melatonin impacts food intake and sleep aspects in patients treated for pineal tumor.

Authors:

Neiva dos Santos Souza¹ José Cipolla Neto²
Fernanda Gaspar do Amaral¹

Affiliations:

¹ Pineal Neurobiology Lab, Department of Physiology, Federal University of São Paulo.

² Department of Physiology and Biophysics, Neurobiology Lab, Institute of Biomedical Sciences, University of São Paulo.

Keywords: Circadian rhythms. Food intake. Melatonin. Pineal gland. Sleep.

Abstract:

Melatonin, produced by the pineal gland, adapts the body to environmental rhythmic variations. Alterations in its production, observed in patients after pineal gland tumors treatments, can lead to physiological and behavioral changes. This prospective cohort study at GRAACC Hospital (n=11, 72.5% male, 16.24±5.71 years, CEP UNIFESP nº 0603/2020, CAAE:

32872620.0.0000.5505) examined how time without endogenous melatonin, after the oncological treatment, impacts quantitative and temporal aspects of food intake [through a 3-day non-consecutive food diary, including weekdays (WD), weekends (WE), total mean (TM)] and sleep variables (self-reported wake-up and sleep times, including the calculation of social jetlag). Descriptive and regression analyses (predicted variable: time without endogenous melatonin; indicators: p-value and R²) were performed using SPSS (version 23.0). Inadequate intake compared to recommended levels was observed for unsaturated fatty acids, calcium, manganese, and vitamin D in 100% of the patients during WD, WE, and TM. Regarding meal timing, dinner had the highest average energy value during WD and WE (470.5 and 493.5 Kcal, respectively), and irregularity in meals was observed during WE (81.8% of patients). Social jetlag (1.25±0.85h) and eating jetlag (1.28±0.87h) were not significantly associated with time without melatonin. However, significant positive association was observed between time without melatonin and the intake of vitamin B1 on WD (R²=0.718, p=0.002) and TM (R²=0.756, p=0.001), monounsaturated fat (R²=0.555, p=0.013), vitamin E (R²=0.620, p=0.007), wake-up time (R²=0.517, p=0.019) and first meal timing (R²=0.626, p=0.006) on WD. These findings highlight the potential influence of time without melatonin on the eating and sleep aspects of patients after the oncological treatment for pineal gland tumors, with implications for future therapeutic strategies.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Effectsofwatermelon(*Citrulluslanatus*)supplementationon metabolic parameters, oxidative stress and bone health in Wistar rats.

Authors:

Deysla Sabino Guarda Figueira¹ Thuane Passos Barbosa Lima²
Carolina de Oliveira Ramos Petra de Almeida² Milla Christie Rodrigues de Lira³
Gabriel de Alcantara Noblat³ Julliana Melengati de Mendonça² Carlos Eduardo de Faria
Cardoso² Matheus Coutinho de Freitas¹
Giovana da Silveira Rezende Ferreira¹ Mariana Sarto Figueiredo¹
Anderson Junger Teodoro¹

Affiliations:

¹ Integrated Food and Nutrition Center, Fluminense Federal University, Niteroi, Brazil.
² Pos-graduated in Food and Nutrition, Faculty of Nutrition, Federal University of the State of Rio de Janeiro, Rio de Janeiro, Brazil.
³ Pos-graduated in Medical Science, Faculty of Nutrition, Fluminense Federal University, Niteroi, Brazil.

Keywords: Bioactive compounds. Metabolic parameters. Tropical fruit. Watermelon supplementation. Wistar rats.

Abstract:

The watermelon (*Citrullus lanatus*) is a tropical fruit rich in water and bioactive compounds as carotenoids, polyphenols and vitamin C suggesting antioxidants and anti-inflammatory activities with potential benefits to human health. This study we evaluated the effects of watermelon supplementation on metabolic parameters, oxidative stress and bone health in Wistar rats. The animals (PN90) received standard diet and were divided in: control group (CG, n=10) received gavage with saline and a watermelon group (WG, n=11) received gavage with 100mg/kg/day during 30 days. Body composition, bone parameters, serum and hepatic analyses were performed. Significant data had $p < 0.05$ (t test). Our protocol was approved by the Ethical Committee (CEUA 9892020623). The animals did not present significant differences in body composition and food consumption. WG showed lower serum levels of total proteins (-12%, 4.92 ± 0.10 , $p < 0.0001$), albumin (-11%, 3.00 ± 0.04 , $p < 0.0001$), cholesterol (-17%, 46.55 ± 1.49 , $p < 0.05$), HDL-cholesterol (-17%, 22.09 ± 0.53 , $p < 0.001$), urea (-10%, 54.36 ± 1.46 , $p < 0.05$), creatinine (-24%, 0.35 ± 0.01 , $p < 0.05$), alkaline phosphatase (-17%, 71.27 ± 4.21 , $p < 0.05$), calcium (-9%, 9.58 ± 0.16 , $P < 0.001$), iron (-22%, 205.80 ± 14.79 , $p < 0.05$) and magnesium (-8%, 2.56 ± 0.04 , $p < 0.05$). In bone parameters we observed higher of distance lateral epichondrium and medial epichondrium (+3%, 7.28 ± 0.02 , $p < 0.001$), radiodensity (+16%, 521.80 ± 11.51 , $p < 0.05$) and lower bone mineral density (-6%, 0.16 ± 0.00 , $p < 0.05$) in left femur. In relation to the analysis of oxidative stress, the WG showed lower serum (-6%, 1.31 ± 0.01 , $p < 0.05$) and hepatic (-10%, 1.42 ± 0.02 , $p < 0.0001$) FOX levels. The data suggest that watermelon supplementation in healthy rats may cause changes in lipid, renal and bone metabolism, in addition cause reduction in lipid peroxidation (serum and hepatic). **Committee number:** 9892020623.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: VLDL-associated miR-122 mediates liver–adipose tissue crosstalk in humans with obesity

Authors:

Carolina Panzarin¹ Laís A. P. Simino¹ Mayara N. Baqueiro¹ Lívia M. Genaro² Raquel F. Leal²
Felipe D. M. Chaim² Elinton A. Chaim²
Helena C. F. de Oliveira³ Marciane Milanski¹ Letícia Ignácio-Souza¹ Márcio A. Torsoni¹ Adriana S. Torsoni¹

Affiliations:

¹ Faculdade de Ciências Aplicadas da UNICAMP, Limeira-SP, Brasil.

² Faculdade de Ciências Médicas da UNICAMP, Campinas-SP, Brasil.

³ Instituto de Biologia da UNICAMP, Campinas-SP, Brasil.

Keywords: miR-122. VLDL. Adipose tissue. Liver. Obesity.

Abstract:

The liver-specific miRNA, miR-122, plays a crucial role in the regulation of lipogenesis and appears to be modulated in hepatocytes, exhibiting elevated circulating levels in the context of obesity. The lipid overload leads to increased exportation of VLDL from the liver to the bloodstream, which primarily transport triglycerides but also carry miRNAs to extrahepatic tissues, particularly adipose tissue. This study aimed to investigate the potential liver–adipose tissue communication axis mediated by miR-122-carrying VLDL in humans, as well as the contribution of miR-122 to adipocyte remodeling in vitro. Participants with obesity (Ob) and controls (C) were recruited after providing informed consent, following approval by the Research Ethics Committee (CAAE: 44054421.7.0000.5404). Liver, adipose tissue and plasma were collected from patients at Hospital das Clínicas – UNICAMP, Brazil. VLDL was isolated from plasma by ultracentrifugation. In vitro experiments were conducted using 3T3-L1 cells, differentiated into mature adipocytes and transfected with miR-122 mimic (10nM) for 48h. miR-122 and mRNAs levels were analyzed by RT-PCR. BMI was positively associated with miR-122 levels in the liver ($R^2=0.59$, $p=0.02$), plasma ($R^2=0.41$, $p=0.02$) and VLDL ($R^2=0.46$, $p=0.007$). miR-122 content in VLDL was also positively correlated with its levels in adipose tissue ($R^2=0.58$, $p=0.001$). Transfection with miR-122 mimic in 3T3-L1 led to lipid droplets accumulation (Oil Red staining), upregulation of inflammatory cytokines (N=3, Tnf FC: 2.5 ± 0.44 $p=0.003$; Il6: FC: 7.46 ± 1.35 $p=0.001$; Il1b: FC: 2.06 ± 0.60 $p=0.04$) and genes involved with lipid synthesis (N=3 Acaca FC: 1.45 ± 0.07 $p<0.001$; Scd1: FC: 1.59 ± 0.19 $p=0.03$; Pparg: FC: 1.33 ± 0.016 $p<0.0001$; Srfb1: FC: $1,245\pm0.056$ $p=0.04$). In conclusion, our findings suggest a potential role for VLDL as a carrier of miR-122 from the liver to adipose tissue and support the hypothesis that miR-122 may contribute to adipocyte dysfunction.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: The effects of maternal obesogenic diet on the hypertrophic capacity of skeletal muscle in adult rat offspring subjected to compensatory hypertrophy

Authors:

Vitor Carlos de Araújo Bandeira³ Carolina Ramos de Mendonça¹ Raíssa Andrade de Araújo Silva¹ Iuri Gabriel Telles Lopes¹
Thaynan Raquel dos Prazeres Oliveira² Anielle Mylena de Medeiros Barbosa³ Ana Helena Lemos da Silva¹
Bianch Alexsandro da Silva²
Brenda Hilary Avelino de Vasconcelos¹ Pedro Henrique Elias da Silva³
Bruna Karoline Alves de Melo Silva³ Fernando Wesley Cavalcanti de Araújo¹ Diogo Antonio Alves de Vasconcelos^{1,2,3}

Affiliations:

¹ Department of Nutrition – Postgraduate Program in Nutrition- UFPE.

² Postgraduate Program in Nutrition, Physical Activity and Phenotypic Plasticity-UFPE. ³ Multicenter Postgraduate Program in Physiological Sciences-SBFis.

Keywords: Obesogenic diet. Metabolic disorders. Muscle ablation. Hypertrophy

Abstract:

Unbalanced maternal diets can lead to metabolic disorders, such as insulin resistance. This condition can impair the maintenance of skeletal muscle mass. Therefore, the present study evaluated the effects of an maternal obesogenic diet on hypertrophic capacity, muscle mass, and skeletal muscle cross-sectional area in adult offspring of rats subjected to compensatory hypertrophy (CEUA UFPE, Process No.0035/2022). The following parameters were evaluated: adipose tissue mass; mass of the extensor digitorum longus (EDL), soleus, and gastrocnemius muscles; and muscle fiber area of the EDL muscle. Ten rats were fed a maternal control diet (DCMat) or a maternal obesogenic diet (DOMat), with dietary manipulation occurring from one week before pregnancy until the end of lactation. The offspring underwent muscle ablation on postnatal day 83 and euthanized on day 90. The experimental groups were established (N: 8): DCMat subdivided into Contralateral Limb (CP) and Hypertrophy Limb (PH); DOMat subdivided into CP and PH. In the mothers, there was an increase in total food intake (14.89%) and caloric intake (16.48%) in the DOMat group only during gestation, with a reduction in protein intake (11.29%) and an increase in lipid intake (104.99%) during gestation, which continued until lactation (25.18% and 73.31%, respectively). In the offspring, an increase in the area under the curve in the glucose tolerance test at 30 days (13.66%) was observed. At 90 days, the DOMat group showed greater retroperitoneal fat accumulation (137.89%), increased wet (10.25%) and dry (14.24%) gastrocnemius weight, increased EDL muscle wet weight in both hypertrophy groups (DCMat: 18.85%, DOMat: 21.33%), and a higher percentage of fibers with larger areas in the DOMat group after compensatory hypertrophy. Based on our results, we demonstrated that maternal obesogenic diet did not impair hypertrophy in adult rats subjected to synergistic EDL muscle ablation, but it was associated with increased adiposity.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Nutritional transition from a low-protein to a high-fat diet reduces the expression of antioxidant enzymes and activates inflammatory pathways

Authors:

Aiany C. Simões-Alves¹
Ana Paula da Fonseca Arcoverde Cabral de Mello¹ Suellen Cristina Barbosa de Amorim¹
Milca Suelen Santos Coutinho¹ Mariana P. Fernandes²
João H. Costa-Silva¹

Affiliations:

¹ Laboratory of Nutrition, Physical Activity and Phenotypic Plasticity, Federal University of Pernambuco, Vitoria de Santo Antão, Pernambuco, Brazil.

² Laboratory of General Biochemistry, Molecular Biology and Exercise, Federal University of Pernambuco-UFPE, Vitória de Santo Antão, Pernambuco, Brazil.

Keywords: Chronic diseases. Hyperlipidic diet. Malnutrition. Nutrition. Obesity.

Abstract:

In the last decades, a new nutritional paradigm has been identified in low- and middle-income countries, whereby undernutrition has been replaced by overnutrition, defining a rapid and drastic nutrition transition. Such nutrition transition is associated to the consumption of westernized diets rich in SFA, leading to increased risk for chronic metabolic diseases in adulthood. Here, we tested the hypothesis that a saturated fatty acid-enriched hyperlipidic diet (HL) affects liver expression of genes of the redox balance and inflammatory pathway in postweaning rat offspring subjected to maternal protein restriction. Pregnant Wistar rats received either a control (C; 19% protein) or low protein (LP; 8% protein) diet during gestation and lactation. At weaning, pups received either C or HL diets up to 90 days of life. All experimental protocols were approved by the Ethical Committee of the UFPE, Brazil (23076.046459/2018-17). The LP+HL group showed an upregulation of transcription of peroxisome proliferator-activated receptor γ (+48%) and peroxisome proliferator-activated receptor γ coactivator α (+96%) compared with the LP+C group ($P < .05$), respectively. Similarly, gene expression of the markers of inflammation, nuclear factor-kappa B1 (+194%) and tumor necrosis factor- α (+192%), was enhanced ($P < 0.05$). Although other antioxidant enzymes were not modified in gene expression, catalase (CAT) was 66% higher in LP+HL compared with LP+C. In contrast, CAT protein content in the liver was 50% lower in LP groups compared with C, and superoxide dismutase 2 (SOD2) was twice as high in LP groups compared with C. Postweaning HL after maternal protein restriction induces hepatic metabolic adaptation characterized by enhanced oxidative stress, unbalanced expression in the antioxidant enzymes SOD1, SOD2 and CAT, and activation of inflammatory pathways but does not impact circulating markers of lipid metabolism and liver function.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Vagus nerve and alteration on iron-hepcidin axis may be involved on the splenic iron deposition in the hypothalamic obese rats

Authors:

Eveline Cristiane Batista Schmidt Helene¹ Caroline de Maman Oldra¹
Giovana Fanhani Tessaro¹
Ellen Carolina Zawoski Gomes¹² Marianela Andrea Díaz Urrutia¹² Amanda Rocha Fujita¹
Beatriz Machado Daudt¹
Elizângela Vanessa da Cruz Hoffmann³ Matheus Dias Martins³
Sabrina Grassioli¹

Affiliations:

¹ Endocrine Physiology and Metabolism Laboratory, Center for Biological and Health Sciences, Stricto Sensu Postgraduate Program in Biosciences and Health, Western Paraná State University, Cascavel, PR, Brazil.

² Medical Sciences, Assis Gurgacz Foundation University Center (FAG), Cascavel, PR, Brazil.

³ Physiotherapy Undergraduates, Univel University Center, Cascavel, PR, Brazil.

Keywords: Spleen. Obesity. Iron.

Abstract:

Introduction: The vagus nerve (VN) and splenic dysfunctions may contribute to immune and metabolic abnormalities present in obesity; a condition that alters iron homeostasis. High hepcidin levels, hypoferrremia and excessive iron intracellular deposition have been demonstrated in pro-inflammatory obese state. **Objective:** Here we analyzed the impact of VN ablation on iron-hepcidin axis and splenic iron deposition on hypothalamic (Ho) obese male rats. **Methods:** Ethical approval (n°15-22). Ho was induced by high doses (4g/kg) of monosodium glutamate administered during the first postnatal days (PND). Control (Ct) received an equimolar solution. At 60 PND, Ho rats underwent subdiaphragmatic vagotomy (SV) or sham surgery (n=8 rats/group). At 90 PND half of rats in each group received hemolytic anemic agent phenylhydrazine (PHZ, 40 mg/kg). At 120 PND were evaluated white adipose tissue (WAT) content and circulating levels of iron and hepcidin. Splenic hemosiderin deposition and Red Pulp (RP) and White Pulp (WP) size were measured. **Results:** Ho-sham show higher WAT, reduced iron (p=0.017) and hepcidin (p=0.018) levels in comparison to Ct-sham. Moreover, Ho-sham rats had smaller spleen (p=0.004), RP (p<0.0001), and WP (p<0.0001) size and elevated splenic hemosiderin deposition (p<0.0001) in relation to Ct-sham. Hepcidin circulating levels was positively correlated to splenic hemosiderin deposition in the Ho-sham group (r=0.976; p<0.0001). However, hepcidin negative correlation with splenic hemosiderin deposition was noted in the Ho-sham^{PHZ} (r=-0.802; p=0.022) and Ho-SV (r=-0.048; p=0.002) groups. The Ho-SV^{PHZ} rats had smaller adiposity (p<0,0001) and augmented hepcidin levels (p<0.001) compared to Ho-sham. **Conclusion:** Hypothalamic obesity results in reduced iron serum levels, accompanied of higher iron deposition on splenic tissue, a response related with elevated levels of hepcidin. VN appears involved in hepcidin and iron splenic deposition in hypothalamic obese rats.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: 7 Month old Alzheimer's disease animal model double transgenic mice (APPSWE/PS1DE9) show differences in metabolic and locomotor activity but age comparably to healthy controls

Authors:

Marcos Yuji Shiroma Graziano¹ Ana Beatriz Henrique-Santos¹ João Victor Silva Nani¹
André de Souza Mecawi¹

Affiliations:

¹ Universidade Federal de São Paulo.

Keywords: Alzheimer's Disease. Aging. Metabolic change. Animal model.

Abstract:

Alzheimer's Disease (AD) is the leading cause of dementia in the elderly population. One of its hallmarks is the deposition of amyloid plaques in the extracellular space of the central nervous system (CNS), mainly in the cortex and hippocampus, which is recapitulated in the well-established animal model to study AD, the double-transgenic mice strain APPswe/PS1dE9 (APP/PS1). In this study, we longitudinally characterized metabolic and locomotor phenotypes in male (n=8) and female (n=5) APP/PS1 mice versus paired wild-type (WT) littermates (males n=8; females n=11) at 3 and 7 months of age. Using indirect calorimetry cages (Oxymax CLAMS), we assessed body mass, food/water intake, O₂ consumption, CO₂ production, energy expenditure, and locomotor activity (horizontal/vertical) over 4 days (2 adaptation, 2 measurement) at both ages (CEUA UNIFESP: 3253150224). Data was analyzed using ANOVA followed by Tukey's post-test. Results were considered significant when $p < 0.05$. We observed significant genotypic effects in food intake per body mass (pbm), which was higher in 7 months old APP/PS1 mice. This group also showed higher O₂ consumption, CO₂ production and greater heat release overall. For their locomotor activity, they displayed greater horizontal activity (HLA) overall and higher vertical activity (VLA) in the dark period. Females of this age group had higher total food intake and also pbm. They showed higher O₂ consumption and CO₂ production in the dark period, but lower respiratory exchange rate (RER) overall. Finally, they displayed greater VLA overall. Taking the passage of time into account, we observed that both genotypes showed an increase in the HLA overall and a decrease in both food/water intake pbm. Only WT, though, showed an increase in RER due to age. These findings share a glimpse of the differences in the baseline metabolism and activity of aged APP/PS1 animals compared to WT, as well as the effects of disease progression in such animals.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Impact of dietary adequacy on redox status in the cardiac ventricle in aged and obese rats

Authors:

Douglas Santos Batista¹ Júlia de Oliveira Borges¹ Raildo da Silva Coqueiro²
Thiago Macêdo Lopes Correia³ Amanda Alves de Almeida¹ Maiara Raulina de Jesus Dias¹
Regiane Ribeiro Dias¹
Samuel Santos Bittencourt Pereira¹ Karine Chaves Silva¹
Telma de Jesus Soares¹
Amélia Cristina Mendes de Magalhães¹

Affiliations:

¹ Instituto Multidisciplinar em Saúde, Universidade Federal da Bahia, Programa de Pós-Graduação Multicêntrico em Ciências Fisiológicas, Vitória da Conquista, Bahia 45029-094, Brazil.

² Universidade Estadual Do Sudoeste Da Bahia, Campus Jequié, 45205-490.

³ Instituto de Ciências da Saúde, Departamento de Biorregulação, Universidade Federal da Bahia, Salvador, Bahia 40110-100, Brazil.

Keywords: Aging. Inflammation. Obesity. Oxidative stress. Sarcopenia.

Abstract:

Morphological and functional cardiac changes are inherent to aging and are aggravated by sedentary lifestyle and high-fat diets. In contrast, caloric restriction modulates pathogenic factors, such as oxidative stress. Despite the significance of this topic, there is a notable lack of studies examining the effects of dietary modifications on the cardiac oxidative profile in the early stages of aging associated with obesity. Thus, this study aimed to evaluate the impacts of dietary adequacy on redox status in the cardiac ventricle of aged Wistar rats with obesity induced by a high-fat diet. Twenty-four male rats (CEUA-UFBA 079/2020) were used and divided into three groups (n=8): Aged + Control Diet (ACD), Aged + High-fat Diet (AHF), and Aged + Dietary Readjustment (ADR). The high-fat diet was introduced at 9 months and was replaced by a normocaloric diet in the last two months in the ADR group. The cardiac ventricle was analyzed by histological and enzymatic techniques to determine the redox profile. The AHF group presented greater body mass (ACD: 555.4±61.1; AHF: 677.3±99.7; ADR: 552.5±59.1)(P<0.0072), increased TBARS (ACD: 0.624±0.11; AHF: 2.238±0.26; ADR: 1.712±0.38)(P<0.0001) and reduced activity of the enzymes SOD (ACD: 2.979±0.35; AHF: 1.852±0.42; ADR: 2.391±0.27) (P<0.001), Catalase (ADC: 0.793±0.10; AHF: 0.465±0.09; ADR: 1.760±0.39)(P<0.0001) and GPx (ACD: 0.064±0.00; AHF: 0.048±0.00; ADR: 0.074±0.00)(P<0.0001). Dietary readjustment reduced body mass and improved the cardiac oxidative profile in all parameters evaluated, indicating a protective effect against oxidative stress associated with obesity and aging. These findings reinforce the importance of nutritional interventions as a strategy to mitigate the adverse effects of obesity on the heart during aging, potentially contributing to the prevention of cardiovascular diseases.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: High-fat maternal diet enriched with omega 3: increases activity and expression of antioxidant enzymes and reduces inflammatory markers.

Authors:

Suellen Cristina Barbosa de Amorim¹ Elionay Gomes dos Santos Silva¹ Mariana P. Fernandes²
Aiany C. Simões-Alves¹
Aline Maria N. L. Gomes-Bloise¹ Adriano F. Alves³
João H. Costa-Silva¹

Affiliations:

¹ Laboratory of Nutrition, Physical Activity and Phenotypic Plasticity, Federal University of Pernambuco, Vitoria de Santo Antão, Pernambuco, Brazil.

² Laboratory of General Biochemistry, Molecular Biology and Exercise, Federal University of Pernambuco-UFPE, Vitória de Santo Antão, Pernambuco, Brazil.

³ Laboratory of Experimental Immunopathology, Federal University of Paraíba, UFPB, Brazil.

Keywords: High-fat diet. Inflammatory markers. Omega 3. Phenotypic plasticity.

Abstract:

The intake of a high-fat diet during pregnancy and lactation has been associated with an increased risk of metabolic disorders in offspring. We hypothesized that omega-3 enrichment in this diet may attenuate metabolic impairments and prevent cardiometabolic diseases. Wistar rat offspring were fed a control diet (C: 19% lipids; $\omega 6:\omega 3$ ratio = 12.66), a high-fat diet (HL: 33% lipids; $\omega 6:\omega 3$ = 21.22), or a high-fat diet enriched with omega-3 (HL $\omega 3$: 33% lipids; $\omega 6:\omega 3$ = 9.45) during pregnancy and lactation. Male offspring were evaluated at 90 and 300 days of age. Murinometric and biochemical data, oxidative stress, gene expression of inflammatory markers and NF- κ B count were analyzed. Protocols approved by CEUA nº 23076.049500/2016-37. At 90 days, the HL group showed an increase in lipid levels, glycemia and transaminases when compared to the other groups. The HL $\omega 3$ group showed lower levels of AST ($\downarrow 24\%$ vs HL; $p=0.0007$) and in the AST/ALT ratio ($\downarrow 17\%$ vs HL; $p=0.0002$). Regarding the antioxidant response, the HL $\omega 3$ group demonstrated greater activity of SOD ($\uparrow 25\%$ vs HL; $p=0.0281$) and GST ($\uparrow 15\%$ vs HL; $p=0.0046$). Regarding gene expression, HL $\omega 3$ showed higher expression of SOD2 ($\uparrow 22\%$ vs HL; $p=0.0414$), in addition to lower expression of IL-1 β ($\downarrow 35\%$ vs HL; $p=0.0212$), when compared to the HL group. The HL $\omega 3$ group showed better antioxidant response and lower expression of hepatic inflammatory markers when compared to the HL group, at 90 days of age. At 300 days of age, no significant differences were observed in inflammatory and oxidative stress markers between the experimental groups. Regarding the NF- κ B count, the HL $\omega 3$ group showed an increase at 90 and 300 days in relation to both groups. Thus, enrichment with omega-3 attenuated the deleterious effects of the high-fat diet until early adulthood, but not at older ages.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: The PKA/CREB pathway stimulates interleukin-6 production by skeletal muscle during atrophy

Authors:

Leticia Cirelli Ruiz¹ Matheus Leonardo Moro¹
João Batista Camargo Neto² Andressa Pereira-Silva² Neusa Maria Zanon¹ Natalia
Lautherbach E. S.¹
Henrique Jorge Novaes Morgan¹ Isis do Carmo Kettelhut²
Luiz Carlos Carvalho Navegantes¹

Affiliations:

¹ Department of Physiology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, SP, Brazil.

² Department of Biochemistry & Immunology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, SP, Brazil.

Keywords: Myokines. Protein metabolism. Ubiquitin-proteasome system.

Abstract:

The molecular mechanisms involved in interleukin-6 (IL-6) production by skeletal muscle under catabolic conditions and its role in regulating the ubiquitin-proteasome system (UPS) remain to be elucidated. We hypothesize that the PKA/CREB pathway plays a key role in this process. To investigate this, we employed both in vitro and in vivo models of muscle atrophy. C2C12 myotubes were incubated in nutrient-deprived medium (HBSS) in the presence or absence of Forskolin (FSK, 10 μ M) and Isobutylmethylxanthine (IBMX, 100 μ M) for 3 hours. Additionally, 11-week-old male C57BL/6 and IL-6 knockout (KO) mice were subjected to bilateral motor denervation via sciatic nerve transection for 3 days. UPS activity was assessed ex vivo in the soleus muscle by measuring tyrosine release into the medium. Culture supernatants, cells, plasma, and gastrocnemius muscles were collected for IL-6 and TNF- α quantification (ELISA), protein analysis (Western blotting), and gene expression assessment (RT-qPCR). Data are presented as mean $\pm$ SEM (n = 4-6), and statistical analysis was performed using Student's t-test or two-way ANOVA (p < 0.05) (CEUA: 1344/2024). As expected, nutrient deprivation with HBSS increased IL-6 production by myotubes (~10-fold). Interestingly, activation of the PKA/CREB pathway with FSK + IBMX also markedly increased IL-6 secretion (~900-fold) and gene expression (~40-fold). Supporting these findings, denervated mice exhibited increased CREB phosphorylation (~50%), IL-6 production in muscle (~5-fold), and its secretion into plasma (~9-fold), without a corresponding increase in the pro-inflammatory cytokine TNF- α . Furthermore, UPS activation in denervated wild-type mice (~20%) was abolished in IL-6 KO animals. Our data suggest that the transcription factor CREB upregulates IL-6 production in skeletal muscle and that this myokine contributes to UPS activation during denervation-induced atrophy.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Role of subdiaphragmatic vagotomy on gastrointestinal motility, feed behavior, autonomic balance and oxidative stress in rats with Crohn's disease

Authors:

Brenda Lois Barros dos Santos¹
Alda Cássia Alves da Silva^{2,4}
Juliana Soares Severo^{3,4}
Heron Silva Soares⁴
Jorrdam Almondes Martins^{4,5}
Rodrigo Soares Pereira Lima^{4,6}
Rebeca Lima Monteiro⁴
Francisco Leonardo Torres Leal^{4,7}
Moisés Tolentino Bento da Silva⁸

Affiliations:

¹ Graduate Program in Pharmaceutical Sciences, Federal University of Piauí, Brazil.
² Graduate Program in Pharmacology, Federal University of Piauí, Brazil.
³ Nutrition Department, Federal University of Piauí³.
⁴ DOMEN, Research Group on Metabolic Diseases, Exercise and Nutrition⁴.
⁵ Multicenter Graduate Program in Physiological Sciences, Federal University of Piauí, Brazil.
⁶ Graduate Program in Food and Nutrition, Federal University of Piauí, Brazil⁶.
⁷ Department of Biophysics and Physiology, Federal University of Piauí⁷.
⁸ Lab. of Physiology. Center for Drug Discovery and Innovative Medicines (MedInUP/RISE-Health), School of Medicine and Biomedical Sciences – University of Porto, Portugal⁸.

Keywords: Crohn's Disease. Vagotomy. Vagus Nerve.

Abstract:

A previous study of our group showed that physical exercise positively modulates GI motility and oxidative markers in rats with Crohn's disease. The current study investigated how the subdiaphragmatic vagotomy (VGX) can modulate GI motility, cardiac function and oxidative stress with Crohn's disease. Male Wistar rats (n=5-10/group), 260-300g, divided into Control (CNT), subdiaphragmatic vagotomy (VGX), Crohn's disease (CD) and VGX+CD. The CD was induced by TNBS intraileally injection. After 7 days of surgical production, we assess the gastric motility, cardiac function vi (ECG), body composition and oxidative stress in all groups. VGX reduced ($P<0.05$) Δ body weight compared to CNT (18 ± 2.9 vs. -9.5 ± 7.4 g). VGX+CD attenuated weight loss compared to Crohn's (-0.7 ± 0.4 vs. -19.8 ± 2.9 g). In body composition, an increase ($P<0.05$) was observed in total body water (46.3 ± 2.1 vs $34.7\pm4.1\%$), extracellular fluid (46.8 ± 0.4 vs $35.2\pm3.9\%$), fat-free mass (63.2 ± 2.9 vs $47.4\pm5.6\%$) and BMI between VGX and CNT. VGX increased ($P<0.05$) gastric retention (42.4 ± 2.8 vs. $29.0\pm2.7\%$) and duodenal retention (36.9 ± 2.7 vs. $24.8\pm1.5\%$) compared to control. VGX+CD increased ($P<0.05$) retention in the ileum compared to CD (28.4 ± 5.9 vs. $14.0\pm0.8\%$). The LF component of heart rate variability increased ($P<0.05$) in the VGX group ($18.120.4$ vs. $24.442.07\%$), and there was a reduction in the LF/HF ratio (0.32 ± 0.03 vs. $0.70\pm0.10\%$) compared to CNT. In the ECG, VGX+CD decreased ($P<0.05$) heart rate (315.4 ± 7.22 vs. 368.6 ± 8.13 bpm) and increased R-R interval (0.188 ± 0.00 vs. 0.164 ± 0.003 s) compared to CD. Brown adipose tissue temperature was lower ($P<0.05$) in the VGX group than in CNT. VGX increased Nox ($289.3\pm8.2\mu$ M vs. $189.6\pm13.9\mu$ M) and MPO (7.7 ± 2.13 vs. 3.7 ± 0.7 U/mg) concentrations in the ileum while decreasing GSH (18.1 ± 0.7 vs. 22.0 ± 1.7 NPSH/mg). Subdiaphragmatic vagotomy alters GI motility, cardiac function, and oxidative stress in healthy or in situations such as Crohn's disease. *The study was approved by CEUA-UFPI n-687/21.*



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Pineapple (*Ananas comosus*) supplementation changes biochemical parameters, oxidative stress and bone health in Wistar rats

Authors:

Deysla Sabino Guarda Figueira¹ Thuane Passos Barbosa Lima²
Carolina de Oliveira Ramos Petra de Almeida² Milla Christie Rodrigues de Lira³
Gabriel de Alcantara Noblat³ Janaina Carvalho Guimarães⁴ Carlos Eduardo de Faria Cardoso²
Mariana Sarto Figueiredo¹ Anderson Junger Teodoro¹

Affiliations:

- ¹ Integrated Food and Nutrition Center, Fluminense Federal University, Niteroi, Brazil.
² Pos-graduated in Food and Nutrition, Faculty of Nutrition, Federal University of the State of Rio de Janeiro, Rio de Janeiro, Brazil.
³ Pos-graduated in Medical Science, Faculty of Nutrition, Fluminense Federal University, Niteroi, Brazil.
⁴ Pos-graduated in Sciences Applied to Health Products, Fluminense Federal University, Niteroi, Brazil.

Keywords: *Ananas comosus*. Bioactive compounds. Pineapple supplementation. Tropical fruit. Wistar rats.

Abstract:

Pineapple (*Ananas comosus*) is a tropical fruit widely cultivated in Brazil, highly valued due to its aroma, sweet flavor and succulence. It is a fruit rich in fiber, organic acids, vitamins, minerals and proteolytic enzymes (bromelain). Due to its high nutritional value, it has potential health benefits such as antioxidant, anti-inflammatory and antimicrobial activities, in addition to improving gastrointestinal function. The present study evaluated the changes in biochemical parameters, oxidative stress and bone health caused by pineapple supplementation in Wistar rats. Healthy adult rats were divided into: control group (CG, n=11) that received gavage with saline solution and a Pineapple group (PG, n=11) that received gavage with 100mg/kg/day of pineapple extract for 30 days. Analyses in serum, liver and bone parameters were performed. Significant data had $p < 0.05$ (t test). Our protocol was approved by the Ethical Committee (CEUA 9892020623). PG showed an increase in brown adipose tissue (+55%, 1.79 ± 0.27) and less left adrenal gland (-17%, 0.01 ± 0.00). In biochemical parameters, the animals presented lower serum levels of total proteins (-12%, 4.94 ± 0.08), albumin (-12%, 2.96 ± 0.04), cholesterol (-18%, 45.82 ± 2.19), HDL-cholesterol (-17%, 22.18 ± 0.76), LDL-cholesterol (-25%, 15.73 ± 1.61), urea (-9%, 55.09 ± 1.87), TGO (-34%, 100.40 ± 8.31), TGP (-18%, 28.10 ± 0.77), alkaline phosphatase (-18%, 70.27 ± 3.90), calcium (-10%, 9.45 ± 0.20), iron (-26%, 196.40 ± 11.01) and magnesium (-14%, 2.40 ± 0.06). In bone parameters we observed higher of distance between lateral epichondrium and medial epichondrium (+2%, 7.18 ± 0.02) and radiodensity (+14%, 510.70 ± 12.84) in the left femur. Regarding the analysis of oxidative stress, PG showed lower serum (-6%, 1.31 ± 0.03) and liver (-20%, 1.25 ± 0.01) FOX levels. Pineapple supplementation improved lipid profile and oxidative stress, also causing changes in hepatic metabolism and bone parameters. **Committee number:** 9892020623



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Combination of benznidazole and benzoylthioureas attenuates tissue lesions caused by chronic *T. cruzi* infection in the esophagus of BALB/c mice

Authors:

Emanuel Perez Floro da Silva¹ Joyce Ribeiro¹
Lucas Felipe Santos¹ Giovanni Bruno Clivati Sodré¹ Stephanie Salgado¹
Camila Cristina Alves Machado¹ Orlando Yesid Esparza Albarracin² Phileno Pingé Filho¹
Eduardo José de Almeida Araújo¹

Affiliations:

¹ State University of Londrina.

² Presbyterian University Mackenzie.

Keywords: Enteric neurodegeneration. America trypanosomiasis. Enteric Nervous System. Anti-*T. cruzi* agents. Acetylsalicylic acid.

Abstract:

Chagas disease (CD), caused by *Trypanosoma cruzi*, impairs gastrointestinal motility through myenteric plexus denervation, leading to megaesophagus and megacolon. Conventional therapies using benznidazole (BZ) and nifurtimox (NFX) exhibit limitations, prompting exploration of novel approaches such as benzoylthioureas (BTUs) and acetylsalicylic acid (ASA), which demonstrate potential neuroprotective properties. This study assessed the efficacy of these therapies in preserving the myenteric plexus of the esophagus in chronically infected BALB/c mice. All procedures adhered to guidelines of the National Council for Animal Experimentation Control (CONCEA) and were approved by the Ethics Committee on Animal Use at the State University of Londrina (CEUA/Uel 023/2022). BALB/c mice were infected with 5×10^2 *T. cruzi* trypomastigotes (Y strain) and treated for four weeks with BZ (25 mg/kg), ASA (25 mg/kg), and BTU-279 (12.5 mg/kg), either alone or in combination. Esophagi were collected 90 days post-infection for histopathological and immunofluorescence analyses. Data were analyzed using ANOVA and complementary statistical tests. *T. cruzi* infection did not alter esophageal tissue mass ($p > 0.05$), nor did treatments affect tissue mass in infected or uninfected mice. Infection increased the thickness of both muscle layers ($p < 0.05$), though only BZ and BTU+BZ prevented circular muscle hypertrophy. ASA increased muscle cell nuclei density per mm² in uninfected mice ($p < 0.05$). Infection reduced total (PGP9.5+) by 39.54%, nitrergic (PGP9.5+/nNOS+) by 37.91% and estimated cholinergic neurons (PGP9.5+/nNOS-) by 48.34% ($p < 0.05$), with no treatment achieving significant neuronal recovery. However, ASA and BZ mitigated nitrergic neuron hypertrophy induced by infection ($p < 0.05$). The BTU and BZ combination effectively preserved esophageal structure and enteric neurons, highlighting its potential as a therapeutic strategy for digestive CD and emphasizing the need for further research.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Angiotensins and a 3D Liver model: A Novel Approach to Investigate Fibrosis

Authors:

Maria Eduarda Souza Favalessa¹ Isadora Zhong Liang Ferreira Feng¹ Izabella Freitas Acipreste¹
Matheus F. Itaborahy¹
Maria Fernanda Silva Amorim¹ Yuri César Capato¹
João Santana da Silva²
Regina Coeli dos Santos Goldenberg² Alfredo M. Goes³
Robson A. S. Santos Cibele Ferreira Pimentel² Marlon Lemos Dias²
M Fátima Leite^{1*}

Affiliations:

¹ Department of Physiology and Biophysics - Federal University of Minas Gerais.

² Precision Medicine Research Center - Federal University of Rio de Janeiro.

³ INCT Hepatologia360°.

Keywords: Angiotensins-based therapy. Hepatic Spheroids. Fibrosis.

Abstract:

In Brazil, 1 in every 33 deaths is due to chronic liver diseases, with fibrosis being prominent in several liver conditions. Therefore, the goal of this work is to evaluate new therapeutic strategies that could reverse fibrosis. For that, we investigated members of the renin-angiotensin system (RAS) that could counteract the actions of Angiotensin II, which promotes fibrosis. We used a 3D experimental model of liver fibrosis to assess the roles of Angiotensin-(1-7), Ac-Angiotensin-(2-7)-NH₂, and Alamandine-(1-5) as potential anti-fibrotic agents. Hepatic spheroids were produced using three distinct human cell types: HepG2, hepatic stellate cells (GRX), and mesenchymal cells (CeSaM). Spheroids were formed in agarose, followed by the administration of 10 ng/mL of TGF- β for 4 days to induce fibrosis. Intracellular Ca²⁺ signaling experiments were performed upon ATP (50 μ M) stimulation in Fluo-4/AM loaded spheroids that had been pretreated for three days with 100 nM of each of the angiotensin peptides. We observed differences in Ca²⁺ signaling profiles among control, untreated fibrotic, and fibrotic spheroids with the peptides. The fibrotic spheroids showed a smaller amplitude of intracellular Ca²⁺ signaling compared to control (Mean $\pm$ SD: 153 $\pm$ 32 vs 301 $\pm$ 20, respectively, $p < 0.0001$, $n > 70$ cells for each condition). They also showed a predominance of oscillatory patterns of Ca²⁺ signal (50%), while cells from control preferentially had a compound type of Ca²⁺ response (68%). Additionally, fibrotic spheroids presented a slower onset to trigger intracellular Ca²⁺ increase upon ATP stimulation. All the peptides used recovered the Ca²⁺ signaling of fibrotic spheroids to similar values and patterns as control, especially the Ac-angiotensin-(2-7)-NH₂. In conclusion, through the developed 3D model of liver spheroids and the evaluation of intracellular Ca²⁺ signaling, we were able to assess the anti-fibrotic potential of the peptides from the alternative axis of the RAS.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Relationship between metabolic syndrome components and sleep quality in adults attended in primary health care

Authors:

José Maurício Lucas da Silva^{1,2,3} Mayara Luclécia da Silva^{1,2} Waleska Maria Almeida Barros^{1,2,4}
Karollainy Gomes da Silva^{1,2}
Ana Patrícia da Silva Souza^{1,2,3}
Maria Eduarda Rodrigues Alves dos Santos^{1,2} Ana Beatriz Januário da Silva^{1,2}
Antonietta Cláudia Barbosa da Fonseca Carneiro^{1,2,3} Robson Feliciano da Silva²
Érica Helena Alves da Silva² Williclecia Walkiria Dias Fereira² Aline Ananias de Lima¹
Letícia Henrique Leite da Silva¹ Sandra Lopes de Souza¹
Viviane de Oliveira Nogueira Souza⁵

Affiliations:

¹ Postgraduate Program in Neuropsychiatry and Behavioral Sciences, Center for Medical Sciences, Federal University of Pernambuco, Recife, Pernambuco, Brazil.

² Integrated Center for Neuroscience Technologies, Osman Lins University Center (UNIFACOL), Vitória de Santo Antão, Pernambuco, Brazil.

³ Department of Physiotherapy, Health Sciences Center, Osman Lins University Center (UNIFACOL), Vitória de Santo Antão, Pernambuco, Brazil.

⁴ Department of Medicine, Health Sciences Center, Osman Lins University Center (UNIFACOL), Vitória de Santo Antão, Pernambuco, Brazil.

⁵ Nutrition Center - Federal University of Pernambuco, Academic Center of Vitória de Santo Antão-PE, Brazil.

Keywords: Adult. Disorders of excessive somnolence. Metabolic syndrome. Sleep.

Abstract:

Metabolic Syndrome (MS) is a cardiometabolic disorder associated with five risk factors: abdominal obesity, reduced high-density lipoprotein cholesterol (HDL-c), elevated triglyceride (TG) levels, systemic arterial hypertension, and glucose intolerance, which indicates insulin resistance. These alterations can directly impact sleep quality. This study aimed to investigate the relationships between MS components, excessive daytime sleepiness (EDS), and sleep quality in adults. This is a cross-sectional study, approved by the Research Ethics Committee of the Federal University of Pernambuco (approval no. 5.902.951), conducted in primary health care in the municipality of Vitória de Santo Antão, PE. Sociodemographic, clinical, and anthropometric questionnaires were applied, along with the Pittsburgh Sleep Quality Index (PSQI) and the Epworth Sleepiness Scale. Blood samples were collected to analyze glucose, HDL-c, and TGs. The sample consisted of 76 individuals, predominantly female ($n = 71$; 93.4%), with most aged between 48 and 55 years ($n = 44$; 57.9%). Overweight was prevalent ($n = 30$; 39.5%), and 51 participants (67.1%) presented a very high accumulation of abdominal fat. According to the International Diabetes Federation guidelines, participants were categorized into two groups: with MS ($n = 33$) and without MS ($n = 43$). Poor sleep quality was observed in both groups: 15 (45.5%) in the MS group and 27 (62.8%) in the non-MS group. In the MS group, there was a correlation between waist circumference (WC) and sleep quality ($r = 0.39$; $p = 0.02$), and between blood glucose and EDS ($r = 0.43$; $p = 0.01$). In the non-MS group, associations were found between glucose and TG levels ($r = 0.90$; $p = 0.001$), and between systolic blood pressure and HDL-c ($r = 0.30$; $p = 0.04$). The study highlights positive correlations between MS and alterations in sleep and EDS, as well as relevant metabolic associations in the non-MS group.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Metabolic Syndrome And Its Association With Mental Health And Executive Functions: A Study In Adults From Vitória De Santo Antão, Pe

Authors:

Mayara Lucécia da Silva^{1,2}
José Maurício Lucas da Silva^{1,2,3} Waleska Maria Almeida Barros^{1,2,4}
Maria Eduarda Rodrigues Alves dos Santos^{1,2} Karollainy Gomes da Silva^{1,2}
Ana Patrícia da Silva Souza^{1,2,3} Ana Beatriz Januário da Silva^{1,2} Érica Helena Alves da Silva²
Robson Feliciano da Silva²
Antonietta Cláudia Barbosa da Fonseca Carneiro^{1,2,3} Williclecia Walkiria Dias Fereira²
Aline Ananias de Lima¹
Letícia Henrique Leite da Silva¹ Sandra Lopes de Souza¹
Viviane de Oliveira NogueiraSouza⁵

Affiliations:

¹ Postgraduate Program in Neuropsychiatry and Behavioral Sciences, Center for Medical Sciences, Federal University of Pernambuco, Recife, Pernambuco, Brazil.
² Integrated Center for Neuroscience Technologies, Osman Lins University Center (UNIFACOL), Vitória de Santo Antão, Pernambuco, Brazil.
³ Department of Physiotherapy, Health Sciences Center, Osman Lins University Center (UNIFACOL), Vitória de Santo Antão, Pernambuco, Brazil.
⁴ Department of Medicine, Health Sciences Center, Osman Lins University Center (UNIFACOL), Vitória de Santo Antão, Pernambuco, Brazil.
⁵ Nutrition Center - Federal University of Pernambuco, Academic Center of Vitória de Santo Antão-PE, Brazil.

Keywords: Adult. Cognition. Mental health. Metabolic syndrome.

Abstract

Metabolic syndrome (MS) consists of metabolic dysregulations, including abdominal obesity, altered glucose metabolism, dyslipidemia, and systemic arterial hypertension. Evidence suggests that its components may negatively impact mental health and executive functions (EFs). This cross-sectional study aimed to investigate the association between MS, mental health, and EFs in adults. Approved by the Ethics Committee of the Federal University of Pernambuco (approval no. 5.902.951). Blood samples were collected for glucose, high-density lipoprotein cholesterol (HDL-c), and triglycerides (TGs) analyses, along with sociodemographic, clinical, and anthropometric questionnaires. Mental health was assessed using the Patient Health Questionnaire-9 and the State-Trait Anxiety Inventory; EFs were evaluated with the Tower of London test. A total of 76 adults (aged 40–59 years) were assessed, 71 (93.4%) of whom were female. Most participants were overweight (n=30; 39.5%) and had very high waist circumference (n=51; 67.1%). Two groups were defined: with MS (n=33) and without MS (n=43). In the MS group, there was a higher prevalence of risk for major depressive episode (MDE) (n=25; 75.8%), moderate trait and state anxiety (n=22; 66.7% and n=16; 48.5%, respectively). In the group without MS: MDE (n=25; 58.1%), moderate trait anxiety (n=21; 48.8%) and low state anxiety (n=24; 55.8%). Execution time in the EF test was lower in the group without MS compared to the MS group (9.77±3.6 vs. 11.52±3.6; p=0.03). In the MS group, correlations were observed between TGs and MDE (r=0.36; p=0.03), MDE and trait anxiety (r=0.41; p=0.01), and state anxiety (r=0.51; p=0.002). In the group without MS, associations were found between HDL-c and execution time (r=0.31; p=0.03), MDE and trait (r=0.62; p=0.001) and state anxiety (r=0.60; p=0.001). In conclusion, individuals with MS performed worse in EFs, and correlations between metabolic indicators and mental health were observed in both groups.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Effect of dapagliflozin administration on masld induced by a high-fat diet and fructose-rich water in C57BL/6 mice

Authors:

Pericles Fabricio Gomes Filho¹ Carolina da Silva Valentim¹
Raíssa Leal de Carvalho dos Santos Cunha¹ Paulo Rogerio Silva de Paiva Júnior²
Rebecca Roosevelt da Conceição Souza Guedes¹ Isaias dos Santos Silva¹
Cherley Borba Vieira de Andrade¹ Kissila Rabelo¹
Luciana Lontro Alves¹ Aluana Santana Carlos² Jorge José de Carvalho¹
Jemima Fuentes Ribeiro da Silva¹

Affiliations:

¹ Universidade do Estado do Rio de Janeiro – UERJ. ² Universidade Iguazu – UNIG.

Keywords: Dapagliflozin. Obesity. High-fat diet. MASLD. SGLT2 inhibitor.

Abstract:

Foods high in fat and sugary drinks lead to lipogenesis, causing inflammation, insulin resistance, and diabetes mellitus. These dysfunctions may lead to the development of metabolic dysfunction-associated steatotic liver disease (MASLD). Dapagliflozin (DAPA), an SGLT2 inhibitor, increases glucose excretion and fatty acid use, potentially altering lipid storage and improving MASLD. We will evaluate DAPA effects under high-fat diet (HFD) and fructose-rich water (HFW). We used 45 male C57BL/6 mice, 3 months old (CEUA No. 010/2024). Three groups (n=15): control (C) received a standard diet, high-fat (HF) and HF+DAPA received HFD and HFW for 16 weeks. In the treatment, C and HF received placebo, and HF+DAPA received 10 mg/kg/day of DAPA for 6 weeks. We evaluated body mass (BM), energy consumption (EC), oral glucose and insulin tolerance tests (OGTT and ITT), and magnetic resonance imaging (MR). The liver mass (LW) was measured, followed by histological processing for OIL RED O (ORO). **BM:** C(n=6) 27.3±2.6g; HF(n=6) 33.8±6.1g (+23.8%vsC, p<0.05); DAPA(n=6) 31.1±3.4g. **EC:** C(n=14) 48.6±6.6kj; HF(n=14) 59.4±11.1kj (+21.5%vsC, p<0.05); DAPA(n=15) 64.5±12.5kj (+11.1%vsHF, p<0.05). **AUC_OGTT:** C(n=14) 49.9±6.4mmol/dl; HF(n=14) 67.9±12.7mmol/dl (+36.1%vs C, p<0.05); DAPA(n=15) 57.4±4.6mmol/dl (-15.6% vs HF, p<0.05). **AUC_ITT:** C(n=12) 19.1±2.5mmol/dl; HF(n=14) 29.8±6.04mmol/dl (+55.4%vsC, p<0.05); DAPA(n=15) 29.3±2.7mmol/dl. **MR:** C(n=8) 10.01±0.7%; HF (n=5) 20.4±2.9% (+104.2%vsC, p<0.05); DAPA(n=6) 17.1±2.1% (-16.1%vsHF, p<0.05). **LM:** C(n=6) 0.99±0.12g; HF(n=6) 1.27±0.15g (+27%vsC, p<0.05); DAPA(n=6) 1.08±0.09g (-15.2% vs HF, p<0.05). **ORO:** C(n=6) 47.04±34.27µm²; HF(n=6) 365±174µm² (+675.9%vsC, p<0.05); DAPA(n=6) 153.8±112.5µm² (-57.9% vs HF, p<0.05). DAPA promoted improved glucose clearance, reduced fat percentage, liver mass, and hepatic lipid accumulation. These findings provide valuable insights into the use of DAPA as a potential adjuvant in the treatment of metabolic diseases such as MASLD.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Antiobesogenic and Antioxidant Effects of Botryosphaeran (a Fungal Exocellular (1-3) (1-6)-B-D-glucan) in Treating Obese and Non-Obese Female Wistar Rats

Authors:

Isabelle Zanata Fabiane¹
Daniel Izidoro Ferreira Da Silva¹ Délcio Francisco Chagas Netto¹ Francine Nunes Ferreira¹
Isabela Petenati De Oliveira¹ Sabrina Leticia Bubans Junges¹ Anna Carolina Vilela¹
Robert F. H. Dekker² Aneli M. Barbosa-Dekker² Pamela Alegranci¹
Valéria Dornelles Gindri Sinhorin³
Eveline Aparecida Isquierdo Fonseca De Queiroz¹

Affiliations:

¹ Núcleo de Pesquisa e Apoio Didático em Saúde (NUPADS), Instituto de Ciências da Saúde, Câmpus Universitário de Sinop, Brasil.

² Beta-Glucan Produtos Farmoquímicos EIRELI, Lote 24^A, Bloco Zircônia, Universidade Tecnológica Federal do Paraná, Câmpus Londrina, Paraná, Brasil.

³ Programa de Pós-Graduação em Ciências Ambientais, Instituto de Ciências Naturais, Humanas e Sociais, Câmpus Universitário de Sinop, Universidade Federal de Mato Grosso, Mato Grosso, Brasil.

Keywords: Beta-glucans. Botryosphaeran. Antiobesogenic. Oxidative stress.

Abstract:

Botryosphaeran, a β -(1 $\rightarrow$ 3) (1 $\rightarrow$ 6)-D-glucan produced by *Botryosphaeria rhodina*, showed hypocholesterolemic, anti-obesogenic, and antioxidant properties demonstrated in male rats, but its effects in female rats remain underexplored. This study evaluated the effect of botryosphaeran on obesity, dyslipidemia, and oxidative stress in obese and non-obese Wistar female rats. Rats were divided into four groups: Control (C), Control+ Botryosphaeran (CB), Obese (O), and Obese+Botryosphaeran (OB). Obesity was induced by a hypercaloric diet for 8 weeks. In the last 15 days, CB and OB groups received botryosphaeran (12 mg/kg/day). Body weight, retroperitoneal adipose tissue, lipid profile and TyG index were assessed. Oxidative stress markers (TBARS and carbonylated proteins) and antioxidant parameters were analyzed in renal, hepatic and muscular tissues. The study was approved by CEUA (protocol 23108.072920/2023-21). Obesity increased body weight (+11.4%; $p < 0.05$), adiposity index (+57.9%; $p < 0.05$), visceral fat (+62.0%; $p < 0.001$), and dyslipidemia. Botryosphaeran reduced body weight (-7.95%; $p < 0.05$), retroperitoneal fat (-29.7%; $p < 0.05$), increased HDL (+23.7%), and reduced triglycerides (-21.3%), total cholesterol (-26.3%), LDL (-34.0%), and VLDL (-18.7%) in obese rats. Insulin sensitivity improved, as reflected in the reduction of the TyG index. In the kidneys, botryosphaeran significantly reduced TBARS and carbonylated proteins, indicating lower lipoperoxidation and oxidative damage, while increasing GPx activity. In the liver and muscle, it reduced TBARS and increased catalase and vitamin C, demonstrating enhanced antioxidant response. Botryosphaeran exhibited antiobesogenic and hypocholesterolemic effects by reducing visceral fat and improving lipid profile. Additionally, it showed antioxidant effects in the kidneys, liver, muscle, and adipose tissue by reducing oxidative stress, being a potential therapeutic agent to treat obesity and its comorbidities.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Antiobesogenic and metabolic beneficial effects of botryosphaeran, an (1-3)(1-6)- β -D-glucan, in offsprings from obese rats during pregnancy

Authors:

Daniel Izidoro Ferreira da Silva¹ Isabelle Zanata Fabiane¹ Adriano Andrade Pio¹
Arthur Michels Avancini¹ Valentina Rodrigues¹ Pâmela Alegranci¹
Júlio Cezar de Oliveira¹
Eveline Aparecida Isquierdo Fonseca de Queiroz¹

Affiliations:

¹ Núcleo de Pesquisa e Apoio Didático em Saúde (NUPADS), Instituto de Ciências da Saúde, Câmpus Universitário de Sinop, Brasil.

Keywords: : β -glucan. Botryosphaeran. Obesity. Pregnancy. Offspring.

Abstract:

Obesity is a chronic, inflammatory and multifactorial disease characterized by an accumulation of white adipose tissue (WAT). Pregnant are also affected by obesity once this pathology can increase the incidence of maternal and neonatal complications, as gestational diabetes *mellitus*. Botryosphaeran is a fungal (1-3)(1-6)- β -D-glucan, that has been demonstrated to promote important antiobesogenic, hypoglycemic and hypocholesterolaemic effects in adult male rats. Thus, the objective of the present study was to demonstrate the metabolic effects of botryosphaeran in the offspring of obese and non-obese female rats. Obese (n=10) and non-obese female wistar rats (n=10) were placed to mate and after the detection of pregnancy they were subdivided into 4 groups: Control (n=5, CG), Control treated with botryosphaeran (n=5, CGB), Obese (n=5, OG) and Obese treated with botryosphaeran (n=5, OGB). The rats from CGB and OGB groups received treatment with botryosphaeran (12 mg/kg/day) for 15 days, from the 8th to the 21st day of pregnancy. After weaning, offspring at 25-days old were euthanized and the tissues, glycemic and lipidic profile were analyzed (protocol CEUA 23108.072920/2023-21). The mother obese rats and their offspring (male and female rats with 25 days of life) presented accumulation of visceral WAT, glucose intolerance, hyperglycemia, hypertriglyceridemia and hypercholesterolemia (total cholesterol and VLDL-cholesterol). Botryosphaeran was effective in reducing significantly the accumulation of adipose tissue, improving the glucose tolerance, and corrected the hyperglycemia, hypercholesterolemia and hypertriglyceridemia, both in the mother and their offsprings. The results demonstrated for the first time the significant antiobesogenic, hypoglycemic and hypocholesterolaemic effects of botryosphaeran in offsprings (female and male) from obese mother rats, demonstrating the potential, safety and beneficial effects of botryosphaeran use during gestational period.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Effect of Hand Immersion in Cold Water on the Thermographic Response of Supraclavicular Skin in Para Athletes

Authors:

Jessé Borges de Oliveira^{1,2,3} Carolina Batista Simões^{1,2,3} Gabriel Adler Gomes-Martins^{1,2,3}
Marcelo Danillo Matos dos Santos³ Eduardo Mendonça Pimenta⁵ Andressa Silva^{4,5}
Marco Túlio de Mello^{4,5}
Dawit Albieiro Pinheiro Gonçalves^{1,2,3}

Affiliations:

¹ Nucleus of Ergogenic, and Systemic and Molecular Physiology (ErgoFis), School of Physical Education, Physiotherapy and Occupational Therapy, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

² Section of Sports Physiology (SFE), Sports Training Center (CTE), Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

³ Sports Training Center (CTE), Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

⁴ Center for Studies in Psychobiology and Exercise (CEPE), School of Physical Education, Physiotherapy and Occupational Therapy, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

⁵ Department of Sports, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

Keywords: Brown adipose tissue. Infrared thermography. Para-athletes. Powerlifting. Thermoregulation.

Abstract:

Introduction: Brown adipose tissue (BAT) in supraclavicular (SC) region has a thermogenic capacity that may be stimulated by cold. Infrared thermography (IT) detects heat loss and may estimate BAT activity. No studies have examined SC temperature (SCT) in athletes with spinal cord injury (SCI) and short stature (ST). The prevalence of obesity is high in both SCI and ST, thus BAT could be a potential therapeutic target. **Objective:** This study aimed to evaluate the effects of cold-water immersion (CWI) on the variation of SCT measured by IT in para athletes. **Methods:** Six para athletes (5 men and 1 woman, 35.7 ± 7.7 years, BMI 26.5 ± 5.6 kg/m²), with competitive experience with SCI and ST were recruited. Each subject remained seated for 20 minutes for acclimation (24°C). The camera was positioned 1 m from the region of interest (ROI), i.e., SC. They underwent 5-min CWI (5–9°C). SCT was recorded every minute during immersion, immediately post-immersion, and for 10 subsequent minutes (1-min intervals; total 35 min). Friedman test was employed. Approved by UFMG Ethics Committee (68427923.8.0000.5149). **Results:** No significant SCT differences occurred across timepoints ($p = 0.1393$). Baseline SCT averaged $33.68 \pm 1.17^\circ\text{C}$. During CWI, mean SCT ranged from

$34.43 \pm 0.31^\circ\text{C}$ (minute 0) to $34.40 \pm 0.25^\circ\text{C}$ (minute 5). Immediately after immersion, SCT ranged from $34.34 \pm 0.37^\circ\text{C}$ to $34.23 \pm 0.27^\circ\text{C}$ (10 min). While group changes were nonsignificant, interindividual variability was significant ($p < 0.01$), indicating divergent thermoregulatory responses. **Conclusion:** CWI failed to increase SCT in para- athletes.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: The impacts of IUGR in hepatic and mesenteric adipose tissue cytokines and its response to acute glucose injection

Authors:

Thais Antonio Jose Mutran¹ Amanda Paula Pedroso¹ Adriana Pereira de Souza¹
Jessica Caroline Alves de Sena Fourniol¹ Diana Dias de Araújo¹
Artur Francisco da Silva Neto¹ Valter Tadeu Boldarine¹
Lila Missae Oyama¹ Eliane Beraldi Ribeiro¹ Mônica Marques Telles¹

Affiliations:

¹ Universidade Federal de São Paulo, São Paulo, Brazil.

Keywords: Energy homeostasis. Fetal programming. Inflammation. Liver. Mesenteric adipose tissue.

Abstract:

The developmental origins of health and disease (DOHaD) suggest that intrauterine growth restriction (IUGR) may predispose metabolic dysfunctions and obesity later in life. Inflammation is associated with metabolic alterations, with the liver and adipose tissues being particularly susceptible to these alterations. Thus, this study aims to investigate the effects of IUGR and acute glucose injection on inflammatory cytokines in the liver and mesenteric adipose tissue before the onset of obesity. To induce IUGR, pregnant Wistar rats were fed *ad libitum* (Control group, C) or received 50% of C intake (Restricted group, R). During lactation, both groups were fed *ad libitum*. The male C and R offspring were fed *ad libitum* from weaning to 3-month-old, then received intraperitoneal injection of either saline (CS and RS) or 2g/Kg glucose (CG and RG), and then euthanized after 45 min. The cytokines levels of IL-6, IL-10 and TNF- α in liver and mesenteric adipose tissue (N=9-10) were measured using commercial kits. The Research Ethics Committee approved the present study (CEUA: 1115260121). Two- way ANOVA with Bonferroni post hoc tests ($p < 0.05$) were used for statistical analysis. In the liver, IL-6 levels was significantly increased by both glucose administration ($F(1,36)$, $p=0.0286$) and IUGR ($F(1,36)$, $p=0.0105$). The antiinflammatory cytokine IL-10 levels were significantly higher, modulated by both IUGR ($F(1,34)$ $p=0.0331$) and by Glucose Injection ($F(1,34)$, $p=0.0164$). TNF- α showed no significant modification by both IUGR or Glucose Injection. In the mesenteric adipose tissue, none of the cytokines showed statistical modulation by IUGR or Glucose Injection. These findings suggest that, with 3-month-old, IUGR impairs inflammation cytokines in the liver but not in the mesenteric adipose tissue. Given the role of hepatic cytokines in modulating insulin sensitivity, these early alterations may contribute to the later development of metabolic disorders.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Maternal malnutrition during pregnancy increases O-GlcNAc in arcuate nucleus astrocytes in adult Offspring.

Authors:

Jéssica Caroline Alves-de-Sena-Fourniol¹ Tânia Leiko Tanaka¹
Iracema Sena de Andrade¹ Amanda Paula Pedroso¹ Thaís Antônio José Mutran¹ Diana Dias Araújo¹
Vater Tadeu Boldarine¹ Wilson Dias Segura² Lila Missae Oyama¹ Eliane Beraldi Ribeiro¹
Monica Marques Telles¹

Affiliations:

¹ Programa de Pós-graduação em Nutrição, Departamento de Fisiologia da Nutrição - Universidade Federal de São Paulo.

² Programa de Pós-graduação em Microbiologia e Imunologia- Universidade Federal de São Paulo.

Keywords: O-GlcNAc. Maternal malnutrition. Energy balance. Hypothalamus. Fetal programming.

Abstract:

Background: Intrauterine growth restriction (IUGR) is directly associated to obesity, hypothalamic proteins alterations and impaired glucose metabolism. O-glycosylation with N-acetylglucosamine (O-GlcNAc) is glucose-dependent and its occurrence in hypothalamic proteins may influence energy balance. This study aimed to evaluate the density of O-GlcNAc-modified proteins and their colocalization with neuronal (NeuN) and astrocytic (GFAP) markers in the arcuate nucleus (ARC) and in the food intake of IUGR animals. **Methods:** Female Wistar rats were subjected to caloric restriction (RG) 50% of the control group (CG) during gestation. At 90 days, male pups (n=3/group) were anesthetized, perfused and their brains collected, frozen at -80°C, sectioned into 30µm coronal slices, and stored at -20°C until processing for immunofluorescence. Sections were incubated overnight with primary antibodies (anti-O-GlcNAc, anti-NeuN, and anti-GFAP) and for 2 hours with fluorescent secondary antibodies (AlexaFluor568, AlexaFluor488). They were then mounted on slides and imaged with a confocal microscope. Food intake was assessed weekly. Fluorescence signals were quantified with Image J software, and statistical analysis was conducted using Student's t-test. CEUA UNIFESP nº1115260121. **Results:** The restriction group (RG) showed higher colocalization of O-GlcNAc with the astrocytic marker GFAP (p<0.001) and lower neuronal (p<0.01) and astrocytic (p=0.05) staining than the control group (CG). There were no differences between the groups regarding O-GlcNAc labeling and colocalization with the NeuN marker (p=0.19). RG presented lower food consumption in the 1st, 3rd (p<0,001), 5th and 6th (p<0,05) week after weaning, with intake equal to that of the CG from the 7th week onwards. **Conclusion:** Hypothalamic astrocytes regulate neuronal nutrition, nutrient transport, and metabolic hormones. The increased glycosylation and reduction of astrocytes and neurons in the ARC promoted by IUGR, and the normalization of food intake between groups, suggest alterations in cellular signaling, which may compromise hypothalamic homeostasis and contributing to obesity.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Effects of sitagliptin on mitochondrial remodeling and glycemic parameters in spontaneously hypertensive rats fed a high-fat diet

Authors:

Paulo Rogerio Silva de Paiva Júnior¹ Pércles Fabrício Gomes Filho² Lucas Silva Bastos¹
Isaías dos Santos Silva¹ Carolina da Silva Valentim²
Raíssa Leal de Carvalho dos Santos Cunha² Cherley Borba Vieira de Andrade²
Jorge José de Carvalho²
Jemima Fuentes Ribeiro da Silva² Aluana Santana Carlos¹

Affiliations:

¹ Universidade Iguaçu – UNIG, Nova Iguaçu – RJ – Brasil.

² Universidade do Estado do Rio de Janeiro – UERJ – RJ – Brasil.

Keywords: High-fat diet. Arterial hypertension. Mitochondria. Rats. Sitagliptin.

Abstract:

Arterial hypertension and dyslipidemia are synergistic risk factors for cardiovascular diseases. Aging, hypertension, and diabetes impair mitochondrial biogenesis, affecting cellular energy metabolism. This study aimed to evaluate glycemic metabolism and mitochondrial biogenesis through morphological and morphometric analyses in spontaneously hypertensive rats (SHR) fed a high-fat diet and treated with sitagliptin. The study was approved by CEUA/PEBIO/UNIG (014/2020). Male SHR rats were divided into three groups: SHR with standard diet (SHR-C), SHR with high-fat diet (SHR-HF), and SHR-HF treated with sitagliptin (SHR-Sita), with 8 animals per group. The high-fat diet was administered from 160 to 210 days of age, and sitagliptin (10 mg/kg/day) was given by gavage from day 180 to 210. The oral glucose tolerance test (OGTT) was performed on days 160, 190, and 200. At 210 days, animals were euthanized and their hearts processed for electron microscopy. Results showed insulin resistance at day 160. On day 190, SHR-C and SHR-HF groups had higher glycemic levels. On day 200, the SHR-Sita group exhibited lower basal glycemia. Ultrastructural analysis revealed an increased number of viable mitochondria, reduced damaged mitochondria, and smaller total mitochondrial area in the SHR-Sita group. It is concluded that sitagliptin promoted beneficial mitochondrial remodeling and improved insulin sensitivity, suggesting antioxidant effects of DPP-4 inhibitors.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Maternal vitamin B9 status may modulate serum biochemical and oxidative stress markers in children

Authors:

Maria Brenda Ellen dos Santos Pereira Nascimento¹² Jéssica Gonzaga Pereira¹²
Anna Bheatriz Gomes Ferreira Marinho¹² Cheid Manuel Chongo¹²
Jéssica de Oliveira Campos¹² Reginaldo Correia da Silva Filho³ Mariana Pinheiro Fernandes³
Béatrice Morio⁴
Aiany Cibelle Simões Alves¹² João Henrique Costa Silva¹²

Affiliations:

¹ Laboratório Multiusuário de Avaliação Física e Processamento de Sinais-LAPS, Centro Acadêmico de Vitória, Universidade Federal de Pernambuco.

² Laboratório Multiusuário em Nutrição, Atividade Física e Plasticidade Fenotípica NAFPF, CAV/UFPE.

³ Laboratório Multiusuário de Bioquímica Geral, Molecular e do Exercício- LABMEX, CAV/UFPE.

⁴ CarMeN laboratory, UMR INSERM U1060/INRAE U1397, Université Claude Bernard Lyon 1, Oullins-Pierre-Bénite, France.

Keywords: Folate. biochemical serum profile. Cardiometabolic health. Oxidative Stress.

Abstract:

Maternal health directly influences child health, especially oxidative balance and infant biochemical profile. Nutritional factors, such as vitamin B9 levels, can affect fetal development and metabolism in childhood through genetic, epigenetic, and behavioral mechanisms. This study evaluates the association between maternal vitamin B9 and markers of infant redox status and biochemicals. A quantitative cross-sectional study, carried out in 2024, with 26 mothers and 29 children (7-11 years old) from public schools. The following parameters were evaluated: Body Mass Index (BMI), Vitamin B9, lipid markers and thiobarbituric acid reactive substances (TABRS), carbonyls, total thiols (TTi), superoxide dismutase (SOD), catalase (CAT), and Glutathione S-Transferase (GST) in erythrocytes. Approved by the Ethics Committee from UFPE (CAAE: 80956824.3.0000.9430). Statistical analysis was performed using Spearman's correlation model. Among mothers, 2 (7.69%) were classified as eutrophic, 11 (42.31%) were overweight and 13 (50%) were obese. Among children, 2 (6.90%) were classified as underweight, 19 (65.52%) as eutrophic, 4 (13.79%) as overweight, 2 (6.90%) as obese and 2 (6.90%) as severely obese. Significant negative correlations were identified between maternal folate levels and markers of infant redox status, such as carbonyls ($r = -0.556$; $p = 0.007$) and TABRS ($r = -0.457$; $p = 0.032$). Furthermore, maternal folate showed a negative correlation with infant LDL ($r = -0.380$; $p = 0.042$). These findings suggest that adequate maternal folate levels may be associated with better redox balance and lipid profile in children, reflecting a possible protective role. Adequate maternal folate levels have been shown to be associated with better redox and lipid markers in children, indicating the influence of maternal nutrition on child metabolic health and highlighting the importance of its adequacy as a preventive strategy against cardiometabolic alterations.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Administration of *Spondias tuberosa* bark extract during pregnancy reverses adipocyte hypertrophy induced by a maternal low- protein diet in adult rats offspring

Authors:

Raquel Leopoldino da Silva¹ Selma Maria de Melo⁴
Raíssa Andrade de Araújo Silva¹ Vitor Carlos de Araújo Bandeira³ Carolina Ramos de Mendonça¹ Anielle Mylena de Medeiros Barbosa³ Bianch Alessandro da Silva²
Brenda Hilary Avelino De Vasconcelos¹ Pedro Henrique Elias da Silva³ Eduardo Carvalho Lira²
Fernando Wesley Cavalcanti de Araújo¹ Diogo Antonio Alves de Vasconcelos^{1,2,3}

Affiliations:

¹ Department of Nutrition – Postgraduate Program in Nutrition- UFPE.

² Postgraduate Program in Nutrition, Physical Activity and Phenotypic Plasticity-UFPE. ³ Multicenter Postgraduate Program in Physiological Sciences-SBFis.

Keywords: Low-protein diet. *Spondias tuberosa*. Adiposity. Fetal development.

Abstract:

Lack or excess of nutrients during critical periods of fetal development causes long-term metabolic disorders. Studies with plants have shown that some have metabolic and antidiabetic effects, such as *Spondias Tuberosa* (UMBU). This study aimed to evaluate the effects of an ethanolic extract of *Spondias tuberosa* bark on adipocyte size in adult offspring of rats subjected to a maternal low-protein diet (CEUA UFPE, Process no. 0086/2021). The experimental groups were established: a control group (17% protein); a low-protein diet group (8% protein); and a low-protein diet group supplemented with *Spondias tuberosa* (ST) bark extract (0.5 g/kg/day). The diets were provided throughout gestation and lactation, whereas the ST extract was administered exclusively during gestation via oral gavage. It was observed that both during lactation and after weaning, the offspring of the DHMat group had a lower weight compared to the DCMat group (41% and 17, respectively). There were changes in the offspring regarding weight, body composition and adiposity in the group with protein deficiency in the maternal diet. At 90 days of age, it was identified that the size of the area of the adipocytes of the epididymal adipose tissue of the DHMat offspring showed an increase compared to the DCMat group, with a higher concentration between 300 and 920 μm^2 , and that the DHMat-Umbu group had a reduction in the area compared to DHMat, with a higher concentration between 50 and 300 μm^2 . Maternal intake of a low-protein diet during gestation and lactation resulted in an increase in adipocyte size in the offspring. However, supplementation with *Spondias tuberosa* bark extract during pregnancy effectively reversed this adipocyte hypertrophy, suggesting a protective effect against the adverse metabolic consequences of early-life protein restriction.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Heat Exposure Combined with Whey Protein Supplementation Enhances Strength and Fatigue Resistance in the Diaphragm Muscle of Mice

Authors:

Pedro William Martins-Pessoa^{1,3} Gustavo de Oliveira Zanetti^{1,3} Rafael Vaccaro Aguiar^{1,2,3} Gabriel Adler Gomes-Martins^{1,2,3} Elisa Santiago Pereira⁴
Lucas Antônio da Silva Dias^{1,3} Luiz C. Navegantes⁵
Walter Luís Garrido Cavalcante⁴ Dawit Albieiro Pinheiro Gonçalves^{1,2,3}

Affiliations:

¹ Ergogenic and Systemic and Molecular Physiology Research Group (ERGOFIS), School of Physical Education, Physiotherapy and Occupational Therapy, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

² Section of Sports Physiology (SFE), Sports Training Center (CTE), Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

³ Exercise Physiology Laboratory (LAFISE), School of Physical Education, Physiotherapy and Occupational Therapy, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

⁴ Laboratory of Pharmacology of the Neuromuscular Junction and Toxinology (LFJT), Institute of Biological Sciences, Belo Horizonte, MG, Brazil.

⁵ Department of Physiology, Ribeirão Preto Medical School, University of São Paulo, São Paulo, Brazil.

Keywords: Muscle adaptations. Heat exposure. mTOR-pS6 signaling. Physical performance. Whey protein.

Abstract:

Introduction: Metabolic and morphological adaptations in muscle mass improve sports performance. Exposure to hot environments (H), such as a sauna, stimulates hypertrophic signaling and an oxidative muscle phenotype. Whey protein (W) supplementation stimulates protein anabolism, promoting muscle hypertrophy. However, the synergistic effects of W and H on muscle and performance remain unexplored. **Aim:** To evaluate the synergistic effects between H exposure and W supplementation on muscle adaptations and physical performance in mice. **Methods:** In experiment (exp)#1, CD1 mice (n=5/group) were supplemented with W or maltodextrin

(M) prior to heat exposure (H, 40°C, 30 min/day, for 17 days) or thermoneutral condition (TN, 28°C) during the nocturnal period. In exp#2, supplementation occurred after heat exposure for 15 days. Exp#3 assessed the mTOR-pS6 signaling pathway after an acute intervention, following the same order of interventions as in exp#1. All groups evaluated in vivo limb strength by using the grip test and ex-vivo test in the diaphragm muscle. Comparisons were made using repeated measures two-way ANOVA followed by Tukey's post-test. Ethics approval: CEUA-UFMG 103/2021 **Results:** In exp #1, MH (maltodextrin + heat) showed an increase of ~16% in strength and ~110% in diaphragm fatigue resistance compared to MC (maltodextrin + control; P<0.05). In exp #2, the HW (heat + whey protein) group demonstrated a ~23% (P<0.05) increase in limb strength compared to CM (control + maltodextrin). However, there were no significant differences in fat mass or muscle mass among the groups (P>0.05). The HM (heat + maltodextrin) group showed an increase in pS6 phosphorylation (P<0.05). **Conclusion:** Heat exposure, rather than the combination with whey supplementation, was responsible for promoting significant gains in muscle strength, diaphragm fatigue resistance, and pS6 phosphorylation, a key regulator of protein synthesis. These findings may contribute to the development of strategies for health and performance.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Impact of the vagus nerve and the spleen ablation on iron metabolism in anemic male Wistar rats

Authors:

Amanda Rocha Fujita¹

Ellen Carolina Zawoski Gomes¹ Giovana Fanhani Tessaro¹

Eveline Cristiane Batista Schmidt¹ Elizângela Vanessa da Cruz Hoffmann¹ Matheus Dias Martins¹

Sabrina Grassioli¹

Affiliations:

¹ Laboratório de Fisiologia Endócrina e Metabolismo, Centro de Ciências Biológicas e da Saúde, Universidade Estadual do Oeste do Paraná, Cascavel, PR, Brasil.

Keywords: Anemia. Liver. Iron metabolism.

Abstract:

Introduction: Hepatic hepcidin modulates iron absorption and bioavailability. Vagus nerve (VN) alters liver immune and metabolic responses via anti-inflammatory vagal reflex, whose effect on iron homeostasis is unknown. **Objective:** This study evaluated the impact of the VN and spleen ablation on the liver histology and axis iron-hepcidin in male Wistar rats postanemia induction. **Methodology:** Ethics committee approval nº 15-22. This is an experimental study of a total of 24 male Wistar rats (n=6/group) (*Rattus norvegicus*). Animals were kept under controlled luminosity and temperature and had free access to regular rodent chow, tap water, and specific boxes (4 rats per box) appropriate for rodents. At 60 days of life, the rats were randomly separated into four experimental groups: subdiaphragmatic vagotomy (VS); splenectomy (SPL); SV+SPL, involving both procedures; and sham surgery (SHAM). At 90 days of life, all groups were subdivided into anemic and non-anemic groups. Hemolytic anemia was pharmacologically induced by administering phenylhydrazine (PHZ) at a dose of 40mg/kg, while non-anemic rats received saline (0.9%). At 120 days of age, following 12 hours of fasting, the rats were euthanized in a CO₂ chamber and subsequently decapitated using a guillotine. After the procedure, biochemical and biometric parameters were collected. Statistical analysis included ANOVA or Generalized Linear Model (Gamma distribution), depending on assumption testing (Shapiro-Wilk, Levene, Durbin-Watson), with significance set at p<0.05. **Results:** The VS reduced BW (p=0.021) in relation to SHAM rats. Metabolic parameters were not modified either by surgeries or PHZ. VS (p=0.014) and PHZ (p=0.001) changes in spleen weight, with VS leading to a spleen weight reduction while PHZ led to a rise. In the SPL-PHZ, rats were noted to have higher iron levels (p=0.013). Plasma hepcidin levels were influenced by VS (p=0.007) and SPL (p=0.015), with both surgeries showing reduced levels, a response accentuated in the VS+SPL (p=0.029). Augmented Kupffer cells were noted in the liver of the SV+PHZ and SV+SPL groups. SV+SPL rats showed a smaller PV size relative to other groups, an effect of interaction of SV versus SPL (p=0.015). The percentage of centrilobular fibrosis was affected by SV (p=0.001), with SV rats presenting high fibrosis in comparison to other groups; an effect also observed in the SPL+PHZ animals (p=0.020). **Conclusion:** The combined surgery and anemic male Wistar rats significantly reduced plasma hepcidin levels. This suggests that vagus nerve ablation and splenectomy cause complex and distinct changes in the metabolism of anemic rodents, impacting the regulation of iron homeostasis and suggesting interconnected mechanisms.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: TRPV1 Channel does not participate in β 3-adrenergic induced UCP1 increase in brown adipose tissue but contributes to the thermoregulatory responses

Authors:

Pedro Lucas Caillaux Luciano¹ Maria Eduarda Farias Pigaiani¹ Daniela Lopes Vieira¹
Ana Luiza Flausino Fazollo¹ Gabriel da Silva Teodoro de Faria¹
Luciana de Souza Brandão Martins¹ Kiany Miranda²
Sílvia Carolina Guatimosim¹
Maria Nathália de Carvalho Magalhães Moraes Figueira Borges³ Maristela de Oliveira Poletini¹

Affiliations:

¹ Universidade Federal de Minas Gerais. ² Universidade Federal do Espírito Santo. ³ Universidade Federal de São Paulo.

Keywords: Brown Adipose Tissue. Energy Metabolism. Thermogenesis. Thermoregulation.

Abstract:

Introduction: TRPV1 channels are calcium-permeable receptors expressed in neuronal and non-neuronal tissues. TRPV1 blockade induces hyperthermia via cold-defense mechanisms involving β 3-adrenoreceptor (β 3AR) signaling, but its role in direct β 3-adrenergic thermogenesis remains unclear. **Objectives:** To investigate the role of TRPV1 in β 3AR-induced thermogenesis. **Methods:** TRPV1-KO and WT mice (C57BL/6) were implanted with telemetry probes to monitor abdominal temperature (T_a). One group of mice received CL-316,243 (CL) (β 3AR agonist, 1 mg/kg) or vehicle (0.9% saline) at 12:00 P.M, with T_a recorded for 3 hours. Separate WT mice were pretreated with the TRPV1 agonist resiniferatoxin (RTX, 500 ng/kg) or vehicle (1%-ethanol in saline) 12:00h. One hour later, they received CL (n=8) or vehicle (n=7). Brown adipose tissue (BAT) was collected for histology and qPCR analysis (UCP-1, β 3AR, clock genes *Bmal-1*/*Per-2*). Statistical differences in T_a were assessed using two-way ANOVA followed by Sidak's post-test, while relative mRNA levels were analyzed by one-way ANOVA. This study was approved by CEUA protocol 87/2024. **Results:** TRPV1-KO mice (n=9) exhibited lower baseline T_a than WT (n=9; $p < 0.0001$) but showed a greater ΔT_a after CL-316,243 administration ($p = 0.0063$). RTX pretreatment abolished the CL-316,243-induced T_a increase ($p < 0.0001$). CL-316,243 increased BAT cell number ($p < 0.05$) and UCP-1 mRNA levels ($p < 0.0001$); RTX+CL maintained UCP-1 upregulation. β 3AR mRNA decreased in Veh+CL ($p < 0.05$), RTX+CL ($p < 0.05$), and RTX+Veh ($p < 0.05$) groups. *Bmal-1* mRNA was increased by Veh+CL ($p < 0.001$) and RTX+CL ($p < 0.01$) but not by RTX+Veh, while *Per-2* was not altered by any treatment. **Conclusion:** TRPV1 absence enhances thermogenic capacity (KO mice responded robustly despite lower baseline T_a). TRPV1 activation (via RTX) suppresses β 3AR-driven thermogenesis without affecting UCP-1 upregulation, suggesting that TRPV1 limits thermogenesis through heat dissipation rather than β 3AR signaling.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Introdução Alimentar, Estado Nutricional e Hábitos Alimentares De Crianças De 2 A 5 Anos do Município da Vitória de Santo Antão

Authors:

Irlen Lilianne de Souza Assis¹ Marcia José do Espírito Santo Silva¹ Natália Virgínia Lira Silva¹
João Henrique da Costa Silva¹ Viviane de Oliveira Nogueira¹

Affiliations:

¹ Universidade Federal de Pernambuco- Centro Acadêmico de Vitória.

Keywords: Alimentação saudável. Consumo alimentar. Estado nutricional.

Abstract:

A introdução alimentar (IA), recomendada a partir dos 6 meses, marca o início da oferta de alimentos complementares ao leite materno. A obesidade infantil tem sido associada à introdução precoce e inadequada da alimentação neste período, de modo que, dietas obesogênicas na infância podem levar ao aumento do risco de doenças crônicas (DCNT) na vida adulta. Este estudo teve como objetivo avaliar a qualidade da IA, o estado nutricional e o consumo alimentar de crianças entre 2 e 5 anos em Vitória de Santo Antão, PE. Trata-se de um estudo descritivo com 56 crianças neurotípicas da faixa etária mencionada, todas residentes no município. A pesquisa foi aprovada pelo Comitê de Ética da Universidade Federal de Pernambuco (parecer nº 6.181.367) e contou com o consentimento dos responsáveis. A coleta de dados ocorreu em um Centro Municipal de Educação Infantil (CMEI), onde foram aplicados questionários validados para avaliar o consumo alimentar atual (Colucci et al., 2005) e a IA (Oliveira et al., 2015). O estado nutricional foi determinado a partir de medições do peso, altura e Índice de Massa Corporal (IMC), analisados conforme as curvas da Organização Mundial da Saúde (OMS). Dos participantes, 50% eram meninas e 50% meninos. Os resultados mostraram que apenas 21,4% das crianças apresentaram uma IA de boa qualidade nutricional, 51,8% moderada e 26,8% baixa. A dieta era majoritariamente composta por alimentos ultraprocessados, ricos em açúcar e gordura. Quanto ao estado nutricional, 50,8% estavam eutróficos, 25,5% em risco de sobrepeso, 16,4% com sobrepeso e 5,5% com obesidade. Os dados revelam uma associação entre IA inadequada e alterações no estado nutricional e no consumo alimentar, ressaltando a importância de práticas alimentares adequadas na infância para a promoção de saúde e prevenção de distúrbios nutricionais.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Fetal programming by high-fat diet induces alterations in somatic growth and delays in the parameters of the neurodevelopment in young rats

Authors:

Jéssica Caroline Alves-de-Sena-Fourniol^{1,2} Clarissa Tavares Dias³
Vanessa Santos Batista² Edicarlos Oliveira Moraes² Jairza Maria Barreto-Medeiros² Cristiano Mendes-da-Silva³
Tereza Cristina Bomfim de Jesus Deiró²

Affiliations:

¹ Programa de Pós-graduação em Nutrição, Departamento de Fisiologia da Nutrição – Universidade Federal de São Paulo.

² Departamento de Ciências da Nutrição, Escola de Nutrição da Universidade Federal da Bahia.

³ Programa de Pós-graduação Interdisciplinar em Ciências da Saúde, Departamento de Fisiologia da Nutrição – Universidade Federal de São Paulo.

Keywords: Maternal overnutrition. Reflex ontogeny. Somatic growth.

Abstract:

Maternal diet during critical periods, such as pregnancy and lactation, can impact the developmental health and disease status of offspring. This study aimed to evaluate the effects of fetal programming induced by a high-fat diet on somatic growth and neurodevelopmental parameters in young rats. **Methods:** Fourteen neonatal male Wistar rats ($n=7/\text{group}$) were used, obtained from mothers fed during pregnancy and lactation with a control diet (CD) (Nuvilab®) or a high-fat diet (DH) containing 23% lipids. The pups were evaluated from the 1st to the 21st postnatal day (PND) for reflex maturation: Palmar Grasp (PP), Recumbent Recovery (RD), Startle Response (RS), Vibrissae Positioning (CV), Negative Geotaxis (GN), Cliff Aversion (AP) and Acceleration (AC) and from the 1st to the 40th day for growth of the laterolateral (ELL) and anteroposterior (EAP) cranial axes, weight and body growth. **Statistical Analysis:** Generalized Estimating Equation - GEE for longitudinal data and Generalized Linear Model - GLM for cross-sectional data. Data expressed as mean $\pm$ standard error, $\alpha<0.05$. CEUA UNIFESP 4600030320. **Results:** The HFD offspring had lower body weight than the CD group in PND1 ($p=0.004$) and PND7 ($p=0.042$), shorter body length, PND1 ($p=0.001$) and PND7 ($p=0.008$) and lower EAP in PND1 ($p<0.0001$) and PND7 ($p=0.005$). However, from PND14 to PND40 there was no difference between the groups regarding these parameters. As for the ELL, the HFD group did not differ from the CD group in PND1 and PND7, but it was higher from PND14 to PND40 ($p\leq 0.01$). The HFD group showed delayed maturation of the Vibrissae Placement, Negative Geotaxis and Acceleration Response reflexes ($p<0.05$) compared to CD. **Conclusion:** Maternal high-fat diet induced reduced birth weight and body length, changes in craniofacial development and delayed reflex maturation of the offspring.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Impacts of protein malnutrition and overnutrition during the lactation period on offspring growth

Authors:

Vanessa dos Santos Batista¹

Jéssica Caroline Alves de Sena Fourniol^{1,2} Edicarlos de Oliveira Moraes¹

Jairza Maria Barreto-Medeiros¹ Adenilda Queirós Santos Deiró¹ Gabriele dos Santos Cordeiro¹

Tereza Cristina Bomfim de Jesus Deiró¹

Affiliations:

¹ Departamento de Ciência da Nutrição – Universidade Federal da Bahia.

² Programação de Pós-Graduação em Nutrição da Universidade Federal de São Paulo. Sistema Digestório, Nutrição e Metabolismo.

Keywords: Protein malnutrition; maternal overnutrition; growth; offspring.

Abstract:

Excess weight and malnutrition are global nutritional challenges, with higher prevalence in low-income populations and peripheral urban areas. This study aimed to evaluate whether protein malnutrition and/or a maternal high-fat diet affect offspring growth parameters. A total of 24 male newborn albino Wistar rats were used, divided into three groups according to the maternal diet during lactation: normally nourished (N, n=8, Nuvilab®), malnourished (DES, n=8, 8% casein), and high-fat (HL, n=8, 23% lipids). Mothers and pups were kept in a controlled environment regarding temperature, humidity, light/dark cycle (12h), and with free access to water and food. From the 1st to the 21st postnatal day (PND), body weight, longitudinal growth, and physical characteristics (auricular pavilion and auditory canal opening, eye opening, and eruption of upper and lower incisors) were evaluated. The project was approved by CEUA: EMEVZ-UFBA No. 33/2019. Data were analyzed using GraphPad Prism software, applying two-way ANOVA with Bonferroni post-test. The DES group exhibited lower weight from the 12th (20.90±3.00g) to the 21st PND (27.01±4.28g) compared to the N group (p<0.05). Regarding the HL group, DES showed lower weight from the 14th to the 21st PND. From the 20th PND onwards, the HL group also had lower weight than the N group (p<0.05). In terms of body growth, the HL group presented lower values on the 5th, 15th, and from the 19th to the 21st PND. The DES group showed reduced growth from the 10th to the 21st PND. On the other hand, the HL group exhibited superior growth compared to the DES group from the 14th to the 21st PND (p<0.05). It was concluded that maternal exposure to unbalanced diets, either due to protein deficiency or excess lipids, during the critical developmental period, compromised body growth, weight progression, and physical development of the offspring, with more evident impacts in the malnutrition group.

Financial support: CNPq, FAPESB.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Title: 6-nitrodopamine is an endogenous mediator of the rat gastric fundus relaxation

Authors:

Flaviano Lorenzon¹

Nayane Carolina Silva Vicentini¹ Bruna Lourençoni Alves¹ Gilberto De Nucci¹

Affiliations:

¹ Pharmacology department, Postgraduate Program in Pharmacology, University of Campinas, Campinas/SP.

Keywords: 6-nitrodopamine. Catecholamines. Smooth muscle. Stomach.

Abstract:

Endothelium-and epithelium-derived 6-nitrodopamine (6-ND) is a novel endogenous catecholamine identified in human and animal tissues. It plays an important role in cardiovascular and urogenital systems. In the rat heart, 6-ND is the most potent endogenous modulator of chronotropic and inotropic activity. In human vas deferens and seminal vesicle, it induces smooth muscle contraction, suggesting a role in ejaculation. However, its function in the gastrointestinal system remains unclear. This study aims to determine whether the rat gastric fundus (RGF) releases 6-ND and its functional relevance. RGF was obtained from 40 male Wistar rats (70 days old). Tissues (n=4/group) were suspended in 3-mL glass chambers with Krebs-Henseleit solution (KHS) at 37°C, gassed with 95% O₂/5% CO₂, and supplemented with ascorbic acid (3mM) to prevent catecholamine oxidation. The tissues were incubated for 30 minutes with the voltage-gated sodium channel blocker TTX (1μM). KHS samples were collected and stored at -80°C for LC-MS/MS analysis. For functional studies, 1.5 cm strips were mounted in 10 mL KHS baths at 37°C, under 10 mN resting tension, and connected to an isometric force transducer (PowerLab, ADInstruments). Strips were pre-contracted with carbachol (1 μM), and cumulative concentration-response curves to 6-ND, dopamine (DA), adrenaline (AD), and noradrenaline (NA) (100 pM-1 mM) were obtained. Ethic committee number: 6581-1/2024. 6-ND was released from RGF at concentrations five times higher than DA and NA (1.95±1.28 vs 0.42±0.14 vs 0.41±0.26 ng/mL). AD was undetectable. TTX reduced DA (0.41±0.14 vs 0.16±0.03 ng/mL) and NA (0.41±0.26 vs 0.12±0.11 ng/mL) but not 6-ND (1.95±1.28 vs 3.17±1.68 ng/mL) levels. 6-ND induced relaxation with pEC₅₀ = 5.4±0.2 and E_{max} = 74.61±1.4%. Potency rank: AD (pEC₅₀=7.2±0.09) > NA (6.7±0.09) > DA (4.95±0.2) =6-ND (5.4±0.2). The findings establish 6-ND as a novel endogenous non-neurogenic mediator of the rat gastric fundus smooth muscle relaxation.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Maternal Low-Protein Diet Impairs Skeletal Muscle Hypertrophic Capacity In Adult Rats

Authors:

Anielle Mylena de Medeiros Barbosa¹ Ana Catherine Neves do Nascimento² Gabriel Vinicius Silva Amadeu²

Raíssa Andrade de Araújo Silva¹ Vitor Carlos Araujo Bandeira² Carolina Ramos de Mendonça² Diogo Antonio Alves de Vasconcelos²

Affiliations:

¹ Multicenter Postgraduate Program in Physiological Sciences – UFPE/SBFis, 55608- 680, Vitória de Santo Antão, PE, Brazil.

² Postgraduate Program in Nutrition, Universidade Federal de Pernambuco, 55670- 901, Recife, PE, Brazil.

Keywords: Low-protein diet. Hypertrophy. Skeletal muscle. Developmental plasticity. Rats.

Abstract:

Low birth weight induced by a low-protein diet during pregnancy and lactation, is associated with metabolic alterations in adulthood. Maternal low-protein diets impair the muscular development and structure of the offspring. This study evaluated the effects of such a diet on the Akt-mTOR pathway in the extensor digitorum longus (EDL) muscle of adult rats subjected to compensatory hypertrophy. Twelve Wistar rats were used, divided into control (19% protein) and low-protein (9%) diet groups during gestation and lactation. After weaning, male offspring were maintained on a standard diet until 90 days of age and underwent muscle ablation surgery at day 83 to induce compensatory hypertrophy. Analyses included wet and dry muscle mass, histology, and Western Blotting. An increase in EDL wet mass was observed in the DCMat-PH group compared to DCMat-PC (25.2%). No significant difference was found between the low-protein diet groups. The DHMat-PC group showed a 21.4% reduction compared to DCMat-PC, while DHMat-PH had a 28% lower mass than DCMat-PH. Dry mass followed a similar pattern: a 14.4% increase in DCMat-PH versus DCMat-PC, and reductions of 22.6% and 26.3% in DHMat-PC and DHMat-PH, respectively. Histological analysis showed larger fiber areas in the DCMat-PH group (200–350 μm^2), whereas low-protein groups presented smaller fibers (100–200 μm^2). p-Akt^{Ser473} content was elevated in the hypertrophied limb of the low-protein group compared to its control limb, whereas no significant difference was found in the control group. Total Akt expression remained unchanged between groups. Regarding rpS6, there was a trend toward increased expression in the limb subjected to overload. In summary, the maternal low-protein diet reduced the hypertrophic capacity of the EDL muscle, resulting in smaller cross-sectional fiber areas, and modulated the plasticity response in the offspring's skeletal muscle without affecting Akt expression. Further studies are needed.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Maternal obesity and placental development: the role of insulin and insulin-like growth factors

Authors:

Ana Raquel Marcon Foltz¹ Ana Paula Varela Sanches¹ Beatriz Piatezzi Siqueira¹
Laís Angélica de Paula Simino¹ Lailton Oliveira da Silva¹ Márcio Alberto Torsoni¹ Adriana
Souza Torsoni¹ Marciane Milanski¹
Letícia Ignácio-Souza¹

Affiliations:

¹ Universidade Estadual de Campinas.

Keywords: Fetal Growth. IGF. Maternal Obesity. Placenta.

Abstract:

Maternal obesity is involved in various adverse outcomes in placental and fetal development, as it causes significant changes in placental functions and metabolism. In this context, insulin and insulin-like growth factors (IGFs) are hormones that promote cell proliferation and migration in this organ, playing an essential role in its development. However, due to obesity, these factors appear to be altered, impacting the baby's health and leading to imbalances in optimal growth. Therefore, this study aimed to investigate the role of the aforementioned pathways in placental and fetal growth in obese animals and to assess the invasion capacity of placental cells treated with an obesogenic medium. For this project, placental tissue samples were collected from Swiss mice under approval from the animal ethics committee (CEUA nº 6390-1/2024). The animals were divided into three groups: Control (n=6), Obesity-Prone (n=3), and Obesity-Resistant (n=4), with the latter two groups receiving a high-fat diet. Western blot analysis was performed to evaluate IGF-1 and mTOR protein levels. In addition, trophoblastic cell invasion assays were carried out using the BeWo cell line, treated with 0.3 mM palmitate to mimic an obesogenic environment. Results were expressed as mean $\pm$ standard deviation, with statistical significance set at $p < 0.05$. Obesity-prone mice showed higher IGF-1 levels compared to the control group, indicating possible modulation of fetoplacental growth. The trophoblastic cell invasion assay revealed a decrease in the invaded area in the palmitate-treated group compared to the control. In conclusion, although preliminary, these results suggest that modulation of the IGF pathway is associated with maternal obesity, influencing fetal growth outcomes, and that an obesogenic environment negatively affects placental cell migration.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Therapeutic Peptides Target Calcium Signaling Dysregulation in Hepatic Steatosis

Authors:

Maria Fernanda Silva Amorim¹ Matheus F Itaborahy¹
Isadora Zhong Liang Ferreira Feng¹ Maria Eduarda Souza Favalessa¹ Yuri César Capato¹
Izabella Freitas Acipreste¹ João Santana da Silva² Regina C Goldenberg² Alfredo M. Goes³
Robson A. S. Santos Cibele Ferreira Pimentel² Marlon Lemos Dias²
M Fátima Leite¹

Affiliations:

¹ Department of Physiology and Biophysics - Federal University of Minas Gerais. ² Precision Medicine Research Center - Federal University of Rio de Janeiro.

³ INCT Hepatologia 360°.

Keywords: Angiotensins-based therapy. Hepatic Spheroids. Steatosis.

Abstract:

Hepatic steatosis, characterized by the abnormal accumulation of triglycerides in hepatocytes, is the earliest and most common manifestation of non-alcoholic fatty liver disease (NAFLD), which affects ~25% of the global population [Younossi et al., 2016]. This high prevalence is partly due to the absence of effective therapies for reversing liver damage. Unlike fibrosis or cirrhosis, steatosis is potentially reversible, offering a critical therapeutic window. Early intervention may prevent progression to steatohepatitis, fibrosis, and hepatocellular carcinoma, while also improving cardiometabolic health. In this study, we evaluated novel therapeutic peptides from the renin-angiotensin system, known to activate the ACE2/Ang-(1-7)/Mas axis and reduce hepatic steatosis by improving insulin sensitivity and lipid metabolism. We used a 3D in vitro liver model to test Angiotensin-(1-7), Ac-Ang-(2-7)-NH₂, and Alamandine-(1-5) for their antisteatogenic effects. Hepatic spheroids were generated using HepG2 cells, hepatic stellate cells, and mesenchymal stem cells. Steatosis was induced by exposure to 2 mM oleic acid conjugated with 10% BSA for 48 h. Calcium signaling responses were analyzed using Fluo-4/AM dye under ATP (50 μ M) stimulation, after treatment with peptides (100 nM, 24 h). Steatotic spheroids showed reduced intracellular Ca²⁺ amplitude compared to controls (159.9 $\pm$ 32.86 vs 231.2 $\pm$ 110.6, p <0.0001, n >70 cells/condition), and a shift toward wave-like signaling patterns (85% vs 63% in controls). Peptide treatment, particularly with Alamandine-(1-5) and Ac-Ang-(2-7)-NH₂, restored Ca²⁺ signaling amplitude and profile, indicating their potential to reverse steatosis and re-establish hepatocellular function.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Early adaptation to an unusual host in the bean weevil *Zabrotes subfasciatus* is associated with changes in body size and reproductive physiology

Authors:

Bruno de Oliveira Cruz¹
Sílvia de Oliveira Miranda¹
Emily Rarissa Crisóstomo Benedito¹
Juliana Ramos Martins¹
Luiz Alberto Beijo²
Ester Siqueira Caixeta Nogueira¹
Lívia Maria Rosatto Moda¹
Talita Sarah Mazzoni¹
Isabel Ribeiro do Valle Teixeira³
Angel Roberto Barchuk¹

Affiliations:

¹ Department of Cellular and Developmental Biology, Federal University of Alfenas, UNIFAL-MG, Alfenas, Brazil.

² Department of Statistics, Institute of Exact Sciences, Federal University of Alfenas, UNIFAL-MG, Alfenas, Minas Gerais, Brazil.

³ Federal Institute of Education, Science, and Technology of Southern Minas Gerais, IFSULDEMINAS, Poços de Caldas, Brazil.

Keywords: Adaptation. Bean Weevil. Experimental evolution. Morphology. Physiology.

Abstract:

In phytophagous insects, such as Bruchinae beetles, the choice of host plant species for development and feeding is associated with complex phenotypic combinations. However, scarce literature does not allow the identification of evolutionary patterns. Even less is known about the adaptive changes during host switching, and the factors governing the reproductive capacity of these insects remain unknown, despite being a critical trait that determines their success in infesting grains. To investigate potential reproductive and physiological changes in the adaptive process during host switching, an artificial selection experiment was conducted using chickpea seeds (*Cicer arietinum*) as a host, with the bean weevil, *Zabrotes subfasciatus*, as a model. Over twelve generations of selection, variations in body and gonadal traits were evaluated using a morphometric approach, along with variations in the expression of vitellogenin (Vg) and its receptor (VgR), a yolk precursor glycoprotein essential for egg nutrition in female insects. Morphometric analyses revealed that body length and width, elytra length, pronotum width, and exposed abdomen length were greater in chickpea populations ($p < 0.05$). Gonads were analyzed under fluorescence with DAPI- phalloidin staining, revealing that females reared on chickpeas produce higher quantities of oocytes compared to females reared on beans and tend to retain them in the ovarian calyx in over 70% of cases ($p < 0.05$). Analyses of Vg showed significant differences between individuals reared on beans and chickpeas in generations F7 e F11 ($p < 0.05$), and between F0 and F11 for VgR analyses ($p < 0.05$). Together, our results suggest that the use of a new host by *Z. subfasciatus* is facilitated by morphological and physiological changes associated with the development of life-history traits, particularly reproductive capacity.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Ovarian activation dynamics in *Zabrotes subfasciatus* (Boh.) (Coleoptera: Chrysomelidae, Bruchinae): The need for seeds and males

Authors:

Sílvia de Oliveira Miranda¹
Bruno de Oliveira Cruz¹
Juliana Ramos Martins¹
Talita Sarah Mazzoni¹
Waner de Oliveira Miranda²
Lívia Maria Rosatto Moda¹
Ester Siqueira Caixeta Nogueira¹
Isabel Ribeiro do Valle Teixeira³
Angel Roberto Barchuk¹

Affiliations:

¹ Department of Cellular and Developmental Biology, Federal University of Alfenas, UNIFAL-MG, Alfenas, Brazil.

² Senior Software Engineer, Microsoft, São Paulo, Brazil; Redmond, Washington, USA.

³ Federal Institute of Education, Science, and Technology of Southern Minas Gerais, IFSULDEMINAS, Poços de Caldas, Brazil.

Keywords: Bean weevil. Gene expression. Host. Oviposition. Reproduction.

Abstract:

The beetle *Zabrotes subfasciatus* is a predatory insect of stored grains, especially beans (*Phaseolus vulgaris*). Understanding the biology of *Z. subfasciatus* is crucial for designing sustainable strategies to control these insects. However, there are few studies on the reproductive biology of this insect. Electroantenographic assays indicate that contact between females, host and males stimulates oogenesis and oviposition. However, the morphological and molecular dynamics of ovarian ontogenesis in response to these stimuli remain unknown. This study aimed to determine how the presence of males and hosts influences the reproductive physiology of female *Z. subfasciatus*. Immature females were subjected to controlled environmental conditions, varying the factors that affect oviposition (males and beans). The number of eggs, daily oviposition and adult emergence were recorded. Subsequently, morphological and molecular analyses of ovarian activation were performed under different conditions. For morphological analyses, ovaries were processed for fluorescence microscopy. For molecular analyses, ovaries and carcasses were used in qPCR assays to measure the transcription levels of the vitellogenin (*Vg*) and its receptor (*VgR*) genes, which indicate reproductive capacity. The results show that isolated variables do not induce oviposition activity. Females reproduce on the first day of adulthood and oviposit mainly during the first six days. Morphological analysis indicates that *Vg* endocytosis begins in early adulthood and decreases over time ($p < 0.002$), while molecular analysis suggests that vitellogenesis is promoted by the host ($p < 0.05$), with males stimulating *Vg* synthesis in the ovaries ($p < 0.05$). In this sense, the host is essential for vitellogenesis, while males act as external stimuli for ovarian activation. This strategy shows the relevance of environmental factors in reproductive success, providing insights for sustainable pest management strategies.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Effects of fipronil contamination on cardiovascular responses and autonomic control of the heart in rainbow trout during aerobic exercise

Authors:

Igor Noll Guagnoni^{1,2}
Luiz Henrique Florindo¹
Tobias Wang²

Affiliations:

¹São Paulo State University (UNESP), Brazil.
²Aarhus University (AU), Denmark.

Keywords: Exercise. Fipronil. fish. *Oncorhynchus mykiss*. GABA.

Abstract:

Fipronil is a pesticide that antagonizes chloride channels regulated by GABA/glutamic acid. Several studies have shown its toxicity to fish, promoting gill damage, disrupting redox balance, and impairing GABAergic signaling in the brain. The widespread presence of these ion channels in the nervous system of vertebrates suggests that fipronil can interfere with cardiovascular control and its responsiveness during activities like aerobic exercise. In this context, we hypothesized that acute fipronil exposure compromises cardiovascular responses in rainbow trout during exercise. To test this, *Oncorhynchus mykiss* were kept in uncontaminated conditions (N=8 for the control group and N=5 for fish treated with bicuculline, simulating GABA-blocking effects) or in water contaminated with fipronil (96 hours at 0.5 $\mu\text{g L}^{-1}$; Regent®800WG; N=7). The fish were instrumented with a ventral aortic cannula to measure cardiovascular responses and autonomic heart control. They were placed in a swim tunnel, and data were collected under undisturbed conditions (0.5 body length per second; $\text{BL}\cdot\text{s}^{-1}$) and during exercise (1.5 $\text{BL}\cdot\text{s}^{-1}$), both before and after parasympathetic blockade (atropine; 1.5 $\text{mg}\cdot\text{kg}^{-1}$), followed by complete autonomic blockade (propranolol; 3 $\text{mg}\cdot\text{kg}^{-1}$). All procedures followed Danish Federal Regulations. Results were analyzed using one-way ANOVA ($p\leq 0.05$), with Holm-Šidák post-hoc tests. Our results reveal that fipronil increased the intrinsic heart rate (52%) and induced a pronounced tachycardia during exercise compared to controls (77%), driven by enhanced vagal withdrawal (145%) and adrenergic stimulation (310%). Furthermore, exercise-induced tachycardia in contaminated (slope = 1.52; $r^2 = 0.68$) and bicuculline-treated fish (slope = 1.72; $r^2 = 0.89$) showed similar linear patterns, contrasting with controls (slope = 0.65; $r^2 = 0.34$) and highlighting fipronil's impact on GABAergic mediation in cardiovascular regulation. Funding: FAPESP grant 2020/14167-8 and 2023/01330-6.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Impact of Settleable Atmospheric Particulate Matter (SePM) on metal bioaccumulation and histopathological changes in the main absorption pathways, gills and intestine, in Nile Tilapia

Authors:

Julia Helena da Cruz de Paula¹
Evelise Nunes Fragoso-Moura²
Carolina Fernandes De Angelis¹
Renato Filogonio¹
Michelly Pereira Soares¹
Israel Luz Cardoso¹
Beatriz Helena Montanari¹
Marisa Narciso Fernandes¹
Cléo Alcantara Costa Leite¹

Affiliations:

¹ Department of Physiological Sciences, Federal University of São Carlos, São Carlos, SP, Brazil.

² Department of Hydrobiology, Federal University of São Carlos, São Carlos, SP, Brazil.

Keywords: Ecotoxicology. Fish. Histopathology. Metals. SePM.

Abstract:

The Tubarão complex (ES-BR) has a big industrial complex with a significant impact on particulate matter (PM) emissions. PM contains metals that can settle (SePM), affecting local water bodies and biota. The estuary, the most impacted environment, undergoes natural physicochemical variations, including temperature shifts. This study assessed how thermal variation (15, 20, and 30 °C) influences metal bioaccumulation and histopathological damage caused by SePM (1g/L, 96 h) in the main absorption pathways, gills and intestine, of Nile tilapia. Tissues were analyzed for damage severity, frequency, morphometric changes, and the Histopathological Alteration Index. Exposure to SePM led to bioaccumulation of 19 metals, with the highest concentrations in the intestine, yet no histological damage was observed there. However, gills exhibited significant alterations, including epithelial hypertrophy, filament hyperplasia, lamellar fusion, capillary dilation, vascular congestion, and lamellar aneurysm (15°C, C:3.16±0.30, E:5±1.61); (20°C, C:2.6±0.24, E:5.6±2.11); (30°C, C:2.5±0.22, E:7.0±0.22). Lamellar height decreased in exposed groups: (15°C, C:51.06±9.49, E:30.43±8.91); (20°C, C:79.14±9.88, E:29.49±8.71); (30 °C, C:48.08±5.78, E:19.02±4.44). Lower temperatures correlated with morphometric alterations in intestine such as, increased perimeter, height, villus thickness, and crypt depth: Perimeter - (15°C, C:2,127.2±255.30, E:2,245.1±370.9) (20°C, C:1,671.8±224.5, E:1,391.1±292.2) (30°C, C:700.2±182.0, E:502.1±53.91); Height - (15°C, C:179.8±24.18, E:114.3±17.27) (20°C, C:104.4±5.29, E:106.3±6.13) (30 °C, C:93.7±6.2, E:91.0±7.5); Thickness - (15°C, C:85.6±10.7, E:40.1±3.65) (20°C, C:48.6±4.36, E:46.5±5.55) (30°C, C:45.6±2.99, E:46.6±6.36); Crypt depth - (15°C, C:23.9±1.67, E:12.8±1.16) (20°C, C:12.9±0.32, E:12.7±1.44) (30°C, C:13.1±0.96, E:14.1±1.19). SePM contamination significantly affects tissues across estuarine temperature ranges, posing a relevant risk to biota. CEUA-UFSCar: 9046240523.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Living Airless: Metabolic and oxidative responses of the Tree Frog *Boana pulchella* under anoxic conditions

Authors:

Gustavo Kasper Cubas¹
Marjoriane de Amaral¹
Leonardo Airton Ressel Simões¹
Jorge Felipe Argenta Model¹
Rafaella Sanfelice Normann¹
Bruna Selau¹
Luiz Carlos Kucharski¹

Affiliations:

¹ Laboratório de Metabolismo e Endocrinologia Comparada, Universidade Federal do Rio Grande do Sul.

Keywords: Anoxia. Anurans. Metabolism. Oxidative balance.

Abstract:

Amphibians have evolved biochemical and physiological strategies that have enabled them to thrive in extreme and inhospitable environments. Survival in freezing conditions, for instance, relies on pre-existing adaptations, such as anoxia tolerance. Hence, this study aimed to investigate metabolic variations and oxidative balance in the brain, skeletal muscle, and plasma of the subtropical tree frog *Boana pulchella* during anoxia and subsequent recovery under normoxic conditions. Fifteen adult male *B. pulchella* were randomly assigned to a control (n=5), anoxia (n=5), or recovery (n=5) group (SISBIO #75475-3; CEUA- UFRGS #39416). Anoxia-exposed frogs underwent 30 minutes of complete oxygen deprivation, followed by either euthanasia or a four-hour recovery protocol. Gluconeogenesis from lactate, catalase (CAT) and superoxide dismutase (SOD) activity, and lipid peroxidation (TBARs) were measured in brain and skeletal muscle, along with muscle glucose oxidation, glycogen synthesis, and plasma lactate and glucose levels. The results showed a significant decrease in brain gluconeogenesis from lactate (one-way ANOVA, $F(2, 10) = 5.277$, $p = 0.027$) in anoxia-exposed frogs ($2290 \pm 470.1 \text{ mmol.g}^{-1}.\text{min}^{-1}$) compared to the control group ($4076 \pm 289.3 \text{ mmol.g}^{-1}.\text{min}^{-1}$) (Tukey post hoc, $p \leq 0.05$). In addition, plasma lactate levels showed a substantial increase in the anoxia group ($7.977 \pm 0.657 \text{ mmol.L}^{-1}$) compared to the control ($4.816 \pm 0.292 \text{ mmol.L}^{-1}$) and recovery ($5.640 \pm 0.237 \text{ mmol.L}^{-1}$) groups (one-way ANOVA, $F(2, 10) = 14.84$, $p = 0.001$; Tukey post hoc, $p \leq 0.05$). Brain and muscle antioxidant activity (CAT, SOD), TBARs, muscle glucose oxidation, glycogen synthesis and plasma glucose remained unchanged across groups (Kruskal- Wallis, $p > 0.05$). These findings suggest that *B. pulchella* employs tissue-specific mechanisms to survive anoxia and recover, highlighting the modulation of anaerobic metabolism and the reliance on lactate during oxygen depletion episodes.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Ventilatory responses in heat dissipation in *Tropidurus catalanensis* (Squamata: Tropiduridae)

Authors:

Ane Guadalupe Silva¹

Melissa Bars-Closel¹

Luciane Helena Gargaglioni¹

Kênia Cardoso Bicego¹

Affiliations:

¹Universidade Estadual Paulista "Júlio de Mesquita Filho", Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal, Departamento de Morfologia e Fisiologia Animal

Keywords: Gaping. Lizards. Panting. Thermoregulation. Ventilation.

Abstract:

Thermoregulation is essential for animal survival, allowing adjustments in body temperature in response to environmental conditions. In lizards, heat dissipation by respiratory mechanisms may occur through panting and gaping, both contributing to brain cooling. Panting involves changes in ventilation, with increased respiratory rate and reduced tidal volume, while gaping consists only in sustained opened mouth, without notorious ventilatory changes. It is not yet clear whether lizards actually alter ventilation during heat dissipation, as occurs in birds and mammals, or whether they only perform gaping. To investigate this issue, we evaluated pulmonary ventilation during these behaviors in *Tropidurus catalanensis*. Ten individuals (5 males and 5 females) collected in São Simão-SP-Brazil, and maintained under controlled conditions were analyzed (SISBIO 94934-1; CEUA 3322/2024). The experimental protocol included gradual warming until panting and/or gaping occurred. Pulmonary ventilation was recorded by pneumotachography, evaluating respiratory rate (fR) and tidal volume (Vc) before and during these behaviors. Additionally, the thermal threshold for both mechanisms was also measured. Preliminary results indicate that *T. catalanensis* exhibits an altered ventilatory response during heat dissipation, characterized by a significant increase in respiratory rate and a reduction in tidal volume, consistent with panting. These findings suggest that *T. catalanensis* engages in panting, as observed in birds and mammals, in addition to gaping. This study contributes to the understanding of thermoregulation in lizards and highlights the complexity of ventilatory responses in the context of extreme environments.

Financial support: FAPESP (2021/10910-0) and CAPES (88887.962291/2024-00).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Functional and morphological characterization of the reproductive cycle of the rodent *Cerradomys goytaca*

Authors:

Gabriele Pinna Machado¹
Luzia Augusta Valeriano Monici¹
Carolina Augusta De Barros Silva¹
Pablo Rodrigues Gonçalves²
Jackson De Souza Menezes¹

Affiliations:

¹ Grupo de Pesquisas em Fisiologia Aplicada e Comparada, Laboratório Integrado de Ciências Morfofuncionais, Instituto de Biodiversidade e Sustentabilidade NUPEM, Universidade Federal do Rio de Janeiro, Macaé, RJ, Brazil.

² Laboratório Integrado de Zoologia, Instituto de Biodiversidade e Sustentabilidade NUPEM, Universidade Federal do Rio de Janeiro, Macaé, RJ, Brazil.

Keywords: *Cerradomys goytaca*. Estradiol. Estrous cycle. Morphometry. Reproduction.

Abstract:

Introduction: *Cerradomys goytaca* was discovered in 2011 in the Jurubatiba National Restinga Park. This species is endemic to the restingas of northern Rio de Janeiro. Its reproductive physiology remains unknown, making it essential to understand its reproductive cycle for species conservation. Methods: The experimental procedures were approved by CEUA-UFRJ (MACAE022 and 159/19) and ICMBio (SISBIO 80719-1). For the reproduction experiments, nine pairs of *C. goytaca* were randomly formed and kept together for seven days. The offspring from these pairs were sexed, and their weight, naso-anal length, and tail length were recorded. The estrous cycle was characterized through vaginal cytology in 10 females using rapid panoptic staining. Plasma estradiol concentration was measured using ELISA. Results were presented as mean $\pm$ standard error of the mean (SEM). Statistical analyses were conducted using Student's t-test to compare *Rattus norvegicus* and *C. goytaca*, while the Shapiro-Wilk test assessed data normality. Differences were considered significant at $p < 0.05$. Results: The vulva-to-anus distance in females is ~ 5 mm, while the penis-to-anus distance in males is ~ 16 mm. Growth between 7 and 21 weeks was similar between sexes ($p > 0.05$). The estrous cycle of *C. goytaca* lasts ~ 6 days, with four phases: metestrus, diestrus, proestrus, and estrus. The duration of each phase was: metestrus, proestrus, and estrus – 24 hours; diestrus – 48 hours. Estradiol concentration varied throughout the cycle, peaking during estrus (1244 ± 26.79 pg/mL). Conclusions: This study provides the first detailed description of the reproductive cycle of *C. goytaca*. These findings are essential for conservation strategies and the optimization of breeding practices in laboratory settings, contributing to future research on the species.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Electroretinography for Retinal Function Evaluation in Pediatric Patients with Nystagmus

Authors:

Milena Leal Borges¹
Andressa Zanini Fantato Quercia¹
Sung Eun Song Watanabe^{1,2}
Glacyelli Pereira Santos Mandaro de Assis¹
Evelyn Santana Benyhe¹
Paula Yuri Sacai^{1,2}

Affiliations:

¹ G6 Visual Electrophysiology Laboratory.
² Federal University of São Paulo.

Keywords: Visual Electrophysiology. Electroretinography. Pediatric Nystagmus. Retina.

Abstract:

The term nystagmus describes oscillatory, rhythmic, and repetitive eye movements associated with various ophthalmological conditions, such as congenital malformations, neuropathies, and retinal disorders, including Leber's Congenital Amaurosis and achromatopsia. These conditions pose a risk to visual development, making early detection essential. Pediatric examinations are challenging, and electrophysiology plays a fundamental role. Full-field electroretinography (ffERG) assesses retinal function and aids in the differential diagnosis of these diseases. This retrospective study analyzed ffERG results in children under seven years with nystagmus evaluated in a visual electrophysiology lab between January 2016 and December 2024, with approval from the Research Ethics Committee (protocol no. 7.316.114). Recordings were obtained using a monopolar contact lens electrode under topical anesthesia, without sedation, following the International Society for Clinical Electrophysiology of Vision criteria and Dark- adapted (DA) and Light-Adapted (LA) amplitude was analyzed. A total of 100 individuals (62% male) aged 0.19 to 6.79 years (mean 1.65 ± 1.67) were included. Delayed neuropsychomotor development was observed in 28 children. The causes of nystagmus were cone dystrophy (n=19), Leber's Congenital Amaurosis (n=14), cone-rod dystrophy (n=3), unspecified retinal dystrophy (n=10), albinism (n=4), ocular malformations (n=6), optic neuropathy (n=5), syndromes (n=5), neurological conditions (n=10), other causes (n=7) and unknown etiology (n=17). The ffERG showed results within normal limits in 33% of the patients and amplitude reduction (μV) in 67%, of whom 7 patients with DA reduced, 21 in LA, 19 in both, and 20 with non-detectable response. These results reinforce the importance of ffERG in pediatric nystagmus investigation, enabling early detection of disorders linked to retinal causes and visual development, assisting in diagnosis and clinical management.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Comparative transcriptomics of anurans' liver: Insights on hepatic metabolism

Authors:

Leandro Amaro da Silva Felix¹
Mariana Leivas Müller Hoff¹

Affiliations:

¹ Federal University of Pernambuco.

Keywords: Frogs. Hepatic physiology. RNA-Seq.

Abstract:

To study transcriptional regulation underpinning physiological responses in non-conventional animals is challenging. Unlike mammals, annotated nucleotide sequences for amphibians in the databases, such as the National Center for Biotechnology Information (NCBI), are scarce. Few RNA-Sequencing (RNA-Seq) data for anurans on NCBI are publicly available. However, a recent study generated RNA-Seq libraries of nine anuran species. The major biological processes and pathways in a given tissue can be inferred by the gene expression levels on its transcriptome. Therefore, this work aimed to assemble the transcriptomes from these nine RNA-Seq data of frog liver samples (PRJNA625657) and determine which are the major hepatic functions in Anura by comparing the gene expression levels among anurans and other vertebrate transcriptomes. RNA-Seq data were uploaded to Galaxy platform, checked for quality by FastQC, filtered by Trimmomatic, checked for quality again and the trimmed reads were used to generate transcriptomes by "de novo" assembly (Trinity). Transcriptomes consisted in a collection of contigs, representing the transcripts. Contigs are being annotated against the SwissProt database (Trinotate). Transcriptome assembly is used as reference to read mapping and counting, which result in gene expression levels, using Salmon. The most expressed contigs in *Pseudis minuta* liver were albumin, biliverdin binding serpin (BBS), vitelline membrane outer layer protein 1-like (VMO1), retinol-binding protein 4, apolipoprotein C-I-like, whereas mice liver expresses albumin, major urinary proteins 9 and 10 and apolipoproteins e and a2. Our preliminary results showed frog liver investment in the expression of BBS, involved in coloration, and VMO1, probably related to reproduction. Gene expression and gene ontology analyses for other species will be conducted and compared among frogs and between frogs and mammalians, elucidating the major hepatic functions in Anura.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Metabolic progression during embryonic development of the red-winged tinamous *Rhynchotus rufescens* (Palaeognathae)

Authors:

Carolina Martini de OLIVEIRA¹
Josineudson Augusto II de VASCONCELOS SILVA²
Ray Brasil BUENO DE SOUZA³
Wilfried KLEIN¹

Affiliations:

¹ Departamento de Biologia, FFCLRP, Universidade de São Paulo (USP), Ribeirão Preto, Brazil.

² Departamento de Melhoramento e Nutrição Animal, FMVZ, Universidade Estadual Paulista (Unesp), Botucatu, Brazil.

³ Programa de Pós-graduação em Biologia Comparada, Departamento de Biologia, FFCLRP, Universidade de São Paulo (USP), Ribeirão Preto, Brazil.

Keywords: Aves. Incubation. Carbon dioxide production. Oxygen consumption. Respirometry.

Abstract:

The Tinamidae family of birds is the only one within Palaeognathae able to fly, yet these animals have the smallest heart to body size ratio of all extant birds. This morphological discrepancy, when compared to other birds, however, is only observed two weeks after eclosion. No data are available regarding metabolic activity in embryonic stages nor hatchlings of *R. rufescens*, necessary to understand the development and function of the cardiorespiratory system in this species. This study aimed to measure oxygen consumption ($\dot{V} O_2$) and carbon dioxide production ($\dot{V} CO_2$) in *R. rufescens* embryos. Eleven eggs were subjected to closed flux respirometry at regular intervals during the 21 day incubation period, approved by the local ethics committee (CEUA/FFCLRP process nº 22.1.255.59.2). Results were normalized by egg mass and show that $\dot{V} O_2$ and $\dot{V} CO_2$ in this species increase exponentially throughout incubation, ranging from 0.002 mL/g.h and 0.004 mL/g.h at the second day of incubation to 0.45 mL/g.h and 49.32 mL/g.h, respectively, at the day before hatching, without ever reaching a plateau. Such a plateau has been observed during the final part of incubation in the domestic chicken, for example, and can be attributed to a limitation in gas exchange due to the eggshell acting as barrier for the diffusion of gases in larger embryos. Future studies quantifying the morphological diffusion capacity of the eggshell or measurements of gas concentrations in the air cell within the egg are needed to confirm a diffusion limitation of the eggshell.

This study was financially supported by FAPESP (2024/05178-7) and CNPq (302999/2022-1)



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Thermoregulatory Mechanisms in the Red Junglefowl, *Gallus gallus*: Metabolic and Ventilatory Adjustments to Cold

Authors:

Ray Brasil Bueno de Souza¹

Wilfried Klein²

Jordi Altimiras³

Affiliations:

¹ Programa de Pós-graduação em Biologia Comparada, Departamento de Biologia, FFCLRP, Universidade de São Paulo (USP), Ribeirão Preto, Brazil.

² Departamento de Biologia, FFCLRP, Universidade de São Paulo (USP), Ribeirão Preto, Brazil.

³ Department of Physics, Chemistry and Biology, Linköping University (LiU), Linköping, Sweden.

Keywords: Barometric method. Birds. Respirometry. Temperature.

Abstract:

The thermoregulatory mechanisms of birds are critical for maintaining homeostasis under thermal extremes, yet their metabolic and ventilatory responses to cold conditions remain incompletely understood. This study aimed to investigate the physiological responses of the Red Junglefowl (*Gallus gallus*), the proposed ancestor of domesticated poultry, when exposed to low ambient temperatures. Ten adult males were subjected to a flow-through respirometry experiment combined with the barometric methods for ventilatory measurements to assess changes in oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), respiratory quotient (RQ), and ventilatory parameters during a gradual exposure to decreasing temperatures, ranging from 22°C to -13°C in a 12 hours overnight experiment. Ethical approval for this study was granted by the local Swedish ethics committee (Dnr 04127-2024). The results demonstrated a threefold increase in oxygen consumption during cold exposure compared to resting conditions, indicating heightened metabolic activity to sustain thermogenesis. A significant reduction in RQ from 0.88 to 0.66 suggested a metabolic shift toward lipid oxidation as the primary energy source under cold stress. Ventilatory adjustments included a 1.7-fold increase in tidal volume (V_T) and a doubling of minute ventilation ($\dot{V}_E$), highlighting enhanced respiratory effort to support elevated metabolic demands. These findings provide valuable insights into the cold-induced metabolic adjustments of the Red Junglefowl, demonstrating its capacity for robust thermogenic and ventilatory plasticity. This study contributes to our understanding of avian thermoregulation and highlights the Red Junglefowl as a model for investigating the evolutionary basis of metabolic and thermoregulatory mechanisms in birds.

This study was financed by FAPESP (2024/02773-1) and CNPq (302999/2022-1).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Investigating seasonal variation in mitochondrial function of erythrocytes in the tegu lizard *Salvator merianae*

Authors:

João Vitor Paris-Xavier¹
Derek Felipe Campos¹
Luciane Helena Gargaglioni¹
Kênia Cardoso Bicego¹

Affiliations:

¹ Universidade Estadual Paulista (UNESP), Campus de Jaboticabal.

Keywords: Erythrocytes. Mitochondria. Reptiles. Oxidative phosphorylation. Thermal physiology.

Abstract:

Oxidative phosphorylation is the primary process for ATP production, using the energy from substrate oxidation. Erythrocytes in non-mammalian vertebrates retain their mitochondria, which play a crucial role in oxygen transport and heat production. Thus, we investigated the mitochondrial function in the erythrocytes of the lizard *Salvator merianae*, which undergoes metabolic changes throughout the year, dormancy in winter and reproductive endothermy in spring. Tegus use mitochondrial function to maintain reproductive endothermy, increasing mitochondrial oxygen consumption and mitochondrial density in skeletal muscle. However, the role of erythrocytes in seasonal plasticity has not been explored. We aimed to evaluate mitochondrial respiration, uncoupling, and oxidative phosphorylation efficiency in erythrocytes during the different seasons of the year, concomitant with metabolic changes in tegus. Blood samples (3ml) were collected from 10 tegus (1.0–4.0kg) in December and February, both post-reproduction (CEUA protocol 4743/2024; SISBIO 67600-2). Mitochondrial function in erythrocytes, such as respiration and uncoupling, was assessed in a high-resolution oxygraph (Oroboros). Statistical analysis compared mitochondrial variables between sexes. No significant differences were found for Routine ($P = 0.22$) and LEAK ($P = 0.28$) respiration, or RCR (respiratory control ratio; $P = 0.49$). However, the electron transport system (ETS) showed a significant difference ($P = 0.017$), with females having higher mean values ($36.00 \text{ pmol O}_2 \cdot \text{h}^{-1} \cdot \text{ml}^{-1}$) than males ($26.27 \text{ pmol O}_2 \cdot \text{h}^{-1} \cdot \text{ml}^{-1}$). No significant difference was observed for any variable. No significant seasonal variations were detected, and individual variations over time did not impact overall results. These preliminary results indicate sex-specific mitochondrial function in the erythrocytes of tegus even after reproduction season. A general conclusion will be possible after the completion of the measurements in the other seasons.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Development and structure of the respiratory system in the lizard *Tropidurus catalanensis* Gudynas & Skuk, 1983 (Squamata, Tropiduridae)

Authors:

Gabriela Covulo¹

Barbara de Godoi Champini²

Wilfried Klein¹

Affiliations:

¹ Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto (FFCLRP), Universidade de São Paulo (USP), Ribeirão Preto, Brazil.

² Programa de Pós-graduação em Biologia Comparada, Departamento de Biologia, FFCLRP, Universidade de São Paulo (USP), Ribeirão Preto, Brazil.

Keywords: Squamata; Respiratory System; Histology

Abstract:

Reptiles are a group of vertebrates, whose diversity is reflected in morphological and physiological structures, such as the respiratory system. The lung can be characterized in uni-, pauqui-, and multicameral, with three types of gas-exchange tissue - trabecular, edicular and faveolar - that can be homogeneous or heterogeneously distributed. Although the macrostructure of the respiratory system of some species of lizards have been described, others lack studies such as the Tropiduridae family, infraorder Iguania. The present research project aims to morphologic, macroscopic and histologic describe the lungs of this lizard, comparing different stages of development - embryos, neonates and adults. The individuals used were obtained from the Herpetological Collection of Ribeirão Preto. The two embryonic stages (~20 and 40 dpo) were digitized using computed microtomography with subsequent digital segmentation, while the neonates and adults were dissected and the respiratory system was removed; histological analyses were, later, performed on these individuals. Preliminary results indicated the presence of a unicameral lung, with dorsal niches, mainly noted in the embryos, and faveolar parenchyma already present in the 40 dpo embryo. Unlike what is found in the literature for the Iguania clade, with the pauquicameral lung type predominating in the families Agamidae, Chamaeleonidae, and Iguanidae, the Tropiduridae shows a unicameral lung type with heterogeneously distributed faveolar parenchyma. Therefore, it is recommended to expand the analysis of more Tropiduridae species to understand which lung type predominates in the family.

This study was financially supported by CNPq (PIBIC:2024-1419 e 302999/2022-1).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: The relationship between structure and function of the avian respiratory system

Authors:

Giovana Rodrigues Portari¹
Ray Brasil Bueno de Souza¹
Wilfried Klein¹

Affiliations:

¹ Departamento de Biologia, FFCLRP, Universidade de São Paulo (USP), Ribeirão Preto, Brazil.

Keywords: Birds. Compliance. Mechanics. Respiratory.

Abstract:

Several aspects of the avian respiratory system are still poorly studied, especially from a comparative perspective and when regarding Neotropical species. The present project proposes to study the morphology and mechanics of the avian respiratory system in order to fill these gaps, through a thorough review of the existing literature, as well as analyzing the respiratory system mechanics of the red-winged tinamou *Rhynchotus rufescens* (inhambu-perdiz). Twelve individuals, with approval by the local ethics committee (CEUA/FFCLRP: 22.1.255.59.2), were submitted to standard procedures regarding breathing mechanics analysis. Volume-pressure curves were constructed in static conditions. These data were recorded using LabChart software (ADInstruments) and were analyzed to determine lung volumes and static compliance, using the formula $C = \Delta V / \Delta P$, where C (mL/cmH₂O) represents static compliance, ΔV (mL) the difference between volumes and ΔP (cmH₂O) the difference between pressures. Results found a maximum respiratory system volume of 108.33 ± 19.12 mL, an inflation C of 7.33 ± 2.09 mL/cmH₂O and a deflation C of 7.14 ± 1.45 mL/cmH₂O, considering a mean body mass $\pm$ SD of 0.69 ± 0.08 kg. Our data were compared with those of other species, showing variation among different bird taxa, which may have resulted from different methods applied, or may be due to morphological differences in the respiratory system among birds. Despite the limited amount of data available, the information allows for an initial comparison of mechanical properties among birds, as related to high-altitude adaptations or poor flight capacity.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Comparative analysis of air-breathing frequency (ABF) and critical thermal maximum (CT_{max}) in relation to physiological and behavioral responses between cave-dwelling and surface-dwelling armoured catfish species of the genus *Ancistrus*

Authors:

Leonardo de Assis¹
Derek Felipe Campos¹
Sofia Lopes Basílio da Silva Matos^{1,2}
Kênia Cardoso Bicego¹
Maria Elina Bichuette²
Luciane Helena Gargaglioni¹

Affiliations:

¹ São Paulo State University, FCAV/UNESP, Jaboticabal – SP.

² Federal University of São Carlos, UFSCar, São Carlos – SP.

Keywords: Air breathing. Cave fish. Hypoxia. Physiological adaptation. Thermal tolerance.

Abstract:

The combination of air breathing frequency (ABF) and critical thermal maximum (CT_{max}) is key to understanding the adaptive strategies of bimodal-breathing fish facing environmental changes. We assessed this relationship in *Ancistrus cryptophthalmus* (troglobitic, endangered) and *Ancistrus* sp. (epigean), focusing on physiological and behavioral responses to thermal and hypoxic stress. Fish were acclimated for 12 h and exposed to O₂ levels of 100%, 50%, 25%, and 5%. ABF was recorded with an infrared camera in a chamber equipped with a pneumotachograph, before and after the agitation temperature (Tag). Water started at 22.5 °C and was heated at 0.2 °C/min. Permits: SISBIO nº 90904-1 and CEUA nº 9520/23. Results showed both groups increased ABF as O₂ dropped, but troglobites had higher ABF even under normoxia (5.76 vs. 1.21 breaths.min⁻¹). Under severe hypoxia (5%), epigeans increased ABF by 18x and troglobites by 4.5x, indicating epigeans change their respiratory pattern more drastically. In the ABF+CT_{max} protocol, epigeans had higher CT_{max} (36.08 °C vs. 34.45 °C), though gill ventilation was similar between groups. ANOVA revealed that both species ($p=0.0002$) and O₂ levels ($p=0.0021$) influenced CT_{max}, with no interaction ($p=0.2455$). No significant correlation was found between CT_{max} and ABF ($p=-0.4286$, $p=0.2992$) or gill ventilation ($p=0.3810$, $p=0.3599$). PCA revealed clear group separation: epigeans were linked to higher CT_{max} and gill ventilation; troglobites, to elevated ABF before (18.25 vs. 1.75) and after hypoxia. We conclude that troglobitic and epigean *Ancistrus* adopt distinct strategies: epigeans show greater thermal resistance and efficient gill ventilation, while troglobites, adapted to hypoxic cave environments, maintain a high baseline ABF and increase it moderately under stress. Understanding these mechanisms is vital for predicting fish responses to climate change and informing conservation strategies.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Morphological diffusion capacity of the eggshell in the red-winged tinamous *Rhynchotus rufescens* (Palaeognathae)

Authors:

Carolina Martini de Oliveira¹

Josineudson Augusto II de Vasconcelos Silva²

Wilfried Klein¹

Affiliations:

¹ Departamento de Biologia, FFCLRP, Universidade de São Paulo (USP), Ribeirão Preto, Brazil. ² Departamento de Melhoramento e Nutrição Animal, FMVZ, Universidade Estadual Paulista (Unesp), Botucatu, Brazil.

Keywords: Aves. CT-Scan. Diffusion distance. Tinamidae.

Abstract:

The shell of an avian egg consists of a calcareous structure composed of calcium carbonate crystals with irregularly distributed pores for gas exchange. Gas exchange and evaporative water loss occur mainly by diffusion and can be calculated using Fick's Law. To this end, one needs to know the physical dimensions of the pores. These data are not yet available for the eggs of *R. rufescens* and were the objectives of this study. Eleven eggshells were obtained after hatching and images were obtained from three regions of eight eggs, the two poles (blunt pole - BP and sharp pole - SP) and the one equidistant between them (equatorial region - ER). The eggs were obtained after the chicks hatched and the samples were scanned using μ CT of the Biodiversity Documentation Center (FFCLRP). Shell fragments were washed with running water, dried for 24 h, and then inserted into microcentrifuge tubes. Images were processed using Avizo 3D software. Animal use was approved by the ethics committee (CEUA/FFCLRP nº 22.1.255.59.2). The pores were unevenly distributed, being more numerous on the BP (0.89 pores/mm²), than SP (0.56 pores/mm²), and ER (0.30 pores/mm²). Pore diameter was similar in all regions (BP 21.1 μ m, SP 19.3 μ m, ER 18.9 μ m), while pore length was lower in the SP region (124.3 μ m), when compared to BP (159.6 μ m) and ER (155.8 μ m) regions. A pore density of about 1 pore/mm² in the blunt region of an egg is important to increase gas exchange in the late embryo, since during incubation an air bubble forms below the blunt pole due to evaporative water loss. The increased diffusion velocity of oxygen and carbon dioxide in air, contributes to maintain embryonic gas exchange despite increasing metabolic demands of the growing chick.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Mitochondrial responses to nutritional and immunological challenges in Japanese quail

Authors:

Livia Saccani Hervas¹
Eskil Elmer²
Kênia Cardoso Bicego¹
Andreas Nord²

Affiliations:

¹São Paulo State University
²Lund University

Keywords: Mitochondria, fasting, immune response

Abstract:

Mitochondria play a central role in essential biological processes, including metabolism, immune activation, and thermoregulation. Nutritional status directly affects ATP production, with fasting inducing metabolic suppression and potentially enhancing mitochondrial efficiency, often accompanied by increased reactive oxygen species (ROS). In contrast, infections increase the demand for both ATP and ROS to sustain immune responses. These opposing pressures suggest a trade-off: energy-saving strategies during fasting may limit the organism's ability to mount effective immune responses due to reduced mitochondrial output. This study aimed to investigate how fasting and immune activation interact to influence mitochondrial function in a non-mammalian vertebrate model, the Japanese quail (*Coturnix japonica*). Animals were divided into two groups and fasted for four days prior to receiving an intramuscular injection of lipopolysaccharide (LPS; 1 mg/kg). Blood samples were collected, and mitochondrial respiration was analyzed at 5 and 24 hours post-injection. After euthanasia, liver samples were also analyzed. All experiments were approved by the local ethics committee. Body temperature was recorded throughout the experiment. LPS induced a rapid reduction in body temperature, triggering hypothermia, while fasting alone had no effect ($p < 0.05$). Mitochondrial respiration in erythrocytes were reduced by 15% ($p < 0.05$) on both fasting and LPS independently, with no additive effect. This reduction appeared at 5 hours and persisted at 24 hours. No significant changes were found in liver samples. Our findings suggest that while both fasting and immune activation individually suppress mitochondrial function in blood cells, their combined effects do not exacerbate this suppression. Furthermore, these effects appear to be tissue-specific, highlighting the complexity of mitochondrial responses under competing physiological demands.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Evaluation of Evoked Visual Potentials in children with tumors affecting the visual pathway

Authors:

Evelyn Santana BENYHE¹
Andressa Zanini Fantato QUERCIA¹
Sung Eun Sung WATANABE^{1,2}
Glacyelli Pereira Santos Mandaro de ASSIS¹
Milena Leal BORGES¹
Paula Yuri SACAI^{1,2}

Affiliations:

¹ G6 Electrophysiology Laboratory.
² Federal University of São Paulo

Keywords: Children. Tumors. Visual electrophysiology. Visual Evoked Potential. Visual Pathway.

Abstract:

Optic pathway tumors in children represent a heterogeneous group of neoplasms affecting different segments of the visual system. Pattern-reversal visual evoked potentials (PR-VEP) and sweep visual evoked potentials (Sweep-VEP) assess the function of the visual pathway, providing essential information for diagnosis, lesion progression, and therapeutic follow-up. This retrospective study analyzed VEP results in children under 5 years old with optic pathway and central nervous system tumors evaluated between August 2013 and February 2025, with approval from the Research Ethics Committee (n. 7.316.114). All examinations followed the protocol of the International Society for Clinical Electrophysiology of Vision (ISCEV) with appropriate optical correction. The sample included 49 children (59% female), aged between 5 months and 5 years 10 months (mean 2.75 ± 1.84 years). The most frequent tumors were glioma (39%), astrocytoma (18%), craniopharyngioma (10%), and medulloblastoma (8%) among others. Lesion locations included the optic chiasm (39%), central nervous system (27%), optic nerve (18%), retrochiasmatic region (4%), adjacent to the chiasm (6%), and 4% unspecified. Among the participants, 16 underwent PR-VEP, 16 Sweep-VEP, and 17 both. In Sweep-VEP, 94% showed reduced visual acuity, and 42% had significant interocular difference (>0.2 log units). Of the 66 eyes assessed by PR-VEP, 29 (44%) showed delayed P100 and reduced amplitude, 11 (17%) reduced amplitude, 10 (15%) delayed latency, and 7 (11%) undetectable response. Nine eyes (14%) were normal in patients with unilateral optic nerve tumors (5), pineal tumor (2) and medulloblastoma (2). These findings emphasize the clinical challenge posed by optic pathway tumors in children due to their heterogeneity and impact on visual development. PR-VEP and Sweep-VEP proved to be fundamental tools for evaluating visual pathway function and supporting lesion monitoring and therapeutic decisions.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Descrição de espécie inédita do gênero *Loksaphorus* Hirschmann, 1979 (Acari: Uropodina: Eutrachytidae)

Authors:

Sofia Refrande de Souza^{1,2,3}

Ademar Ferreira da Silva¹

Ingrid Benevides Machado¹

Affiliations:

¹ Laboratório de Carrapatos e Outros Artrópodes Ápteros – LAC, IOC/FIOCRUZ.

² Programa de Vocação Científica – PROVOC/FIOCRUZ.

³ Escola Politécnica Joaquim Venâncio - EPSJV/FIOCRUZ.

Keywords: Ácaro. *Check list*. Mesostigmata

Abstract:

Os ácaros da Família Eutrachytidae pertencem à coorte Uropodina. Esses ácaros são comumente encontrados em matéria orgânica em decomposição. Embora tais ácaros exerçam papel de suma importância para o equilíbrio das populações da acarofauna, há, no entanto, diversas lacunas taxonômicas a estes relacionados. Neste contexto, estão incluídas as espécies pertencentes à Família Eutrachytidae, sobre as quais, é incipiente o estudo relacionado à descrição de novas espécies. Assim, o presente estudo visa prover um *Chek list* atualizado das espécies do gênero *Loksaphorus* e descrever espécie inédita para este mesmo gênero, por meio de exemplares coletados no período de janeiro a novembro de 2013 em matéria orgânica de solo, no município do Rio de Janeiro - RJ, Brasil. Foram realizadas pesquisas de artigos de descrição de espécies da família Eutrachytidae e gênero *Loksaphorus*, posteriormente elaboradas ilustrações da espécie inédita com base no futuro holótipo. Seguidamente, foram nomeadas as cerdas presentes no corpo do ácaro, diferindo-o da espécie tipo do gênero (*Loksaphorus loksaisimilis*), outrora um gênero monotípico. Recentemente, Kontschán em 2024 realocou duas espécies do gênero *Deraiphorus* para *Loksaphorus*, o qual passa a reunir as seguintes espécies: *Loksaphorus loksai*, *Loksaphorus loksaisimilis* e *Loksaphorus orghidani*. Dessa forma, aqui são apresentados os caracteres de discriminação de *Loksaphorus* sp. nov. em relação *L. loksaisimilis*. *Loksaphorus* sp. nov. ostenta cerdas *ad1*, *ad2* e *ps* diferindo de *L. loksaisimilis* com a combinação *ad1* e *ad2*, somente. Além disso, *L. loksaisimilis* apresenta cerda *st4* posicionada na margem da base da placa genitoventral, enquanto *Loksaphorus* sp. nov. ostenta cerda *st6* abaixo da placa genitoventral, sendo antecédida por um poro, posicionado na margem da base da placa. Posteriormente, as duas espécies recentemente transferidas para o gênero *Loksaphorus*, também terão seus caracteres considerados para comparação com *Loksaphorus* sp. nov.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Effect of varespladib on the reversal of crotoxin-induced neuromuscular blockade

Authors:

Elisa Santiago Pereira¹ Leticia Santos Bueno¹ Paula Ladeira Ortolani²
Consuelo Latorre Fortes Dias² Walter Luís Garrido Cavalcante¹

Affiliations:

¹ Departamento de Farmacologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais (UFMG), Brasil.

² Centro de Pesquisa e Desenvolvimento, Fundação Ezequiel Dias (FUNED), Brasil.

Keywords: Crotoxin. *Crotalus durissus terrificus*. Neuromuscular Junction. Varespladib. Venom

Abstract:

Snakebite envenomation is a neglected disease that primarily affects economically vulnerable populations. Despite the efficacy of antivenom, its limited availability and adverse reactions highlight the need for complementary treatments. This study evaluated varespladib, a synthetic molecule, in reversing the neuromuscular blockade induced by crotoxin (CTX, from *Crotalus durissus terrificus* venom) in mouse phrenic nerve-diaphragm preparations (CEUA 25/2021). The preparations were maintained in Ringer's solution (carbogen, 35°C). Myographic recordings used indirect electrical stimulation (0.5 ms, 0.2 Hz, 3 V). Three protocols were conducted: (a) CTX (5 µg/mL) followed by varespladib (5 µg/mL) at 10, 30, or 60 min (n=4-7); (b) CTX with washing at 30 or 60 min (n=5-8); (c) CTX, washing (30/60 min), followed by varespladib (n=3-7). Morphological analysis assessed tissue integrity and myonecrosis index (n=4-5). The data (mean) were analyzed by Student's t-test and ANOVA (p<0.05). CTX caused an irreversible blockade, promoting 96% inhibition of twitches at 180 min (p<0.01 vs. control). Varespladib prevented the blockade when administered at 10 minutes after CTX (p>0.05 vs. control), showing partial effects at 30 and 60 minutes with 42% and 33% inhibition, respectively (p<0.01 vs. control). Washing performed 30 minutes after CTX exposure prevented blockade progression, maintaining 50% contractile activity, while at 60 minutes the blockade was irreversible (p<0.01 vs. control). Varespladib administered after washing promoted the reverse of blockade (100% recovery) at both 30 and 60 minutes (p>0.05 vs. control). Therefore, Varespladib prevented and reversed CTX-induced blockade without causing direct muscular effects, demonstrating its potential as a complementary or first-aid therapy in snakebite envenomations.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Gestational Exposure to Low Doses of Glyphosate-Based Herbicide Induces Transient Neurodevelopmental Delays in Rats Offspring

Authors:

Victoria C Malinski-Nery¹ Aline Barbosa Lima¹ Natalia S Tonet¹ Morgana Contini¹
Alex Rafacho¹

Affiliations:

¹ Laboratory of Investigation in Chronic Diseases- LIDoC, Department of Physiological Sciences, Center of Biological Sciences (CCB), Federal University of Santa Catarina (UFSC), Florianópolis, Brazil.

Keywords: Glyphosate. Developmental Milestones. Behavioral Assessments. Endocrine-disruptors.

Abstract:

Glyphosate, the active ingredient in glyphosate-based herbicides (GBHs), is widely used and may pose health risks during gestation due to its endocrine-disrupting properties. However, data on the effects of low-dose GBH exposure during pregnancy on offspring (neuro)development remain scarce. This study aimed to evaluate whether environmental doses of GBH administered during gestation affect (neuro)developmental outcomes in rats. Pregnant 90-day-old Wistar rats (n=12 per group) received daily orogastric doses of either 0.5 mg/kg or 50 mg/kg of RoundUp Mais® (GBH 0.5 and GBH 50) or water (control) throughout gestation. After birth, offspring were assessed for (neuro)developmental milestones, including fur growth, incisor eruption, eye opening (days), righting reflex, negative geotaxis, grasp reflex, and startle reflex (seconds). Data were analyzed using ANOVA with Tukey's post hoc test ($p < 0.05$). All procedures were approved by CEUA-UFSC (protocol no. 3797220921). GBH exposure did not significantly affect fur growth, incisor eruption, eye opening, grasp, or startle reflexes. However, on postnatal day 5, increased righting reflex latency was observed in GBH

50 females (8.5 ± 7.8 s) and GBH 0.5 males (6.8 ± 8.9 s) compared to controls (females: 3.4 ± 3.5 s; males: 3.5 ± 3.6 s). No differences were observed in GBH 50 males or GBH 0.5 females. Additionally, negative geotaxis latency was higher on day 7 in GBH 50 females but normalized in subsequent evaluations. We conclude that gestational exposure to low doses of GBH induced only mild and transient alterations in neurodevelopmental reflexes. These early delays did not persist but given the role of neonatal reflexes as indicators of brain maturation, further studies are needed to assess potential long-term neurodevelopmental effects.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Ruthenium (II) complex with venilpyridine induces oxidative stress, cell cycle arrest and apoptosis in melanoma cells

Authors:

Guilherme Álvaro Ferreira-Silva¹ Yasmin dos Santos Louzano¹ Henrique Vieira Reis Silva²
Marília Imaculada Frazão Barbosa² Antônio Carlos Doriguetto²
Marisa Ionta¹

Affiliations:

¹ Laboratory for Evaluation of antitumor Prototypes, Institute of Biomedical Sciences, Federal University of Alfenas, Alfenas-MG, Brazil.

² Institute of Chemistry, Federal University of Alfenas, Alfenas-MG, Brazil.

Keywords: Apoptosis. Melanoma. Oxidative stress. Ruthenium(II) complex

Abstract:

Melanomas are aggressive malignant tumors of the skin which cause approximately 60,000 deaths per year worldwide. Current therapeutic approaches are partially effective for melanoma, especially for advanced cases, and the low efficacy of the drugs is closely associated with tumor resistance. Thus, the search for new anticancer drugs is continuous to improve melanoma treatment strategies, and our laboratory has explored the antitumor potential of ruthenium(II) complexes. In the present study, ruthenium complexes harboring different pyridines (named C1, C2 and C3) were assayed against cells derived from melanoma (CHL-1 and SK-MEL-147). The cell viability was assessed by sulforhodamine B assay; while cell cycle analysis, pro-apoptotic and pro-oxidant activities were investigated by flow cytometry. Protein expression profiles were assessed by immunocytochemistry and western blot. The experiments were performed in triplicate, and statistical analyses were conducted using GraphPad Prism 8.0 software. Since the data were considered parametric, ANOVA followed by Dunnett post-test or t-student test were applied. CHL-1 cell line was more responsive to the treatments compared to SK-MEL-147. IC₅₀ values were 8.37, 10.59, and 9.16 μ M to C1, C2, and C3, respectively. Despite the high cytotoxicity of these metallic complexes on CHL-1 cells, their effects were minimal on primary skin fibroblasts (FPM). The CHL-1 cultures treated with C1 and C3 exhibited increased frequencies of Sub-G1 population. We demonstrated that the cytotoxic effects of the C1 and C3 were associated with their ability to induce oxidative stress and, in turn, apoptosis. The reactive species of oxygen levels were higher in CHL-1 cultures treated with C3. Our findings indicate that nitrosyl ruthenium (II) complexes containing pyridine and venilpyridine have great cytotoxic activity on CHL-1 cells and represent promising prototypes for further investigations.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Antihypertensive effects of the aqueous extract (AE) of the leaves of *Saccharum officinarum* L. (sugarcane) in a model of primary (SHR) and secondary (2K1C) hypertension

Authors:

Giovanna Cartillone dos Santos¹ Augusto Luis Salsa Vasquez Duarte² Juliana Corsini Zirolodo³
Maria Thereza Gamberini⁴

Affiliations:

Faculdade de Ciências Médicas da Santa Casa de São Paulo.

Abstract:

Systemic arterial hypertension (SAH) is classified as primary or secondary associated with endocrine and non-endocrine diseases, such as renovascular hypertension. A previous study demonstrated the hypotensive potential of an aqueous extract (AE) from sugarcane (*Saccharum officinarum* L.) leaves in normotensive rats, yet its effects in hypertensive animals remain unconfirmed. Thus, this study aimed to evaluate the antihypertensive responses of AE in spontaneously hypertensive rats (SHR; a primary hypertension model) and in rats with renovascular hypertension (two-kidney, one-clip Goldblatt model: 2K1C).

Methods: Systolic (SBP), diastolic (DBP), and mean arterial pressure (MAP) were monitored in SHRs and Wistar rats subjected to left renal artery occlusion with a 0.2-mm silver clip 2K1C, as well as in sham-operated and non-operated Wistar rats, using tail plethysmography. After stabilization, the animals received a single oral dose of water (1.5 mL/kg; control group) or AE (0.5, 1, or 10 mg/kg). The data were expressed as mean±SEM and analyzed by ANOVA followed by Tukey's test ($p<0.05$) (CEUA nº 2023/02). **Results:** In the SHR, AE (1 mg/kg, $n=11$) reduced SBP by 10% and MAP by 8% compared to controls (SBP 189.4 ± 1.5 mmHg, MAP 127.8 ± 2.3 mmHg, $n=9$). In the 2K1C protocol, after 5 weeks, baseline values were SBP 148.0 ± 1.8 mmHg, DBP 95.8 ± 1.5 mmHg, and MAP 113.2 ± 1.2 mmHg ($n=22$). AE (1 mg/kg) reduced SBP by 9%, while all doses lowered DBP for approximately 14%. MAP decreased for approximately 12% with AE (1 and 10 mg/kg). No changes were observed in sham-operated rats. In non-operated animals, AE (1 mg/kg, $n=4$) reduced SBP by 13% versus controls (142.5 ± 4.8 mmHg, $n=4$). **Conclusion:** This study demonstrates the antihypertensive effect of AE in both primary and secondary hypertension models, supporting further investigation into its mechanism of action and the identification of sugarcane's active compound(s).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: GlyT1 inhibition promotes neuroprotection in the middle cerebral artery occlusion model through the activation of GluN2A-containing NMDAR

Authors:

Daniel Pereira Cavalcante¹ Antonio Ítalo dos Santos Nunes¹ Eduardo Rosa da Silva¹
Gustavo Almeida de Carvalho¹ Mauro Cunha Xavier Pinto¹

Affiliations:

¹ Department of Pharmacology, Institute of Biological Sciences, Federal University of Goiás, Goiânia, GO, Brazil.

Keywords: Ischemia. NMDA receptors. Neuroprotection.

Abstract:

Cerebral ischemia, characterized by impaired blood flow to the brain, often results in cognitive and motor decline. Glycine and GlyT1 neuroprotection can be related to glutamatergic neurotransmission regulation and NMDA receptors. Objective: investigate the potential neuroprotective effect of GlyT1 blockade and identify the underlying mechanisms in mouse models of cerebral ischemia. Methods: The study utilized C57BL/6 mice, treating them with various compounds including NFPS (a GlyT1 inhibitor), Eliprodil, and TCN-201 (NMDAR inhibitors), and C646 (a CREB inhibitor). The treatment was conducted 24 hours before inducing ischemia using the middle cerebral artery occlusion (MCAO) model. Three days post-induction, motor deficits were evaluated with the cylinder test (CT) and limb clasp (LC), while ischemia size was assessed using triphenyltetrazolium chloride (TTC) staining and proteins expression was evaluated by western blot. The mice were divided into five groups (Sham, MCAO, NFPS or NFPS/Inhibitor, Inhibitor) with 8 animals per group. Data was analyzed using one-way ANOVA. All procedures were approved by the Ethics Committee on Animal Use (Protocol No. 041/19). Results: The study found that NR2B NMDAR inhibitors effectively decreased motor deficits in CT, LC ($81.2 \pm 4.8\%$; 1.6 ± 0.9) and ischemia size ($16.1 \pm 6.1\%$). However, in CT, LC and ischemia size the inhibition of NR2A NMDAR ($76.4 \pm 5.3\%$; 2.0 ± 0.8 ; $16.8 \pm 4.9\%$, respectively) or the CREB protein ($79.1 \pm 8.6\%$; 2.4 ± 0.7 ; $22.1 \pm 7.9\%$, respectively) did not show any significant effects on these parameters. Interestingly, the neuroprotective effect of NFPS was affected in the presence of NR2A NMDAR and CREB inhibitors, but not in the presence of NR2B NMDAR inhibitors. Conclusion: These findings suggest that GlyT1 inhibition mediates neuroprotection through activation of GluN2A-containing NMDARs and the GluN2A/CaMKIV/CREB signaling cascade, thereby modulating the balance between GluN2A and GluN2B subunits.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Neurorepair Induced by treatment with Sarcosine in an Animal Model of Cerebral Ischemia

Authors:

Antonio Ítalo dos Santos Nunes¹ Ingrid Martins Faria¹
Joaquina Victoria Teodoro Muassabo¹ Amanda Rodrigues Quintanilha¹ Eduardo Rosa da Silva¹
Daniel Pereira Cavalcante¹ Gustavo Almeida de Carvalho¹ Mauro Cunha Xavier Pinto¹

Affiliations:

¹ Department of Pharmacology, Institute of Biological Sciences, Federal University of Goiás, Goiânia, GO, Brazil.

Keywords: Glycine transporter type 1. NMDA receptor. Stroke.

Abstract:

Ischemic stroke involves excitotoxic mechanisms mediated by excessive glutamate release and NMDAR overactivation. Therapeutic strategies targeting neural repair after the ischemic event are essential for functional recovery. Sarcosine, a GlyT1 inhibitor, modulates NMDARs and may promote neuroplasticity and regeneration following ischemic injury. Objective: To evaluate the reparative effects of sarcosine using a treatment regimen in a mouse model of permanent middle cerebral artery occlusion (MCAO). Methods: Male Swiss mice (8 weeks old) were subjected to permanent MCAO and subsequently treated with sarcosine (125, 250, and 500 mg/kg) for five consecutive days. The ischemic region was analyzed using 2% TTC staining. Functional assessment was performed using the Cylinder Test and the Limb Clasping Test. Five experimental groups were included (n = 9 per group). One-way ANOVA was used for statistical analysis. The study protocol was approved by the Animal Ethics Committee (Protocol No. 041/19). Results: Sarcosine treatment promoted significant reparative effects, improving motor performance and postural stability. A significant reduction in infarct volume was observed, particularly with the 500 mg/kg dose ($104.4 \pm 2.3\%$; 0.62 ± 0.26 ; $09.13 \pm 1.92\%$, respectively). Conclusion: Post-ischemic administration of sarcosine facilitated neural repair and functional recovery in a murine model of stroke, indicating its therapeutic potential in promoting regeneration after ischemic injury. GlyT1 inhibition represents a promising target for post-stroke interventions.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Neurorestorative effect of a new L-proline transporter inhibitor (MCX13) in an animal model of cerebral ischemia (MCAO)

Authors:

João Elias Pinheiro Sousa de Paula¹ Gustavo Almeida de Carvalho^{1,2} Daniel Pereira Cavalcante¹
Lucas Rodrigues Couto Nascimento¹ Antonio Ítalo dos Santos Nunes¹ Amanda Rodrigues Quintanilha¹ Onésia Cristina de Oliveira-Lima¹ Mauro Cunha Xavier Pinto¹

Affiliations:

¹ Departamento de Farmacologia, Instituto de Ciências Biológicas, Universidade Federal de Goiás, Goiânia - GO, Brazil.

² Centro Universitário de Mineiros - UNIFIMES - Campus Trindade, Goiânia - GO, Brazil.

Keywords: Discovery of new drugs. L-proline transporter. PROT/SLC6A7. Stroke.

Abstract:

Cerebral ischemia is a serious neurological disorder associated with high mortality and long-term disability. A common mechanism involved in cerebral ischemia and other neurological conditions is excitotoxicity, making the modulation of glutamatergic neurotransmission a relevant pharmacological target. This study investigated the neurorestorative effect of MCX13, a selective inhibitor of the L-proline transporter PROT/SLC6A7, in a mouse model of middle cerebral artery occlusion (MCAO). C57BL/6 mice were subjected to MCAO and treated with MCX13 (1.25, 2.5, or 5 mg/kg) for five consecutive days. Motor performance was assessed using Limb Clasping and Cylinder tests, and infarct volume was analyzed via TTC staining (n = 10 per group). All procedures were approved by the Institutional Animal Ethics Committee (Protocol no. 059/24). In silico analysis showed a Gibbs free energy of binding of -7.707 kcal/mol for the PROT/MCX13 interaction, similar to LX6171 (-8.063 kcal/mol). Pharmacokinetic predictions indicated the ability of MCX13 to cross the blood-brain barrier, and molecular dynamics simulations confirmed complex stability through RMSD, RMSF, and SASA parameters. MCX13 treatment significantly reduced infarct size, particularly at 5 mg/kg ($4.70 \pm 0.91\%$), and improved motor performance in the open field test at 5 mg/kg (421.9 ± 13.56). In the Y-maze, mobility events increased at 2.5 mg/kg (19.22 ± 1.7), supporting experimental consistency. Motor score analysis revealed improvements at 2.5 mg/kg (99 ± 3) and 5 mg/kg (100 ± 2), and Limb Clasping test results improved at 5 mg/kg (1.00 ± 0.25), all compared to the control group. These results highlight the neurorestorative potential of MCX13 for ischemic stroke therapy.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: GlyT1 inhibition by NFPS post-treatment promotes neuroprotection in the middle cerebral artery occlusion model

Authors:

Daniel Pereira Cavalcante¹ Geane Reis dos Santos¹
Antonio Ítalo dos Santos Nunes¹ Eduardo Rosa da Silva¹
Gustavo Almeida de Carvalho¹ Mauro Cunha Xavier Pinto¹

Affiliations:

¹ Department of Pharmacology, Institute of Biological Sciences, Federal University of Goiás, Goiânia, GO, Brazil.

Keywords: Ischemia. Glycine. Neuroprotection.

Abstract:

Cerebral ischemia, characterized by reduced cerebral perfusion, often induces neurological deficits. The neuroprotective roles of glycine and GlyT1 inhibition is already showed in NFPS pre-treatment. Objective: To evaluate the neuroprotective potential of GlyT1 blockade with a NFPS post-treatment. Methods: The study utilized C57BL/6 mice, treating them with NFPS (a GlyT1 inhibitor) in 1.25, 2.5 and 5.0 mg/kg doses. The treatment was conducted for three days after inducing ischemia using the middle cerebral artery occlusion (MCAO) model. Three days post-induction, motor deficits were evaluated with the cylinder test (CT) and limb clasping (LC), while ischemia size was assessed using triphenyltetrazolium chloride (TTC) staining and free radical content was evaluated with reactive oxygen species (ROS) and nitric oxide (NO). The mice were divided into five groups (Sham, MCAO, 1.25, 2.5 and 5.0 mg/kg) with 6-8 animals per group. Data was analyzed using one-way ANOVA. All procedures were approved by the Ethics Committee on Animal Use (Protocol No. 041/19). Results: The study found that NFPS effectively decreased motor deficits in CT, LC with 5.0 mg/kg dose ($47.5 \pm 1.04\%$; 1.16 ± 0.40) and ischemia size with 2.5 and 5.0 mg/kg doses ($29.5 \pm 4.1\%$; $11.8 \pm 2.2\%$), in free radical content was showed a decreased in ROS and NO by 5.0 mg/kg dose ($96.8 \pm 9.0\%$; $98.5 \pm 7.3\%$). These results showed a neuroprotection caused by NFPS reducing motor impairment and oxidative stress damage. Conclusion: Interestingly, the neuroprotective effect of NFPS corroborate with GlyT1 inhibition studies and a post-treatment showed similar results of NFPS pre-treatment neuroprotection.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: 3D cell culture establishment to better understand crotoxin cytotoxic effect on breast tumor cell lines

Authors:

Carlos Frederico Lima Gonçalves¹ Tamires Cunha Almeida¹
Gisele Picolo¹

Affiliations:

¹ Pain and Signaling Laboratory, Butantan Institute.

Keywords: Cancer. Crotoxin. Spheroids. 3D cell culture.

Abstract:

Natural molecules have attracted attention for centuries due to their therapeutic properties. Crotoxin (CTX), the main toxin found in the venom of *Crotalus durissus terrificus*, has been described as having cytotoxic effects on several 2D cancer cell lines. But, 2D models present several limitations, such as homogeneous oxygen exposure, uniform nutrient availability, and a low or absent extracellular matrix (ECM), which may compromise the evaluation of antitumoral agents. So, 3D models have emerged as an alternative for drug screening in preclinical studies. Our project aims to establish 3D spheroid tumor cultures, derived from immortalized cell lines, to evaluate the antitumoral effects of CTX. We used agarose-coated (AGC) plates to allow cells in suspension to aggregate and form spheroids. Breast tumor MCF7a and MDA-MB-231 (MDA) cell lines were maintained in a 2D format using the same medium condition. CTX toxicity was evaluated by spheroid shape and size, using diameter average from ten measures circling the spheroid, in addition to resazurin assay (reduction to resorufin). At AGC plates, MCF7 formed spheroids two days after seeding at baseline condition. On the other hand, MDA cells only formed spheroids with the addition of rat collagen type I (6 µg/mL) at the medium, within 72h. In 2D viability assay using MTT, CTX was tested in 12.5 - 500µg/mL against those cells and we observed that 12.5µg/ml and 200µg/ml decreased MCF7 and MDA viability, respectively, however only after 72h. Then, a greater concentration (250µg/ml) was used for 3D tests. It was observed that growth and shape were affected, also MDA spheroids viability decreased at about 20% on resazurin reduction assay, after 48h. The implemented protocol will enable a more comprehensive evaluation of the antitumoral effects of CTX, being able to be applied to other antitumor compounds too. Ultimately, it may contribute to the development of more effective cancer treatments in the future.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Effects of *Delonix regia* galactomannan on neuroinflammation and microglial activation in animal model of osteoarthritis

Authors:

Francisco Glerison da Silva Nascimento¹ Ana Maria Sampaio Assreuy¹
Rondinelle Ribeiro Castro¹ Mário Rogério Lima Mota²

Affiliations:

¹ UECE, Instituto Superior de Ciências Biomédicas.

² UFC, Departamento de clínica odontológica.

Keywords: Intra-articular injection. Neuroinflammation. Polysaccharide.

Abstract:

Osteoarthritis (OA) represents the most prevalent form of human arthropathy and its pharmacological therapy is limited due the adverse effects elicited by systemic or intra-articular agents. In order to establish new therapeutic approaches from natural products, galactomannan from *Delonix regia* (GM-DR) have been previously tested in experimental OA, which effects were antinociception and reduction of cartilage damage. Objective: To investigate the mechanisms involved in the antinociceptive effect by GM-DR on rats subjected to monoiodoacetate-induced OA. Material and methods: The seeds were collected in Limoeiro do Norte (CE). The identification of the species was carried out at the Angiospermae Taxonomy Laboratory (Department of Biology/Federal University of Ceará-UFC), exsiccate (57952) deposited in Prisco Bezerra Herbarium, SISGEN (No. A54ED41). Male Wistar rats were used (170-200 g, CEUA/UFC 9994050421, n=6). OA-induced animals received sodium monoiodoacetate in the left tibiotarsal joint (1mg/25 µL), whereas the control group received sterile saline. Single treatment with GM-DR (100 µg/25 µL) was performed at different timepoints (3, 7, 14, 21 and 28) in OA-induced animals. At 56 day, dorsal root ganglia (DRG) and dorsal horn spinal cord were dissected for histopathological analysis and immunohistochemistry for TNF-α, IL-1β, c-Fos and Iba-1 (L4-L5). Results The intra-articular GM-DR treatment reduced immunoexpression of TNF-α ($p=0.0175$), IL-1β ($p=0.0005$) and c-Fos ($p=0.0083$) in DRGs, indicating that intra-articular GM-DR reduces the inflammatory state and neuronal hyperactivity in DRGs. Furthermore, GM-DR attenuated the immunoexpression of IL-1β ($p=0.0002$) and microglial activation (Iba1) ($p=0.004$) in dorsal horn of spinal cord. Conclusion: Intra-articular GM-DRR reduces neuroinflammation and microglial activation.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Impacto óleos essenciais encapsulados em levedura no desenvolvi

Authors:

Ana Julia de Oliveira Santos¹ Daisy Aline Azevedo Brito¹

Affiliations:

¹ Instituto Oswaldo Cruz, Laboratório de Bioquímica e Fisiologia de insetos.

Abstract:

O *Aedes aegypti*, vetor de doenças como dengue, Zika e chikungunya, é um desafio para a saúde pública em regiões tropicais e subtropicais, devido à sua fácil adaptação a ambientes urbanos. O principal método de controle, o uso de pesticidas neurotóxicos sintéticos, enfrenta limitações como impactos à saúde humana e animal e o desenvolvimento de resistência pelos mosquitos. Diante disso, alternativas como inseticidas botânicos, especialmente óleos essenciais, vêm ganhando destaque por sua composição complexa e ação defensiva natural. No entanto, sua aplicação direta apresenta limitações, como rápida degradação e baixa estabilidade. Uma alternativa promissora é o encapsulamento dos óleos essenciais em leveduras, técnica que aumenta a estabilidade, permite liberação controlada e prolonga sua eficácia. Estudos anteriores mostraram sucesso com o óleo essencial de laranja encapsulado, que apresentou alta atividade larvicida inclusive contra populações resistentes. Observações preliminares indicaram alterações na coloração de larvas após duas horas de exposição a concentrações subletais, sugerindo um possível mecanismo de ação relacionado à melanização. Este projeto busca aprofundar essas observações, avaliando alterações morfológicas em diferentes estágios do desenvolvimento do *Aedes aegypti* após tratamento com óleos essenciais encapsulados. Larvas de terceiro estágio serão expostas a diferentes concentrações, e os estágios larval, pupal e adulto serão documentados fotograficamente para análise morfológica detalhada. Os resultados serão comparados a um grupo controle não tratado, permitindo avaliar o potencial dos óleos essenciais encapsulados como alternativa eficaz no controle vetorial.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Isolation and characterization of mangrove fungi and prospecting for bioactive polypeptides

Authors:

Felipe Ricardo de Mello¹ Leticia Fontes Gama¹
Laura Almeida Silva Castro¹ Cristiane Angélica Ottoni¹ Leandro Mantovani de Castro¹

Affiliations:

¹ Unesp - Instituto de Biociências - Câmpus do Litoral Paulista (IB-CLP).

Keywords: Antimicrobial. Biological activity. Bioprospecting. Fungi. Mangrove.

Abstract:

Mangroves are productive ecosystems but are vulnerable to urbanization and pollution. Their soils, rich in organic matter, depend on microbiota, including filamentous fungi, for nutrient cycling. Adapted to extreme conditions, these fungi produce metabolites and enzymes essential for survival, some of which have recognized biotechnological and pharmaceutical potential. This study aimed to isolate, characterize, and identify filamentous fungi from mangrove soils in São Paulo, Brazil, and evaluate the antimicrobial, antioxidant, and antitumor activity of their protein and peptide extracts. Fungi were isolated from sediment and characterized macro- and micromorphologically, followed by molecular identification using DNA barcoding. The identified species were *Trichoderma harzianum*, *Aspergillus fumigatus*, *Aspergillus tubingensis*, and *Aspergillus niger*. After identification, the fungi were cultivated in liquid medium to obtain biomass and secretome. Biomass was subjected to proteomic screening for protein and peptide profile analysis. The secretome was obtained in aqueous and enriched media, and the aqueous fraction was filtered using 10 kDa membranes to separate the retained and permeated fractions. *T. harzianum* showed the best results in antimicrobial tests, leading to further evaluation of its extract at concentrations of 10, 20, 40, 80, 100, and 200 µg. Antimicrobial activity was assessed by disk diffusion and minimum inhibitory concentration (MIC) against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Antimicrobial sensitivity tests were performed in four independent experiments conducted on separate days and analyzed after 24 hours of incubation. The results showed higher activity against Gram-positive bacteria, with larger inhibition zones and lower MIC values, while Gram-negative bacteria showed no inhibition. This study highlights the antimicrobial potential of mangrove-derived fungi, especially *T. harzianum*, and supports the future isolation and characterization of its bioactive compounds based on proteomic analyses.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: The impact of a mixture of contaminants (TBT, Hg, and Cd) exposure in the reproductive axis of female rats.

Authors:

Flávia Caroline Farias dos Santos¹ Cidália De Fátima Januário^{1,2} Jones Bernardes Graceli^{1,3}

Affiliations:

¹ Federal University of Espírito Santo, Brazil.

² Zambeze University, Mozambique.

³ Southern Illinois University, USA.

Keywords: Co-exposure. Female reproductive axis abnormalities. Heavy metals. Ovary. Tributyltin.

Abstract:

We are exposed to diverse environmental contaminants every single day. Bioaccumulation of contaminants in food/water leads to public health concerns from potential ingestion of a mixture of contaminants. However, the reproductive consequences of co-exposure to tributyltin (TBT) + cadmium (Cd) + mercury (Hg) (MIX) are not well known. Thus, this study aims to test the hypothesis that exposure to MIX alters reproductive parameters in female rats. Adult Wistar rats were exposed to a mixture of contaminants, such as TBT (100ng/kg/day), Cd (100 mg/L) and Hg (1st dose: 4.6 mg/kg, subsequent doses: 0.07 mg/kg/day) for 15 days. All protocols were approved by CEUA-UFES (04/2023). The data were shown as mean $\pm$ SEM, t-test. 20 rats/group in total. We observed an increase in serum FSH levels in MIX rats compared to CON (CON: 1.00 ± 0.09 ; MIX: 1.19 ± 0.15 μ g/mL, $p < 0.05$). MIX rats spend less time in the estrous phase (CON: 1.50 ± 0.11 ; MIX: 1.03 ± 0.11 days, $p < 0.05$). MIX exposure led to a reduction in corpora lutea number and an increase in preantral follicles number (CON: 1.37 ± 0.05 ; MIX: 1.16 ± 0.07 and CON: 0.91 ± 0.10 ; MIX: 1.37 ± 0.19 follicle/mm², $p < 0.05$, respectively). In addition, mast cell number increases in ovarian of MIX rats compared to CON (CON: 0.014 ± 0.003 ; MIX: 0.04 ± 0.005 cells/mm², $p < 0.05$). The protein ER α expression in the ovary stroma of MIX rats was reduced compared to CON rats (CON: 4.24 ± 0.54 ; MIX: 3.15 ± 0.53 cells/ μ m², $p < 0.05$). While the protein expressions of PR in the ovarian stroma and FAS in the antral follicles of MIX rats were increased (CON: 5.99 ± 0.87 ; MIX: 11.81 ± 1.14 and CON: 5.16 ± 0.88 ; MIX: 8.16 ± 1.66 cells/ μ m², $p < 0.05$, respectively). After ovariectomy (OVX), MIX-OVX rats showed lower serum FSH levels compared to CON-OVX rats (MIX-Intact: 25.9 ± 4.0 ; MIX-OVX: 14.9 ± 1.9 μ g/mL, $p < 0.05$). These data suggest that exposure to a mixture of contaminants induces blunted estrogen-negative feedback and, impaired ovary morphophysiology in female rats.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Chronic exposure to a mixture of phthalates changes white adipose tissue morphology in female mice

Authors:

Flávia Caroline Farias dos Santos¹ Jody Anne Flaws²
Jones Bernardes Graceli^{1,3}

Affiliations:

¹ Federal University of Espírito Santo, Brazil.

² University of Illinois, USA.

³ Southern Illinois University, USA.

Keywords: Endocrine disruptors. Estrogen. Phthalates. White adipose tissue.

Abstract:

Phthalates are synthetic compounds widely used in plasticizers. These contaminants can be found in several plastic products, including packing foods, contributing to diverse human exposure routes. Phthalates are obesogenic chemicals, and their exposure causes metabolic disorders. We hypothesize that chronic exposure to a mixture of phthalates for 12 months changes white adipose tissue (WAT) morphology in female mice. Female CD-1 mice (6 weeks) were fed with a mixture of phthalates, composed of DEP (35.2%), DEHP (21.0%), DBP (14.9%), DiBP (8.6%), DiNP (15.1%), and BzBP (5.1%) for 12 months.

Three levels of exposure for a mixture were utilized: 0.15 ppm, 1.5 ppm, and 1500 ppm. All protocols were approved by IACUC and WAT morphological parameters were assessed. The data were shown as mean $\pm$ SD. Sample number 3 - 4. A lower gonadal weight was observed in 0.15 ppm exposure compared to CON (CON: 1.22 ± 0.39 ; 0.15 ppm: 0.29 ± 0.14 g, $p < 0.05$). In histological analyses, we observed a decrease in adipocyte diameter at 0.15 ppm exposure compared to the CON (CON: 48.5 ± 1.7 ; 0.15 ppm: 31.4 ± 6.4 μ m, $p < 0.05$). Furthermore, shift areas in WAT with apparent brown adipose tissue (BAT) aspect were observed in 0.15 ppm exposure. The number of granulomas increased at 0.15 ppm exposure compared to the CON (CON: 0.052 ± 0.038 ; 0.15 ppm: 0.903 ± 0.716 number/mm² $\times 10^6$, $p < 0.05$). Moreover, a borderline increase of mast cells was observed in 0.15 ppm exposure compared to CON (CON: 0.24 ± 0.07 ; 0.15 ppm: 2.47 ± 2.27 cells/mm², $p = 0.054$). In addition, adipocytes surrounded by macrophages similar crown-like structures (CLS) were present in the mixture of phthalate exposure groups as well. In the 0.15ppm group, we observed a borderline increase in ER α expression compared to CON (CON: 0.96 ± 0.43 ; 0.15 ppm: 4.65 ± 3.66 positive cells/mm², $p = 0.08$). These data suggest that exposure to a mixture of phthalates promotes abnormal WAT morphology in female rats, which may be related to estrogen signaling.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Probenecid Potentiates cGMP-Mediated Relaxation in Isolated Rat Corpus Cavernosum

Authors:

Guilherme Ruiz Leonardi¹ Mariana Burille Moretti¹
João Victor Corrêa de Barros¹ Jeniffer Maia de Lima¹
Edson Antunes¹ Fabiola Zakia Mónica¹

Affiliations:

¹ Department of Pharmacology, Faculty of Medical Sciences, University of Campinas, Campinas, Brazil.

Keywords: Corpus cavernosum. Erectile dysfunction. Probenecid.

Abstract:

Erectile dysfunction is a common male sexual dysfunction that is characterized by the recurrent inability to attain and maintain an erection sufficient for satisfactory sexual performance, and it has a significant impact in social and financial quality of life. Drug repurposing is a strategy that aims to redirect already approved drugs for clinical use, exploring new therapeutic indications. This study aims to evaluate the effect of probenecid in promoting the relaxation of smooth muscle in the corpus cavernosum (CC). Experiments were conducted on isolated CC from rats (ethic committee approval at CEUA/UNICAMP: 6401-1/2024). Concentration-response curves (CRCs) were carried out in an isolated organ bath and the potency (pEC₅₀) and maximal response values (E_{max}) were determined. For statistical analysis, the unpaired student's t-test was used. Probenecid promoted a relaxation in CC of rats with an E_{max} close to 50%. This response was significantly reduced in the presence of inhibitors of guanylyl cyclase (ODQ, 100 μ M, 26.8% $\pm$ 4.3, n=5, P<0.01) or eNOS (L-NAME, 100 μ M, 24.2% $\pm$ 7.0, n=5, P<0.01) and significantly increased in the presence of phosphodiesterases inhibitor (IBMX, 100 μ M, 57.3% $\pm$ 22.2 vs 93.3% $\pm$ 8.8, n=5, P<0.05). But, in the presence of adenylyl cyclase inhibitor (SQ 22,536, 100 μ M) no significant difference was observed in E_{max} and pEC₅₀. We also observed that probenecid (1 mM; 30 min) can increase the E_{max} of acetylcholine (E_{max}: 46.5% $\pm$ 6.7 vs 70.5% $\pm$ 8.0, n=5, P<0.01) and sodium nitroprusside (SNP, E_{max}: 75.3% $\pm$ 5.8 vs 96.2% $\pm$ 4.4, n=5, P<0.01) in a CRC. We also observed a leftward shift in the relaxing response induced by tadalafil (pEC₅₀: 3.88 $\pm$ 0.20 vs 4.28 $\pm$ 0.24; n=5, P<0.05) and SNP (pEC₅₀: 5.22 $\pm$ 0.15 vs 5.49 $\pm$ 0.24; n=5, P<0.05) in presence of probenecid (1 mM; 30 min). In conclusion, probenecid relaxes CC and enhances responses to key relaxant agents, possibly through NO-sGC-cGMP pathway. However, further studies are needed to elucidate better these mechanisms.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Pharmacologic Characterization of Probenecid in the Prostate of Healthy Rats: An In Vitro Study

Authors:

Mariana Burille MORETTI¹ Fabíola Taufic Monica IGLESIAS¹ Guilherme Ruiz LEONARDI¹
Jeniffer Maia de LIMA¹
João Victor Corrêa de BARROS¹

Affiliations:

¹ Universidade Estadual de Campinas.

Keywords: β -adrenergic agonists. Benign Prostatic Hyperplasia. Probenecid.

Abstract:

Benign Prostatic Hyperplasia (BPH) affects approximately 50% of men aged 50–61. Defined by prostate enlargement due to cellular proliferation that often leads to lower urinary tract symptoms (LUTS) and negatively impacts quality of life, BPH is a multifactorial condition mainly associated with aging, but also influenced by hormonal changes, metabolic syndrome, and inflammation. This study aims to characterize the effects of Probenecid, a competitive inhibitor of the organic anion transporter (OAT), on the prostate of healthy rats, exploring its potential to enhance relaxation of prostatic smooth muscle when combined with other drugs. In vitro experiments were conducted using prostate strips from rats, following approval by UNICAMP's Ethics Committee on Animal Use (CEUA – 6459-1/2024). Strips were mounted in organ chambers containing Krebs solution (37°C). Isometric force was monitored via PowerLab 400™. After a 45-minute equilibration period, concentration-response curves (CRCs) were generated for Isoproterenol (100 pM–100 μ M), Salbutamol (100 pM–100 μ M), and Sodium Nitroprusside (SNP; 100 pM–100 μ M). Relaxation responses were expressed as a % of Phenylephrine-induced contraction (10 μ M). Maximum response (Emax) and potency (pEC50) were determined. All data were expressed as mean $\pm$ SEM (n), analyses were performed using unpaired Student's t-test and one-way ANOVA. $p < 0.05$ was considered statistically significant. Results showed that the incubation of Probenecid (1 mM, 300 μ M, 100 μ M; 30 minutes) significantly increased pEC50 of β -adrenergic agonists Isoproterenol ($p < 0.05$; pEC50: 6.59 ± 0.18 vs 7.35 ± 0.18 ; N=5) and Salbutamol ($p < 0.05$; pEC50: 4.56 ± 0.07 vs. 5.08 ± 0.08 ; N=6). However, only the CRC for Salbutamol demonstrated an increase in Emax as well ($p < 0.05$; Emax: 64.48 ± 5.01 vs. 89.72 ± 10.73 ; N=6). SNP showed a significant difference in Emax only when tissues were incubated with the highest concentration of Probenecid ($p < 0.05$; Emax: 47.51 ± 12.82 vs. 64.02 ± 14.61 ; N=8). In conclusion, our study suggests that incorporating Probenecid into BPH treatment could enhance symptom relief and/or reduce the required dose of β -adrenergic agonists, potentially minimizing unwanted side effects.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Effects of the Biopesticide Spinosad on Epididymal Morphology and Sperm Activity

Authors:

Letícia Pazin Bomfim¹ Ivana Regina da Costa¹ João Antônio Cyrino Zequi²
Glaura Scantamburlo Alves Fernandes¹

Affiliations:

¹ Laboratory of Toxicology and Metabolic Disorders of Reproduction, Center for Biological Sciences, State University of Londrina, Londrina, PR.
² Department of Animal and Plant Biology, State University of Londrina, Londrina, PR.

Keywords: Endocrine disruptor. Pesticide. Reproduction. Toxicology.

Abstract:

The increasing use of pesticides, led by Brazil, drives the search for less toxic alternatives. Spinosad, a biopesticide with low environmental risk, acts on the nervous system of insects, causing hyperexcitation, paralysis, and death. Despite its effectiveness, its potential as an endocrine disruptor in non-target species requires further studies, especially regarding reproductive development. For this purpose, 45 male Wistar rats from the Central Animal Facility of the State University of Londrina were used. The experimental protocol was approved by CEUA-UEL (nº. 073.2023). The animals were divided into three groups (n=15/group) and exposed via gavage (PND 25-65): Control (Vehicle); SPI_{0.2} (0.2 mg/kg Spinosad); and SPI₂ (2 mg/kg Spinosad). On PND 66, the animals were anesthetized and euthanized for epididymis and sperm collection. Sperm motility was analyzed, and mitochondrial activity was assessed. Epididymal histopathology was performed through qualitative analysis, and stereology followed Weibel's method. Statistical analysis was carried out using the ANOVA test according to distribution, significance was set at p<0.05 and results were presented as mean ± standard error. Stereological analysis revealed a lumen reduction in the caput portion in the SPI_{0.2} group (26,58%). Histopathology indicated an increased number of cells in the lumen of the experimental groups, suggesting alterations in epididymal cell dynamics. Mitochondrial assessment showed a reduction in the proportion of sperm classified with maximum mitochondrial activity (8,14%), indicating potential energy impairment, with the SPI₂ group showing the most pronounced mitochondrial dysfunction. These results indicate that exposure to Spinosad may impact sperm mitochondrial activity without directly affecting motility. Overall, the findings indicate that Spinosad can influence male reproductive physiology, affecting epididymal morphology and sperm bioenergetics.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Synthesis and study of single crystal 1,10-phenanthroline complexed with Cu(II) and Zn(II) for antibacterial actions

Authors:

Anna Rackel Pinheiro Valerio¹ Vitor Martins de Andrade¹ Kátia da Conceição¹
Rossano Lang¹

Affiliations:

¹ Instituto de Ciência e Tecnologia - Universidade Federal de São Paulo.

Keywords: Antibacterial activity. Complexes based on Cu(II) and Zn(II). 1,10-phenanthroline.

Abstract:

In the 20th century, the period from the 1930s to the 1960s was marked by the immense production of antibiotics, such as sulfonamides and penicillin, which were quickly incorporated into clinical practices. However, the indiscriminate and incorrect use of these and other antimicrobials contributed to the rise of bacterial resistance, leading to a serious public health issue that persists today. In this context, the use of metallic compounds associated with polypyridines as therapeutic agents and the understanding of the pharmacological properties of these molecules have become a focus of scientific research. Since then, there has been growing interest in crystals complexed with transition metal ions and organic molecules due to their chelating properties and cellular interactions. The goal is to identify potential activities with greater efficacy, fewer side effects, and minimized resistance in organisms, which are crucial for antimicrobial actions. This study aims to contribute to the field of biologically applicable materials through the synthesis and study of mixed crystals of 1,10-phenanthroline complexed with Cu(II) and Zn(II). After successful crystal growth, five material characterizations were performed, including structural analysis by X-ray diffraction (XRD), elemental analysis by Energy Dispersive X-ray Spectroscopy (EDX), thermal analysis by Differential Thermal Analysis by Thermogravimetry (TG-DTA), vibrational analysis by Fourier Transform Infrared Spectroscopy (FT-IR), and optical analysis by UV-VIS-NIR Spectroscopy, as well as one preliminary biological tests on the Gram-negative bacterium *E. Coli* and the Gram-positive bacterium *S. Aureus*. The XRD pattern refined by the Rietveld method revealed that the complexed crystal has monoclinic symmetry with the space group C2/c, where the Cu(II) and Zn(II) atoms are coordinated by two phenanthroline molecules and a chlorine anion in a distorted trigonal bipyramidal geometry. The estimated elemental ratio between Cu(II) and Zn(II) was 0.85:0.15 (EDX analysis), and the number of H₂O molecules present in the crystal was 4 (TG-DTA analysis), resulting in a stoichiometry of Cu_{0,85}Zn_{0,15}(Phen)₂Cl(H₂O)₄. The FT-IR spectrum shows vibration modes between 500 and 1750 cm⁻¹, with wavenumber shifts compared to free monohydrated 1,10-phenanthroline, confirming the complexation of transition metals with phenanthroline ligand molecules. The UV-Vis-NIR absorbance spectrum exhibits a transmission window between 410 and 610 nm. It absorbs photons in other optical windows: 200-410 nm and 610-1000 nm, covering the UV and NIR spectral regions, which significantly differs from the monohydrate 1,10-phenanthroline ligand. The results of the biological assays for *E. Coli* and *S. Aureus* were distinct. The complex dissolved in deionized water eradicated 100% of *E. Coli* cells - a bactericidal effect compared to controls - across all concentrations tested (0.05 mg/mL, 0.1 mg/mL, and 0.2 mg/mL). In contrast, for the *S. Aureus* culture, the complex inhibited bacterial growth by approximately 80% to 99% at the same concentrations. Conversely, cellular proliferation was observed in the control groups of metal chlorides.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Synthesis and study of single crystal 1,10-phenanthroline complexed with Cu(II) and Zn(II) for antitumoral actions

Authors:

Anna Rackel Pinheiro Valerio¹ Uilla Barcick¹
André Zelanis Palitot Pereira¹ Rossano Lang¹

Affiliations:

¹ Instituto de Ciência e Tecnologia - Universidade Federal de São Paulo.

Keywords: Antitumoral activity. Complexes based on Cu(II) and Zn(II). 1,10-phenanthroline.

Abstract:

Cancer is a term used to describe a group of more than 100 diseases that share common characteristics, such as the disorderly and abnormal growth of mutated cells. Neoplasms originate in different tissues and can spread to distant organs from the primary site, an event known as metastasis. Melanoma is a type of skin cancer that begins in melanocytes, the cells responsible for producing the pigment melanin. Although its incidence is not very high, this type of cancer has attracted the attention of researchers due to its aggressiveness and potential to progress to metastasis. Cis-diamminedichloroplatinum, commonly known as Cisplatin, is a drug used in the treatment of various types of cancer, such as testicular, ovarian, bladder, prostate, and others. Despite its widespread use in chemotherapy treatments, Cisplatin has adverse effects that limit its dosage and application, with nephrotoxicity and neurotoxicity being the most significant. However, it remains the gold-standard drug used for comparison purposes in numerous studies. In this context, new compounds have been developed as alternatives to Cisplatin, particularly crystals complexed with transition metal ions and organic molecules, due to their chelating properties and cellular interactions. The goal is to identify potential activities with greater efficacy, fewer side effects, and minimized resistance in the organism, especially using metals that are naturally present in the body, such as copper, zinc, iron, and others. This study aims to contribute to biologically applicable materials by synthesizing and studying mixed crystals of 1,10-phenanthroline complexed with Cu(II) and Zn(II). After successful crystal growth, five material characterizations were performed, including structural analysis by X-ray diffraction (XRD), elemental analysis by Energy Dispersive X-ray Spectroscopy (EDX), thermal analysis by Differential Thermal Analysis by Thermogravimetry (TG-DTA), vibrational analysis by Fourier Transform Infrared Spectroscopy (FT-IR), and optical analysis by UV-VIS-NIR Spectroscopy, as well as one cell viability assay using the MTT reagent performed on the A375 cell line (human malignant epithelial melanoma). The XRD pattern refined by the Rietveld method revealed that the complexed crystal has monoclinic symmetry with the space group C2/c, where the Cu(II) and Zn(II) atoms are coordinated by two phenanthroline molecules and a chlorine anion in a distorted trigonal bipyramidal geometry. The estimated elemental ratio between Cu(II) and Zn(II) was 0.85:0.15 (EDX analysis), and the number of H₂O molecules present in the crystal was 4 (TG-DTA analysis), resulting in a stoichiometry of Cu_{0,85}/Zn_{0,15}(Phen)₂Cl(H₂O)₄. The FT-IR spectrum shows vibration modes between 500 and 1750 cm⁻¹, with wavenumber shifts compared to free monohydrated 1,10-phenanthroline, confirming the complexation of transition metals with phenanthroline ligand molecules. The UV-Vis-NIR absorbance spectrum exhibits a transmission window between 410 and 610 nm. It absorbs photons in other optical windows: 200-410 nm and 610-1000 nm, covering the UV and NIR spectral regions, which significantly differs from the monohydrate 1,10-phenanthroline ligand. The cell viability assay was conducted with the A375 cell line at five different concentrations (0.25, 0.125, 0.0625, 0.03125, and 0.015625 mg/mL). Although the IC₅₀ of the complex has not been determined, it exhibits a cell growth inhibition rate approximately 12% higher than the Cisplatin gold-standard.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Pharmacological characterization of Pannexin-1 channels in rat corpus cavernosum: an in vitro study

Authors:

João Victor Corrêa de Barros¹ Jeniffer Maia De Lima¹ Guilherme Ruiz Leonardi¹ Gabriela Reolon Passos¹ Mariana Burille Moretti¹ Edson Antunes¹
Fabiola Taufic Mônica Iglesias¹

Affiliations:

¹ Universidade Estadual de Campinas – UNICAMP.

Keywords: Erectile dysfunction. Pannexin-1. Corpus cavernosum.

Abstract:

Approximately 70% of men with lower urinary tract symptoms (LUTS), such as urgency, incontinence, and post-void discomfort, also have erectile dysfunction. These symptoms negatively impact social, sexual, and financial quality of life. Pannexins are channel-forming proteins, with Pannexin-1 (Pnx1) modulating purinergic signaling through ATP release. Pnx1 activation occurs through stimuli such as membrane stretching and depolarization, activating purinergic P2Y2R receptors, which recruit PKC α and stimulate TRPV4 channels, increasing Ca²⁺ influx and promoting vasodilation. This project focuses on characterizing Pnx1 channels in the corpus cavernosum (CC) and how they contribute to smooth muscle relaxation, enabling penile erection. The experiments are being conducted on rat CC. We performed the Western Blot (WB) technique to assess the presence of Pnx1 in the CC. Concentration-response curves (CRCs) were carried out, and potency (pEC₅₀) and maximum response (E_{max}) values were determined. All protocols were approved by the Ethics Committee (Protocol in CEUA/UNICAMP: 6559-1/2024). Using WB, we successfully identified the presence of Pnx1 channels in rat CC. We observed that 10Pnx (50 μ M; 30 minutes) enhanced CC relaxation in response to the relaxant agents acetylcholine (ACh) and sodium nitroprusside (SNP), with a leftward shift in the relaxation response (pEC₅₀: ACh: 5.61 $\pm$ 0.34 vs. 6.26 $\pm$ 0.30; N=4; SNP: 5.55 $\pm$ 0.29 vs. 6.69 $\pm$ 0.38; N=5). Meanwhile, the relaxation response to tadalafil (TDL) showed an increased E_{max} in the presence of 10Pnx (control: 58.8% $\pm$ 13.8 vs. 10Pnx: 82.1% $\pm$ 10.7; N=5) and a trend toward a leftward shift in pEC₅₀, though without statistical significance. In conclusion, Pnx1 channels are present in rat CC, and their inhibition enhances responses to key relaxant agents such as acetylcholine, SNP and TDL. Further studies are needed to elucidate the pathways and mechanisms involved.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Correlation Between Cardiac, Hepatic and Renal Oxidative Stress Markers in Wistar Rats Subjected to an Acute Dose of Doxorubicin

Authors:

Felipe Berretta¹ Juliana Silva Siqueira¹
Thiago Luiz Novaga Palacio¹
Dijon Henrique Salomé de Campos¹ Jordanna Cruzeiro¹
João Guilherme Correa Marcolino¹ Carlos Thiago Roder Petronilio¹ Camila Renata Corrêa Camacho¹

Affiliations:

¹ Universidade Estadual Paulista Júlio de Mesquita Filho.

Keywords: Chemotherapy. Oxygen species. Liver-heart-kidney correlation

Abstract:

Doxorubicin (Dox) is a chemotherapy drug used in cancer treatment; however, among its side effects, it induces systemic oxidative stress (OS), compromising organs such as the heart, liver, and kidneys. Malondialdehyde (MDA), protein carbonylation (CBO), and advanced oxidation protein products (AOPP) assess this impact, but studies rarely consider organ interdependence in the OS response. This study aimed to determine the correlation between markers of cardiac, hepatic and renal oxidative stress in Wistar rats subjected to an acute dose of doxorubicin. Male Wistar rats (n=20) were distributed into two groups: control + vehicle (C+V, n=10) and Dox (n=10 with 4 mg/kg of Dox intraperitoneally). Forty-eight hours after administration, the animals were euthanized for OS biomarker analysis in cardiac, hepatic, and renal tissues (MDA and CBO, nmol/mg protein; AOPP, μ mol equiv. Chloramine T/L/mg protein), (CEUA:1429/2023). Data were analyzed using simple linear regression. The results indicate that the liver plays a central role in the oxidative stress response, showing significant correlations with the heart and kidneys for MDA, CBO, and AOPP. Liver and heart MDA, CBO and AOPP ($r=0.5397$, $p=0.0140$; $r=0.6755$, $p=0.0011$, $r=0.5567$, $p=0.0108$); Liver and kidney MDA, CBO and AOPP ($r=0.5189$, $p=0.0191$; $r=0.4948$, $p=0.0368$; $r=0.5189$, $p=0.0191$). This suggests that the liver may influence or reflect oxidative stress in these organs, possibly due to its role in metabolism and redox balance regulation. In contrast, the relationship between the heart and kidney was less consistent, suggesting a weaker interdependence or one mediated by other factors. Heart and kidney MDA, CBO and AOPP ($r=0.3070$, $p=0.1879$; $r=0.4592$, $p=0.0552$; $r=0.3920$, $p=0.0874$). These findings highlight the need to analyze oxidative stress systemically, considering the interaction between organs.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Exposure to atomoxetine hydrochloride decreases testosterone biosynthesis, colony formation, induces genotoxicity and cell death in TM3 Leydig cells

Authors:

Débora Hipólito Quadrelli^{1,2} Andresa Hiromi Sakaiz
Julia Ayumi Ikeda Kawasaki² Juliana Mara Serpeloni²
Glaura Scantamburlo Alves Fernandes²

Affiliations:

¹ Department of Immunology, Parasitology and General Pathology, Londrina State University, Londrina, PR.

² Department of General Biology, Londrina State University, Londrina, PR.

Keywords: ADHD. Cell death. Male reproductive system. Toxicity.

Abstract:

The symptoms of Attention Deficit/Hyperactivity Disorder (ADHD) can cause personal, social and academic harm. One way to mitigate these symptoms and enhance the quality of life for patients is through medication. Among them, atomoxetine increases the availability of neurotransmitters, which also act on male genital system's receptors. Leydig cells are critical for reproductive function and studies on the effect of this drug on these cells are scarce. Thus, this study aims to evaluate whether the use of atomoxetine hydrochloride harms Leydig cells *in vitro*. For this purpose, TM3 cells were exposed to atomoxetine hydrochloride (250, 1000 or 1750 ng/mL) for 24 hours, and assessed for cell viability, death, comet and clonogenic assays, in addition to testosterone and cytokine levels. Data represented as mean $\pm$ standard deviation ($n=3$). ANOVA and Dunnett's test, statistically different from control when $p \leq 0.05$. Regarding cell viability, no significant differences were observed in the treatments compared to the control group, as well as in the levels of cytokines TNF- α and IL-1 β . In contrast, there was a significant decrease in 1000 and 1750 ng/mL groups concerning testosterone levels and a significant increase in the DNA damage index at the 1000 ng/mL concentration when compared to the control group. In addition, a significant reduction in viable cells and a significant increase in apoptotic cells were observed at all treatments. At the highest concentrations, there was a significant increase in necrotic cells when compared to the control group. Finally, in the three experimental concentrations, the colony formation capacity decreased significantly when compared to the control group. The results indicates that atomoxetine hydrochloride impaired Leydig cells, inducing genotoxicity, cell death, DNA damage, reduced testosterone levels and decreased colony formation, even without significantly altering cell viability and levels of pro-inflammatory cytokines TNF- α and IL-1 β .



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: The insecticide malathion causes oxidative stress in the placenta and uterus of Wistar rats and increases the number of micronuclei at different gestational ages

Authors:

Gabrielle Chalupa Faria¹ Giovanna Facchetti Frigoli¹
Karen Gabriela Fávaro de Souza¹ Beatriz Mascarenhas Matildes² Ivana Regina da Costa¹
Phelipe de Oliveira Favaron¹ Juliana Mara Serpeloni¹
Glaura Scantamburlo Alves Fernandes¹

Affiliations:

¹ Department of Biological Sciences, State University of Londrina - Londrina, PR.

² Department of Biology, Positivo University - Londrina, PR.

Keywords: Gestational Age. Genotoxicity. Organophosphates. Placentation. Reactive Oxygen Species.

Abstract:

In 2024, Brazil recorded more than 6 million suspected cases of dengue. Malathion is the insecticide used to control the mosquito that transmits dengue. Maternal exposure to the insecticide poses a significant risk because its metabolites can cross the placenta and amniotic fluid, causing potential damage to the placenta and fetal development. In this context, the aim of this project was to evaluate the effects of low doses of malathion at different gestational ages. For this purpose, 42 Wistar rats were used (CEUA 019/2023). On gestation day 0 (GD), after confirmation of pregnancy, the animals were divided into three groups: C (0.9% saline), M10 (10 mg/kg) and M50 (50 mg/kg), by gavage. The doses chosen were based on 0.5% (M10) and 2.5% (M50) of the oral LD₅₀ (2000 mg/kg) for rats. The treatment lasted for two gestation periods: GD15 and GD20. Due to the number of false positives, the number of pregnant rats per group was adjusted to C (n=8); M10 (n=4) and M50 (n=10) on GD15 and C (n=7); M10 (n=5) and M50 (n=8) on GD20. Euthanasia was performed after anaesthesia by puncture of the hepatic portal vein. Five placenta and one uterus fragment per animal were used for the oxidative stress analyses (TBARS, GST, GSH, GSSG, GT, SOD and catalase). The micronucleus test was performed on maternal blood; amniotic fluid was collected at DG20 and biochemical parameters (Total Proteins, Urea, Glucose, Calcium, Alkaline Phosphatase and Lactate) were analysed. Fetal weight and morphometric measurements were obtained from all fetuses. Statistical analysis was performed by one-way ANOVA or Kruskal-Wallis test. On GD15 in the uterus TBARS presented a decrease in M10 and M50 ($p < 0.0001$). While the levels of GSSG in M10 ($p < 0.0001$) and M50 ($p = 0.0039$) and GT in M50 ($p = 0.0068$) increased. On the other hand, on GD20 TBARS decreased in M50 ($p = 0.0181$), while the levels of GSH in M50 ($p = 0.0395$) and GST in M10 ($p = 0.0010$) and M50 ($p < 0.0001$) presented a decrease. In the placenta, on GD15 TBARS increase in M50 ($p = 0.0003$). While on GD20 the SOD activity increases in M10 ($p = 0.02$). The number of micronuclei increased in the groups receiving the highest dose of malathion at both DG15 ($p = 0.0047$) and DG20 ($p = 0.0019$) indicating the mutagenic potential of the insecticide. However, the biochemical parameters in the amniotic fluid, fetal weight and morphometry showed no statistically significant change ($p > 0.05$). These results indicate that malathion was sufficient to induce oxidative stress in the rat placenta and uterus without affecting fetal and placental development.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Nicotine reward sensitivity in high sugar-induced binge-eating mice and the role of cannabidiol in food intake

Authors:

César Augusto Manduca e Silva¹ Anna Paula Marçal¹
Rayssa Brianis Costa¹ Daniele Cristina de Aguiar¹

Affiliations:

¹ Laboratory of Neuropsychopharmacology, Pharmacology Department, Institute of Biological Sciences, Federal University of Minas Gerais.

Keywords: Cannabinoids. Eating disorders. Nicotine addiction. Psychiatric disorders.

Abstract:

Binge Eating Disorder (BED) is characterized by recurrent episodes of excessive food consumption within a specific period, accompanied by feelings of loss of control and guilt. Hyperpalatable foods rich in fats, carbohydrates, and sodium are commonly consumed during binge episodes. Several brain systems are involved in BED, often overlapping with psychiatric disorders such as Substance Use Disorder (SUD). Nicotine, although legal, causes substantial harm to individuals and society, with consumption increasing mainly due to e-cigarette use. Our study aimed to investigate whether binge-eating animals show altered nicotine-reinforcing effects in the Conditioned Place Preference (CPP) test and whether cannabidiol (CBD), which is known to reduce drug-seeking behavior, could decrease the binge-eating mice's food consumption. Ninety-eight C57BL/6J mice (M=48; F=50), aged six-seven weeks (CEUA 50/2024) were divided into two groups: high-refined-carbohydrate diet (HC) and standard diet (SD). They underwent a binge eating induction protocol on intermittent days for over four weeks. Subsequently, they were subdivided for the intraperitoneal administration of CBD (30 mg/kg) or vehicle. Finally, they were subjected to the CPP test with subcutaneous nicotine (M=0.5 mg/kg, F=0.25 mg/kg) to evaluate the drug's reinforcing effects. Intermittent HC diet exposure promoted binge eating in both sexes. However, CBD did not alter feeding behavior. No statistical differences were observed between the control and nicotine groups in CPP tests. These data suggest that CBD (30 mg/kg) did not influence binge eating in C57BL/6 mice. Experiments with varying doses and timings are ongoing to obtain more robust data. Due to the statistical variability in nicotine preference tests, it is unclear whether binge eating affects the drug's rewarding effects.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Investigation of the effects of mesotherapy with yohimbine in mice

Authors:

Christiano Thadeu de Souza Menezes¹ Stéphanie Christine Sinder Mello¹ Caroline Fernandes-Santos¹
Thereza Cristina Lonzetti Bargut¹

Affiliations:

¹ Núcleo de Estudos em Metabolismo, Nutrição e Histopatologia (NEMENUTH) - Instituto de Saúde de Nova Friburgo (ISNF).

Keywords: Beige adipocyte. Lipolysis. Mesotherapy. White adipocyte. Yohimbine.

Abstract:

Mesotherapy is a non-surgical method to eliminate localized fat, and yohimbine (YO) is used to stimulate lipolysis in these regions. We investigated the effects of a single subcutaneous YO application in female Swiss mice. Three groups (n=6-8 each) received a single subcutaneous injection in the right inguinal region of saline or YO (80 µg or 800 µg). Body mass (BM), biochemistry, morphology, and protein expression analyses were performed 2h or 24h after the intervention (CEUA 4232071220/2021). Same-time comparisons were made by one-way Anova and Tukey's post-hoc test, pre vs post by paired t-test, and 2h vs 24h by t-test. When comparing BM and blood glucose before and after application, there was a decrease in YO-800-24h (-3.8%), and in YO-800-2h (-16%), respectively. Total cholesterol was reduced in YO-800-24h compared to the control (-59%), and triglycerides were lower in YO-800-24h compared to YO-800-2h (-46%). For glycerol, YO-800-24h was higher than the control (+110%) and YO-80-24h (+88%). Brown adipose tissue (BAT) and right inguinal white adipose tissue (iWAT) weight in YO-80-24h were lower than YO-80-2h (-22% and -26%). Right iWAT was also reduced in YO-800-24h compared to YO-800-2h (-35%) and increased in YO-800-2h compared to the control (+55%). As for the adipocyte diameter in iWAT, YO-80-24h was smaller than YO-800-2h (-22%), and in YO-800-24h compared to control (-58%), YO-80-24h (-44%) and YO-800-2h (-53%). YO-80-24h was also smaller compared to control-24h (-24%) and YO-80-2h (-34%). The volume density (Vv) of white adipocytes in iWAT was lower in YO-800-2h compared to YO-80-2h. Conversely, beige adipocytes Vv was higher in YO-800-2h than YO-80-2h, and in YO-80-24h compared to YO-80-2h. UCP-1 immunolabeling confirmed the presence of this protein in the tissue. A single subcutaneous YO application led to metabolic changes local iWAT reduction and induction of beige adipocytes. Yohimbine should be used with caution and further studies are needed.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Behavioral effects of neonatal aluminum exposure in *Caenorhabditis elegans*

Authors:

Caroline Falabreti¹ Gabriela Mota Tibola¹ João Vitor Martins¹ Wagner Antonio Tamagno² Aline Pompermaier³
Carla Alves¹

Affiliations:

¹ Instituto Federal de Educação, Ciências Tecnologia do Rio Grande do Sul - Câmpus Sertão.
² School of Health Sciences, Purdue University, West Lafayette, IN, United States.
³ Laboratório de Ecologia e Conservação, Universidade Federal da Fronteira Sul, Erechim, RS, Brasil.

Keywords: Biomarker. Ecotoxicology. Nematode.

Abstract:

With the advancement of industrialization, waste generation has become an eminent problem, especially with heavy metals. Aluminum (Al) is widely used metal in industrial processes due to its characteristics of conductivity, malleability, and lightness. However, studies have shown that Al is potentially associated with the development of neurodegenerative diseases. Exposure to Al can potentiate physiological and behavioral changes. In addition, the impacts of neonatal exposure to Al and its possible effects on development are not yet fully understood. To investigate toxicity from neonatal exposure to Al, the *C. elegans* model was exposed to 0 mg.L⁻¹, 5.5 mg.L⁻¹, 8 mg.L⁻¹, and 10.5 mg.L⁻¹ of Al. We used the N2 strain (wild type), which was synchronized, to obtain eggs at the same stage. Then the eggs (n=500) were exposed to Al concentrations for approximately 24h, until hatching. Afterward, they were transferred and maintained in a nematode growth medium and fed with *Escherichia coli* (OP50) at 20 °C, until they reached the adult stage L4. The markers of body bends rate (change of axis during movement, body bends.min⁻¹), and pharyngeal beating (contraction of the pharyngeal muscle, pumping.min⁻¹) were evaluated in the L4 stage. Preliminary results show a significant decrease in the pharyngeal beating rate of the worms when exposed to the 3 concentrations of Al in the neonatal period (156±8.73; 152±12.08; 152±7.88), when compared to the control group (198.3±1.64). In contrast, the movement of the worm decreased at the Al concentration of 5.5 mg.L⁻¹ (44.12±2.62) and increased at the dose of 10.5 mg.L⁻¹ (82.83±3.19) when compared to control (59.71±2.29). Therefore, exposure to aluminum even in the embryonic stage of the worm has a significant influence on the adult life of the animal, causing changes in its behavior. These results together with biochemical analyses will provide clearer evidence of the toxicological effects of exposure to Al in the early stages of development.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: *Ilex paraguariensis* post-exposure to Al reestablishes sociability patterns in zebrafish

Authors:

Gabriela Mota Tibola¹ Caroline Falabreti¹ João Vitor Martins¹
Wagner Antonio Tamagno² Aline Pompermaier³
Carla Alves¹

Affiliations:

¹ Instituto Federal de Educação, Ciência e Tecnologia do Rio Grande do Sul - Câmpus Sertão, Sertão, RS, Brazil.
² School of Health Sciences, Purdue University, West Lafayette, IN, United States.
³ Laboratório de Ecologia e Conservação, Universidade Federal da Fronteira Sul, Erechim, RS, Brazil.

Keywords: Behaviour. Pollution. Toxicology.

Abstract:

Aluminum (Al) is one of the most commonly used metals in industries because of its conductivity, malleability, and lightweight characteristics. However, research indicates that aluminum may be associated with the development of neurodegenerative diseases due to its neurotoxic properties. Yerba mate (*I. paraguariensis*) is culturally present in the daily lives of several communities in southern Brazil. It is known that *I. paraguariensis* has antioxidant properties that can prevent or remedy oxidative stress. In this study, we used the zebrafish (*Danio rerio*) to investigate the effects of Al and the potential neuromodulatory properties of yerba mate (CEUA/IFRS: 2229110319). Therefore, fish were initially exposed to Al: 0 mg.L⁻¹, 5.5 mg.L⁻¹, 8 mg.L⁻¹, and 10.5 mg.L⁻¹ for four days. The animals were then treated with microencapsulated *I. paraguariensis* extract (MIPE) (maltodextrin encapsulated of the infusion of 7 g of yerba mate in 100 mL of Milli-Q water at 70 °C for 15 min) for eleven days. After exposure, the social behavior test was performed, where the fish were individually transferred to a test aquarium between an empty tank and another tank with a group of conspecifics. Social behavior was filmed for 2 min. The aquarium was divided into three segments, and the number of entries, crossings, and time spent in each area. The Al concentration of 5.5 mg.L⁻¹ decreased time spent in the segment closest to the shoal (3.25±1.49), but when exposed to yerba mate, the fish returned to the interaction zone with its conspecifics. All Al concentrations increased the time spent in the empty zone of the tank (1.28±1.68; 4.7±3; 6.18±4.16; 4.8±4.26). These demonstrate the negative effects of Al on the social behavior of fish, which can cause reproduction difficulties and an anxiety-like effect. However after using these extracts, an improvement in social interaction was observed, demonstrating a possible neuromodulatory effect.

Financial Support: CNPq, FAPERGS e IFRS.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: *Ilex paraguariensis* post-exposure to aluminum reestablishes biomarkers in *Caenorhabditis elegans*

Authors:

Carla Alves¹ Caroline Falabreti¹

Gabriela Mota Tibola¹ Wagner Antonio Tamagno² Aline Pompermaier³

Affiliations:

¹ Instituto Federal de Educação, Ciências Tecnologia do Rio Grande do Sul - Câmpus Sertão.

² School of Health Sciences, Purdue University, West Lafayette, IN, United States.

³ Laboratório de Ecologia e Conservação, Universidade Federal da Fronteira Sul, Erechim, RS, Brasil.

Keywords: Biomarker. Ecotoxicology. Nematode.

Abstract:

Industries use aluminum (Al) in various processes, and it is known for its excellent properties. However, studies indicate that Al may be linked to neurodegenerative conditions due to its neurotoxic effects. *I. paraguariensis* is a plant significant in the everyday lives of numerous residents in southern Brazil, recognized for its antioxidant properties that can help mitigate oxidative processes. Here, we utilized Al to analyze the possible advantages of extract *I. paraguariensis*. For this work, the wild-type (N2) *C. elegans* model was exposed from L1 to L4 life stages to 0 mg.L⁻¹, 5.5 mg.L⁻¹, 8 mg.L⁻¹, and 10.5 mg.L⁻¹ of Al. Following this, the worms received treatment with microencapsulated *I. paraguariensis* extract (MIPE), (7g of extract with 100 mL of Milli-Q water at 70 °C for 15 minutes) over a 48h period (From L2 to L4). We used the N2 strain (wild type), which was synchronized, to obtain eggs at the same stage. Afterward, they were transferred and maintained in a nematode growth medium and fed with *Escherichia coli* (OP50) at 20 °C, until they reached the adult (L4) stage. The markers of the defecation cycle (time between the defecations, sec), and pharyngeal beating (contraction of the pharyngeal muscle, pumping.min⁻¹) were evaluated after the exposure. The results show that Al in all concentrations causes a decrease in pharyngeal pumping (201±3.99; 187.2±9.17; 183.47±7.98), compared to control (225±10.19). But with the post-treatment with MIPE, all exposures increased pharyngeal pumping (243.73±5.72; 245±10.3; 243.33±7.8), compared to control (235±6.84) and Al exposure. The defecation cycle increased when exposed to concentrations of Al (53.86±3; 76.33±3.2; 61.73, compared to control (48.73±3.9). And with the post-treatment with MIPE, we have no differences in the time to all exposures (44.53±1.99; 45.4±2.66; 47.33±2.44) and control (47±2.44). These results highlight the potential of the extract to reverse the harmful effects of Al due to the neuromodulatory effect.

Financial Support: CNPq, Fapergs e IFRS.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Exposure to Low Dose NDMA Causes Structural and Inflammatory Changes in the Prostate of Adult Rats in a Transgenerational Effect, in the Context of the Paternal Origins of Health and Disease (POHaD)

Authors:

Karen Gabriela Favaro de Souza¹ Gabrielle Chalupa Faria¹
Débora Hipólito Quadrelli¹ Ivana Regina da Costa¹ Bárbara Campos Jorge² Livia Trippe
Nagaoka² Julia Stein²
Arielle Cristina Arena²
Glaura Scantamburlo Alves Fernandes¹

Affiliations:

¹ State University of Londrina - UEL, Londrina - PR.

² São Paulo State University Júlio de Mesquita Filho - UNESP, Botucatu - SP.

Keywords: Environmental Contaminant. Nitrosamine. DOHaD. Prostate. Toxicity.

Abstract:

NDMA is a nitrosamine with mutagenic and carcinogenic potential, frequently found in food and water. Few studies have assessed its adverse effects on the prostate, especially at doses considered safe by regulatory agencies. This study investigated the impact of low dose NDMA exposure in male Wistar rats (F0 and F1 generations) within the concept of the Paternal Origins of Health and Disease (POHaD). F0 males were exposed to vehicle (1 mL/kg/day) or NDMA (7.2 ng/kg/day) from PND 60 to 104 and then mated with unexposed females. The experimental groups were: Control (unexposed) and paternal NDMA (exposed males). One male per litter was euthanized at PND 70 (indirect exposure), while two others continued exposure from PND 22 to 70 (one with vehicle, one with NDMA) and were euthanized at PND 120. The study was approved by the CEUA of UNESP-Botucatu (protocol 4704260423). Parametric (Student's t-test, ANOVA with Dunnett) or nonparametric tests (Mann-Whitney, Kruskal-Wallis with Dunn) were used according to data distribution and homoscedasticity (Brown-Forsythe test) (n=6-10). F0 males showed a significant increase in prostate weight, with no changes in F1 at PND 70 or 120. Stereology revealed epithelial increase and luminal reduction in F0. At PND 70, F1 showed reduced interstitial tissue; at PND 120, paternal exposure led to epithelial and interstitial expansion and reduced lumen. Collagen quantification showed decreased types I and III at PND 70 and increased type I at PND 120 in the directly exposed group. Histopathology revealed inflammatory infiltrates and increased mast cell degranulation in directly exposed animals. No significant changes were observed in TBARS, GST, CAT, or GSH, but SOD activity increased, and GT and GSSG decreased in F0. In conclusion, NDMA induced direct and transgenerational prostate toxicity, with structural, inflammatory, and oxidative alterations, even at low dose.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Alteration of stimulated flow rate, biochemical composition, and salivary redox state in male Wistar rats caused by the antiepileptic drug Carbamazepine

Authors:

Rafaela Yumi Gregório Fuzishima¹ José Vitor Furuya de Lima¹
Renan José Barzotti^{1,2} Larissa Victorino Sampaio^{1,2} Rayara Nogueira de Freitas^{1,3}
Guilherme Eduardo Rocha Silva^{1,3} Leonardo dos Anjos Teruel¹
Gabriela Borba Crispim de Carvalho¹ Yasmin Andrade Dos Santos^{1,3} Antonio Hernandez Chaves Neto^{1,2,3}

Affiliations:

¹ Department of Basic Sciences, São Paulo State University (Unesp), School of Dentistry, Araçatuba.

² Multicenter Graduate Program in Physiological Sciences, São Paulo State University (Unesp), School of Dentistry, Araçatuba.

³ Graduate Program in Sciences, São Paulo State University (Unesp), School of Dentistry, Araçatuba.

Keywords: Carbamazepine. Oxidative stress. Saliva.

Abstract:

Carbamazepine (CBZ) is an antiepileptic drug known for its high prevalence of dry mouth sensation among patients. Therefore, this study aimed to evaluate the possible effects of CBZ on the flow rate, biochemical composition, and salivary redox state in rats. For this purpose, young male Wistar rats (6 weeks old) were randomly divided into three groups (n = 10/group): Control (C, saline solution), CBZ25 (25 mg/kg/day), and CBZ50 (50 mg/kg/day) (Tegretol® 10 mg, Novartis, Taboão da Serra, Brazil). After 28 days of treatment via intragastric gavage, pilocarpine-induced saliva was collected and subjected to biochemical analyses (CEUA FOA/UNESP No. 393-2024). Data were analyzed using one-way ANOVA followed by Tukey's post-hoc test, with $p < 0.05$ considered significant. CBZ treatments did not affect salivary pH or buffering capacity. However, CBZ25 ($p < 0.05$) increased the salivary flow rate normalized by salivary gland weight. Total protein concentration was higher in animals treated with CBZ25 ($p < 0.01$) and CBZ50 ($p < 0.05$) compared to C. In contrast, CBZ50 reduced salivary amylase activity compared to the C ($p < 0.05$) and CBZ25 ($p < 0.05$) groups. Additionally, CBZ25 ($p < 0.0001$) and CBZ50 ($p < 0.05$) increased salivary magnesium secretion, while CBZ25 also elevated chloride concentration compared to C ($p < 0.05$). On the other hand, calcium and phosphate concentrations were lower in the CBZ50 group compared to C ($p < 0.05$). Sodium and potassium concentrations did not differ among groups. Furthermore, CBZ50 increased total oxidant capacity compared to the CBZ25 group ($p < 0.05$). Simultaneously, CBZ50 promoted greater oxidative damage to salivary lipids and proteins compared to the C ($p < 0.05$) and CBZ25 ($p < 0.05$ and $p < 0.01$) groups. Finally, both doses reduced total antioxidant capacity compared to C ($p < 0.05$). Therefore, CBZ may be a risk factor for oral health, inducing salivary gland dysfunction in a dose-dependent manner (FAPESP Process No. 2024/15065-5).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: *Trans*-Aconitic Acid, bioactive compound isolated to sugarcane, enhances dopaminergic activity in VTA subregions related to emotional and motor circuits in Wistar male rats submitted to forced acute physical activity

Authors:

Juliana Corsini Ziroldo¹ Isabella Marques da Silva² Julia Ferreira Mendonça³ Maria Thereza Gamberini⁴

Affiliations:

¹ Faculdade de Ciências Médicas da Santa Casa de São Paulo.

Keywords: Dopaminergic. Rats. Sugarcane. Trans-aconic acid. Ventral tegmental área.

Abstract:

The mesolimbic pathway, consisting of ventral tegmental área(VTA), nucleus accumbens (NAc) and ventral pallidum, is related to incentives and rewards, although its involvement in motor control is postulated, since projections from VTA extend to substantia nigra(SN), which has efferent fibers to basal ganglia. A previous preclinical study with *trans*-aconitic acid(AA), active compound identified in sugarcane, demonstrated increased motor performance and anxiolytic effect in Wistar male rats evaluated in rotarod and elevated plus-maze. As a hypothesis, it was postulated that stress reduction to improve motor performance of rats. Since dopaminergic pathways are involved in motivational and motor responses, it was investigated dopaminergic areas of VTA by quantifying tyrosine hydroxylase-positive(TH+) neurons in interfascicular (IF), paranigral nucleus(PN) and parabrachial área(PBP) subregions. Brains of rats subjected to rotarod after treatment with H₂O(control, n=6) and (AA 0.05µg/kg, n=6) were sectioned, labeled with anti-tyrosine hydroxylase primary antibody and revealed with fluorescent secondary antibody(594 nm)(CEUA001/21). The results expressed as mean±standard error were evaluated by t-test(p<0.05) for comparisons between the responses of control and treated groups in each area analyzed separately. The results revealed an increase in TH+ neurons in IF (1961±253 versus 724±87) and PBP (4203±749 versus 2344±262) in rats treated with AA compared to control. However, there was no variation in PN. All areas evaluated have connections with shell and core portions of NAc involved in emotional and motor responses. Connections between NAc core and nigrostriatal system through SN are described, which would function as an interface between the limbic-motor system. So, we postulates that the effects of *trans*-aconitic acid may be related to dopaminergic activation of VTA and areas related to emotional and motor behaviors, which would justify the increase in motor performance of rats.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Effects of unsaturated fatty acid supplementation during gestation: delays in motor development and alterations in muscle characteristics in offspring rats

Authors:

Pedro Henrique da Silva Braga¹ Luiza Alves Amorim¹

Douglas Moritz Moreira^{1,2} Mariana Inocêncio Manzano¹ Mônica Maciel²

Katya Naliwaiko² Matheus Felipe Zazula^{1,2}

Affiliations:

¹ Grupo de Pesquisa em Gestação e Doenças Metabólicas, Universidade Cesumar – Curitiba, Paraná, Brasil.

² Laboratório de Plasticidade Morfofuncional, Departamento de Biologia Celular, Universidade Federal do Paraná – Curitiba, Paraná, Brasil.

Keywords: Fatty acid supplementation. Motor development. Muscle characteristics. Gestation and lactation.

Abstract:

The supplementation of unsaturated fatty acids during pregnancy can have significant impacts on the development of offspring, affecting aspects such as behavior, motor development and muscle characteristics. This study investigated the effects of supplementation in rats during lactation and its impacts on their offspring. Thirty-two rats were used, divided into 4 groups: control, omega-3, omega-6, and omega-9, which were supplemented with unsaturated fatty acids for 30 days before mating and throughout gestation and lactation with 4 g/kg daily. Their offspring (male) were analyzed for motor development, and muscle characteristics at various points during the postnatal period (CEUA 1459/2022 Data were analyzed using anova for repeated measures, survival analysis, and Chi-square (χ^2) test of accession, depending on the variable assessed). Offspring of supplemented rats exhibited delays in geotaxis, escape from the abyss reflex, and eruption of the upper incisors (time in days, in all). The supplemented groups also showed delays in ear detachment and eye opening. Regarding weight gain (%), offspring from supplemented rats showed an increase at DPN21, but the weight gain pattern was similar across groups over time. Concerning body growth, the supplemented offspring exhibited shorter nasoanal length (cm) at some stages, although the growth pattern was similar across groups. The Lee index was higher in supplemented offspring starting from DPN05, indicating a higher proportion of body mass. Muscle characteristics showed increased density in the diaphragm, soleus, long extensor of the toes, and tibialis anterior muscles of supplemented offspring. However, muscles such as the plantar and soleus (g/cm³, in both) showed lower weight and volume, suggesting a different distribution in extensor digitorum longus development. Supplementation with unsaturated fatty acids during pregnancy had significant impacts on motor development, and muscle characteristics in the offspring. These results indicate that supplementation can have positive effects on physical development and body composition of offspring in the early stages of life, with repercussions on muscle development and long-term health.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: **Sperm mitochondrial activity was impaired by *in vitro* exposure to polystyrene nanoplastics.**

Authors:

Beatriz Teixeira de Oliveira¹ Melissa Helena Garcia Matheus¹ Cristiana Livramento Oliveira Pinto¹ Nicolý Caixêta Gonçalves¹
Raul Silva Santarem^{2,3} Ana Paula Ramos³ Hugo Felix Perini¹
Gláucia Eloisa Munhoz de Lion Siervo¹

Affiliations:

¹ Universidade Federal do Triângulo Mineiro ² ETEC José Martimiano da Silva

³ Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo

Keywords: Microplastics. Plastic nanoparticles. Reproduction. Toxicity.

Abstract:

Global plastic production is increasing, leading to the accumulation of particles in landfills, seas, and oceans, and to contamination of water and food. These pollutants may affect sperm, potentially impairing their function. This study evaluated the *in vitro* effects of different concentrations of polystyrene nanoplastics (NP-PS) on sperm mitochondrial activity. The project was approved by the Research Ethics Committee (CEP-UFTM – CAAE 71552323.7.0000.5154). Volunteers (n = 7) were informed of the study's objectives and criteria. Semen was evaluated prior to exposure, and sperm were isolated using the *swim-up* technique in BWW medium. Isolated cells (2×10^6 cells/well) were exposed to 2, 20, and 200 $\mu\text{g/mL}$ of NP-PS (180 nm, zeta potential -27 mV) for 30 and 120 minutes. Mitochondrial activity was assessed using DAB staining of the midpiece, classifying sperm as fully stained, partially stained, or unstained. Data were tested for normality and variance homogeneity. The Kruskal–Wallis and Dunn's *post hoc* tests were used, with significance at $p < 0.05$. Exposure to 20 and 200 $\mu\text{g/mL}$ of NP-PS significantly reduced the percentage of fully stained sperm after 30 min ($p = 0.0108$; $p = 0.0001$) and 120 min ($p = 0.0108$; $p = 0.0001$) compared to the control. At 120 min, 200 $\mu\text{g/mL}$ also differed from 2 $\mu\text{g/mL}$. Partially stained sperm increased at 2 and 20 $\mu\text{g/mL}$ after 30 min ($p = 0.0252$; $p = 0.0010$) and 120 min ($p = 0.0193$; $p = 0.0013$). Unstained sperm increased at 20 and 200 $\mu\text{g/mL}$ after 30 min ($p = 0.0398$; $p = 0.0001$) and 120 min ($p = 0.0457$; $p = 0.0001$), but not at 2 $\mu\text{g/mL}$. These results suggest that NP-PS can impair mitochondrial activity in human sperm, potentially affecting fertility.

This study was supported by FAPEMIG (APQ-00221-22).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Intra-Articular Oxygen Reduces Temporomandibular Joint Arthritis-Related Pain and Inflammation

Authors:

Ana Carolina dos Santos Machado¹ Tássia Tillemont Machado¹ Catarine Massucato Nishijima¹
Carlos Amílcar Parada¹
Cláudia Herrera Tambeli¹

Affiliations:

¹ Center for Pain Studies, Department of Structural and Functional Biology, Institute of Biology (IB), University of Campinas (UNICAMP).

Keywords: Endocannabinoid system. Inflammatory pain. Opioid system. Oxygen therapy. Temporomandibular joint arthritis.

Abstract:

Temporomandibular joint (TMJ) arthritis-related pain impacts quality of life. Oxygen therapy shows promise in pain modulation, especially via hyperbaric chambers. This study evaluated intra-articular Oxygen's analgesic and anti-inflammatory effects in TMJ synovitis. Fifty-eight adult female Wistar rats received intra-articular monosodium iodoacetate (MIA; 2 mg/30 μ L) or saline (30 μ L). Oxygen (30 μ L) was co-administered with MIA or given 3 days later ($n=6-8$ /group). Mechanical hyperalgesia was measured via electronic von Frey on baseline and days 3, 5, 7, and 10 post-MIA or saline injection. ELISA was used to analyze Cytokines (IL-1 β , TNF- α , IL-10) in periarticular tissue on days 5 and 10 ($n=5-6$ /group). To assess receptor involvement, CB1 (AM251), CB2 (AM630), or κ -opioid (nor-BNI) antagonists (100 μ g) were injected intra-articularly on day 5 post-treatment ($n=6$ /group). Ethics approval: CEUA-UNICAMP #5795-1/2021. Oxygen prevented hyperalgesia, with a higher mechanical nociceptive threshold by $29.88\% \pm 4.52\%$ vs. MIA-only ($p=0.0006$), and reversed established hyperalgesia with a $26.01\% \pm 9.61\%$ increase ($p=0.0431$). Oxygen anti-inflammatory effects were confirmed by 2.5 $\times$ lower IL-1 β ($p=0.0155$) at day 5 and 3.6 $\times$ lower TNF- α ($p=0.0023$) at day 10, with IL-10 increased 1.7 $\times$ on days 5 ($p=0.0124$) and 10 ($p=0.0005$). CB1, CB2, and κ -opioid antagonists reversed the Oxygen-induced analgesia, reducing thresholds by $21.32\% \pm 4.77\%$ ($p=0.0005$), $20.28\% \pm 4.80\%$ ($p=0.0010$), and $25.67\% \pm 4.80\%$ ($p<0.0001$), respectively. Data were analyzed using Two-Way ANOVA or Mixed-effects with Dunnett's test ($p<0.05$). These findings suggest that intra-articular Oxygen therapy alleviates TMJ pain via endocannabinoid and opioid activation and modulates cytokine profiles. These data support its potential as an innovative treatment for TMJ inflammatory pain.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Bisphenol S and High Fat Diet-Induced Brown Adipose Tissue Plasticity

Authors:

Luiza da França Losito^{1,2} Gabriel Ximenes de Oliveira¹ Lyss Feliz Meirelles¹
Ana Beatriz Proença^{1,2} Ana Livia Ladislau^{1,2}
Guilherme dos Santos Reis^{1,2} Beatriz Alexandre-Santos³ Nathalia Silva Carlos Oliveira³
D'angelo Carlo Magliano² Eliete Dalla Corte Frantz^{1,2}

Affiliations:

¹ Exercise Science Laboratory, Fluminense Federal University, Niteroi, Brazil.
² Morphology and Metabolism Research Center, Fluminense Federal University, Niteroi, Brazil. ³ Integrated Unit of Specialized Pathology, Fluminense Federal University, Niteroi, Brazil.

Keywords: Bisphenol S. Brown Adipose Tissue. Obesity.

Abstract:

Bisphenol S (BPS) is the main substitute for Bisphenol A (BPA) in the plastic industry and induces obesogenic effects. Adipose tissue plays a central role in obesity and is composed of two tissues with opposing functions in energy metabolism. Brown adipose tissue (BAT) has a multilocular morphology and abundant mitochondria, responsible for dissipating energy through thermogenesis. Obesity impairs BAT plasticity, inducing a whitening process. To investigate the impact of BPS exposure on the morphology and metabolism of BAT, C57BL/6 male mice were divided into 4 groups (n=12): control (SC), SC+BPS (SCB), high-fat diet (HF), and HF+BPS (HFB). BPS was administered in drinking water (25 µg/Kg/day) for 12 weeks. Body mass (BM), adiposity index, lipid content, nuclear and vascular density, and thermogenesis markers were evaluated in BAT. SCB showed an increased BM (P=0,0089) and adiposity index (P=0,0147) relative to the SC group, while HF (P<0,0001);(P<0,0001) and HFB (P<0,0001);(P<0,0001) had an additional increase in BM compared to SC and SCB. Both HF (P<0,0001);(P<0,0001) and HFB (P<0,0001);(P<0,0001) groups displayed increased BAT mass compared to SC and SCB, along with higher lipid content (HF: P=0,0009; HFB: P=0,0106) compared to the SC group. The SCB (P=0,0234);(P=0,0464), HF (P<0,0001);(P=0,0001) and HFB (P=0,0042);(P<0,0001) groups showed a decrease in the number of nuclei and vessels per area relative to the SC group, with the high-fat diet groups (HF and HFB) also presenting a significant reduction in vessel density compared to the SCB group (HF: P=0,05; HFB: P=0,0382). Regarding UCP-1 protein expression, the HF (P=0,0302) and HFB (P=0,0401) groups had lower levels than SC. Both the HF diet and BPS exposure led to increased body mass and adiposity, reduced brown adipocytes and vessels per area, suggesting a whitening process and impaired thermogenesis in BAT. CEUA/UFF: 1929240521.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Analysis of the proliferative effect of *Lavandula dentata* essential oil on HaCaT keratinocytes

Authors:

Marcela Bego Nering de Sousa¹ Eduardo Purgatto²
Karin Vicente Greco³ Vanessa Moreira¹

Affiliations:

¹ Departamento de Farmacologia, Escola Paulista de Medicina, Universidade Federal de São Paulo

- UNIFESP, São Paulo/SP.

² FORC-CEPID - Faculdade de Ciências Farmacêuticas - Universidade de São Paulo - USP, São Paulo/SP.

³ University College London - UCL, Londres, Reino Unido.

Keywords: Brazilian lavender. keratinocyte. skin regeneration

Abstract:

Skin regeneration is often impaired by conditions like psoriasis, dermatitis, keloids, and diabetes, which are linked to chronic inflammation and fibrosis. Lavender essential oil has gained attention for its anti-inflammatory, antioxidant, analgesic, and pro-regenerative properties. While *Lavandula angustifolia* is well studied, *Lavandula dentata*, cultivated in Brazil, remains totally unexplored regarding its pro-regenerative effects. Its unique terpene profile may offer synergistic bioactive effects, highlighting the need for further investigation. To assess its potential, HaCaT keratinocytes were cultured in DMEM with supplements at 37°C/5% CO₂. For viability assays, 5×10³ cells (N=4) were seeded in 96-well plates and treated with *L. dentata* nanoemulsion (NEL) (0.5–128 µg/mL) for 24 h before mitochondrial activity assessment. Proliferation and migration were analyzed using a scratch assay in 24-well plates (1×10⁵ cells/well, N=4). After ~90% confluence, a scratch was made, and images were captured at 0 h and 24 h after NEL (0.5–4 µg/mL) treatment. BrdU-based proliferation assays were performed in 96-well plates (4×10⁴ cells/well) using NEL (0.125–4.0 µg/mL). NEL at 8 µg/mL concentration significantly reduced mitochondrial activity by 9% (p<0.005), prompting the use of lower concentrations in further experiments. In scratch assays, cells treated with NEL (0.5–4 µg/mL) showed a 16–21% increase (p<0.05) in proliferation compared to control, with no concentration-dependent differences. BrdU assays confirmed NEL-induced proliferation, with a concentration-dependent response between 0.125 µg/mL (51,72%) and 0.5 µg/mL (90,80%), respectively (p<0,001). Concentrations of 1.0 µg/mL (80.46%) and 2.0 µg/mL (35.63%) also differed significantly from control (p<0.001). These preliminary results suggest that essential oil of *L. dentata* promotes keratinocyte proliferation and regeneration, highlighting the need for further studies on its mechanisms and pharmacological targets.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Impact of Exposure to N-Nitrosodimethylamine (NDMA) on Fertility and Embryo Fetal Development in Wistar Rats

Authors:

Luara Magalhães¹ Julia Tavares Alves¹
Bárbara Campos Jorge¹ Julia Stein¹
Lívia Trippe Nagaoka Gonçalves¹ Julia Vitória Francisco de Aquino¹ Giovanna Ferrari Rosalem¹
Otavio Galassi Vitale¹ Giovanna Kugel Marraschi¹
Reggina Lorena Mellado Castro¹ Sara Tawany Caetano dos Santos¹ Arielle Cristina Arena¹

Affiliations:

¹ Department of Structural and Functional Biology - Morphology Sector; Institute of Biosciences of Botucatu (IBB) – UNESP.

Keywords: Embryo-fetal. Embryotoxicity. Environmental contaminants. Nitrosamines. Rats.

Abstract:

Nitrosodimethylamine (NDMA) is a compound belonging to the nitrosamines class, which are environmental contaminants found in various products, including food and water. The presence of NDMA has been observed in widely used drugs, which has drawn the attention of regulatory agencies. Nitrosamines are known for having mutagenic and carcinogenic effects in high doses, in addition to evidence of an embryotoxic potential. Despite this, there is a lack of studies investigating the possible adverse effects on embryo-fetal development after exposure to NDMA. Therefore, our aim was to evaluate fertility parameters and embryo-fetal development following combined preconceptional and gestational exposure to NDMA in Wistar rats. For this study, 12 male and 24 female Wistar rats were divided into two groups: (1) Control (control males x control females) and (2) NDMA (NDMA males x NDMA females). The males were exposed to NDMA (0 and 7,2 ng/kg/day; gavage) from the preconception period (postnatal day-PND- 60 to 90) until mating (PND 91 to 104). The females were exposed to the same doses through the preconception, mating and gestational periods (gestational day -GD- 0 to 20). The males were euthanized after mating, and the females on GD 20 for fertility analysis and fetal evaluation. The fetuses were destined for morphological, visceral and skeletal analysis to evaluate if there were any anomalies. The fertility test showed a tendency toward reduction in the placental weight in the male offspring ($p=0.07$) and an increase in the proportion of large for the gestational age (LGA) fetuses in the female offspring ($p=0.05$). The skeletal analysis showed a significant increase in the number of fetuses with supernumerary ribs ($p=0.016$). The other analysis did not show any significant differences. These data suggest that NDMA exposure may affect intrauterine development in subtle ways, highlighting the need for studies at different points in gestation. (CEUA:9891060624)



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Norepinephrine and angiotensin II stimulation alter the cell proliferation in human oral squamous cell carcinoma and keratinocytes by different mechanisms.

Authors:

Beatriz Travalon Fabricio¹

Lucas Henrique Francisco Custódio^{1,2} Juliane Stephanie Mendonça Rodrigues^{1,2} Daniel Galera Bernabé³

Sandra Helena Penha Oliveira^{1,2}

Affiliations:

¹ Department of Basic Sciences, São Paulo State University (UNESP), School of Dentistry, Araçatuba, SP, Brazil.

² Multicenter Graduate Program in Physiological Sciences.

³ Department of Diagnosis and Surgery, São Paulo State University (UNESP), School of Dentistry, Araçatuba, SP, Brazil.

Keywords: Angiotensin II. Cell proliferation. Keratinocytes. Norepinephrine. Squamous cell carcinoma.

Abstract:

Squamous cell carcinoma (SCC) is a common malignant neoplasm among oral cancer cases. Inflammation and stress contribute to cancer development through the release of angiotensin II (Ang II) and norepinephrine (Nor). Thus, the objective of this study was to evaluate the role of Ang II in Nor-induced stress in a tongue squamous cell carcinoma cell line (SCC-9 and SCC-25) and keratinocytes (NOK-SI) regarding cell proliferation. Cells were treated with Nor (0.1 μ M, 1 μ M, and 10 μ M) or angiotensin II (Ang II) (100 nM). Additionally, cells were pre-treated with Ang II and, after one hour, stimulated with Nor. Cell proliferation was assessed using the MTT assay. Results were analyzed by one-way ANOVA ($p \leq 0.05$). Exposure to Nor at a concentration of 10 μ M increased the proliferation of SCC-9 and SCC-25 cells compared to control. In SCC-9 cells, this effect occurred six hours after Nor exposure ($p < 0.01$). In SCC-25 cells, this effect persisted for 24 and 48 hours ($p < 0.05$, $p < 0.0001$ respectively). In NOK-SI, the stimulation of Nor did not induce any modification in the proliferation activity. These results suggest that Nor is capable of inducing cell proliferation in these cells, corroborating with literature data, which showed increased cell proliferation at a concentration of 10 μ M. Additionally, Nor, combined with Ang II, reduced constitutive proliferation in NOK-SI. Additionally, a decrease in NOK-SI cell proliferation was observed in the group stimulated with Ang II + Nor (10 μ M) compared to the Ang II group at 24 and 48 hours ($p < 0.01$, $p < 0.001$ respectively). These results suggest that Ang II, combined with Nor, reduces the constitutive cellular metabolism of the NOK-SI cell line, possibly involving the activation of β -adrenergic receptors and/or AT1R in their energy metabolism. Therefore, our data suggests that Nor induces proliferation in SCC-9 and SCC-25 cell lines, while in the NOK-SI cell line, only Nor combined with Ang II decreases constitutive cell proliferation.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Spike protein induces cytokines production mediated by RAS components expression on human three-dimensional bronchiole co-culture

Authors:

Lucas Henrique Francisco Custódio^{1,2}
Juliane Stephanie Mendonça Rodrigues^{1,2} Beatriz Travalon Fabricio¹
Sandra Helena Penha Oliveira^{1,2}

Affiliations:

¹ Department of Basic Sciences, São Paulo State University (UNESP), School of Dentistry, Araçatuba, SP, Brazil.

² Multicenter Graduate Program in Physiological Sciences.

Keywords: Renin Angiotensin System. SARS-CoV-2. Three-dimensional cell culture.

Abstract:

COVID-19 is a respiratory disease caused by SARS-CoV-2, which enters cells via its Spike protein (Sp) binding to angiotensin-converting enzyme 2 (ACE2). This study assessed if Sp induces expression of renin angiotensin system (RAS) components and inflammatory cytokines. A three-dimensional bronchiole co-culture (TBCC), formed using three human lung cell lines, including fibroblasts (MRC-5), smooth muscle cells (BSMC), and epithelial cells (A-549), was pre-treated with losartan (LOS, 10 μ M), AVE 0991 (AVE, 0.1 μ M), diminazene aceturate (DIZE, 0.1 μ M), and A779 (0.1 μ M) and stimulated by Sp (100 nM). Expression of renin, angiotensinogen (AGT), angiotensin-converting enzyme (ACE), ACE2, angiotensin II receptor type 1 (AT1R) and Mas receptor (MASR) was evaluated 6h after by RT-qPCR. Cytokines production (TNF- α , CCL-2, IL-8, and IL-1 β) was evaluated 6h after by ELISA. Results were analyzed by one-way ANOVA followed by Tukey post hoc ($p \leq 0.05$). Sp induced AT1R and MASR expression at 6 h ($p \leq 0.05$, $p < 0.01$, respectively), which was augmented by A779 ($p < 0.01$, $p < 0.001$, respectively). AVE and DIZE have also increased MASR expression ($p < 0.0001$). AVE and DIZE did not change the AT1R expression. LOS inhibited the Sp-induced AT1R expression ($p \leq 0.05$). TBCC constitutively expressed ACE2 and Sp did not alter its expression. LOS, DIZE, and AVE pre-treatments reduced ACE2 constitutive expression ($p < 0.01$). The inherent expression of ACE, AGT, and Renin in the TBCC suggested the potential for endogenous angiotensin II synthesis. Sp increased the release of TNF- α , CCL-2, IL-8, and IL-1 β ($p < 0.001$, $p \leq 0.05$, $p \leq 0.05$, $p \leq 0.05$, respectively). LOS reduced Sp induced CCL-2 and TNF- α secretion ($p \leq 0.05$, $p < 0.0001$, respectively) and AVE had the same effect ($p < 0.01$, $p < 0.0001$, respectively). DIZE reduced CCL-2 release ($p < 0.001$). Our data show that Sp elicits both inflammation and RAS component upregulation in TBCC, shedding light on RAS involvement in SARS-CoV-2 and potential therapies.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Spinosad exposure during puberty impairs testis morphophysiology in Wistar rats

Authors:

Letícia Pazin Bomfim¹ Ivana Regina da Costa² João Antônio Cyrino Zequi³
Glaura Scantamburlo Alves Fernandes⁴

Affiliations:

¹ Department of Physiological Sciences, Biological Sciences Center, State University of Londrina – Londrina, PR.

² Department of Immunology, Parasitology and Pathology, Biological Sciences Center, State University of Londrina – Londrina, PR.

³ Department of Animal and Vegetal Biology, Biological Sciences Center, State University of Londrina – Londrina, PR.

⁴ Department of General Biology, Biological Sciences Center, State University of Londrina – Londrina, PR.

Keywords: Pesticide. Postnatal development. Spermatogenesis. Spermatozoa. Seminiferous tubules.

Abstract:

Spinosad is a naturally derived pesticide used in several countries as a promising insecticide for controlling pests that threaten public health. The peripubertal period is a critical phase in postnatal reproductive system development, making it highly susceptible to toxic agents. This study aimed to evaluate whether Spinosad exposure during this period affects testicular morphophysiology in rats. Forty-five Wistar rats were divided into three experimental groups ($n=15/\text{group}$): Control, SPI(0,2), and SPI(2,0). The SPI(0,2) and SPI(2,0) groups received Spinosad via gavage at doses of 0,2 mg/kg and 2,0 mg/kg, respectively, diluted in saline for 40 consecutive days (PND 25–65). The control group received only the vehicle during the same period. On PND 66, the animals were weighed, anesthetized, and submitted to euthanasia. The testes were collected for spermatogenesis kinetics, histopathological analysis, and sperm count, while sperm from the vas deferens was collected for morphology analysis. For statistical analysis, the ANOVA test was used and the significance level was set at 5%. Data are presented as mean $\pm$ SEM. Animal handling procedures were approved by the Ethics Committee on the Use of Animals at the State University of Londrina (CEUA/Uel – No. 073.2023). Daily sperm production (DSP) decreased in the SPI (0,2) group ($5,743\pm0,860$; $p=0,0143$) but increased in the SPI (2,0) group ($12,220\pm0,864$; $p=0,0002$). Spermatogenesis kinetics revealed a rise in seminiferous tubules at stages IX–XIII in SPI (0,2) ($30,800\pm2,059$; $p=0,024$) and at stages VI–VIII in SPI (2,0) ($11,400\pm1,288$; $p=0,035$). Additionally, both Spinosad-exposed groups showed an increased number of morphologically abnormal seminiferous tubules (SPI (0,2): $13,000\pm1,427$; $p<0,0001$; SPI (2,0): $17,670\pm1,481$; $p<0,0001$). However, sperm morphology remained unchanged across all groups ($p=0,324$). These findings suggest that Spinosad exposure during puberty disrupts testicular structure and function in male Wistar rats.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Virtual screening of natural products for identifying novel antiviral candidates targeting the VP39 protein of the Mpox virus.

Authors:

Guilherme Landim de Rezende¹ Leonardo Pereira de Araújo¹ Evandro Neves Silva¹
Letícia Barbosa Silva¹ André Luiz Caliari Costa¹ Sávio Rezende Souza¹
Thiago Caetano de Andrade Belo¹ Patrícia Paiva Corsetti¹
Leonardo Augusto de Almeida¹

Affiliations:

¹ Laboratory of Molecular Biology of Microorganisms, Universidade Federal de Alfenas (UNIFAL), Alfenas, Minas Gerais, Brazil.

Keywords: Molecular docking. Monkeypox. Virtual screening. VP39.

Abstract:

Monkeypox Virus (Mpox), classified as a global health emergency in 2022 and 2024 due to mutations and outbreaks that spread worldwide, belongs to the *Orthopoxvirus* genus of the *Poxviridae* family. This double-stranded DNA virus causes symptoms such as fever, muscle pain, fatigue, and skin pustules, with its hallmark feature being the formation of lesions across various parts of the body. Current treatments include Tecovirimat (ST246), which inhibits viral particle formation, and Brincidofovir (CMX001), which targets viral DNA replication. However, both drugs exhibit significant side effects, underscoring the urgent need for new therapeutic compounds with improved safety profiles. In this study, the VP39 protein, involved in viral replication and capsid formation, was identified as a promising molecular target. Using compounds from the Zinc20 database, drug repurposing was conducted, coupled with binding energy and interaction analyses using Autodock Vina, Pymol, and LigPlot+. Additionally, the ADME-Tox technique was applied to predict the absorption, distribution, metabolism, excretion, and toxicity properties of the selected compounds. The compound Amentoflavone (ZINC03984030) emerged as a potential VP39 inhibitor, demonstrating a binding energy of -11.2 kcal/mol, superior to the -8.1 kcal/mol observed for the co-crystallized control compound. Notably, Amentoflavone interacted with the same active site amino acids as the control compound and exhibited no unfavorable ADME-Tox predictions. Amentoflavone, a compound found in *Hypericum perforatum* and *Ginkgo biloba*, could thus represent a promising therapy for Mpox. However, *in vitro* and *in vivo* testing are required to validate these findings and establish its efficacy as a therapeutic alternative against the virus.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Integrating bioisosterism and virtual screening in the search for Adenosine A2A Receptor therapeutics.

Authors:

André Luiz Caliari Costa¹ Leonardo Pereira de Araújo¹ Evandro Neves Silva¹ Guilherme Landim de Rezende¹ Thiago Caetano Andrade Belo¹ Leticia Barbosa Silva¹
Sávio Rezende Souza¹ Patrícia Paiva Corsetti¹
Leonardo Augusto de Almeida¹

Affiliations:

¹ Laboratory of Molecular Biology of Microorganisms, Federal University of Alfenas (UNIFAL), Alfenas, 37130-001, Minas Gerais, Brazil.

Keywords: A2A receptor. Drug repositioning. Molecular docking. Neurodegenerative diseases.

Abstract:

The adenosine A2A receptor (A2Ar), a G protein-coupled transmembrane protein, stands out as a promising therapeutic target by exhibiting functional duality. Compounds with agonistic characteristics to A2Ar trigger anti-inflammatory effects, while antagonists demonstrate positive effects in the treatment of neurodegenerative diseases. Thus, the aim of this study was to identify in-silico, new drug candidates with potential to act on A2Ar. For this purpose, a systematic review was conducted, and the target deposited in the Protein Data Bank under code 5NM4 was selected, in addition to a library containing 84,215 compounds derived from natural products from the ZINC20 database was selected for screening. Redocking analyses for validation, molecular docking for virtual screening, bioisosterism, and predictions of absorption, distribution, metabolism, excretion, and toxicity (ADME-Tox) were performed. Adenosine, a compound with agonist capacity of A2Ar, was selected for the bioisosterism process, in which approximately 710 bioisosteres were designed with promising results ranging from binding energy between -9.76 kcal/mol and -8.12 kcal/mol. For the antagonist analysis, compounds ZINC04015296 and ZINC05220992 presented binding energies of -14.9 kcal/mol and -14.6 kcal/mol respectively, superior results to the control used in redocking. Compounds ZINC05220992 and Ad_bio_2 antagonist and agonist, respectively, showed ideal ADME-TOX characteristics and good interaction results with A2Ar. Therefore, it is crucial to conduct in vitro and in vivo tests to validate the potential results demonstrated here, aiming to benefit the population affected by related diseases, which negatively impact both physical and mental quality of life.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Vasorelaxant effects of *trans*-3-methoxy- β -nitrostyrene in rat thoracic aorta: involvement of soluble guanylate cyclase

Authors:

Yeimer Antonio Guevara¹
Alefe Isleyker Aguiar Santiago¹ Lucas Rodrigues Melo¹
Renata Fereirra Carvalho Leitão¹ Glória Pinto Duarte²
Joyce Karen Lima Vale³ Rosivaldo Santos Borges³ Pedro Jorge Caldas Magalhães¹ Saad Lahlou¹

Affiliations:

¹ Federal University of Ceará.
² Federal University of Pernambuco.
³ Federal University of Pará.

Keywords: NO/cGMP pathway. Soluble guanylate cyclase. *Trans*-3-methoxy- β -nitrostyrene.

Abstract:

Vasorelaxant effects of *trans*-4-methoxy- β -nitrostyrene (T4MN) in rat aorta were mediated through stimulation of soluble guanylate cyclase (sGC) stimulation. The present study investigated the mechanisms underlying the vasorelaxant effects of the stereoisomer, *trans*-3-methoxy- β -nitrostyrene (T3MN) in rat aortic ring preparations. In endothelium-intact preparations, T3MN (IC_{50} = 0.66 [0.11-2.73] μ M) was 100 times more potent as vasorelaxant agent than T4MN. This effect was unchanged by indomethacin (IC_{50} = 0.26 [0.10-0.58]), MDL-12,330A (IC_{50} = 0.98 [0.38-1.97]) or glybenclamide (IC_{50} = 1.52 [0.16-7.20]) but was significantly (P < 0.05, by Mann-Whitney U-test) reduced by endothelium removal (IC_{50} = 2.35 [0.90-5.32]), L-NAME (IC_{50} = 124.29 [28.18-307.00]), ODQ (IC_{50} = 124.29 [28.18-307.00]), LY294002 (IC_{50} = 124.29 [28.18-307.00]), TEA (IC_{50} = 18.51 [6.60-40.11]), 4-AP (IC_{50} = 36.85 [14.83-83.26]), or apamin (IC_{50} = 31.36.19 [8.60-66.94]). Under Ca^{2+} -free conditions, T3MN did not alter contractions evoked by caffeine, but significantly reduced the contractions induced by (i) the protein kinase C activator phorbol-12,13-dibutyrate (IC_{50} = 162.48 [103.00-300.00] μ M), (ii) the tyrosine phosphatase inhibitor sodium orthovanadate (IC_{50} = 447.85 [132.00-1000.00] μ M), (iii) the exogenous calcium through receptor- or voltage-operated Ca^{2+} channels and (iv), in an ODQ-preventable manner, those evoked by PHE or Ca^{2+} influx through stores-operated Ca^{2+} channels activated by thapsigargin-induced Ca^{2+} store depletion. In conclusion, T3MN induced a potent vasorelaxant effect that seems to be mediated partly by an endothelium-dependent mechanism involving activation of Akt/eNOS/NO pathway and partly by an endothelium-independent mechanism through activation of sGC/cGMP/PKG pathway in vascular smooth muscle, leading to inhibition of Ca^{2+} influx from the extracellular milieu and IP_3 -sensitive intracellular Ca^{2+} release as well as activation of potassium channels. CEUA (1677030622).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Prophylactic effect of Ascorbic Acid or Ketamine on Anxiety-Related Behavior induced by DSS

Authors:

Gabrieli Muller Duarte¹ Laura de Araujo Borba¹ Bruna Reque Kouba¹ Willk Felipe Lopes Silva¹
Pedro Borges de Souza¹
Ana Lúcia Severo Rodrigues¹

Affiliations:

¹ Department of Biochemistry, Universidade Federal de Santa Catarina, Florianópolis, Brazil.

Keywords: Anxiety. Ascorbic acid. Prophylactic treatment.

Abstract:

Anxiety disorders are prevalent and burdensome conditions whose management is needed. Ascorbic acid (AA), a potent antioxidant in the brain, has been shown to elicit antidepressant and anxiolytic effects. However, its potential to exert a prophylactic antidepressant and anxiolytic effect against an inflammatory challenge remains to be investigated. Male Swiss mice (CEUA protocol N° 4508220221) received a single administration of AA (1 or 5 mg/kg, p.o.), or ketamine (5 mg/kg, i.p.). After 7 days, mice were exposed to Dextran Sulfate Sodium (DSS) 1.5% for seven days in the drinking water. On the 8^o day the behavioral tests were conducted. Statistical analysis was performed by two-way ANOVA followed by Newman Keuls post hoc test when appropriate (significance when $p < 0.05$). Results were expressed as percentage of the mean $\pm$ standard deviation of the mean (control group = 100%). In the open field test (OFT), AA 5 mg/kg increased number of entries in the center of the apparatus as compared to control group ($p < 0.05$; $161.1\% \pm 12.4\%$). In mice subject to DSS, AA (1 and 5 mg/kg) and ketamine increased the number of entries as compared to DSS group ($p < 0.01$; $153.3\% \pm 26.0\%$; $p < 0.05$; $139.3\% \pm 13.7\%$, $p < 0.01$; $154.1\% \pm 37.0\%$, respectively). Regarding the number of groomings in the OFT, DSS increase this parameter as compared to control group ($p < 0.05$; $212.5\% \pm 52\%$). However, this effect was prevented in mice pretreated with AA 1 mg/kg ($p < 0.05$; $144\% \pm 59\%$), AA 5 mg/kg ($p < 0.01$; $81\% \pm 45.7\%$), and ketamine ($p < 0.05$; $92.5\% \pm 57.3\%$). Regarding the behavioral tests designed to evaluate depressive-related parameters (tail suspension test and splash test) no significant effects were observed in any group ($p > 0.05$) and no locomotor alterations were observed in the OFT. The results suggest that a single administration of AA or ketamine elicits an anxiolytic-like effect in mice.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Omega-3 and omega-6 in metabolic regulation: dose-dependent effects on body, plasma, and inflammatory parameters

Authors:

Matheus Felipe Zazula¹ Mônica Maciel¹
Hellen Yukari Ito Beirauti¹ Ana Carla Costa e Silva¹ Beatriz Borges Mattes¹ Luiz Claudio
Fernandes² Katya Naliwaiko¹

Affiliations:

¹ Laboratório de Plasticidade Morfofuncional, Departamento de Biologia Celular, Universidade Federal do Paraná – Curitiba, Paraná, Brasil.

² Laboratório de Metabolismo Celular, Departamento de Fisiologia, Universidade Federal do Paraná – Curitiba, Paraná, Brasil.

Keywords: Polyunsaturated fatty acids. Dyslipidemia. Macrophage activation. Body composition.

Abstract:

The consumption of n-6 unsaturated fatty acids has increased significantly in developing countries, while the intake of n-3 sources remains insufficient. As an alternative, n-3 supplementation through capsules has gained prominence, but no clear guidelines on dosage or safe intake. This study assessed the dose-response curve of n-3 and n-6 fatty acids in reproductively mature female rats. Seventy-two Wistar rats were divided into nine groups: control and supplemented with n-3 or n-6 at doses of 1, 2, 3, or 4 g/kg (n = 8 per group). Daily oral supplementation was provided for four weeks, and body characteristics, glucose and lipid homeostasis, leukocyte profile, and peritoneal macrophage activity were analyzed (CEUA 1459/2022). Data were analyzed using two-way ANOVA (dose, supplementation) followed by Tukey's post hoc test. The data were also evaluated using the Integrated Biological Response index version 2 (IBRV2). Results revealed that moderate doses of n-3 (1 g and 2 g/kg) positively impacted lipid homeostasis, and maintaining a metabolic profile similar to the control (p < 0.0001). Additionally, these doses showed potential immunomodulatory effects, enhancing peritoneal macrophage activity (p < 0.0001). These findings suggest that balanced n-3 supplementation may help regulate metabolism and modulate the immune system (p < 0.0001). However, higher n-3 doses (3 g and 4 g/kg), as well as all doses of n-6, resulted in significant adverse effects (p < 0.0001). Excessive intake of these fatty acids led to weight gain, increased fat deposition, and the development of dyslipidemia. Additionally, exacerbated activation of peritoneal macrophages was observed, indicating a potentially harmful inflammatory response (p < 0.0001). These effects suggest that while n-3 is recognized for its benefits, excessive consumption can negate its advantages and cause negative metabolic impacts similar to n-6. Thus, the results highlight the importance of balanced fatty acid supplementation. While moderate doses of n-3 provided metabolic and immunological benefits, higher doses—potentially as harmful as excessive n-6 intake—induced detrimental effects, underscoring the need for careful control and monitoring of nutrient intake.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: β -Caryophyllene derived from clove oil, carried in a self-emulsifying drug delivery system, potentiates antirheumatic effects in rats

Authors:

Any Carolina Chagas-Almeida¹ Ana Paula Ames-Sibin¹
Ana Maria Canassa Pinheiro¹ Maara Cristina Ramos¹
Ana Paula Marques Andrade¹ Ana Paula Desiree de Oliveira¹
Anacharis Babeto de Sá-Nakanishi¹ Lívia Bracht¹
Jurandir Fernando Comar¹

Affiliations:

¹ Universidade Estadual de Maringá.

Keywords: β -caryophyllene. Clove oil. Rheumatoid arthritis. Adjuvant-induced arthritis. SNEDDS. Self-emulsifying systems.

Abstract:

β -Caryophyllene (β -Cary), a sesquiterpene compound, is recognized for its anti-inflammatory properties. It can be derived from specific oils, notably clove oil, which is rich in eugenol. The remaining fraction after eugenol extraction, containing over 90% β -Cary, presents a promising alternative for drug development. This study prepared a self-nanoemulsifying drug delivery system containing β -Cary and evaluated its effects upon oral administration on inflammation and systemic oxidative stress of Holtzman rats with adjuvant-induced arthritis. The formulation (β -SNEDDS) was developed in the Master's dissertation by Almeida et al. (2021). β -SNEDDS was composed of β -Cary, surfactant and lipophilic base and was evaluated as size and morphology. Arthritic and healthy rats received orally once a day for 18 days β -SNEDDS or unformulated β -Cary, both at doses of 50 and 100 mg/kg. All procedures were approved by the Ethics Committee for Animal Experimentation of UEM (Protocol number: 2008040222). β -SNEDDS formed a set of uniform spherical droplets (<100 nm diameter) in aqueous media. During simulated digestion, β -SNEDDS formed emulsifying small droplets in the gastric phase and very tiny droplets in the intestinal stage. β -SNEDDS (100 mg/kg) prevented weight loss of arthritic rats, reduced paw edema by 50%, delayed the onset of secondary lesions, and restored the number of leukocyte in the joint cavity. In both plasma and liver, protein carbonylation levels were reduced by 21% and 55%, respectively. Myeloperoxidase (MPO) activity and reactive oxygen species (ROS) levels were also decreased, reaching values comparable to those of the control rats. The unformulated β -Cary (100 mg/kg) was only partially effective against arthritis. In conclusion, β -SNEDDS was effective against adjuvant-induced arthritis in rats, and it could serve as an alternative agent in the treatment of rheumatoid arthritis.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: p-Syneprine inhibits adipogenic differentiation and induces UCP-1 expression in 3T3-L1 preadipocytes

Authors:

Marcellie Silva Nascimento Lima¹ Mariana Maciel Pereira¹ Andressa Guimarães²
Tayanne Malafaia¹
Erica Patricia Garcia de Souza² Christina Barja-Fidalgo¹
Elaine Oliveira²
Simone Vargas da Silva¹

Affiliations:

¹ Laboratório de Farmacologia Celular e Molecular, Departamento de Biologia Celular, Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro.
² Laboratório de Fisiologia da Nutrição e Desenvolvimento, Departamento de Ciências Fisiológicas, Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro

Keywords: 3T3-L1. *Citrus aurantium*. Obesity. Preadipocytes. p-Syneprine.

Abstract:

Obesity is a disease characterized by excessive body fat accumulation, the leading risk factor for developing associated comorbidities such as cardiovascular disease, type 2 diabetes mellitus, and certain types of cancer. *Citrus aurantium* L. has been used as an alternative to weight loss, with fewer adverse effects than most other pharmacological therapies. Commercially, *C. aurantium* is standardized in p-syneprine, its active ingredient with $\beta 3$ adrenergic receptor agonist action, used to reduce adiposity and stimulate thermogenesis. In this study, we evaluated the adipogenic response of 3T3-L1 preadipocytes to isolated synephrine. The results show that p-syneprine did not affect cell viability and proliferation in any of the doses studied. After 96 hours of differentiation, p-syneprine (10 μ M) inhibited the differentiation of 3T3-L1 preadipocytes and, after 48 hours, p-syneprine seems to inhibit the accumulation of triglycerides in these cells. We demonstrate that 3T3-L1 preadipocytes express the $\beta 3$ adrenergic receptor (ADBR3), and the induction of differentiation did not alter the expression of this receptor in cells in all times analyzed. We evaluated the effect of p-syneprine on the protein expression of the two main browning markers (PGC-1 α and UCP-1). Although p-syneprine does not alter the expression of PGC-1 α , the presence of the differentiation medium was able to increase its protein expression. After 48 hours of incubation, p-syneprine induced an increase in the protein expression of UCP-1 in the absence of the differentiation medium. We also analyzed the gene expression of leptin, adiponectin, and TNF- α . P-syneprine seems to decrease leptin gene expression after 24 hours of differentiation while not altering adiponectin and TNF- α expression in any studied group and time. Until the moment, we concluded that p-syneprine acts by inhibiting the differentiation of 3T3-L1 preadipocytes. However, more research is needed to unravel the mechanisms involved in this event.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Evaluation of the isoflurane effects on the inflammatory response and redox state of the mice liver fed a high-fat diet

Authors:

Ana Carolina Lourenço Ferreira¹ Luiza Ramos De Camargos¹ Pedro Alves Machado-Junior¹
Gleidson Guilherme Carvalho da Silva¹ Maria Laura da Cruz Castro²
Ana Beatriz Farias de Souza¹ Daniela Caldeira Costa² Frank Silva Bezerra¹

Affiliations:

¹ Laboratory of Experimental Pathophysiology (LAFEx), Department of Biological Sciences (DECBI), Institute of Exact and Biological Sciences (ICEB), Federal University of Ouro Preto, Ouro Preto, MG, Brazil.

² Laboratory of Metabolic Biochemistry (LBM), Department of Biological Sciences (DECBI), Institute of Exact and Biological Sciences (ICEB), Federal University of Ouro Preto (UFOP), Ouro Preto, Minas Gerais, Brazil.

Keywords: Inflammation. Isoflurane. NAFLD. Obesity. Oxidative stress.

Abstract:

The isoflurane induces lung inflammation and oxidative stress, a key factor in non-alcoholic fatty liver disease (NAFLD). This is associated to obesity, NAFLD often necessitates surgical interventions requiring anesthetics like isoflurane. This study aimed to assess the effects of isoflurane exposure on inflammatory and oxidative parameters in the liver of mice fed a high-fat diet. It was approved by the UFOP Ethics Committee - CEUA nº2910240322. 32 C57BL/6 mice of 9-10 weeks were used and received standard diet (SD) or high-fat diet (HFD) for 10 weeks. After the experimental protocol, they were divided into 4 groups (n=8): standard diet control group exposed to ambient air (**SDC**), isoflurane 1.9% group exposure (**ISO**), high-fat diet group exposed to ambient air (**HFD**), and high-fat diet + isoflurane group exposed to isoflurane 2.6% (**HFD-ISO**). The groups were exposed for 3 hours. After 24 hours, the animals were euthanized and blood and liver were collected. In the liver function, AST was increased in the HFD-ISO (63.86 ± 8.43) and HFD (62.57 ± 11.89) compared to the SDC (46.14 ± 6.67) ($p < 0,05$). The SOD activity increased in HFD (185.2 ± 12.81) and HFD-ISO (179.6 ± 13.09) compared to SDC (160.2 ± 11.08); CAT activity was higher in HFD+ISO (4594 ± 466.00) compared to SDC (3353 ± 961.50) and ISO (3506 ± 557.50); TBARS levels were higher in HFD+ISO (93.04 ± 16.38) compared to SDC (60 ± 10.00), ISO (72.79 ± 12.64) and HFD (62.40 ± 4.23) ($p < 0,05$). CCL2 liver levels were higher in the HFD+ISO (4665.00 ± 502.50) and HFD (4232.00 ± 284.70) compared to the SDC (3074.00 ± 507.50) and ISO (3401.00 ± 395.00) ($p < 0,05$). Inflammatory cell count was higher in the HFD (103.3 ± 2.69) and HFD+ISO (93.86 ± 1.04) compared to SDC (79.18 ± 2.99) and ISO (82.20 ± 1.41) ($p < 0,05$). IL-6 levels were higher in HFD (1496 ± 114.5), HFD+ISO (1447 ± 162.4) and ISO (1147 ± 137.6) compared to SDC (945.7 ± 95.73) and ISO ($p < 0,05$). Preliminary results show that isoflurane alters hepatic lipid metabolism, promoting inflammation and oxidative stress.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Application of Dandelion in Ovarian Cancer: A Promising Natural Approach

Authors:

Julia Amanda Rodrigues Fracasso¹, Luisa Taynara Silvério da Costa, Débora Barros Barbosa³,
Vinicius Augusto Simão¹, Lucinéia dos Santos¹

Affiliations:

¹ Faculty of Science and Letters/Unesp; Assis/São Paulo; Brazil.

² School of Dentistry/ UNESP; Araçatuba/ São Paulo; Brazil.

Keywords: Antioxidant. Plants. Cancer.

Abstract:

Ovarian cancer ranks among the most lethal gynecological neoplasms and constitutes a significant global public health concern due to its high mortality rate and frequent diagnosis at advanced clinical stages. Although it accounts for approximately 3% of all cancers affecting women, it is the fifth leading cause of cancer-related death among the female population. The nonspecific nature of its early symptoms, combined with the lack of effective screening methods, contributes to late diagnosis, limiting therapeutic success and reducing survival rates. In this context, there is growing interest in the development of new therapeutic alternatives, particularly those based on bioactive compounds of natural origin. *Taraxacum officinale* (dandelion) is a medicinal plant traditionally recognized for its anti-inflammatory and antioxidant properties. Recent studies indicate its potential antitumor activity. The present study aimed to evaluate the antioxidant and cytotoxic effects of a hydroethanolic extract of *T. officinale* (EHT) on human epithelial ovarian adenocarcinoma cells (SKOV-3) and healthy murine fibroblasts (NIH3T3). The extract was obtained by maceration in 70% ethanol. The total phenolic content was quantified by UV-Vis spectrophotometry using the Folin-Ciocalteu method. Antioxidant activity was determined through lipid peroxidation (LPO) inhibition and DPPH radical scavenging assays. Cytotoxicity was assessed using the MTT assay at concentrations ranging from 100 to 600 µg/mL over 24, 48, and 72 hours in both cell lines. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test, with the significance level set at $p < 0.05$. Phytochemical analysis revealed a total phenolic content of 190 mg gallic acid equivalents (GAE)/g of dry extract. Lipid peroxidation inhibition was $48.78 \pm 0.00\%$ and $54.74 \pm 0.00\%$ at concentrations of 400 and 600 µg/mL, respectively. DPPH radical scavenging activity was $57.22 \pm 0.00\%$ and $74.47 \pm 0.00\%$ at the same concentrations. The viability of NIH3T3 fibroblasts remained above 70% at all tested concentrations, suggesting low cytotoxicity to healthy cells. In contrast, SKOV-3 cells showed viability below 70%, with effects dependent on both concentration and exposure time, particularly after 48 and 72 hours, with statistically significant differences compared to the negative control (saline solution). The hydroethanolic extract of *T. officinale* demonstrated selective cytotoxic activity against ovarian cancer cells while preserving the viability of normal fibroblasts, in addition to showing significant antioxidant capacity. These findings support the potential use of *T. officinale* as a complementary therapeutic strategy based on natural compounds in the management of ovarian cancer.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Investigation of molecular interactions between agmatine and its potential targets in depression, anxiety and suicide

Authors:

Sofia Alves Rodrigues¹ Glorister Alves Altê¹
Ana Lúcia Severo Rodrigues¹

Affiliations:

¹ Federal University of Santa Catarina.

Keywords: Agmatine. Major depressive disorder. Molecular docking.

Abstract:

Major Depressive Disorder (MDD) is a prevalent psychiatric disorder whose symptoms often co-exist with anxiety disorders. Moreover, patients with MDD are considered to be at risk for suicidal behavior. Agmatine, an endogenous polyamine, is a pleiotropic molecule that exhibits antidepressant and anxiolytic effects in animal models, but the mechanisms underpinning its effects are not fully understood. This study employs molecular docking to verify the possibility of interactions between agmatine and its potentially relevant molecular targets related to MDD, anxiety, and suicide, with a focus on the nature of these interactions. The proteins were extracted from the Protein Data Bank (PDB) and processed in the Chimera v1.12 software. The agmatine three-dimensional structure was obtained from Pubchem. Molecular docking was carried out in DockThor v.2.0. Intermolecular interactions were analyzed in Chimera v.1.12 and Ligplot v.2.2.8. The affinities between agmatine and the targets revealed by molecular docking were -8.542 kcal/mol for serotonin 1E receptor (HTR1E), -7.073 kcal/mol for muscarinic 3 receptor (CHRM3), -7.003 kcal/mol for sigma non-opioid intracellular receptor 1 (SIGMAR1) and -6.523 kcal/mol for serotonin 1A receptor (HTR1A). The interaction between agmatine and HTR1E includes hydrogen bonds involving the amino acid residue Asp102 (2.92 Å and 2.30 Å) and Trp304 (3.20 Å), while between agmatine and CHRM3 includes one hydrogen bond with Asp148 (3.16 Å) and Asn508 (3.30 Å), SIGMAR1 presented one with Asp126 (2.64 Å) and two with Glu172 (3.03 Å and 3.14 Å), and finally, HTR1A included one with Ser199 (2.55 Å) and Tyr195 (3.16 Å). Several other hydrophobic interactions involving amino acid residues were also demonstrated. The results indicated that serotonin 1E receptor may be the best-fitting ligand with agmatine, thereby likely contributing to its antidepressant and anxiolytic effect, which may be confirmed in *in vivo* experiments.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Effect of Chlorothalonil on the gene expression of mouse (*Mus musculus*) brain cortex

Authors:

Pedro Henrique Santos de Jesus¹
Leandro Amaro da Silva Felix¹
Mariana Leivas Müller Hoff¹

Affiliations:

¹ Federal University of Pernambuco.

Keywords: Agrochemicals. Nervous system. Pesticides. RNA-Seq. Transcriptome.

Abstract:

Biocides are vastly used in agriculture, specially in monoculture, and affect the environment, causing soil impoverishment and biodiversity loss, as well as causing health problems. Chlorothalonil (CTL) is a broad-spectrum pesticide, primarily used as a fungicide to protect crops, vegetables, and ornamentals. Exposure to agrochemicals has been linked to the development of neurodegenerative diseases affecting the central nervous system. A transcriptome study reported that primary cell culture of mouse cortex treated with CTL did not show gene expression patterns related to neurodegeneration and autism. This screening did not inform, however, which transcriptome alterations were caused by CTL treatment in primary cultured neurons. Therefore, we aim to identify the transcriptional alterations in mouse neurons that result from CTL exposure using bioinformatic tools on RNA sequencing (RNA-Seq) data available on the National Center for Biotechnology Information (NCBI). No additional animal was used for this work. Two RNA-Seq runs of primary culture of mouse neurons exposed to CTL (PRJNA288135: SRR2078356 and SRR2078355) were uploaded from NCBI to Galaxy platform, checked for quality with Falco analysis, trimmed using Trimmomatic, and checked for quality with Falco again. Quality control check indicated the trimmed reads' quality was satisfactory to proceed to mapping and gene expression counting analyses. Mapping and counting will be conducted on RNA STAR, using mouse genome as reference. The same workflow will be performed on RNA-Seq runs of primary culture of mouse neurons exposed to CTL vehicle (also available from PRJNA288135) and then the transcriptomes will be compared for gene expression levels, overrepresentation and enrichment analyses. These comparisons will allow us to determine the CTL effects on neurons and guide future research on the CTL neurotoxicity.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Structural Characterization and Biological Activity of a Potential toxin-peptide Extracted from the Sea Anemone *Bunodosoma caissarum*

Authors:

Bianca Menezes Torres Alves¹ Felipe Ricardo de Mello¹ Leandro Mantovani de Castro¹

Affiliations:

¹ Universidade Estadual Paulista "Júlio de Mesquita Filho" – UNESP.

Keywords: Biological activity. *Bunodosoma caissarum*. Ion channels. Sea anemones. Toxins.

Abstract:

Advances in omics technologies have fueled the bioprospecting of toxins, leading to the discovery of new compounds. A recent study with the sea anemone *Bunodosoma caissarum* identified 430 polypeptides in the tentacles and mucus, with 23 showing similarities to toxins found in other organisms, while 45% displayed no similarity to any annotated genes. In this context, our objective was to characterize the biological activity of a specific sequence containing amino acid residues in positions resembling those found in known toxins. First, the tertiary structure was predicted in silico using the AlphaFold Server, followed by secondary structure analysis via circular dichroism. Biological assays included hemolytic tests, cytotoxicity assays in neuroblastoma cell lines, and behavioral tests. AlphaFold structure prediction indicated the formation of α -helix and β -sheet structures. Circular dichroism analysis revealed a pronounced minimum at approximately 204 nm (-5.9 ellipticity units), suggesting the presence of β -sheet structures. Additionally, a positive peak near 194 nm (5.4 ellipticity units) might be associated with α -helices, while a second peak around 220 nm (0.73 ellipticity units) further suggested α -helix formation. The peptide did not cause significant hemolysis (<2%) at any tested concentration; however, agglutination was observed at 100 μ M. In behavioral assays, it significantly reduced (~50%) the average speed and distance traveled by nauplii at 100 μ M and 200 μ M. No cytotoxicity was observed in neuroblastoma cell lines across all tested concentrations. Although the peptide did not exhibit significant cytotoxicity or hemolysis, its impact on nauplii behavior and its predicted interaction with sodium-iodide symporter channels suggest a potential biological role that merits further investigation. These findings contribute to the ongoing exploration of marine toxins as candidates for therapeutic development.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: IL-6/JAK/STAT3 pathway and its possible role in ketamine antidepressant and antisuicidal effects

Authors:

Glorister Alves Altê¹

Ana Lúcia Severo Rodrigues¹

Affiliations:

¹ Federal University of Santa Catarina.

Keywords: Arketamine. Esketamine. Major depressive disorder. Suicide. Bioinformatics.

Abstract:

Major depressive disorder (MDD) is common and complex psychiatric disease with a multifactorial etiology that alterations in neurotransmission and neuroplasticity mechanisms, neuroinflammation, neurodegeneration, and redox system imbalance. Ketamine is an emerging molecule with great results in treatment resistant depression and suicide ideation. The most known ketamine's mechanism of action is the NMDA receptor blockage. However, ketamine could have a polipharmacological profile that could be responsible by its rapid and sustainable antidepressant effect. Therefore, this study aimed to predict new targets of ketamine enantiomers related to their antidepressant and antisuicidal effects. The genes targets associated with depression and suicidal behavior were obtained from DisGeNET database using the keywords "Major Depressive Disorder; CUI: C1269683" and "completed suicide; CUI: C0852733". The protein-protein network was constructed using STRING and the statistical analysis of the network and the final layout were performed in Cytoscape. Molecular docking was performed in DockThor and molecular interactions were analysed in Ligplot+. Thus, the integrated bioinformatics analysis provides an overview of the key targets of ketamine, which may be involved in its antidepressant and anti-suicidal properties. As main results, IL-6 is a central node in MDD and Suicide in network pharmacology analysis. After filtering the nodes with Cytoscape's diffusion algorithm, we performed molecular docking and both ketamine enantiomers may interact with JAK1, JAK2 and IL-6R. This finding emphasizes the possible involvement of the IL-6/JAK/STAT3 pathway in the antidepressant and anti-suicidal effects of ketamine enantiomers.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Multigenerational Study of Paternal F2 Generation Male Rats: Evaluations of Reproductive Development after Exposure of the F0 Generation to Nitrosodimethylamine

Authors:

Giovanna Kugel Marraschi¹
Livia Trippe Nagaoka¹
Julia Stein¹
Barbara Campos Jorge¹
Otávio Galassi Vitale¹
Sara Tawany Caetano dos Santos¹
Luara Magalhaes¹
Reggina Lorena Mellado Castro¹
Giovanna Ferrari Rosalem¹
Julia Tavares Alves¹
Julia Vitória Francisco de Aquino¹
Arielle Cristina Arena^{1,2}

Affiliations:

¹ Botucatu Biosciences Institute - UNESP Botucatu.

² Center for Toxicological Information and Assistance (CIATOX) - UNESP Botucatu.

Keywords: Environmental contaminants. Male rats. Multigenerational inheritance. Reproductive toxicology.

Abstract:

Exposure to environmental contaminants can occur at any time of life. When directly exposed in adulthood, F0 individuals can show adverse effects, as can their indirectly exposed offspring (F2), configuring a multigenerational inheritance. Nitrosodimethylamine (NDMA), one of the most abundant substances in nitrosamines class, has a daily exposure limit of 96 ng/day due to its carcinogenic potential. This study aims to evaluate the impacts of F0 low-dose NDMA exposure on the reproductive development of male rats from the paternal lineage of F2 generation (PF2). Male and female rats of F0 generation were divided into 2 experimental groups (control and NDMA) and exposed to vehicle (deionized water) or NDMA (1 mL/kg/day and 7.2 ng/kg/day; by gavage) (8 rats/group/sex) during the pre-conception, mating and gestational/lactational periods. These animals mated within their own group to generate the F1 generation, where only male rats were selected (1 male/litter) and, as adults, mated with external females giving rise to the PF2. Male PF2 offspring were assessed for early development and puberty onset parameters and, after this period, were euthanized to collect organs at postnatal day (PND) 70 and 110. No significant changes were observed in early development (PND 1, 13, 22) or puberty onset. Organ weights at PND 70 remained unaltered; although, at PND 110, the relative weights of the testes ($p = 0.0051$, $430.9\text{g} \pm 5.810$ vs. $394.2\text{g} \pm 10.65$), epididymis ($p = 0.0077$, $151.1\text{g} \pm 3.533$ vs. $135.1\text{g} \pm 4.289\text{g}$), prostate ($p = 0.0262$, $89.38\text{g} \pm 5.003$ vs. $73.63\text{g} \pm 4.505$) and ducts ($p = 0.0356$, 25.98 ± 1.093 vs. 22.32 ± 1.252) were reduced. These alterations may indicate compound-related disruptions in seminiferous tubules, sperm production and androgenic signaling. Thus far, our findings suggest that F0 exposure to NDMA is capable of implying multigenerational effects on the PF2 male reproductive organs. However, ongoing analyses will be essential to complement our hypothesis. (CEUA: 4704260423).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Consequences of Nitrosodimethylamine (NDMA) Exposure on Reproductive and Toxicological Parameters in Adult Male Wistar Rats

Authors:

Otávio Galassi Vitale¹
Lívia Trippe Nagaoka¹
Bárbara de Campos Jorge¹ Júlia Stein¹
Giovanna Kugel Marraschi¹
Sara Tawany Caetano dos Santos¹
Luara Magalhaes¹
Reggina Lorena Mellado Castro¹
Giovanna Ferrari Rosalem¹
Júlia Tavares Alves¹
Júlia Vitória Francisco de Aquino¹ Arielle Cristina Arena^{1,2}

Affiliations:

¹ Institute of Bioscience of Botucatu – UNESP.
² Center for Toxicological Information and Assistance.

Keywords: Endocrine disruption. Male reproductive health. Nitrosodimethylamine. Reproductive toxicology. Environmental contaminants.

Abstract:

N-nitrosamines are mutagenic and carcinogenic agents found in food, cigarettes, water, and medications, raising concerns for regulatory agencies like FDA. Few studies have assessed their reproductive effects at low doses. This study evaluates the effects of N-Nitrosodimethylamine (NDMA), one of the most prevalent in the environment, on toxicological and reproductive parameters in male Wistar rats. 44 males were divided into two groups (n = 22/group): control (1 mL/kg/day deionized water) and NDMA (7.2 ng/kg/day), both orally exposed by gavage for 45 days (PND 60–105). Males were euthanized on PND 105 for organ and blood collection. NDMA exposure showed no clinical toxicity, with no significant changes in food/water intake, body weight, or biochemical parameters. However, the NDMA group had a higher relative prostate weight (104.7 vs. 122.5 mg/100 g, p = 0.05). No morphological sperm differences were found, but NDMA-exposed males had fewer mature spermatids (111.70 ± 2.43 vs. $120.20 \pm 1.70 \times 10^6/g$, p = 0.011) and lower daily sperm production (18.31 ± 0.39 vs. $19.70 \pm 0.28 \times 10^6/g/day$, p = 0.011). Sperm transit was delayed, increasing total transit time (10.65 ± 0.25 vs. 9.53 ± 0.21 days, p = 0.001). Motility parameters (%) showed reduced total motile sperm [88.32 (87.04–92.47) vs. 82.82 (77.96–89.29), p = 0.04], increased progressive motility [13.38 (7.53–18.53) vs. 19.43 (16.34–22.52), p = 0.04], and more slow-moving [8.13 (1.23–13.98) vs. 17.30 (10.81–25.56), p = 0.02] and static sperm [11.68 (7.53–12.96) vs. 17.18 (10.71–22.04), p = 0.04]. Sperm kinetics changed, with lower ALH [22.21 μm (21.70–23.68) vs. 20.83 μm (20.66–21.22), p = 0.008] and WOB [41.10% (38.25–42.36) vs. 38.44% (37.12–39.11), p = 0.011]. NDMA may act as an endocrine disruptor, imbalancing steroid hormones impairing spermatogenesis. Future analyses will assess hormone levels to clarify toxicity mechanisms. Even at an acceptable dose, NDMA exposure significantly affected male reproductive health. CEUA: 4704260423.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Role of full-spectrum *Cannabis sativa* oil in lymphoid organs of rats exposed to crack cocaine during pregnancy

Authors:

Zaíne Marcyelle Freire do Nascimento Omena¹ Amanda de Barros Coutinho¹
Fernanda Maria Araujo de Souza¹ Eloísa Bortolote Oliosi³
Edite Santos Siqueira¹
Júlia Luíza Gomes dos Santos² Amanda Larissa Dias Pacheco¹ Olagide Wagner de Castro¹
Igor Santana de Melo¹

Affiliations:

¹ Institute of Biological and Health Sciences (ICBS), Federal University of Alagoas (UFAL). ² Faculty of Dentistry, Federal University of Alagoas (UFAL).
³ Institute of Pharmaceutical Sciences, Federal University of Alagoas (UFAL).

Keywords: Cannabidiol. Crack cocaine. Gestational.

Abstract:

Gestational exposure to crack pyrolysis products causes psychotropic and neurotoxic effects and modulation of the immune system. This study investigated the immunological effects of prenatal exposure to crack pyrolysis in Wistar rats and the possible therapeutic role of full-spectrum *Cannabis sativa* oil (OFSCS) on the exposed offspring. All experiments were approved by CEUA (#28/2021). Pregnant females were exposed daily to 200 mg of crack smoke, from the 5th to the 21st day of gestation. At birth, the pups were distributed equally between the mothers and monitored until postnatal days P30 and P60. Part of the offspring received oral treatment with OFSCS (14 days), and the thymus and spleen were subsequently collected for weight measurement and determination of thymocyte and splenocyte counts. Flow cytometry was used to analyze T subpopulations (CD4+ and CD8+). The data were analyzed using either an unpaired t-test or a three-way ANOVA. Gestational exposure to crack cocaine did not significantly alter the body weight of the mothers or the thymus; only the weight of the spleen and the CD8+ T lymphocyte subsets showed a significant reduction. In the P30 offspring, treatment with OFSCS did not affect these parameters. At P60, it reversed weight loss in males exposed to crack and had an impact on thymic cellularity and spleen weight in unexposed males. Furthermore, gestational exposure to crack decreased the number of total splenocytes in females. These results suggest that gestational exposure to crack induces discrete immunological alterations, with a sex-specific effect. Treatment with OFSCS showed sex- and time-dependent effects, suggesting a potential modulator of immune alterations related to prenatal exposure to drugs.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Effects of Incretin-Based Therapies on NeuroCardiovascular Dynamic Changes Induced by High Fat Diet in Rats

Authors:

Silvio Rodrigues Marques Neto^{1,2} Raquel Carvalho Castiglione¹ Iasmim Bento Leal¹
Rayana Guagliardi Brito¹ Ana Beatriz Castro Ribeiro¹
Giovanna de Andrade de Barros e Vasconcellos¹ Eliete Bouskela¹

Affiliations:

¹ Universidade do Estado do Rio de Janeiro.

² Universidade Salgado de Oliveira.

Keywords:

Obesity. Mitochondrial swelling. High-fat diet. Liraglutide.
Cerebral microcirculation.

Abstract:

Obesity promotes cardiac and cerebral microcirculatory dysfunction that could be improved by incretin-based therapies. However, the effects of this class of compounds on neuro-cardiovascular system damage induced by high fat diet remain unclear. The aim of this study was to investigate the effects of incretin-based therapies on neuro-cardiovascular dysfunction induced by high fat diet in Wistar rats. We have evaluated fasting glucose levels and insulin resistance, heart rate variability quantified on time and frequency domains, cerebral microcirculation by intravital microscopy, mean arterial blood pressure, ventricular function and mitochondrial swelling. High fat diet worsened biometric and metabolic parameters and promoted deleterious effects on autonomic, myocardial and haemodynamic parameters, decreased capillary diameters and increased functional capillary density in the brain ($p < 0.05$). Biometric and metabolic parameters were better improved by glucagon like peptide-1 (GLP-1) compared with dipeptidyl peptidase-4 (DPP-4) inhibitor ($p < 0.01$). On the other hand, both GLP-1 agonist and DPP-4 inhibitor reversed the deleterious effects of high fat diet on autonomic, myocardial, haemodynamic and cerebral microvascular parameters ($p < 0.05$). GLP-1 agonist and DPP-4 inhibitor therapy also increased mitochondrial permeability transition pore resistance in brain and heart tissues of rats subjected to high fat diet ($p < 0.01$). Incretin-based therapies improve deleterious cardiovascular effects induced by high fat diet and may have important contributions on the interplay between neuro-cardiovascular dynamic controls through mitochondrial dysfunction associated to metabolic disorders.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Neuroprotective Effect of Citicoline in the Alzheimer's Disease Model of *Caenorhabditis elegans* CL2006

Authors:

Emilha Uzun Papaya¹
Matheus Henrique Franco Alves¹ Isabella Giunco Estigarribia¹ Daniel Ferreira Leite²
Paola dos Santos da Rocha¹ Jaqueline Ferreira Campos¹ Edson Lucas dos Santos¹ Kely de Picoli Souza¹

Affiliations:

¹ Universidade Federal da Grande Dourados.

² Universidade Federal de Mato Grosso do Sul.

Keywords: Biochemical compound. Neurodegeneration. Oxidative stress

Abstract:

Alzheimer's disease (AD) is the leading cause of dementia in the elderly, characterized by the accumulation of β -amyloid, mitochondrial dysfunction, and oxidative stress, factors that contribute to neuronal degeneration and cognitive impairment. Citicoline is a phospholipid precursor and a source of choline for acetylcholine synthesis, being considered a potential therapeutic agent in neurodegenerative diseases. Although studies have demonstrated neuroprotective effects of citicoline in experimental models with features of AD, there are, to date, no specific studies evaluating its mechanism of action in Alzheimer's disease. In this context, the aim of this study was to investigate the neuroprotective effects of citicoline in the β A1-42-induced AD model in *Caenorhabditis elegans* CL2006. To this end, the effect of citicoline was evaluated on β A1-42-induced toxicity in *C. elegans* CL2006, a transgenic model for AD. Synchronized nematodes (L4 stage) were pre-treated for 1 hour at 15 °C with citicoline (25, 250, and 500 μ g/mL) and then exposed to 25 °C for 22 hours to induce paralysis. Assessments were performed every 2 hours over a 10-hour period, considering individuals responsive to mechanical stimuli as viable. Paralysis reversal was assessed after a 24-hour recovery period at 20 °C. The assay was conducted in triplicate, and results are considered preliminary. At the end of the evaluation period, the control group showed $8.17 \pm 2.12\%$ of non-paralyzed nematodes. In contrast, groups treated with citicoline at concentrations of 25, 250, and 500 μ g/mL exhibited $22.46 \pm 2.51\%$, $26.79 \pm 5.47\%$, and $33.38 \pm 4.41\%$ non-paralyzed animals, respectively. After the recovery period, animals in the untreated control group showed no recovery. Conversely, the citicoline-treated groups demonstrated $12.02 \pm 1.99\%$, $25.12 \pm 4.05\%$, and $31.72 \pm 2.19\%$ of non-paralyzed nematodes, respectively. These findings indicate that citicoline was able to reduce β A1-42-induced paralysis, maintaining a higher number of non-paralyzed individuals in *C. elegans* CL2006, suggesting its therapeutic potential in neurodegenerative conditions such as Alzheimer's disease.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Modulatory Effects of Omega-3-Derived Lipid Mediators and Acetylsalicylic Acid on Skeletal Muscle Regeneration and Fatigue Response in a Snake Venom-Induced Injury Model

Authors:

Gabriela Grassmann Roschel¹ Eduardo Purgatto²
Vanessa Moreira¹

Affiliations:

¹ Universidade Federal de São Paulo.

² Universidade de São Paulo.

Keywords: Lipid mediators. Muscle regeneration. Omega 3 fatty acids.

Abstract:

Muscle regeneration is regulated by myogenic factors and inflammatory mediators. Lipid mediators (LM) derived from omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) may promote regeneration and are synthesized by enzymes acetylated by acetylsalicylic acid (ASA). However, their role in skeletal muscle regeneration remains unclear. Male Swiss mice (n=4/group, 6 weeks, CEUA5123050421) were treated intraperitoneally (i.p.) with a fish oil nanoemulsion (FON; 6.0 µg/g DHA and 10.2 µg/g EPA in 500 µL) or 0.9% saline for 7 days. Then, *Bothrops asper* venom (Bav; 2.5 mg/kg in 50 µL) was injected intramuscularly (i.m.) into the left gastrocnemius muscle, and saline was injected into the contralateral side (control). A distinct group received i.p. ASA (117 mg/kg in 500 µL) every 3 days. At 24 h, 3, 7, and 28 days after Bav, muscles were collected for western blot analysis of PAX-7 and α-smooth muscle actin (α-SMA). Fatigue resistance was assessed 28 days post Bav using a 15-min tetanic isometric stimulation. PAX-7 expression increased in FON (66.36%) and FON+ASA (65.41%) groups compared to saline after Bav (p<0.05) and remained elevated in FON+ASA (56.29%) at 3 days (p<0.05). However, PAX-7 decreased in FON alone at 3 and 7 days post Bav (53.85% and 72.28%, p<0.05). α-SMA was significantly reduced in FON+ASA (86.73%) at 7 days post Bav (p<0.05). In the fatigue test, FON+ASA group showed reduced resistance at 5 min (33.6%) and 6 min (39.4%) compared to Bav i.m. (p<0.05), with values similar to the control. These findings suggest that EPA and DHA-derived lipid mediators modulate key myogenic and contractile markers during regeneration, and their effects may be potentiated by ASA. However, the reduced fatigue resistance observed with combined FON and ASA treatment indicates that the balance between structural regeneration and functional recovery should be further investigated.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Antimelanoma Potential and Selectivity of Cerumen and Propolis from *Nannotrigona testaceicornis* (Hymenoptera, Apidae, Meliponini)

Authors:

Pedro Henrique Patrício Barbosa¹ Natália Guedes Jorge¹
Alécio da Silva Soutilha¹ Paola dos Santos da Rocha¹ Edson Lucas dos Santos¹ Kely Picoli de Souza¹ Jaqueline Ferreira Campos¹

Affiliations:

¹ Universidade Federal da Grande Dourados – UFGD.

Keywords: Cancer. Cell culture. Cytotoxicity. Stingless bee. Natural products.

Abstract:

Melanoma, known as skin cancer, develops in cutaneous melanocytes located in the deeper layers of the epidermis. Melanoma is considered one of the most aggressive types of cancer and has high mortality rates due to its metastatic profile. Given the increase in skin cancer incidence in recent decades, and the side effects and low selectivity of chemotherapy treatments, the search for new therapies has been under investigation. Among these, the search for natural products derived from Brazilian biodiversity stands out. In this context, this study evaluated the effect of the ethanolic extract of cerumen (EEC-Nt) and propolis (EEP-Nt) from the stingless bee *Nannotrigona testaceicornis* against murine melanoma (B16F10-Nex2), human melanoma (SK-Mel-19), and normal cells (human peripheral blood mononuclear cells: PBMC, CEP nº 7.169.283). The selectivity index was calculated by comparing cytotoxicity in PBMCs to the cytotoxicity of the specific cell line. The cytotoxic effects of the extracts were assessed using the MTT assay (n=1). EEC-Nt showed an IC₅₀ of 277.59 ± 2.44 µg/ml against the B16F10-Nex2 line, with a selectivity index of 2.94. The IC₅₀ of EEC-Nt in SK-Mel-19 was 533.92 ± 2.72 µg/ml, with a selectivity index of 1.53. EEP-Nt presented an IC₅₀ of 182.76 ± 2.26 µg/ml against B16F10-Nex2, with a selectivity index of 3.97. For SK-Mel-19, the IC₅₀ of EEP-Nt was 526.07 ± 2.72 µg/ml, and the selectivity index was 1.38. The results demonstrate the anticancer potential of EEC-Nt and EEP-Nt against cancer cell lines, showing good selectivity for the B16F10-Nex2 line compared to PBMC cytotoxicity, with values above 1. Finally, these data open new perspectives for the chemical characterization of the extracts, investigation of cell death mechanisms, preclinical model testing, and the appreciation and conservation of Brazilian biodiversity.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Antioxidant Properties of *Xanthosoma sagittifolium* (Taioba) Constituents Related to the Endogenous System of the Yeast *Saccharomyces cerevisiae*

Authors:

Isabel João de Deus Amilcar¹ Nathalia Soares Camargo¹ Nayana de Figueiredo Pereira²
Mariele Rondon Santos Gonçalves³ Douglas Siqueira de Almeida Chaves² Mário Geraldo de
Carvalho³
Cristiano Jorge Riger¹(Orientador)

Affiliations:

¹ Laboratório de Estresse Oxidativo em Microrganismos (LEOM). Universidade Federal Rural do Rio de Janeiro (UFRRJ), Seropédica (RJ), Brasil.
² Laboratório de Química de Produtos Naturais (LQPN), Instituto de Química (IQ). Universidade Federal Rural do Rio de Janeiro (UFRRJ), Seropédica (RJ), Brasil.
³ Laboratório de Química de Bioativos Naturais (LQBioN), Instituto de Ciências Biológicas e da Saúde (ICBS). Universidade Federal Rural do Rio de Janeiro (UFRRJ), Seropédica (RJ), Brasil.

Keywords: *Xanthosoma sagittifolium*. *Saccharomyces cerevisiae*. Antioxidants. Reactive oxygen species

Abstract:

The excessive generation of reactive oxygen species (ROS) can lead to oxidative stress, resulting in cellular damage and compromising biological functions. The presence of antioxidant compounds in the diet helps prevent and reduce the damage caused by these species, contributing to cellular homeostasis. *Taioba* (*Xanthosoma sagittifolium*) is a non-conventional edible plant (PANC), rich in bioactive compounds with potential antioxidant properties; however, its effect on the endogenous antioxidant defence system in eukaryotic organisms requires further investigation. The yeast *Saccharomyces cerevisiae* is a suitable biological model for toxicity and antioxidant capacity studies and is widely used in biomedical and biotechnological research. The present study analyses the antioxidant properties related to the chemical constituents of the aerial parts and roots of *Xanthosoma sagittifolium* L. Schott (*Taioba*) in relation to the endogenous system of *Saccharomyces cerevisiae*. Aqueous extracts from the stem (STCaq) and leaf (STFaq), as well as their respective ethyl acetate fractions (STCA and STFA), were initially prepared at the Laboratory of Natural Bioactive Chemistry (LQBioN). At the Laboratory of Natural Products Chemistry (LQPN), methanolic and dichloromethane extracts were produced from the tuber. From the leaves, seventeen different flavonoids were identified using HPLC and UV spectra, with three initially identified using reference standards. Two *S. cerevisiae* strains were used: a wild-type control (BY4741) and an isogenic mutant lacking an antioxidant transcription factor ($\Delta yap1$). To determine non-cytotoxic extract concentrations, growth curve and resazurin-based metabolic activity assays were performed. None of the extracts exhibited toxicity at concentrations below 0.5 mg·mL⁻¹ for either strain. The antioxidant analyses included cell viability tests and measurements of intracellular oxidant levels. In both strains, cells were exposed to hydrogen peroxide (2.0 mM) to induce oxidative stress. Results showed a 29% increase in viability for the control strain and 46% for the mutant strain when treated with the aqueous leaf extract. Regarding intracellular oxidation levels, a reduction in oxidative stress (42.7%) was observed only in the control strain with the same extract. This study highlights the antioxidant potential of *X. sagittifolium*, indicating possible pharmacological applications related to its chemical constituents.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Protective Activity of Chalcone Derivatives in a Parkinsonian Model of *Saccharomyces cerevisiae*

Authors:

Danniel Cosme Neves Grillo¹ Lucas Caruso Araújo Bezerra² Arthur Eugen Kümmerle² Cristiano Jorge Rigger¹

Affiliations:

¹ Laboratory of Oxidative Stress in Microorganisms (LEOM), Department of Biochemistry, Institute of Chemistry, Federal Rural University of Rio de Janeiro (UFRRJ), Seropédica, RJ, Brazil

² Laboratory of Molecular Diversity and Medicinal Chemistry (LaDMol-QM), Department of Organic Chemistry, Institute of Chemistry, Federal Rural University of Rio de Janeiro (UFRRJ), Seropédica, RJ, Brazil

Keywords: Alpha-synuclein. Chalcones. Parkinson's disease. *Saccharomyces cerevisiae*.

Abstract:

Parkinson's disease (PD) is the second most common neurodegenerative disorder worldwide, with increasing prevalence due to population aging. It is characterized by the loss of dopaminergic neurons in the substantia nigra and by Lewy bodies and neurites, mainly composed of the protein α -synuclein (α -syn). Given the lack of therapies that modify disease progression, we investigated chalcone derivatives, bioactive compounds with antioxidant and anti-inflammatory properties, which show potential for treating neurodegenerative diseases. To model α -syn toxicity, we used a *Saccharomyces cerevisiae* strain expressing α -syn-GFP and a control strain with empty vector. The cytotoxicity of four chalcones was assessed via growth curves (OD₅₇₀) and viability assays (plating/CFU assay) after 2 and 4 hours of treatment. Antioxidant capacity was evaluated with the 2',7'-dichlorofluorescein assay, and fluorescence microscopy was used to assess α -syn aggregation. Tested concentrations were 10 μ M and 30 μ M. α -syn expression reduced cell viability by 72.63% $\pm$ 5.8% (2h) and 71.4% $\pm$ 4.2% (4h). Chalcones showed no toxicity. After 2h, all increased viability, especially compound 3 (126.85% $\pm$ 12.30%) and compound 1 (144.23% $\pm$ 6.13%) at 30 μ M. After 4h, only compounds 3 (120.89% $\pm$ 9.66%) and 4 (125.37% $\pm$ 12.26%) maintained effects. Microscopy confirmed that compounds 3 and 4 (10 μ M, 2h) reduced α -syn aggregation, while compounds 1 and 2 showed antioxidant activity, decreasing fluorescence by 17.87% $\pm$ 5.44% and 38.32% $\pm$ 12.14%, respectively. In conclusion, chalcone derivatives improved viability and reduced α -syn toxicity and aggregation in yeast, supporting their potential as promising compounds for future therapeutic approaches in Parkinson's disease.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Influence of Cu(II) toxicity in eukaryotic study models expressing neurodegenerative disease peptides

Authors:

Thaina Fonseca Bueno¹ Rodrigo de Paulo Osório¹ Cristiano Jorge Riger¹

Affiliations:

¹ Laboratory of Oxidative Stress in Microorganisms (LEOM), Department of Biochemistry, Institute of Chemistry, Federal Rural University of Rio de Janeiro (UFRRJ), Seropédica, RJ, Brazil.

Keywords: Alzheimer. Copper. Parkinson. *Saccharomyces cerevisiae*.

Abstract:

Alzheimer's and Parkinson's diseases are the most common neurodegenerative diseases in elderly people, linked to the progressive loss of neurons, directly affecting the quality of life of these individuals. The imbalance in the homeostasis of metals, such as copper, is a potentiator of oxidative stress, one of the factors that contributes to the progression of these diseases, due to an increase in the formation of reactive oxygen species (ROS) favoring the aggregation of beta-amyloid (A β) and alpha-synuclein (α -syn) peptides, and increasing toxicity in nerve cells. This study aims to evaluate whether the Cu (II) ion increases cellular toxicity using as a simple eukaryotic model yeast cells *Saccharomyces cerevisiae* expressing the peptides (A β) or (α -syn). Copper toxicity was evaluated through spot test, with CuSO₄ at concentrations of 1, 5, 8, 10, 20 and 50 mM in different incubation times (4 and 72 hours). Both strains transformed with plasmids and the control strain exposed to concentrations of 20 and 50 mM showed significant inhibition of cell growth, which was intensified in strains expressing A β and α -syn. Intracellular oxidation was analyzed with dichlorofluorescein (DCFH-DA) in the 3 strains at a concentration of 50 mM CuSO₄, with 72 h of treatment; showing high fluorescence compared to the control $298.56 \pm 8.37\%$ (PV), $127.45 \pm 5.53\%$ (A β), 229.06

$\pm 4.89\%$ (α -syn). Cell viability assay (plating) with 5 mM CuSO₄ in SD glucose and SD glycerol media, after 4 hours of treatment, also showed a reduction in cell viability of $30.57 \pm 18.28\%$ (PV), $28.59 \pm 12.41\%$ (A β), $14.27 \pm 13.47\%$ (α -sin), with the formation of petites in the medium with glycerol. These results suggest that Cu(II) induces oxidative stress and implies a decrease in cell viability, especially at high concentrations and long exposure time, indicating its neuronal toxicity associated with the generation of peptides in neurodegenerative diseases.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Influence of antioxidant compounds derived from chalcones and coumarins in *Saccharomyces cerevisiae* cells with autophagic dysfunction

Authors:

Nathália Valle Costa¹ Karine Falcão dos Santos¹ Arthur Eugen Kümmerle² Cristiano Jorge Riger¹

Affiliations:

¹ Laboratory of Oxidative Stress in Microorganisms (LEOM). Departament of Biochemistry, Institute of Chemistry. Federal Rural University of Rio de Janeiro (UFRRJ), Seropédica (RJ), Brazil.

² Laboratory of Molecular Diversity and Medicinal Chemistry (LaDMol-QM). Departament of Organic Chemistry, Institute of Chemistry. Federal Rural University of Rio de Janeiro (UFRRJ), Seropédica (RJ), Brazil.

Keywords: Autophagic. chalcones. coumarins. yeast.

Abstract:

Autophagy plays a fundamental role in cellular recycling, involving several mechanisms that remove dysfunctional cytoplasmic components. Neurodegenerative diseases and cancer may be associated with problems involving this degradation pathway. Chalcones and coumarins are bioactive compounds with antioxidant and anti-inflammatory properties, with therapeutic potential, that is, they can aid in the self-digestion process of misfolded proteins. The objective of this work is to investigate the influence of derivatives of these compounds on the autophagic system, using as a model *Saccharomyces cerevisiae* with its intact genome (BY4741) and the isogenic mutant in a gene related to the autophagic pathway (Δ atg8). A screening was performed, with the metabolic activity marker resazurin, at concentrations of 2.5 μ M and 5.0 μ M of the compounds, where there was no toxicity for both strains in 24 hours. Cell viability in a culture medium that activates the autophagy process in yeast demonstrated that at 2.5 μ M for the chalcone derivative and at 5.0 μ M for the coumarin derivative, the cells had a survival of $95.79 \pm 2.87\%$ and $118.51 \pm 59.41\%$ compared to the mutant strain (Δ atg8), respectively. At higher concentrations, such as 50 μ M, chalcones in nitrogen-restricted medium for 24 h caused a $57 \pm 8.48\%$ loss of autophagic viability in the mutant strain (Δ atg8), compared to the control strain (BY4741). The metabolic indicator 2,3,5-triphenyltetrazolium chloride, used qualitatively, forms the formazan product by weak enzymatic action, that is, the toxicity may be a reflection of greater impairment in cellular respiration, already impaired in the mutant strain. These results show that concentrations of 50 μ M are toxic to the chalcone derivative and affect its respiratory function. Concentrations of 5.0 μ M for the chalcone derivative and 2.5 μ M for the coumarin derivative have a considerable survival percentage compared to the control and mutant strains, and are not toxic.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Development of active compounds against A β 42 peptide aggregation: seeking alternatives for the treatment of Alzheimer's.

Authors:

Rodrigo de Paulo Osorio¹
Larissa de Almeida Peixoto Ferreira² Nathalia Fonseca Nadur²
Cristiano Jorge Riger¹ Arthur Eugen Kummerle²

Affiliations:

¹ Universidade Federal Rural do Rio de Janeiro; Departamento de Bioquímica.
² Universidade Federal Rural do Rio de Janeiro; Departamento de Química Orgânica.

Keywords: Alzheimer. Beta-amyloid. Coumarins. *Saccharomyces cerevisiae*.

Abstract:

Alzheimer's disease is a progressive neurodegenerative disease. Some of the main characteristics of this pathology are oxidative stress, hyperphosphorylation of tau protein and accumulation and aggregation of beta-amyloid peptide, especially A β 42 peptide. Since it is a multifactorial disease, multitarget compounds may be an important alternative in the search for efficient therapeutic interventions. Therefore, different coumarin derivatives have been developed aiming at cellular protection against some of the main toxicity mechanisms of Alzheimer's disease. Cell viability (plating), intracellular oxidation (2,7-dichlorofluorescein), mitochondrial potential (JC-1) and amyloid aggregation (Thioflavin T) assays were performed using the eukaryotic model of *Saccharomyces cerevisiae* transformed for the expression of A β 42. The treatments were performed with coumarin compounds at a concentration of 50 μ M for 4 hours. The results are presented as a mean and standard deviation of three independent experiments. ANOVA followed by Tukey's test was used. The difference was considered statistically significant when $p < 0.05$. The cell viability assay showed that M1 caused a recovery of $22 \pm 9.05\%$ in cell viability for cells expressing A β 42, while M2 and M3 showed cell viability similar to the untreated control. The level of intracellular oxidation was not attenuated by M1 and M3, but M2 showed a reduction of $24.43 \pm 5.18\%$ in the level of intracellular oxidation. Compound M1 caused a $79.18 \pm 7.37\%$ increase in mitochondrial membrane potential. In addition, compound M1 was able to reduce the level of protein aggregation by $9.14 \pm 4.52\%$. The results indicate that M1 was able to attenuate the cellular toxicity caused by the amyloid peptide through an anti-aggregation effect in the humanized yeast model.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Neuroprotective effect of sarcosine in an animal model of Alzheimer's disease

Authors:

Raphaella Almeida Chiareli^{1,2} Amanda Rodrigues Quintanilha¹ Gustavo Almeida de Carvalho¹
Raul Izidoro Ribeiro¹
Andris Figueiroa Bakusis¹ Rodrigo Mello Gomes¹ Mauro Cunha Xavier Pinto¹

Affiliations:

¹ Universidade Federal de Goiás.

² Universidade Estadual de Goiás.

Keywords: Neurodegeneration. Neuroprotection. Memory.

Abstract:

Introduction: Sporadic Alzheimer's disease (AD) is characterized by alterations in insulin signaling, memory impairment, neuroinflammation, and synaptic dysfunction. Sarcosine is an inhibitor of glycine transporter type 1, an agonist of glycine receptors, and a co-agonist of the NMDA receptor. The aim was to investigate the neuroprotective effect of sarcosine in an animal model of Alzheimer's disease. The project was approved by the Ethics Committee for Animal Use of the Federal University of Goiás, under protocol number 042/2019. Methods: Swiss mice were used to induce the model by intracerebroventricular administration of STZ and were treated with sarcosine at doses of 125, 250 and 500 mg/kg for 28 days. After 25 days, tests were performed to assess motor activity and memory. On day 28, magnetic resonance imaging was performed to assess the hippocampus and cerebral cortex, followed by immunofluorescence to assess neuronal density and neuroinflammation. Golgi staining was used to quantify dendritic spines. Results: Treatment with sarcosine for 28 days at doses of 125 ($59.85 \pm 2.25\%$), 250 ($52.29 \pm 2.87\%$), and 500 mg/kg ($60.71 \pm 2.22\%$) reversed spatial memory deficits, preserved hippocampal areas at doses of 250 ($12.12 \pm 0.20\%$) and 500 mg/kg ($13.02 \pm 0.96\%$) and the cerebral cortex at doses of 125 (49.74 ± 0.81), 250 (53.24 ± 0.66) and 500 mg/kg (52.90 ± 1.41). Neuronal density was preserved at 500 mg/kg (108 ± 4.18), as were dendritic spines at 500 mg/kg (31.13 ± 1.47). It also reduced neuroinflammation by decreasing astrogliosis when animals were treated with 125 (28140 ± 3233), 250 (27662 ± 3885), and 500 mg/kg sarcosine (27220 ± 2675). Conclusions: Taken together, these results demonstrate the potential neuroprotective effect of sarcosine in an animal model of AD.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Adolescent Binge Drinking Withdrawal Leads to Long-lasting Spinal Cord Neuron Damage and Oxidative Stress in Rats

Authors:

Diane Cleydes Baía da Silva¹ Enzo Gabriel Lacerda Rodrigues¹ Maria Luísa Lopes da Silva¹
João Carlos do Nascimento Martins¹ Felipe Ewerton Gonçalves da Silva¹, Maria Vitoria Oliveira
Rebelo¹ Isabella Maia Carmo e Silva¹
Chan David Ribeiro¹
Paulo Fernando Santos Mendes² Daiane Claydes Baia da Silva² Sabrina de Carvalho
Cartágenes¹ Rafael Rodrigues Lima²
Luanna de Melo Pereira Fernandes¹

Affiliations:

¹ Laboratory of Neuropharmacology and Behaviour, Center for Health and Biological Sciences, State University of Pará, Belém, Pará, Brazil.
² Laboratory of Functional and Structural Biology, Institute of Biological Sciences, Federal University of Pará, Belém, Pará, Brazil.

Keywords: Adolescence. Alcohol withdrawal. Binge-like ethanol. Motor neuron. Spinal cord.

Abstract:

The rising ethanol (EtOH) consumption among adolescents poses a concerning risk of damage to the central nervous system (CNS). Alcohol binge drinking (BD) withdrawal can lead to oxidative imbalance and glutamatergic excitotoxicity, triggering apoptotic mechanisms and potential functional impairments in CNS. Although the spinal cord is essential for executive motor function, the short- and long-term effects of adolescent BD - along with the cellular and molecular mechanisms of EtOH toxicity and withdrawal - remain poorly understood. This study aims to investigate the short- and long-term biochemical and histological effects of adolescent BD withdrawal on the spinal cord. After approval by the Animal Experimentation Ethics Committee of the Federal University of Pará (CEUA/UFPA), under protocol number 1395290822, forty 35-day-old male Wistar rats underwent intermittent EtOH (3g/kg/day, 20%w/v) exposure via intragastric gavage, simulating a binge-like pattern for 3 consecutive days, following a 3-day on/4-day off cycle, over 4 weeks. The animals were euthanized one and thirty days post-withdrawal. Spinal cord samples were collected to quantify motor neurons and analyze oxidative biochemical parameters. Prolonged abstinence led to a reduction in motor neurons across all spinal cord segments - cervical, thoracic, and lumbar. Reduced glutathione levels were consistently low at both withdrawal time points. Additionally, prolonged abstinence was associated with decreased total antioxidant capacity and increased thiobarbituric acid reactive substances (TBARS) levels, while nitric oxide levels were elevated in both immediate and prolonged abstinence. These findings provide insight into the spinal cord effects of EtOH withdrawal, highlighting motor neuron loss and oxidative stress as key mechanisms. The observed motor neuron degeneration following prolonged withdrawal suggests a potential long-term risk of adolescent binge EtOH consumption.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Adolescent Binge Ethanol Consumption Impairs Motor Function and Induces Long-Lasting Oxidative Stress in Female Rats

Authors:

Jéssica Rayssa Cardoso Teixeira^{1,2} Enzo Gabriel Lacerda Rodrigues¹ João Carlos do Nascimento Martins¹ Felipe Ewerton Gonçalves da Silva¹ Maria Luisa Lopes da Silva¹ Wallacy Watson Pereira Melo³ Jofre Jacob da Silva Freitas⁴ Rafael Rodrigues Lima³ Sabrina de Carvalho Cartágenes^{1,2} Antônio Rafael Quadros Gomes¹ Cristiane Socorro Ferraz Maia^{2,4} Luanna de Melo Pereira Fernandes^{1,4}

Affiliations:

- ¹ Laboratory of Neuropharmacology and Behaviour, Center for Health and Biological Sciences, State University of Pará, Belém, Pará, Brazil.
- ² Laboratory of Pharmacology of Inflammation and Behavior, Institute of Biological Sciences, Federal University of Pará, Belém, Pará, Brazil.
- ³ Laboratory of Functional and Structural Biology, Institute of Biological Sciences, Federal University of Pará, Belém, Pará, Brazil.
- ⁴ Amazon Preclinical Studies Center, Center for Biological and Health Sciences, State University of Pará, Belém, Pará, Brazil.

Keywords: Adolescence. Alcohol abstinence. Binge Drinking. Motor function. Oxidative stress.

Abstract:

Ethanol (EtOH) is one of the most widely consumed psychoactive substances worldwide, largely due to its availability and social acceptance. The binge drinking pattern, characterized by excessive alcohol intake in a short period followed by abstinence, is increasingly common, especially among young women. Adolescent alcohol consumption can have long-term consequences, as EtOH affects multiple brain regions, particularly those involved in motor control. This study aims to investigate the immediate and prolonged effects of binge EtOH consumption from adolescence to adulthood on motor behavior and oxidative biochemistry in the motor cortex, striatum, and cerebellum. After approval by the Animal Experimentation Ethics Committee of the State University of Pará (CEUA/UEPA), in license BIO-15/2023, 48 female Wistar rats were divided into 4 groups (n=12/group) and received EtOH (3g/kg/day, 20%w/v) or distilled water via intragastric gavage for 3 consecutive days, following a 3-day on/4-day off cycle, over 8 weeks. Evaluations occurred 24 hours and 14 days post-exposure to assess withdrawal effects. Motor function was analyzed using the open field, pole, beam walking, and rotarod tests. Oxidative biomarkers, including thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH), and Trolox equivalent antioxidant capacity (TEAC), were analyzed in brain tissues. Binge EtOH withdrawal reduced spontaneous locomotion, induced bradykinesia, and impaired motor coordination and balance but did not cause persistent deficits in motor learning. EtOH withdrawal also increased lipid peroxidation in the motor cortex, cerebellum, and striatum, even after 14 days of abstinence, and persistently decreased GSH levels in these regions. These findings suggest that binge-like ethanol withdrawal induces lasting motor dysfunction, with oxidative stress as a key underlying mechanism.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Virtual screening of natural products targeting TNF- α for psoriatic symptomology modulation

Authors:

Sávio Rezende Souza¹ Leonardo Pereira de Araújo¹ Evandro Neves Silva¹
Thiago Caetano de Andrade Belo¹ André Luiz Caliar Costa¹ Guilherme Landim de Rezende¹
Letícia Barbosa Silva¹
Patrícia Paiva Corsetti¹ Leonardo Augusto de Almeida¹

Affiliations:

¹ Laboratory of Molecular Biology of Microorganisms, Federal University of Alfenas (UNIFAL), Alfenas, 37130-001, Minas Gerais, Brazil.

Keywords: Bioisosterism. Molecular Docking. Psoriasis. TNF- α . Virtual Screening.

Abstract:

Tumor necrosis factor-alpha (TNF- α) is a pro-inflammatory cytokine that regulates the inflammatory response and performs various functions in immune system cells. Dysregulation of this response can lead to autoimmune diseases such as psoriasis. Commercial TNF- α inhibitors are expensive biological compounds like recombinant proteins and monoclonal antibodies. He and collaborators (2005), reported a chemical compound capable of inhibiting TNF- α . However, its structural instability poses a significant challenge for in vivo studies and commercial applications. Thus, this study aimed to identify, through an in silico approach, new drug candidates with potential activity against the TNF- α receptor. A systematic review was conducted, selecting the target deposited in the Protein Data Bank under the code 2AZ5, along with a library of 84,215 natural product-derived compounds from the ZINC20 database for screening. Validation analyses were performed using redocking, followed by molecular docking for virtual screening, bioisosterism, and absorption, distribution, metabolism, excretion, and toxicity (ADME-Tox) predictions. The methodology was validated through redocking, where the co-crystallized compound described by He and collaborators with the 2AZ5 protein showed a binding energy of -8.9 kcal/mol. In virtual screening, the compound ZINC70699837 demonstrated a binding energy of -11.3 kcal/mol, superior to the control, but exhibited predictions of hepatotoxicity, AMES toxicity, and a maximum tolerated dose below zero. Consequently, 212 bioisosteres were designed to discover new structures without these adverse predictions. The compound TNF_bio_1 exhibited ideal pharmacokinetic and pharmacodynamic properties. Therefore, it is crucial to perform in vitro and in vivo tests to validate the promising results demonstrated, aiming to benefit the population affected by inflammatory diseases, which negatively impact both physical and mental quality of life.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: The potential of decanoic acid as a neurorepair agent in an animal model of cerebral ischemia

Authors:

Antonio Ítalo dos Santos Nunes¹ Eduardo Rosa da Silva¹
Daniel Pereira Cavalcante¹ Amanda Rodrigues Quintanilha¹ Gustavo Almeida de Carvalho¹
Jacqueline Alves Leite¹
Mauro Cunha Xavier Pinto¹

Affiliations:

¹ Department of Pharmacology, Institute of Biological Sciences, Federal University of Goiás, Goiânia, GO, Brazil.

Keywords: AMPA receptors. Fatty acids. Inflammation. Stroke.

Abstract:

Introduction: Cerebral ischemia is a neurological disorder caused by the abrupt interruption of cerebral blood flow, leading to cognitive and motor impairments. Decanoic acid has been proposed as a potential therapeutic agent for neurological diseases. Objective: To evaluate the neurorepair potential of decanoic acid in a cerebral ischemia model. Methods: Cerebral ischemia was induced in 8-week-old male Swiss mice by permanent distal occlusion of the middle cerebral artery and common carotid artery. Animals received decanoic acid (62.5, 125, 250, 500, and 1000 mg/kg) via oral gavage for seven days post-induction. Ischemic area morphology was assessed using TTC staining, while motor asymmetry, postural tone, and spatial and contextual memory deficits were evaluated through the Cylinder test, Limb Clasping test, Object Location Test (OLT), and Novel Object Recognition Test (NORT), respectively (n=10 per group). Mechanisms involved in neurorepair were explored using ELISA for inflammatory biomarkers and Western blotting for AMPA receptor pathways. Statistical analyses were performed using one-way ANOVA. All procedures were approved by the Ethics Committee on Animal Use (Protocol No. 048/23). Results: After seven days of treatment, decanoic acid promoted neurorepair effects, reducing the ischemic area and improving motor and memory impairments. The 250 mg/kg dose was the most effective ($4.06 \pm 1.85\%$; $100.40 \pm 1.13\%$; $99.35 \pm 3.49\%$, respectively). Furthermore, it decreased pro-inflammatory cytokine expression, though this effect was not associated with changes in AMPA receptor expression. Conclusion: Decanoic acid exhibits therapeutic potential for ischemic lesions, enhancing functional and cognitive recovery while reducing inflammation.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: *In vitro* study of leucine supplementation and acerola (*Malpighia emarginata* DC.) hydroethanolic extract effects on the modulation of cancer cachexia.

Authors:

Gabriella Gonçalves da Silva Rezende¹ Ianca Carneiro Ferreira¹
Marcos José Salvador²
Maria Cristina Cintra Gomes-Marcondes¹

Affiliations:

¹ Nutrition and Cancer Laboratory, Department of Structural and Functional Biology, Institute of Biology, University of Campinas (UNICAMP).

² Vegetal Metabolism and Symbionts Laboratory, Department of Vegetal Biology, Institute of Biology, University of Campinas (UNICAMP).

Keywords: Acerola. Amino acid. Cancer. Cachexia. Treatment.

Abstract:

Cancer-associated cachexia induces progressive muscle degradation, affecting up to 80% of patients, which contributes to approximately 30% of cancer-related deaths. Considering this, the present study evaluated, *in vitro*, the effects of leucine and hydroethanolic extract of acerola BRS Sertaneja (HEA) on muscle cells, mimicking the cachectic condition. L6 myoblast cells were cultured and treated for 72 hours with culture medium enriched with leucine and/or HEA, exposed or not to ascitic fluid from Walker 256 tumour-bearing rats, simulating *in vivo* conditions. We assessed cell viability using MTT and Neutral Red (NR) assays, proliferation by counting viable cells over time, and oxidative stress by measuring the GSH, GST, and alkaline phosphatase activity. Our results showed that cell viability was preserved in the leucine-treated group, maintaining cellular integrity without significant alterations. Treatment with HEA exhibited cytotoxicity at higher concentrations (2000, 3000, and 4000 µg/mL), reducing viability by ~ 25% (MTT) and 45% (NR). Treatment with ascitic fluid (at 10%) also decreased viability (~35% MTT and ~20% NR), suggesting cell damage mediated by inflammation and oxidative stress. Ascitic fluid exposure significantly impaired the cell proliferation, reducing it by ~86% compared to the control group, while HEA partially attenuated this effect (~63%). Leucine alone stimulated proliferation (1.6-fold increase), whereas HEA alone showed no difference compared to control. In the oxidative stress analysis, GSH levels had no variation between groups, but a significant increase in GST was observed in the group treated with Extract+Leucine ($P = 0.0026$) and in the Leucine group ($P = 0.011$), compared to the control. These findings suggest that the combination of HEA and leucine enhances the antioxidant and proliferative capacity of L6 muscle cells, potentially conferring a protective effect against oxidative stress and cell degeneration induced by cachexia.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Aqueous extract of *Vernonia condensata* flowers prevents DNA oxidation and protects *Caenorhabditis elegans* from oxidative stress

Authors:

Ígor Vítor da Silva¹ Alex Santos Oliveira¹ Vitor Simon Araújo¹
Matheus Henrique Franco Alves¹ Paola dos Santos da Rocha¹ Edson Lucas dos Santos¹
Jaqueline Ferreira Campos¹
Kely de Picoli Souza¹

Affiliations:

¹ Research Group on Biotechnology and Bioprospecting Applied to Metabolism (GEBBAM), Federal University of Grande Dourados.

Keywords: Boldo-bahiano. *C. elegans*. Oxidative stress. Natural Products. RENISUS.

Abstract:

Oxidative stress is an imbalance between reactive oxygen species production and endogenous antioxidants, leading to biomolecule oxidation, such as DNA. This condition is related to diseases like cancer and Alzheimer's. Exogenous antioxidants, especially those from plants, may help prevent this damage and reduce disease development. *Vernonia condensata* (boldo-bahiano) contains compounds with antioxidant potential. This study aimed to evaluate the antioxidant capacity of the aqueous extract of *V. condensata* flowers (EAVc-FI) using *in vitro* and *in vivo* assays. Flowers were collected, sanitized, dried, ground, and subjected to exhaustive aqueous infusion followed by lyophilization. To assess the protective effect of EAVc-FI on biomolecule oxidation, genomic DNA from cultured cells was incubated with different extract concentrations (10–1000 µg/mL) and 10% H₂O₂, then exposed to UV transilluminator for 5 min. To evaluate the *in vivo* effect, *Caenorhabditis elegans* (N2 strain) was incubated with the extract (250–1000 µg/mL) for 1 h and exposed to Juglone (250 µM). Viability was monitored for 6 h, with non-responsive nematodes considered dead. Gel analysis was performed using ImageLab software and statistical analysis with GraphPad Prism 9. Analysis of variance (ANOVA) followed by Dunnett's test for comparison between two or more groups was performed and the results were considered significant when $P < 0.05$. EAVc-FI prevented DNA oxidation at higher concentrations, notably at 1000 µg/mL, which showed 17±1.4% oxidation compared to total oxidation induced by H₂O₂. *In vivo*, the extract protected the animals from oxidative stress, with 1000 µg/mL maintaining 38.09±2.06% viability at 4 h versus 25.62±2.1% with Juglone alone. These results suggest antioxidant potential of *V. condensata* flowers *in vitro* and *in vivo*, reinforcing the relevance of natural products in managing oxidative stress and related diseases.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Formulation Strategies to Enhance Cannabidiol Solubility and Bioavailability: A Preliminary Scoping Review

Authors:

Yasmin Bastos Campos¹ Lívia Maria Ribeiro Rosário² Rafela Figueiredo Rodrigues¹ Flávia Chiva Carvalho¹

Affiliations:

¹ Faculty of Pharmaceutical Sciences, Federal University of Alfenas – MG. ² Institute of Experimental Biology, Federal University of Alfenas – MG.

Keywords: Cannabidiol. Drug compounding. Nanotechnology. Solubility. Solvent system.

Abstract:

Cannabidiol (CBD) exhibits anti-inflammatory, antipsychotic, analgesic, antidepressant, and anxiolytic properties. Although its mechanism of action is not yet fully elucidated, it has been widely studied and used in various clinical settings. In Brazil, ANVISA has approved 38 Cannabis-based medicinal products, all intended for oral administration. This is due to the physicochemical characteristics of CBD, which is poorly soluble in aqueous media and has low oral bioavailability resulting from extensive first-pass metabolism—factors that hinder the achievement of desired therapeutic outcomes. In response, new strategies are being explored to enhance aqueous solubility and bioavailability, such as the application of nanotechnology. However, the progress and implementation of these promising approaches in pharmaceutical formulations remain uncertain. This scoping review investigates the challenges and expectations surrounding these strategies, focusing on alternative routes of administration. Following the Joanna Briggs Institute Methodology for Scoping Reviews, the guiding research question is: “What technologies are being used to improve the solubility of cannabidiol for alternative routes of administration?” Studies were identified using MeSH/DeCS descriptors and Boolean operators in PubMed, Web of Science, Virtual Health Library (BVS), and Embase, yielding 21,040 articles imported into Rayyan. After removing duplicates, 12,658 remained. The current phase involves a blinded, two-step manual screening by two reviewers; a third resolves disagreements. The process follows PRISMA guidelines and is registered in the Open Science Framework (OSF). Preliminary findings show few studies specifically address CBD solubility in water, with most focusing on nanotechnological techniques. This review aims to clarify which technologies are being employed to improve CBD solubility, supporting the development of new formulations and enhancing its therapeutic application.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Larvicidal potential of native seeds against *Callosobruchus maculatus*: A physiological and biochemical evaluation for sustainable agricultural pest management using *Apuleia leiocarpa*

Authors:

João Victor Barbosa Santana¹ Maria Eduarda Alves Pinheiro¹ Andreza Figueira Soares¹
Kayan Eudorico Ventury Baptista² Antônia Elenir Amâncio Oliveira² Elane da Silva Ribeiro¹
Flávia Borges Mury¹

Affiliations:

¹ Instituto de Biodiversidade e Sustentabilidade (NUPEM/UFRJ).
² Universidade Federal do Norte Fluminense (LQFPP/UENF).

Keywords: *Apuleia leiocarpa*. *Callosobruchus maculatus*. Pest control. Natural defense. Proteins.

Abstract:

Agriculture relies on pest control to maintain the quality and quantity of food production. One of the most common methods is the use of chemical pesticides, which can pose health risks to humans and animals. However, plants also have natural defense mechanisms against predators, including the production of bioactive compounds, particularly in seeds, that can serve as alternative pesticide sources. One of the main pests affecting legume production in Brazil is the bean weevil (*Callosobruchus maculatus*), a beetle of the order Coleoptera and subfamily Bruchinae. Due to its high reproductive rate and infestation capacity, this insect causes significant losses in stored grain, reducing the commercial value of crops by up to 95%. Effective control of this pest is therefore essential for sustainable agriculture. In this context, this project aims to evaluate the larvicidal activity of *Apuleia leiocarpa* seeds on the development of *Callosobruchus maculatus*. The seeds were ground, and the resulting flours were tested for repellency. The protein extracts were visualized by SDS-PAGE. Preliminary results showed an approximately 95% reduction in oviposition on *Apuleia leiocarpa* seeds compared to cowpea (*Vigna unguiculata*). In addition, no adult emergence was observed in *Apuleia leiocarpa*, whereas cowpea seeds produced an average of 7.4 adults per seed. Artificial seeds containing 5% *Apuleia leiocarpa* seed meal completely repelled oviposition. These results highlight the potential of *Apuleia leiocarpa* as a promising natural resource for pest control. Increased knowledge of the biochemical properties of this native Brazilian species may lead to the development of sustainable alternatives to chemical pesticides, contributing to safer agricultural practices and environmental conservation.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Investigation of alternative therapeutic strategies for treatment of oral squamous cell carcinoma employing ruthenium(II) complex

Authors:

Gabrielle dos Santos¹ Caio Cesar Cândido² Tamara Luiza Cabral¹ Thamires Mazzola¹
Simone da Silva Lamartine Hanemann³ Felipe Fornias Sperandio⁴
Antonio Carlos Doriguetto²
Marilia Imaculada Frazão Barbosa² João Adolfo Costa Hanemann¹ Marta Miyazawa¹
Marisa Ionta³

Affiliations:

- ¹ College of Dentistry, Federal University of Alfenas (UNIFAL-MG), Alfenas, Minas Gerais, Brazil.
² Institute of Chemistry, UNIFAL-MG, Alfenas, Minas Gerais, Brazil.
³ Laboratory for evaluation of antitumor prototypes (LAPAN), UNIFAL-MG, Alfenas, Minas Gerais, Brazil.
⁴ College of Dentistry, University of Saskatchewan, Saskatoon SK, Canada.

Keywords: Cell cycle. MAPK/ERK. Oral cancer. Beta-catenin. Ruthenium complex.

Abstract:

Squamous cell carcinoma (SCC) is the most common type of oral cancer. The therapeutic approaches are limited, especially when the disease is diagnosed at an advanced stage, which contributes to the high mortality rate associated with this type of cancer. Thus, it is important to expand therapeutic strategies for SCC. The present study aimed to explore the chemopreventive and chemotherapeutic effects of a ruthenium(II) complex containing a cinnamic acid derivative in its structure, [Ru(*trans*-4-(trifluoromethyl)cinnamic acid)(dppb)(bipy)]PF₆, hereafter referred to as *trans*CINNAM. Oral dysplasia (DOK) and squamous cell carcinoma (SCC-9 e SCC-25) cell lines were used to evaluate the influence of this metal complex on proliferative behavior and oncogenic signaling pathways. Sulforhodamine B cell viability assay, clonogenic capacity, and cell cycle analysis were performed to characterize the cytotoxic and antiproliferative activities of *trans*CINNAM; while Western blot was used to determine protein expression levels of cell cycle regulators and modulation of oncogenic signaling pathways. The results revealed that *trans*CINNAM exhibited significant cytotoxicity on cancer cells, with IC₅₀ values of approximately 3 µM. The cytotoxic effect of *trans*CINNAM was lower for dysplastic and fibroblasts (IC₅₀ around 5 and 6 µM), respectively, compared with oral cancer cells. Increased expression of p-ERK was observed in SCC-25 cells in response to treatment, along with a significant reduction in β-catenin levels. The expression of p-AKT was not affected by the treatment in SCC-25 cells. Preliminary data suggest that *trans*CINNAM has higher cytotoxic activity against oral tumor cells compared to dysplastic and gingival fibroblasts. Further studies will be performed to understand the relationship between the cytotoxic activity of *trans*CINNAM and MAPK/ERK pathway activation.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: *Tillandsia loliacea*: Antimelanoma activity, selectivity, and in vivo toxicity

Authors:

Natália Guedes Jorge¹ Alécio da Silva Soutilha¹
Pedro Henrique Patrício Barbosa¹ Matheus Henrique Franco Alves¹ Maria Victória Benites Rodrigues¹ Emilha Uzum Papaya¹
Helder Freitas dos Santos¹ Paola dos Santos da Rocha¹ Jaqueline Ferreira Campos¹ Kely de Picoli Souza¹
Edson Lucas dos Santos¹

Affiliations:

¹ Universidade Federal da Grande Dourados.

Keywords: *Caenorhabditis elegans*. Cancer. Cell culture. Cytotoxicity. Natural products.

Abstract:

Melanoma is a type of skin cancer responsible for high mortality rates due to its metastatic capacity. Thus, melanoma represents a therapeutic challenge that demands new strategies. This study aimed to investigate the chemical composition and effect of the dichloromethane:methanol (1:1) extract of *Tillandsia loliacea* (E1:1TL) on melanoma cells, normal cells, and the preclinical model *Caenorhabditis elegans*. Phenolic and flavonoid contents of E1:1TL were quantified using the Folin-Ciocalteu and AlCl₃ complexation methods, respectively (n=3). Cytotoxic activity on melanoma cells was assessed in the SK-Mel-103 human melanoma cell line via cell viability assay (MTT method, 24h, n=3), while selectivity was evaluated in normal human PBMC cultures (CEP nº 6.166.432) using the same method. Safety was tested in the *C. elegans* preclinical model (N2 strain, n=3) through a subchronic toxicity assay at 125-1000 µg/mL (one-way ANOVA). E1:1TL showed 104.75 ± 1.09 mg gallic acid equivalents/g for phenolic and 32.51 ± 2.48 mg quercetin equivalents/g for flavonoids, with 31% of phenolics being flavonoids. In SK-Mel-103, E1:1TL exhibited a dose-dependent effect with an IC₅₀ of 79.08 ± 18.63 µg/mL and maximum inhibition of 67% at 400 µg/mL. In PBMC, E1:1TL maintained 100% viability up to 200 µg/mL, without reaching IC₅₀ at 400 µg/mL, preventing selectivity index (SI) calculation; this profile suggests high specificity for cancer cells, minimizing toxicity to healthy cells. In *C. elegans*, E1:1TL showed no subchronic toxicity (p>0.05). These results suggest that E1:1TL phenolics and flavonoids may inhibit melanoma proliferation, with high selectivity and no toxicity in biological models, showing promise versus current therapies nonspecific cytotoxicity. This opens prospects for phytochemical, cell death, and preclinical studies, positioning *T. loliacea* as an innovative source of antimelanoma agents.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Impact of Bergamot Leaf and Fruit Extracts on Metabolic Syndrome Parameters in Wistar Rats

Authors:

Caíque Manentti Caccia¹ Juliana Silva Siqueira¹ Thiago Luiz Novaga Palacio¹ Jordanna Cruzeiro¹
Nubia Alves Grandini¹ Taynara Aparecida Vieira¹
Marina de Paula Salomé dos Santos¹ Camila Renata Corrêa¹

Affiliations:

¹ São Paulo State University (Unesp), Medical School, Botucatu/SP, 18618687, Brazil.

Keywords: Antioxidant. Nutraceuticals. Polyphenols.

Abstract:

High sugar-fat diets are associated with metabolic syndrome (MS), which is characterized by hypertension, impaired blood glucose (BG) and dyslipidemia. *Citrus bergamia* (Bergamot) contains bioactive compounds with the potential to modulate metabolic parameters. However, differences exist between its leaf and fruit, particularly in flavonoid concentrations. This study aimed to compare the effects of bergamot leaf and fruit extracts on MS-related parameters. Male Wistar rats were randomly assigned to two dietary groups (n=21/group): Control (C) and High Sugar-Fat (HSF). After 20 weeks, MS-related parameters were assessed, and the HSF group was further subdivided into three groups (n=7/group): HSF, HSF supplemented with bergamot leaf extract (HSF+LF), and HSF supplemented with bergamot fruit extract (HSF+FR). Following an additional 10-week intervention, the rats were anesthetized and euthanized, and MS markers were analyzed: systolic blood pressure (SBP), BG, high-density lipoprotein (HDL), triglycerides (TG), and adiposity index (AI). Statistical comparisons were performed using an independent *t*-test at week 20 and one-way ANOVA followed by Tukey's post-hoc test ($\alpha = 0.05$) at the end of the experimental protocol (CEUA=1337/2019). After 20 weeks, the HSF group exhibited significantly higher MS markers compared to the C group, including increased SBP ($p < 0.001$), BG ($p < 0.001$), and TG ($p = 0.002$). After 10 weeks of treatment, the HSF+LF group, compared to the HSF group, demonstrated a significant increase in HDL (mg/dL; HSF: 18.429 ± 4.276 vs. HSF+LF: 23.571 ± 2.370 , $p = 0.024$) and decrease in TG (mg/dL; HSF: 111.000 ± 22.083 vs. HSF+LF: 78.200 ± 17.024 , $p = 0.009$). No significant differences were observed in the HSF+FR group. These findings indicate that the HSF diet effectively induced MS in Wistar rats, while bergamot leaf extract improved specific dyslipidemia parameters, suggesting potential metabolic benefits.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Green Propolis prophylaxis improves liver function in diabetic rats

Authors:

Aline Barbosa Ribeiro¹ Clara Martins Barth¹

Maria Eduarda Bonaldi Cardoso¹ Felipe Aparecido Feloni Birer¹ Vanessa Leiria Campo¹

Affiliations:

¹ Barão de Mauá University Center.

Keywords: Diabetes mellitus. Dyslipidemia. Propolis.

Abstract:

Propolis has biological and therapeutic effects in several pathological and clinical conditions, such as type 2 diabetes. However, its potential as a prophylactic or therapeutic agent in experimental models of type 1 diabetes-induced hyperglycemia remains underexplored. The present study aimed to evaluate the effects of both therapeutic and prophylactic administration of propolis in diabetic rats. The experimental protocol was conducted following the approval of the Ethics Committee for Research and Animal Experimentation of the Barão de Mauá University Center (Process: 527/24). Rats were divided into two groups and treated with ethanolic extract of propolis (100 mg/kg by gavage) for seven days. On the eighth day, after fasting for 8 to 12 hours, the rats received a single injection of STZ (50 mg/kg) intraperitoneally. The development of diabetes was confirmed after 48 hours by verifying hyperglycemia (> 200 mg/dL) from blood collection. Following the confirmation of diabetes, the rats received either propolis or an ethanolic vehicle by gavage for an additional seven days. The following day, the rats were anesthetized, and blood and liver were collected. As expected, diabetes effectively promoted a significant reduction in the assessed body weight, and the prior administration of propolis increased delta body weight (-10.6 ± 1.7 vs. -31 ± 6.7 in ethanolic vehicle). Moreover, the prophylactic treatment affected lipid metabolism due to changes in the contents of total triacylglyceride (53.2 ± 28.8 vs. 303.9 ± 82.5 in the ethanolic vehicle) and cholesterol (141.4 ± 35.5 vs. 248.4 ± 12.6 in the ethanolic vehicle). Data showed no significant differences in the analyzed variables to therapeutic treatment. These findings suggest that green propolis may prevent the progression of diabetes by mitigating body and liver weight loss. Further studies are needed to show whether the anti-inflammatory and antioxidant properties of propolis are involved in its prophylactic effect.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Alcohol metabolism in a repeated binge drinking pattern induces biochemical alterations in adolescent female rats: focus on liver enzymes and lipid profiles

Authors:

Maria Vitoria Oliveira Rebelo^{1,2} Thais Pereira Torres Magno¹ Maria Luisa Lopes da Silva¹ Enzo Gabriel Lacerda Rodrigues¹ Isabella Maia Carmo e Silva¹
João Carlos do Nascimento Martins¹ Felipe Ewerton Gonçalves da Silva¹ Luan Nascimento Pereira de Amorim¹ Rafael Hendrick Freitas de Paula¹ Chan David Ribeiro¹
Larissa Pillar Gomes Martel¹ Anderson Bentes de Lima²
Luanna de Melo Pereira Fernandes¹

Affiliations:

¹ Laboratory of Neuropharmacology and Behaviour, Center for Health and Biological Sciences, State University of Pará, Belém, Pará, Brazil.

² Laboratory of Experimental Pharmacology, Center for Health and Biological Sciences, State University of Pará, Belém, Pará, Brazil.

Keywords: Adolescent. Binge Drinking. Liver. Lipid Metabolism.

Abstract:

Excessive alcohol consumption has already become a public health problem, especially among young women, who are notable for exhibiting a consumption pattern known as Binge Drinking (BD), characterized by blood alcohol concentrations equal to or greater than 0.08 g/dL. This pattern of consumption tends to cause significant damage to various organs and tissues, particularly the liver, which is responsible for metabolizing substances such as lipids and enzymes. The project was approved by the Institutional Animal Care and Use Committee (CEUA, No. 16/2023). Female Wistar rats (n=54) were used and received either ethanol (3 g/kg/day, 20% w/v) or distilled water via orogastric gavage for three consecutive days per week for up to eight weeks. After 4 weeks of BD, 8 weeks of BD, and 8 weeks of BD followed by 14 days of abstinence, serum biomarkers of liver damage — including AST, ALT, and alkaline phosphatase (ALP) — as well as total cholesterol, HDL, LDL, VLDL, and triglycerides, were assessed. The results showed a significant increase in ALT levels ($P=0.015$), AST/ALT ratio ($P=0.0035$), ALP ($P<0.0001$), total cholesterol ($P=0.042$), VLDL ($P<0.0001$), HDL ($P=0.005$), and triglycerides ($P<0.0001$) after 4 weeks of BD. After 8 weeks of BD, ALT ($P<0.0001$), the AST/ALT ratio ($P=0.0093$), ALP ($P=0.0004$), VLDL cholesterol ($P=0.0182$), and triglycerides ($P=0.0027$) remained elevated, accompanied by increased AST levels ($P=0.0005$). Notably, even fourteen days after 8 weeks of BD, ALT ($P<0.0001$), VLDL ($P=0.0005$), and triglycerides levels ($P=0.0002$) remained significantly elevated. This experimental study contributes to the scientific community by demonstrating the impacts of intermittent and episodic alcohol consumption and its repercussions, suggesting that binge drinking during adolescence induces alterations in liver markers and increases fat accumulation in the bloodstream. Furthermore, these changes persist into adulthood, even after 14 days of ethanol withdrawal.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Long-term hepatic oxidative imbalance and tissue damage induced by binge drinking from adolescence to adulthood in female rats

Authors:

Thais Pereira Torres Magno¹ Maria Vitoria Oliveira Rebelo¹ Jessica Rayssa Cardoso Teixeira^{1,2}
Adria Paes de Souza Costa¹
Maria Luisa Lopes da Silva¹ Isabella Maia Carmo e Silva¹ Luiz Carlos Figueiredo Filho¹ Tiago
Monteiro Batista¹ Georgia Liz Castelo Lima¹ José Nazareno Cunha Negrão³
Cristiane do Socorro Ferraz Maia^{2,3} Jofre Jacob da Silva Freitas³
Luanna de Melo Pereira Fernandes^{1,3}

Affiliations:

¹ Laboratory of Neuropharmacology and Behaviour, Center for Biological and Health Sciences, Pará State University.

² Inflammation and Behavior Pharmacology Laboratory, School of Pharmacy, Institute of Health Sciences, Federal University of Pará.

³ Amazon Preclinical Studies Center, Center for Biological and Health Sciences, Pará State University.

Keywords: Adolescent. Binge Drinking. Lipid Peroxidation. Liver Steatosis. Oxidative Stress.

Abstract:

Excessive ethanol (EtOH) consumption during adolescence contributes to social, economic, and health problems worldwide, especially among women. The most common pattern of consumption is known as binge drinking (BD), characterized by intermittent and episodic intake that raises blood EtOH concentrations to 0.08 g/dL. This pattern disrupts homeostasis, particularly in the liver, which is responsible for EtOH metabolism and the generation of reactive oxygen species and acetaldehyde, a highly hepatotoxic metabolite. This process generates reactive oxygen species and acetaldehyde, a primary metabolite with high hepatotoxic potential. However, it is still not fully understood how different cycles of BD during adolescence affect the liver in both the short and long term, nor what mechanisms are involved. This study aimed to evaluate the effects of repeated BD episodes from adolescence to adulthood on oxidative biochemistry and liver morphology. After CEUA approval (No. 16/2023), 62 adolescent female Wistar rats were subjected to a three-day EtOH protocol (3 g/kg/day; 20% w/v) via intragastric gavage, or water (control), for 8 weekly cycles. Liver samples were collected 24 hours and 14 days after the final administration to assess short-and long-term effects. Results showed a significant increase in lipid peroxidation, MDA levels, ($P=0.0002$) and catalase activity ($P=0.0085$), and a decrease in superoxide dismutase (SOD) activity ($P=0.0068$) after 8 BD cycles. These biochemical changes were accompanied by moderate steatosis, necrosis, and inflammatory infiltrate. Long-term, the biochemical alterations persisted, with mild steatosis, pyknotic nuclei, cytoplasmic acidophilia, and only partial restoration of normal tissue architecture. These findings suggest that BD may induce significant liver damage during adolescence, which can persist into adulthood. Further studies are warranted to elucidate the underlying mechanisms involved.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Neuroprotection Induced by Pre-treatment with Sarcosine in an Animal Model of Cerebral Ischemia

Authors:

Antonio Ítalo dos Santos Nunes¹ Joaquina Victoria Teodoro Muassabo¹ Ingrid Martins Farias¹
Amanda Rodrigues Quintanilha¹ Eduardo Rosa da Silva¹
Daniel Pereira Cavalcante¹ Gustavo Almeida de Carvalho¹ João Elias Pinheiro Sousa de Paula¹ Mauro Cunha Xavier Pinto¹

Affiliations:

¹ Universidade Federal de Goiás.

Keywords: Glycine transporter type 1. NMDA receptor. Stroke.

Abstract:

Stroke is characterized by an interruption of cerebral blood flow, leading to oxygen and glucose deprivation in neural tissues. Cerebral ischemia triggers excessive glutamate release, abnormal activation of N-methyl-D-aspartate receptors (NMDAR), and excitotoxicity. Sarcosine, a GlyT1 (glycine transporter type 1) inhibitor, modulates NMDAR function and has been proposed as a promising neuroprotective strategy for stroke. Objective: To investigate the neuroprotective potential of sarcosine through a pretreatment regimen in a mouse model of permanent middle cerebral artery occlusion (MCAO). Methods: Male Swiss mice (8 weeks old) were pretreated with sarcosine (125, 250, and 500 mg/kg) for five consecutive days prior to MCAO induction. The ischemic region was morphologically assessed using 2% triphenyltetrazolium chloride (TTC) staining. Motor performance was evaluated using the Cylinder Test (motor asymmetry) and the Limb Clasp Test (postural stability). The study included five experimental groups (n = 6 per group). Statistical analysis was performed using one-way ANOVA. All procedures were approved by the Animal Ethics Committee (Protocol No. 041/19). Results: Pretreatment with sarcosine led to significant neuroprotective effects, as evidenced by improvements in motor asymmetry and postural regulation. TTC staining revealed a marked reduction in infarct volume, with the 500 mg/kg dose showing the most pronounced effect ($98.33 \pm 4.0\%$; 0.20 ± 0.22 ; $16.62 \pm 3.76\%$, respectively). Conclusion: Prophylactic administration of sarcosine conferred significant neuroprotection in a murine model of ischemic stroke, reducing infarct size and improving neurological deficits. These findings support the potential of GlyT1 inhibition as a preventive therapeutic strategy for cerebral ischemia.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Evasins EVABm14B and EVABm1A from *Rhipicephalus microplus* as anti-inflammatory potential and interaction with HIV gp120 protein

Authors:

Renato Bernabé¹

Vitor Cândido Oliveira¹ Júlia David¹

Helga Gomes² Glória Braz⁴ Itabajara Vaz Jr^{3,5} Carlos Logullo² Jorge Moraes^{1,2}

Affiliations:

¹ Laboratório Integrado de Bioquímica Hatisaburo Masuda - Instituto de Biodiversidade e Sustentabilidade - NUPEM, Universidade Federal do Rio de Janeiro; Rio de Janeiro – RJ; 21941-902; Brazil.

² Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro, RJ, Brazil.

³ Instituto Nacional de Ciência e Tecnologia - Entomologia Molecular, Rio de Janeiro, Brazil.

⁴ Laboratório de Tecido Conjuntivo, Hospital Universitário Clementino Fraga Filho.

⁵ Centro de Biotecnologia and Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.

Keywords: Rhipicephalus. Ticks. Evasin. Chemokine. anti-inflammatory. Hiv.

Abstract:

Ticks are ectoparasites that feed on the blood of humans and animals, acting as disease vectors. They secrete proteins called Evasins in their saliva, which modulate the host's immune system by binding to chemokines, enabling prolonged feeding. These molecules also exhibit potential effects on viral infections. This study uses transcriptomic sequences from the tick *Rhipicephalus microplus* to model Evasin structures, validate them using Ramachandran plots, and perform molecular docking with chemokines and the HIV gp120 protein. Additionally, Evasins (EVABm1A and EVABm14B) were expressed in plasmids for purification and anti-inflammatory testing. Fifteen Evasins were identified: 12 from Class A, 1 from Class B, and 2 unclassified. Two Evasins were structurally modeled and validated, with docking assays revealing strong interactions with chemokines and gp120. EVABm14B was expressed, purified, and demonstrated anti-inflammatory activity. Purified Evasins may inhibit chemokines in inflammatory processes and, EVABm1A by mimicking the CCR5 receptor, could interfere with HIV gp120-CD4 binding, offering potential for novel HIV therapies.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Innovating Antibiotic Research and Development Through Brazil's Biodiversity

Authors:

Taise Tomie Hebihara Fukuda¹ Silvia Helena Cestari de Oliveira¹ Eduardo Penteado Gottardo¹ Monique Samaan¹
German Enrique Wassermann¹ Spartaco Astolfi Filho²

Affiliations:

¹ Cristália Pharmaceutical Chemicals. ² Federal University of Amazonas.

Keywords: Antibiotics. Biodiversity. Natural Products.

Abstract:

The discovery of new antimicrobial compounds is a critical global challenge, especially considering the WHO's prediction that by 2050, up to 8 million people worldwide could die from antibiotic-resistant infections. Brazil, known for its extraordinary biodiversity, is home to over 20% of the world's species, making it the most biodiverse nation on Earth and placing it at the top among the 18 megadiverse countries. Leveraging this natural wealth, Cristália has launched an innovative program to explore rare Actinobacteria from Brazil's ecosystems in search for new antibiotics. These bacteria produce secondary metabolites, many of which act as chemical defenses with potent antimicrobial properties. Cristália's multidisciplinary team has developed its own methodology, leading to the successful isolation of microorganism strains from soil samples. During the screening of their extracts, they discovered that the strain *Dactylosporangium sp.* CRIS0015 exhibited inhibitory activity against human pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA). By combining bio-guided fractionation, high-resolution mass spectrometry, and tandem mass spectrometry, the active compound responsible for this effect was identified as Fidaxomicin. Furthermore, the genomic DNA of *Dactylosporangium sp.* CRIS0015 was sequenced using Oxford Nanopore's next-generation sequencing (NGS) technology. After assembling the genome through two distinct pipelines, the team identified and characterized the biosynthetic gene cluster (BGC) responsible for Fidaxomicin production. Today, many pharmaceutical companies have abandoned antibiotic discovery programs due to low profitability, allowing bacteria to evolve resistance faster than new treatments are developed. This reality highlights the need for efficient screening programs to uncover antibiotics from Brazil's rich biodiversity. Cristália's pipeline has already delivered promising results, exemplified by the discovery of Fidaxomicin.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Clinical and obstetric profile of normotensive and pre-eclamptic pregnant women attended at the Botucatu Maternity - SP, Brazil

Authors:

Sara Tawany Caetano dos Santos¹ Bárbara Campos Jorge¹
Júlia Polotto da Silva¹ Joelcio Francisco Abbade² Valéria Cristina Sandrim¹ Arielle Cristina Arena¹

Affiliations:

¹ Institute of Bioscience of Botucatu – UNESP/Botucatu – São Paulo, Brazil. ² The Medical School of Botucatu – UNESP/Botucatu – São Paulo, Brazil.

Keywords: Preeclampsia. Gestation. Environmental compound. Clinical profile.

Abstract:

Pre-eclampsia (PE) is a disease that affects many pregnant women and can lead to death for both mother and child. It is mainly characterized by an increase in blood pressure together with an increase in proteinuria and/or significant damage to target organs such as the brain and kidneys. The origin of PE has already been discussed and explored, with the two main mechanisms being abnormal placentation and maternal endothelial dysfunction; however, in another part of our study, we will also investigate the involvement of endocrine disruptors in this pathophysiology. To this point, 286 patients were recruited from the Maternity Hospital of the Botucatu Medical School, 183 of whom were patients with a suspicion or diagnosis of PE and 103 samples from patients classified as normotensive (NT). All the patients were informed and agreed to the objectives of the study and access to their medical data, signing an informed consent form and having a blood sample taken for hormone analysis. Pregnant women with PE had a higher pre-gestational BMI than NT women, as well as an increased final gestational weight, and pregnant women with PE had a lower gestational age at delivery (37 weeks) compared to healthy pregnant women (39 weeks). The blood pressure of pregnant women with PE was also increased (140x90 mmHg vs 116 x 73 mmHg), which was expected. The cesarean rate was 80% in the PE group and 32% in the NT group. Among the pregnant women with PE who had complications, 56.5% had hypertensive crisis, 15.9% had eclampsia and 44% of the fetuses had growth restriction. The estradiol and progesterone levels of these pregnant women were also analyzed. The concentration of progesterone was 25% lower in PE pregnant women compared to NT pregnant women, with no difference in estradiol levels. Pregnant women with PE showed significant clinical and hormonal changes compared to healthy pregnant women and our next step is to assess whether the endocrine disruptors present in the urine of these pregnant women may contribute to the pathophysiology of PE. Ethics committee: 6677222 FMB/UNESP.

Financial support: FAPESP (2023/12666-5; 2023/06651-5 e 2021/12010-7).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Metabolic Effects of *Baccharis dracunculifolia* DC on an Innovative Animal Model of Cardiometabolic Syndrome

Authors:

Rafaela Caroline Santa Clara-Amorim¹ Gustavo Ratti da Silva²
Lucas Pires Guarnier³ Arisa Namie Higashijima¹
Francislaine Aparecida dos Reis Lívero¹

Affiliations:

- ¹ Federal University of Paraná.
² Paranaense University.
³ University of São Paulo.

Keywords: Alecrim-do-campo. Diabetes. Metabolic syndrome. Preclinical study. Smoking.

Abstract:

Cardiometabolic syndrome (CMS) is a global health challenge involving interconnected risk factors and mechanisms, such as impaired energy metabolism and oxidative stress. However, many preclinical models fail to fully replicate the multifactorial complexity of human CMS. This study aimed to validate an innovative, translational animal model of cardiometabolic syndrome combining type 2 diabetes (streptozotocin 30 mg/kg), dyslipidemia (0.5% cholesterol-enriched diet), and tobacco exposure (9 cigarettes/day, 5 days/week) and to elucidate metabolic effects of *Baccharis dracunculifolia*. Female Wistar rats were divided into six groups and treated by gavage from day 14: Basal, negative control (C-), *Baccharis dracunculifolia* extract at 30, 100, or 300 mg/kg (BD30, BD100, BD300), and positive control [C+, simvastatin (v.o.) + insulin (s.c.)]. On day 28, animals were euthanized, and samples were collected for biochemical, histological, and oxidative stress analyses. The protocol was approved by the Ethics Committee on Animal Experimentation of Paranaense University (protocol 1003/2022). Phytochemical analysis of the *B. dracunculifolia* extract revealed seven phenolic acids and six flavonoids as major constituents. Notably, treatment with BD30 demonstrated dose-dependent hepatoprotective effects, with complete reversal of alanine and aspartate aminotransferase levels compared to C-. Similar improvements were observed in liver weight and in both plasma and hepatic cholesterol and triglyceride percentages. An hepatoprotective antioxidative effect was also observed in the BD30 group, with reduced glutathione, superoxide dismutase, and lipid peroxidation levels maintained near basal values. Higher doses of the extract and the C+ group showed only partial improvements across all experiments. These findings underscore the ethnopharmacological relevance and safety of *B. dracunculifolia*, particularly at lower doses, and support this pioneering preclinical model as a promising tool for CMS research that closely mimics human pathophysiology.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Toxicological evaluation of yellow passion fruit seed oil (*Passiflora edulis f. flavicarpa*) nanocarried in microemulsion-gel in zebrafish (*Danio rerio*) embryos

Authors:

Nayara Cecilia do Couto Guedes¹ Thais Morais de Brito¹
Julia Braz Gimenes¹ Renata Jurema Medeiros¹
João Vitor Vicente-da-Silva^{2,3,4,5} Flávia Almada do Carmo^{3,5} Magno Maciel-Magalhães¹
Beatriz Ferreira de Carvalho Patricio^{2,4}

Affiliations:

¹ Laboratório de Fisiologia; Departamento de Farmacologia e Toxicologia; Instituto Nacional de Controle de Qualidade em Saúde; Fundação Oswaldo Cruz.
² Laboratório de Inovação Farmacêutica e Tecnológica; Departamento de Ciências Fisiológicas; Instituto Biomédico; Universidade Federal do Estado do Rio de Janeiro.
³ Laboratório de Tecnologia Industrial Farmacêutica; Departamento de Fármacos e Medicamentos; Faculdade de Farmácia; Universidade Federal do Rio de Janeiro.
⁴ Programa de Pós-Graduação em Biologia Molecular e Celular; Instituto Biomédico; Universidade Federal do Estado do Rio de Janeiro.
⁵ Programa de Pós-Graduação em Ciências Farmacêuticas, Faculdade de Farmácia; Universidade Federal do Rio de Janeiro.

Keywords: Microemulsion-gel. Nanotechnology. *Passiflora*. Zebrafish.

Abstract:

Yellow passion fruit seed oil (YPSO) - *Passiflora edulis f. flavicarpa* - exhibits promising properties for skin healing due to its high content of polyunsaturated fatty acids. Microemulsion-gel combines the characteristics of hydrogels with nanocarriers, providing unique efficacy. This study assessed the toxicity of a microemulsion-gel formulation containing YPSO, compared to a formulation without oil, at various concentrations (96 µg.mL⁻¹, 240 µg.mL⁻¹, 600 µg.mL⁻¹, 1500 µg.mL⁻¹, and 3750 µg.mL⁻¹), using the Fish Embryo Toxicity Test (FET) by OECD 236 guidelines. Parameters such as embryo coagulation, number of deformities, body length, eye diameter, and heart rate were evaluated. According to Information Note No. 01/2024, from the National Council for the Control of Animal Experimentation (CONCEA), ethical licensing is not mandatory when experiments are conducted exclusively with embryos up to the limit of 120 hpf. The results indicated significant changes in the toxicity score of both formulations at concentrations of 600 µg.mL⁻¹ and 1500 µg.mL⁻¹, with a higher number of deformities. Regarding body length, the only significant change occurred with the white gel at 240 µg.mL⁻¹, where larvae exhibited greater length than the control group. For eye diameter, significant changes were observed at the 240 µg.mL⁻¹ concentration of both the white and YPSO gel. Regarding heart rate, a significant alteration was seen only with the YPSO gel at 240 µg.mL⁻¹. The 600 µg.mL⁻¹ and 1500 µg.mL⁻¹ concentrations did not provide a valid sample size for other evaluations beyond deformity scoring. The observed changes at the higher concentrations suggest that while YPSO may hold promise for cosmetic applications, further evaluation is essential to determine the safe and effective concentration for human use. We highlight that assays with adult zebrafish may be a potential alternative for tests involving microemulsions. The study also indicates the importance of the zebrafish toxicity model for screening cosmetic formulations, enabling a detailed analysis of adverse reactions across various physiological parameters.

Acknowledgments: Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) - E-26/211.757/2021, E-26/200.210/2023 e E-26/210.034/2024; Fundação Oswaldo Cruz Programa Inova Ideias Inovadoras - VPPIS-004-FIO-22-2-52.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Exploring the antiproliferative activity of essential oil from lemon myrtle using HEPG2 cells in 2d and 3d cultures

Authors:

Guilherme Álvaro Ferreira-Silva¹ Juliana De Andrade Santiago² Maria Das Graças Cardoso²
Marisa Ionta¹

Affiliations:

¹ Universidade Federal de Alfenas.

² Universidade Federal de Lavras.

Keywords: Apoptosis. Cell cycle arrest. Essential oil. Hepatocellular carcinoma. Spheroid.

Abstract:

Hepatocellular carcinoma (HCC) is the most common type of primary liver tumor, accounting for approximately 90% of cases, and is often diagnosed at advanced stages. Despite recent advancements in systemic therapies for HCC, such as kinase inhibitors and monoclonal antibodies, the mortality rate remains high. In this context, identifying new anticancer drugs is crucial to expanding therapeutic strategies for HCC. Research on essential oils highlights their potential therapeutic benefits, including antitumor activity. This study aims to investigate the effects of essential oil from Lemon Myrtle (LMEO) leaves on proliferative behavior of HepG2 cell line. Cell viability assays (MTS assay and Trypan blue exclusion test) and cell cycle analysis were performed to evaluate antiproliferative activity of LMEO. The expression and activation of critical regulators of cell cycle were analyzed by Western blot. DNA damage induction and pro-apoptotic activity were evaluated by flow cytometry. Additionally, clonogenic assay and spheroid formation were conducted to assess the long-term effects of LMEO on HepG2 proliferation. LMEO efficiently reduced the proliferation of HepG2 cells under experimental conditions tested. Treatment led to G2/M cell cycle arrest, with downregulation of cyclin B and upregulation of p21. Notably, ERK activation was reduced in response to LMEO. Moreover, LMEO decreased the clonogenic capacity of HepG2 cells and inhibited long-term proliferation of HepG2-spheroids. LMEO also induced DNA damage and apoptosis in HepG2 cells. Taken together these findings demonstrate the antitumor potential of LMEO against HCC and support further investigations.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Selecting molecular targets to investigate the role of glyphosate exposure in the pathogenesis of Alzheimer's disease using the Comparative Toxicogenomics Database

Authors:

William Possamai Teles^{1,2} Luã Henrique Urrutigaray^{1,2} Diovana Gelati de Batista^{1,2,3} Caroline Brandão Quines^{1,2} Thiago Gomes Heck^{1,2,3,4} Vitor Antunes de Oliveira^{1, 2}

Affiliations:

¹ Grupo de Pesquisa em Fisiologia (GPeF).

² Universidade Regional do Noroeste do Estado do Rio Grande do Sul – UNIJUÍ.

³ Programa de Pós-Graduação em Modelagem Matemática e Computacional (PPGMMC).

⁴ Programa de Pós-Graduação em Atenção Integral à Saúde (PPGAIS).

Keywords: Alzheimer disease. Biomarkers. Environmental exposure. Glyphosate. Molecular pathology.

Abstract:

Reactive oxygen species metabolic process (ROSMP), apoptotic process (AP), and cell population proliferation (CPP) are key phenotypes associated with Alzheimer's disease (AD) development according to the Comparative Toxicogenomics Database (CTD). Pollutant exposure may hasten AD progression, however, it remains unclear whether glyphosate-based (GLY) pesticides contribute to AD by affecting these phenotypic pathways. This study aims to identify target genes that may be useful to investigate a possible connection between Gly exposure, AD development and ROSMP, AP, and CPP phenotypes. We used the CTD platform (<https://ctdbase.org/>) which allows manual selection of data from published research on chemicals, genes, pathways, phenotypes, diseases, taxa, anatomy, and exposures. Genes involved in ROSMP (GO:0072593), AP (GO:0006915), and CPP (GO:0008283) interacting with GLY (MeSH ID, PubChem D000097797) and AD (MeSH ID D000544, OMIM ID 104300) were identified using the chemical-phenotype interaction query (A) and disease-phenotype tab (B), focusing on GLY as the chemical and AD as the disease. The results from A were then compared with B to identify common genes. A total of 27 genes were linked to GLY and ROSMP (GLY-ROSMP-set), 184 to GLY and AP (GLY-AP-set), and 87 to GLY and CPP (GLY-CPP-set). Among AD genes, 2 were related to ROSMP (AD-ROSMP-set), 10 to AP (AD-AP-set), and 6 to CPP (AD-CPP-set). The comparison of GLY-ROSMP-set, GLY-AP-set, and GLY-CPP-set (GLY-ROSMP-AP-CPP-set) revealed 3 common genes, while the comparison of AD-ROSMP-set, AD-AP-set, and AD-CPP-set (AD-ROSMP-AP-CPP-set) identified 1 common gene (BCL2). Also, the intersection of GLY-ROSMP-AP-CPP-set and AD-ROSMP-AP-CPP-set contained 1 shared gene: BCL2. Since BCL2 gene expression is down-regulated in AD and encodes a protein that blocks apoptosis by regulating mitochondrial protein release and caspase activation, we consider BCL2 as a potential target for investigating if GLY exposure is involved in AD.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: The XDH gene of the population of São Paulo

Authors:

Juliana de Oliveira Fernandes¹ José Eduardo Tanus dos Santos²

Affiliations:

¹ Faculdade Anhanguera de Sumaré – Enfermagem.

² Faculdade de Medicina de Ribeirão Preto Universidade de São Paulo.

Keywords: Pharmacogenetics. São Paulo. xanthine dehydrogenase. xanthine oxidase. XDH gene.

Abstract:

The XDH gene encodes the enzyme XOR, which is transcribed as xanthine dehydrogenase (XDH, EC 1.1.1.204). Xanthine oxidase (XO, EC 1.1.3.22) is the result of post-translational modifications of XOR. Superoxide and hydrogen peroxide produced by XO are associated with oxidative stress, endothelial dysfunction, gout (uric acid), hypertension, and other pathological conditions of the cardiovascular system. However, under specific conditions, the XDH isoform also generates reactive oxygen species (ROS). The low specificity for substrates allows XOR to metabolize several endogenous metabolites and a variety of exogenous compounds, including drugs. Arterial hypertension is a multifactorial disease associated with high production of ROS in vascular tissue. It is known that patients present variations in response to pharmacological treatment and, among the various factors involved, the genetic variability of the population has its contribution. Therefore, the objective is to identify genetic variants and allele frequencies that will help in choosing clinical candidate variants for new drugs or treatments with existing drugs. Whole genome sequencing (WGS) data from cohorts of the population of São Paulo, available in the ABraOM repository, allow us to better understand the variants of the entire genome, including the XDH gene. Admixed populations harbor specific variants that are underrepresented in genomic studies. Currently, the repository presents two cohorts that together total 1,780 unrelated individuals. The first cohort of 609 healthy, elderly individuals presented 397 variants of the XDH gene. The second, with 1,171 individuals, presented 2,084 variants. The hypertension-related single nucleotide polymorphisms (SNPs) rs1884725 (frequency 0.763877), rs1054889 (frequency 0.405209) and rs1042039 (frequency 0.410333) were found in these populations. These data are important for pharmacogenetic and pharmacogenomic studies of the Brazilian population involving the XDH gene.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Effects of oral administration of Brazilian Red and Faveleira propolis on acute nephrotoxicity induced by cisplatin in juvenile Wistar rats

Authors:

João Gustavo Pontes Marcena¹ Geovanna Sousa Coelho¹ Gabriel Prata Bacil¹
Leticia Cardoso Valente² Isadora Penedo de Souza¹ Guilherme Ribeiro Romualdo² Claudia
Helena Pellizzon¹
Luis Fernando Barbisan¹

Affiliations:

¹ São Paulo State University (UNESP), Department of Structural and Functional Biology - IBB/UNESP

² São Paulo State University (UNESP), Experimental Research Unity (UNIPEX) - FMB/UNESP

Keywords: Cisplatin. Nephrotoxicity. Propolis.

Abstract:

Propolis is a natural wax-like resin that bees produce and widely used in popular medicine as an antibacterial, anti-inflammatory, and antioxidant. Cisplatin (CIS) is a chemotherapy used to treat several malignant neoplasms, but with several side effects, including nephrotoxicity. Thus, this animal study aims to analyze the potential protective activity of these two variants, Brazilian red (BRP) and Faveleira (FP) propolis, against nephrotoxicity induced by a single dose of CIS. Juvenile female Wistar rats were allocated into eight experimental groups: G2, G3, G4, G6, and G7 received a single intraperitoneal dose of cisplatin (20 mg/kg), and G1, G5, and G8 received only vehicle at postnatal day (PND) 21. Groups G3 to G5 and G6 to G8 received 100 and 200 mg/kg of BRP and FP by gavage, respectively, at PND 19 to 21. Group G1 received only saline (vehicle) as a control. Blood samples were collected through the caudal vena 4 and 24 hours after CIS or vehicle administration at PND 21 and 22, respectively, for DNA damage analysis and analyzed using the comet assay. All the animals were euthanized at PND 22. The kidneys were surgically removed and weighed, and some samples were fixed in buffered formalin (10%) for 24 hours for histology processing or frozen at - 80°C for further antioxidant analysis. The findings of the comet assay (tail intensity parameter) indicate that oral treatment with BRP ($p = 0.3831$) or FP ($p = 0.9633$) did not induce DNA damage in peripheral leukocytes. In addition, the oral treatments with CIS and BRP or FP did not alter ($p > 0.05$) body weight and relative left kidney ($p = 0.2378$) and right kidney ($p = 0.3136$) weights in relation to control and CIS groups (G1 and G2). Serum creatinine levels did not show differences among all groups. In conclusion, the results obtained so far demonstrate the safety of using BRP and FP in the experimental model. (CEUA No. 1896140225 / SISGEN AF234D8).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Evaluation of the effects of *in vitro* exposure to different concentrations of polystyrene nanoplastics on human sperm motility

Authors:

Beatriz Teixeira de Oliveira¹ Gabriela Sofia de Oliveira¹ Cristiana Livramento Oliveira Pinto¹
Nicoly Caixêta Gonçalves¹
Raul Silva Santarem^{2,3} Ana Paula Ramos³ Hugo Felix Perini¹
Gláucia Eloisa Munhoz de Lion Siervo¹

Affiliations:

¹ Universidade Federal do Triângulo Mineiro. ² ETEC José Martimiano da Silva.

³ Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo.

Keywords: Plastic. Semen. Toxicology.

Abstract:

With the growing global production of plastics, millions of tons of waste are generated. In the environment, these particles degrade into micro- and nanoplastics, which can accumulate in rivers, seas, and soil, contaminating water and food. The literature already indicates that these particles can affect reproductive aspects in rodents and wild animals; however, their effects on human reproduction remain poorly understood. Therefore, this study aimed to evaluate the effects of *in vitro* exposure to different concentrations of polystyrene nanoplastic (NP-PS) on human sperm motility. The project was approved by the Research Ethics Committee (CEP-UFTM - CAAE 71552323.7.0000.5154). The volunteers were informed about the objectives, risks and benefits of the research, as well as inclusion and exclusion criteria. Sperm were isolated from semen and exposed to three concentrations (n=7/group) of NP-PS (180nm, zeta potential -27mV) in BWW medium: 2, 20 and 200 µg/mL, for 30 and 120 minutes. Following exposure, the gametes were transferred to a Neubauer chamber, where 100 sperm were counted and classified as progressively motile, non-progressively motile, or immotile. A 30-minute exposure resulted in a significant reduction in the percentage of motile sperm (progressive and non-progressive) at concentrations of 20 µg/mL and 200 µg/mL compared to the control group (p=0.038 and p=0.0009, respectively). After 120 minutes of exposure, only the 200 µg/mL concentration induced a significant reduction in motility compared to the control group (p=0.0008). In conclusion, *in vitro* exposure of sperm to NP-PS impairs motility, which may compromise their functionality. These findings highlight the importance of further studies investigating the risks associated with environmental exposure to nanoplastics and their potential link to infertility. This work was supported by the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) through grant APQ-00221-22.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Binge Drinking on Adolescence: Neurobehavioral and Gut-Brain Axis Alterations in Female Rats

Authors:

Maria Luisa Lopes da Silva¹
Enzo Gabriel Lacerda Rodrigues¹ João Carlos do Nascimento Martins¹ Felipe Ewerton
Gonçalves da Silva¹ Ádria Paes de Souza Costa¹
Maria Vitoria Oliveira Rebelo¹ Isabella Carmo Maia E Silva¹ Thaís Pereira Torres¹
Jofre Jacob da Silva Freitas² Sabrina de Carvalho Cartágenes¹
Luanna de Melo Pereira Fernandes^{1,2}

Affiliations:

¹ Laboratory of Neuropharmacology and Behaviour, Center for Health and Biological Sciences, State University of Pará, Belém, Pará, Brazil.

² Amazon Preclinical Studies Center, State University of Pará, Belém, Pará, Brazil.

Keywords: Adolescence. Anxiety-like behavior. Brain-Gut Axis. Binge Drinking.

Abstract

Female adolescents had an increase in the episodic intermittent alcohol consumption - binge drinking (BD) - pattern and are prone to take risky behaviors due to the prefrontal cortex immaturity. Hypothalamic-pituitary-adrenal axis can be impaired by BD, leading to high cortisol levels that corroborates to anxiety. Also, BD and cortisol cause higher intestinal permeability that permits the cross of molecules that promote brain dysfunction via the vagus nerve, inflammation and endocrine pathways, and these interactions are part of the brain-gut axis (BGA). This study explored the effects of adolescent BD consumption in female rats on the BGA. After approval by the Animal Experimentation Ethics Committee (CEUA/UEPA), n. 16/2023, 24 female Wistar rats were divided into ethanol and control and received EtOH (3g/kg/day, 20%w/v) or distilled water via intragastric gavage following a 3-day on/4-day off, for 4 cycles. Anxiety-like behavior was assessed 24 hours post the last dose by using open field (OF) for distance travelled, and elevated T-maze (ETM) and marble burying (MB). After behavioral tests, animals were euthanized and tissue samples of blood serum and ileum were collected to estimate cortisol levels and histomorphological changes with Hematoxylin & Eosin to verify the crypts/villi total length (CVTL) and Toluidine Blue stain for mast cell count (MC). Adolescent BD showed a locomotion reduction in OF ($p=0.0044$), latency to leave closed arm increased ($p<0.0001$) and number of entries on open arm reduced ($p=0.0194$) in ETM, cortisol level elevated ($p=0.03$), CVTL reduced ($p<0.0001$) and MC number elevated ($p<0.0001$). No difference was found in the total marbles buried. The results indicate that adolescent BD induces inflammation and morphological damage to the intestine accompanied by an anxiogenic state and increased cortisol levels. Thus, this protocol was able to BGA impairment and future research will disclose the differences between adolescents and adults.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Novel apocynin analogues improve endothelial function in aorta rings

Authors:

Júlia M. do Nascimento Arruda¹ Sofia S. Ramos¹
Samuel A. M. Campos¹ Mariana S. Quirino² Cristina Antonial² Ricardo Menegatti³ Mirele R. Machado⁴ Rita C. Tostes⁴
Simone R. Potje¹

Affiliations:

- ¹ Department of Biomedical Sciences and Health, Minas Gerais State University – UEMG.
- ² Department of Basic Sciences, São Paulo State University – UNESP.
- ³ Faculty of Pharmacy, Federal University of Goiás – UFG.
- ⁴ Department of Pharmacology, University of São Paulo – USP.

Keywords: Acetophenone derivative. Apocynin. Endothelial function.

Abstract:

Oxidative stress arises from excess ROS, mainly from mitochondria, NOXs, and uncoupled NOS, contributing to cardiovascular diseases. Antioxidants such as apocynin help mitigate the damage caused by ROS excess. This study compared apocynin with acetophenones LQFM355 and LQFM189. Animal Use Ethics Committee (UNESP nº 865-2023; UEMG nº 003/2023). Thoracic aortas from male Wistar rats (10 weeks) were cut (4 mm rings), mounted in Krebs solution (pH 7.4, 37°C, 95% O₂, 5% CO₂), stabilized, and tested for viability with KCl (120 mmol/L). Contraction was induced with phenylephrine (Phe, 1 µmol/L), and the presence of endothelium was confirmed by relaxation (70%) produced by acetylcholine (ACh, 10 µmol/L). The rings were incubated for 30 min with apocynin, LQFM355, or LQFM189 (100 µmol/L), pre-contracted with Phe and exposed to cumulative ACh concentrations (0.1 nmol/L-0.1 mmol/L). NO levels were assessed by the Griess reaction. The MTT assay was used to confirm cell viability after exposure to the tested compounds. The results were expressed as mean ± standard deviation (SD) and compared between groups (ANOVA, p<0.05). The acetylcholine agonist promoted endothelium-dependent relaxation in aortic rings (Control, pD₂: 6.31 ± 0.27, n=5; Emax: 98.41 ± 0.86, n=5). When the rings were incubated with apocynin (pD₂: 7.28 ± 0.08, n=5; Emax: 99.66 ± 0.17, n=5), LQFM355 (pD₂: 6.96 ± 0.14, n=6; Emax: 96.29 ± 1.03, n=6) or LQFM189 (pD₂: 7.15 ± 0.17, n=8; Emax: 97.35 ± 0.67, n=8) there was an increase in the potency of ACh, but there was no change in the maximum effect. Apocynin (AU: 0.1357 ± 0.0119, n=4) and LQFM189 (AU: 0.1518 ± 0.017, n=5) increased NO bioavailability, while LQFM355 (AU: 0.1302 ± 0.008, n=4) showed no difference in comparison to control (AU: 0.1109 ± 0.007, n=5). The results show that the compounds LQFM355 and LQFM189, as well as apocynin, improved the vasodilatory effect of ACh, suggesting that these compounds possibly increase the bioavailability of nitric oxide.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Binge Drinking and Motor Function: A Global Bibliometric Analysis and Mapping of Experimental Studies

Authors:

Maria Luisa Lopes da Silva¹ Chan David Ribeiro¹
João Carlos do Nascimento Martins¹ Enzo Gabriel Lacerda Rodrigues¹ Maria Vitoria Oliveira Rebelo¹ Isabella Carmo Maia e Silva¹
Rafael Hendrick Freitas de Paula¹ Luan Nascimento Pereira de Amorim¹ Sabrini Carvalho da Silva¹
Sabrina de Carvalho Cartágenes¹ Luanna de Melo Pereira Fernandes^{1,2}

Affiliations:

¹ Laboratory of Neuropharmacology and Behaviour, Center for Health and Biological Sciences, State University of Pará, Belém, Pará, Brazil.

² Amazon Preclinical Studies Center, State University of Pará, Belém, Pará, Brazil.

Keywords: Bibliometric. Binge drinking. Motor function.

Abstract

Binge drinking (BD) is linked to neurotoxic effects that impair the brain's structure and function, including motor function (MF). Main deficits in MF involve incoordination, imbalance and lack of fine motor control. This study conducted a global bibliometric analysis of original papers related to BD and MF. Data were collected from the Web of Science Core Collection database in January 2025 using MeSH and entry terms for "binge drinking" and "motor function". Two blind researchers read and selected all articles that discussed both MF and BD the extraction of the files for bibliometric analysis, VOSviewer and graphical construction. Reviews, conference articles and author's notes were excluded. From a total of 741 articles, 225 were included, where the journal with the most publications was Alcohol Clin Exp Res (44); the article with highest citation and density citation was by Pascual; of 937 authors, Boehm had the most documents (18), Goodlet the most citations (868) and Guerri had most citation per document (122,6). Moreover, 551 author's keywords were used to make VosViewer link strength graphs with the keywords by years; words like adolescence, sex differences, inflammation and hippocampus have appeared in the last few years despite having a lower occurrences when compared to cerebellum and fetal alcohol spectrum disorders, that appear in older documents with high occurrences. Most studies were experimental with animal models (85.3%), followed by cohort (8%) and others (6,7%). Experimental studies used mostly locomotion and motor coordination on rodents to assess MF; 59 utilized substances other than alcohol (i.e. caffeine, cocaine) while 68 discussed therapies (i.e. physical activity, environmental enrichment). This suggests that experimental works on BD and MF may be shifting to studies on age groups and that recent works tend to explore inflammatory pathways and the differences on gender, although they seem to lack clinical and other study designs on humans.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Orchiectomy Induces Selective Memory Deficits and Dendritic Spine Loss in Mice

Authors:

Amanda Rodrigues Quintanilha¹ Raphaela Almeida Chiareli^{1,2} Antonio Ítalo dos Santos Nunes¹ Gustavo Almeida de Carvalho^{1,3} João Elias Pinheiro Sousa de Paula¹ Mauro Cunha Xavier Pinto¹

Affiliations:

- ¹ Universidade Federal de Goiás.
- ² Universidade Estadual de Goiás.
- ³ Centro Universitário de Mineiros.

Keywords: Androgens. Cognitive impairment. Testosterone.

Abstract:

Introduction: Testosterone performs essential physiological functions by interacting with specific receptors (ARs). In the central nervous system, it modulates signaling pathways that influence cognition, neuroprotection, and cell survival. Studies in humans and animal models associate its deficiency with cognitive decline, although the underlying mechanisms are not fully understood. Therefore, the aim of this study is to investigate the temporal progression of cognitive deficits induced by orchiectomy in mice at 2 postoperative intervals. This project was performed in accordance with the ethical principles of animal experimentation approved by the Animal Use Ethics Committee (CEUA/UFG) under protocol 023/2020. **Methods:** 20 male Swiss mice (8-10 weeks old) were divided into ORQ (n=10) and sham (n=10) groups. Behavioral tests (Y-maze and open field) were performed at 30 and 90 days, passive avoidance only at 30 days. At the end (90 days), dendritic spines (hippocampal CA1) were quantified by Golgi and neurons (NeuN+/DAPI+) in the CA1 cortex/hippocampus were analyzed by immunofluorescence. **Results:** After 30 days, ORQ did not alter motor activity (9.19 ± 0.93 distance traveled; 298.9 ± 9.5 mobile time; 66.05 ± 9.14 immobile time) but impaired working memory ($54.26 \pm 4.49\%$ alternation) and aversive learning (91.91 ± 24.57 latency). After 90 days, the memory deficit persisted ($48.29 \pm 3.71\%$) without motor changes (9.17 ± 0.79 distance traveled; 228.0 ± 11.84 mobile time; 137.0 ± 12.67 immobile time). There was a significant reduction in dendritic spines in the ORQ (11.25 ± 0.41 vs. sham; $p < 0.05$), but no significant difference was found between total cells and neurons in immunofluorescence in the cortex (110.6 ± 6.9) or hippocampus (114.1 ± 4.8). **Conclusions:** This study shows that orchiectomy selectively impaired working memory and aversive learning and was associated with a reduction in dendritic spines, suggesting specific impairments in neuronal plasticity mediated by testosterone.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Involvement of adenosinergic and cholinergic systems in the antinociceptive activity of the hybrid compound 9-(3,7-dimethyl-oct-6-en-1-yl)-9H-purin-6-amine

Authors:

Daiane Barreto Suzano dos Santos¹

Bruno Guimarães Marinho¹

Affiliations:

¹ Laboratório de Farmacologia da Inflamação e Nocicepção, Programa de Pós-Graduação Multicêntrico em Ciências Fisiológicas, Universidade Federal do Rio de Janeiro.

Keywords: Hybrid compound. Adenosine. Nociceptive.

Abstract:

In order to maximize the benefits and minimize the damage induced by traditional analgesics, presenting additional effects on behavioral aspects, and with less toxicity, we propose a novel hybrid molecule, the compound RI-06, a union of the citronellol molecule, a natural acyclic monoterpene found in citronella oil, with adenine, one of the four main nitrogenous bases found in DNA and RNA, obtained through the molecular hybridization method. In view of this, we investigated the antinociceptive activity of the hybrid compound in in vivo models. Male Swiss mice from the Bioterium of the Department of Physiological Sciences - UFRRJ were used, and they were divided into 5 experimental groups: Saline, indomethacin or morphine (3 mg/kg) and the compound RI-06 at doses of 1, 5 and 10 mg/kg, all orally. The antinociceptive effects of the compound 9-(3,7-dimethyl-oct-6-en-1-yl)-9H-purin-6-amine (RI-06) were evaluated by the acetic acid-induced abdominal writhing, tail-flick and open field tests. The procedures having been approved by CEUA-UFRRJ under number 05/2024. Statistical analysis was performed through ANOVA accompanied by the Bonferroni post-test, with p-values less than 0.05 being considered statistically significant. The compound RI-06 was able to reduce the number of abdominal writhing by 38.4% when compared to the control group, demonstrating antinociceptive activity at the highest dose. In the tail-flick test, the compound was able to increase the area under the curve by 1118% and 2197% at doses of 5 and 10 mg/kg, showing neurogenic activity. This neurogenic antinociceptive effect was inhibited by prior administration of naloxone, atropine, caffeine and 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX). The compound was not able to reduce the number of squares covered in the open field test. The present study demonstrated the neurogenic antinociceptive effect of the RI-06, with the involvement of the opioid, muscarinic cholinergic and adenosinergic systems.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Effects of oral administration of Brazilian Red and Faveleira Propolis on acute hepatotoxicity induced by dimethylnitrosamine in juvenile Wistar rats

Authors:

Geovanna Sousa Coelho¹

João Gustavo Pontes Marcena¹ Gabriel Prata Bacil¹

Leticia Cardoso Valente² Isadora Penedo de Souza¹ Guilherme Ribeiro Romualdo² Luis

Fernando Barbisan¹ Claudia Helena Pellizzon¹

Affiliations:

¹ São Paulo State University (UNESP), Department of Structural and Functional Biology - IBB/UNESP.

² São Paulo State University (UNESP), Experimental Research Unity (UNIPEX) - FMB/UNESP.

Keywords: Carcinogen. Hepatotoxicity. Propolis. Wistar rats.

Abstract:

Propolis is a natural wax-like resinous from bees with numerous biological properties, such as antibacterial, anti-inflammatory, and antioxidant. Dimethylnitrosamine (DEN) is a hepatocarcinogen widely used in rodent models with known liver toxicity activity. Thus, this animal study aims to analyze the potential protective activity of two propolis variants, Brazilian red BRP and faveleira (FP) propolis against hepatotoxicity induced by a single dose intraperitoneal of DEN. Juvenile male Wistar rats were allocated into eight experimental groups: groups G2, G3, G4, G6, and G7 received a single dose of DEN (5 mg/kg body weight), groups G1, G5, and G8 (n=06 each) received DEN vehicle (0.9%NaCl) at postnatal day (PND) 21. Moreover, the groups from G3 to G5 and G6 to G8 (n= 06 each) received 100 and 200 mg/kg doses (gavage) of BRP and FP respectively, during PND 19 to 21. The control group (G1) received only 0.9% NaCl. Blood samples were collected through the caudal vena at 4 and 24 hours after DEN or vehicle administration at PND 21 and 22 respectively, for DNA damage analysis using the comet assay technique (genotoxicity). All the animals were euthanized at PND 22 under anesthesia. The liver was surgically removed and weighed and samples of left lobe were fixed in buffered formalin (10%) for histology processing. Other samples were frozen at -80°C for further antioxidant analysis. The findings of comet assay (tail intensity parameter) indicate that oral treatment with BRP (p =0.3831) or FP (p=0.9633) did not induced DNA damage in peripheral leukocytes. The DEN acute treatment (G2) reduced (p =0.0368) the relative liver weight when compared with the control group (G1). The oral treatments with BRP or FP did not alter (p> 0.05) the body weight and the relative liver weights when compared with only DEN group (G2). In conclusion, oral treatment with BRP and FP was safe in the used dose in this study (CEUA No. 1896140225/SISGEN AF234D8).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Participation of the NO/GMC/K+ATP pathway in the antinociceptive effect of the hydroethanolic extract of *Megaskepasma erythrochlamys* flowers

Authors:

Miriam Evelyn Castanheira de Faria¹

Rodolfo Guimarães Alexandre Vasques Pedroso² Áurea Echevarria Aznar Neves Lima²

Bruno Guimarães Marinho¹

Affiliations:

¹ Laboratório de Farmacologia da Inflamação e Nocicepção, Departamento de Ciências Fisiológicas, Universidade Federal Rural do Rio de Janeiro.

² Núcleo de Síntese e Química Medicinal, Programa de Pós-Graduação em Química, Universidade Federal Rural do Rio de Janeiro.

Keywords: Opioid. Nitric oxide. Megaskepasma. Nociceptive.

Abstract:

The use of medicinal plants as a therapeutic method has accompanied humanity throughout time, forming part of its culture. The widespread use of medicinal plant-based medicines and popular knowledge itself bring with them the need for experimental studies to clarify and confirm information about the actions of plants and characterize biologically active substances, aiming to minimize adverse and toxicological effects. In view of this, we investigated the antinociceptive activity of the hydroethanolic extract of *Megaskepasma erythrochlamys* flowers in in vivo models. Male Swiss mice from the Animal Facility of the Department of Physiological Sciences - UFRRJ were used and divided into 5 experimental groups: Saline, morphine (3 mg/kg) and the extract at doses of 10, 50 and 100 mg/kg, all orally. The antinociceptive effects of the extract were evaluated by abdominal contortions induced by acetic acid, tail flick and open field tests. The procedures were approved by CEUA-UFRRJ under number 12/2024. Statistical analysis was performed using ANOVA accompanied by the Bonferroni post-test, with p-values less than 0.05 being considered statistically significant. The extract was able to reduce the number of abdominal contortions by 63.1% and 60.4% at doses of 50 and 100 mg/kg, demonstrating antinociceptive activity. In the tail-flick test, the compound was able to increase the area under the curve by 394% and 1382% at doses of 50 and 100 mg/kg, demonstrating neurogenic activity. This neurogenic antinociceptive effect was inhibited by prior administration of naloxone, L-NAME, ODQ, and glibenclamide. The compound was not able to reduce the number of squares covered in the open field test. The present study demonstrated the neurogenic antinociceptive effect of the extract, with the involvement of the NO/cGMP/K+ATP pathway.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Study of the immunomodulatory activity of components of crude *Pouteria caimito* seed extract

Authors:

Fabiana Teixeira e Silva¹

Mariana Thomaz de Oliveira e Silva¹ Jessyca Rodrigues Silva¹

Ana Luisa da Silva Narcizo¹ Mauricio Afonso Vericimo¹ Renata de Almeida¹

Affiliations:

¹ Universidade Federal Fluminense.

Keywords: Antibody. Immunomodulation. *Pouteria caimito*.

Abstract:

Pouteria caimito is a tropical tree of the Sapotaceae family, popularly known as abieiro, native to the Amazon region. It is a species native to Brazil in all areas of the country. Some plants of the *Pouteria* genus have been studied due to their great potential for use in phytotherapeutic formulations to combat pathogens and treat numerous diseases. Recent studies in our laboratory have demonstrated that the crude extract of *P. caimito* seeds (EBPC), combined with chicken ovalbumin (OVA) during mouse immunization, inhibits antibody production. Our objective was to fractionate the crude extract of *Pouteria caimito* seeds and evaluate the immunomodulatory activity of its fractions. In this study, crude extracts were obtained from dried and delipidated seeds using 0.1 M sodium borate buffer pH

8.8. The extracts were then subjected to chromatography on a Sephadex G-50 column, and the collected fractions were analyzed for protein content, activity on mouse red blood cells, and the populations of spleen and bone marrow cells. The results demonstrated that fractions with a molecular mass lower than 12 kDa exhibited hemolytic activity, with moderate cytotoxic effects on lymphoid cells, associated with OVA, they drastically inhibited the production of anti-OVA antibodies. The non-hemolytic fractions, with a molecular mass of 60-80 kDa, showed the ability to sensitize red blood cells to adhere on blood leukocytes and resident cells of the mouse peritoneal cavity. This activity was observed to be inhibited by galactose and remained active at temperatures ranging from 40° C to 80° C for 30 minutes. The data set suggests that EBPC component(s) act(s) by selectively inhibiting the humoral response and exhibiting lectin-like activity. This study was submitted to the Ethics Committee for Animal Use of Universidade Federal Fluminense, and approved with the number 3132130819.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Single-dose oral treatment with field rosemary (*Baccharis dracunculifolia*) extract fails to produce anxiolytic effects in Wistar rats

Authors:

Amanda Campos Botrel¹ Renata Maria Leal de Souza¹ Marcos Adriano Carlos Batista²
Leonardo Máximo Cardoso²
Sandra Aparecida Lima de Moura² Carla Speroni Ceron²
Lisandra Brandino de Oliveira¹

Affiliations:

¹ Universidade Federal de Alfenas.

² Universidade Federal de Ouro Preto.

Keywords: Animal model. Anxiety. Anxiolytic. *Baccharis dracunculifolia*.

Abstract:

Anxiety is among the most prevalent mental disorders globally, with Brazil ranking first in worldwide prevalence, according to the World Health Organization, 2022. Conventional treatments are effective, but present limitations including delayed onset of action, adverse effects, and low adherence. This has led to increasing interest in natural compounds with anxiolytic potential, improved safety, and fewer side effects. *Baccharis dracunculifolia* (BD - commonly known as *field rosemary*) has been highlighted for its neuroprotective and antioxidant properties. Previous studies have demonstrated that chronic administration of its extract induces anxiolytic effects in Wistar rats, associated with enhanced antioxidant enzyme activity and stabilization of oxidative stress markers in brain tissue. However, its acute anxiolytic potential remains unclear. This study aimed to investigate the acute anxiolytic effect of intragastrically administered BD extract, in male Wistar rats, using Elevated T-Maze, Open Field and Light-Dark tests. The animals were randomly assigned to three groups: control (water, n=10), BD (50 mg/kg, n=11), and fluoxetine (10 mg/kg, n=11), and behavioral tests were conducted 1, 6 or 24 h after intragastric treatment. All procedures were approved by the Ethics Committee on the Use of Animals of UNIFAL-MG (protocol: 0014/2023). Statistical analysis was performed using one or two-way ANOVA, with significance set at $p < 0.05$. Considering the parameters analyzed, ANOVA showed no statistically significant difference among treatments. These findings suggest that *B. dracunculifolia* extract does not produce acute anxiolytic effects, indicating that chronic administration or a higher dose may be required to observe its potential anxiolytic activity.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Multigenerational Impacts of Nitrosodimethylamine on the Adult Life of Female F1 Generation Rats

Authors:

Giovanna Ferrari Rosalem¹ Julia Stein¹
Livia Trippe Nagaoka¹ Bárbara Campos Jorge¹
Julia Vitória Francisco Aquino¹ Giovanna Kugel Marraschi¹ Otávio Galassi Vitale¹
Arielle Cristina Arena¹

Affiliations:

¹ São Paulo State University.

² Institute of Biosciences of Botucatu, São Paulo, Brazil.

Keywords: NDMA; Nitrosamines; Pollutants; Toxicology

Abstract:

Infertility, influenced by environmental and lifestyle factors, affects about 15% of the global population. Environmental pollutants, especially their genotoxic and carcinogenic effects, have been widely studied, with exposure at any stage of life. Lately, many contaminants have shown adverse effects on reproductive health and development. Among these, Nitrosodimethylamine (NDMA), a nitrosamine found in food, water and the environment, is the focus of this study. We aimed to study the effects of paternal, maternal, and combined NDMA exposure, as well as continuous exposure of F1 female offspring, on toxicological and reproductive parameters in Wistar rats. F0 males and females were divided in two groups: NDMA (7.2 ng/kg/day) and control (1 mL/kg/day distilled water), exposed by gavage from post-natal day (PND) 60 to 90 and during mating. Mating groups were: control (male/female control), maternal (female NDMA), paternal (male NDMA), and combined (male/female NDMA). Females were exposed during pregnancy and lactation and F1 generation were exposed from PND 22 to 60, to control or NDMA. Social behavior was assessed between PND 40-45. In adulthood, estrous cycle and sexual behavior were analyzed. Females with positive sperm (7 females/group) were euthanized on gestational day 20 for fertility test and hemato-biochemical analyses. 5 females/group were euthanized to follicle count. Social behavior was reduced in the combined-control group compared to control-control. No changes were found in estrous cycle, sexual behavior, organ weight, hematological parameters and histological analysis. Biochemical analysis showed reduced alkaline phosphatase in the paternal-treated group. Uterus plus fetuses weight and the number of live fetuses were reduced in the combined-control group. These results support evidence that low-dose NDMA exposure can impair reproductive health and development, raising environmental concerns. (Ethics committee 470426042)

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Effect of the hydroalcoholic extract from *Calycophyllum spruceanum* barks on mice anxiety and depression behaviors

Authors:

Gabriela Fernandes Oliveira Marques¹ Maria Edith Holanda Banhos¹
Ítalo da Silva Barbosa¹ Renata Gomes Cruz da Silva¹ Márcia Coelho Lopes²
Renildo Moura da Cunha³ Ana Maria Sampaio Assreuy² Alana de Freitas Pires² Edson Lopes da Ponte¹

Affiliations:

¹ Centro Universitário Christus.
² Universidade Estadual do Ceará.
³ Universidade Federal do Acre.

Keywords: Anxiety. Depression. Experimental Models. Medicinal plants. Oxidative stress.

Abstract:

Neurobehavioral disorders like anxiety and depression are global health challenges, with limited therapeutic options that can cause several adverse effects. Natural plant products have been extensively researched for the medicinal properties. *Calycophyllum spruceanum* is used by native Amazonian people as anti-inflammatory. Experimental data have demonstrated antinociceptive, anti-inflammatory and antioxidant effects in mice. Based on the literature reinforcement, this study evaluated the hydroalcoholic extract from *C. spruceanum* barks (EHCS_c) in anxiety and depression models. Swiss mice (25-30 g) received EHCS_c (1, 10, 100 mg/kg p.o.) or controls (saline, diazepam, imipramine), underwent behavioral tests and had removed brain areas to quantify the oxidative stress markers (approved by Institucional Ethics Committee nº 022/2017). Expressed data: Mean ± SEM (n=8); analysis: ANOVA, Bonferroni (p<0.05). EHCS_c (100 mg/kg, only) reduced crossings (45.25 ± 38.63 vs. saline: 83.87 ± 38.63), however there were no changes in the rearing and grooming. The behaviour of the head dips showed increased frequency (32.57 ± 13.14 vs. saline: 19.42 ± 13.14), as well as the exploration time (19.12 ± 10.84 vs. saline: 8.28 ± 10.84s). In the anxiety standards avaliation, the mice treatment revealed longer open-arm time (98.28 ± 65.71 vs. saline: 32.57 ± 65.71s) and fewer closed-arm entries (8.35 ± 7.8 vs. saline: 16.25 ± 7.8), similarly to Diazepam. Already in the antidepressant effect evaluation, the mice immobilization time was not changed in the treated groups (63.71±12.69s vs. saline: 50.28±5.13s). Additionally, brain oxydative evaluation demonstrated MDA increase in prefrontal cortex (345.1 ± 191.9 vs. saline: 153.2 ± 191.9 nmol/g) and elevated GSH in striatum (683.3 ± 118.1 vs. saline: 565.2 ± 118.1 µg/g). From these data, it can be suggested that EHCS_c exerts anxiolytic effects involving oxidative modulation of specific brain areas, without eliciting antidepressant activity.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Single-dose administration of *Baccharis dracunculifolia* extract does not elicit antidepressant effects in Wistar rats

Authors:

Yasmim de Castro Freire¹ Renata Maria Leal de Souza¹ Marcos Adriano Carlos Batista²
Leonardo Máximo Cardoso²
Sandra Aparecida Lima de Moura² Carla Speroni Ceron²
Lisandra Brandino de Oliveira¹

Affiliations:

¹ Universidade Federal de Alfenas.

² Universidade Federal de Ouro Preto.

Keywords: Animal model. Antidepressants. Depression. Field-Rosemary.

Abstract:

Depression is a mental disorder with a significant impact on global public health, representing a major challenge due to its high prevalence and psychosocial consequences. According to the World Health Organization (2017) the prevalence of depression in primary care is 10.4%, making it the fourth leading cause of disease burden and the foremost in terms of psychosocial disability. Regarding conventional treatments, many patients do not respond adequately, highlighting the need for new therapeutic alternatives. In this context, *Baccharis dracunculifolia* (BD) has emerged as a promising medicinal plant with potential antidepressant effects. Previous studies have demonstrated that chronic administration of its extract produces an antidepressant-like effect in females, but not in male Wistar rats. However, its acute antidepressant effects remain to be elucidated. Therefore, this study investigated the acute antidepressant effect of intragastric BD extract in male and female Wistar rats. Animals were randomly allocated into 3 experimental treatments within each sex: control (water, n=10 for male and 10 for female), BD-treated (50 mg/kg, n=11 for male and 13 for female), and fluoxetine-treated (10 mg/kg, n=11 for male and 13 for female). The forced swimming test was performed at 1, 6, or 24 hours following intragastric administration. All procedures were approved by the Ethics Committee on the Use of Animals of UNIFAL-MG (protocol: 0014/2023). Statistical analysis was performed using one-way ANOVA with significance set at $p < 0.05$. ANOVA showed no statistically significant differences among treatment groups in any of the behavioral parameters (swimming and immobility time) analyzed, suggesting that *B. dracunculifolia* extract does not exert acute antidepressant-like effects under the conditions evaluated in this study, regardless of the sex of the animal.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Effect of metformin on behavioral and biochemical changes in female mice induced to autism spectrum disorder by valproic acid

Authors:

Gabriela Fernandes Oliveira Marques¹ Madna Costa Freitas²
Renê Felipe de Freitas²
Iara Késsila Milhome Vasconcelos² Isadora Porto de Andrade²
Maria Helena da Silva Pitombeira² Pedro Otávio de Freitas Alves² Vitória Soares Diógenes²
David Ribeiro Fontenele² Ana Maria Sampaio Assreuy² Gislei Frota Aragão²

Affiliations:

¹ Centro Universitário Christus.
² Universidade Federal do Ceará.

Keywords: Neurodevelopment. ASD. Females. Oxidative Stress. Acetylcholinesterase (AChE).

Abstract:

Autism Spectrum Disorder (ASD) is characterized by social deficits, repetitive behaviors, and cognitive impairments. The mTOR signaling pathway has been associated to several ASD-related symptoms. This study investigated the effects of metformin, modulator of mTOR, on behavioral and biochemical alterations in female mice exposed to ASD by valproic acid (VPA). Pregnant mice received VPA (450 mg/kg) on the 12.5 gestational day. After weaning (PND21), female offspring were randomized into groups: Control, VPA, and VPA+MET (400 mg/kg; p.o.; 15 days) for behavioral evaluation and remotion of brain areas to quantify oxidative stress markers, acetylcholinesterase (AChE) activity, and total protein (Institucional Ethics Committee NUP 31032.000519/2023-49). Data expressed by Mean $\pm$ SEM; ANOVA and Tukey tests ($p < 0.05$). The metformin-treatment in females in the open field test increased the crossing n^o (115.3 ± 8.0 vs. VPA: 86.9 ± 9.1), indicating improved locomotor activity, and reduced the rearing n^o (4.5 ± 0.4 vs. VPA: 17.5 ± 2.1), suggesting decreased anxiety-like behavior. It also reduced the n^o (6.7 ± 0.6 vs. VPA: 14.0 ± 1.9) and duration (55.3 ± 7.4 vs. VPA: 108.3 ± 7.4 s) of self-grooming, indicating attenuation of repetitive behaviors. In the Y-maze, the entries into the chamber (11.6 ± 0.8 vs. VPA: 8.3 ± 0.9) and the interaction time (140.0 ± 7.7 vs. VPA: 114.1 ± 5.6 s) improved the work memory performance (70.2 ± 3.3 vs. VPA: $48.7 \pm 3.2\%$). Metformin also reduced MDA in cortex (255.6 ± 54.8 vs. VPA: $383.3 \pm 50.6 \mu\text{g/ml}$) and hippocampus (404.5 ± 73.2 vs. VPA: $1108 \pm 167.6 \mu\text{g/ml}$), and increased GSH in cortex (602.7 ± 29.0 vs. VPA: $449.1 \pm 28.2 \mu\text{g/ml}$) and hippocampus (757.9 ± 45.6 vs. VPA: $458.8 \pm 38.3 \mu\text{g/ml}$), and restored cortical total protein (506.6 ± 66.2 vs. VPA: $169.2 \pm 18.2 \text{ mg/ml}$) and reduced AChE activity (53.8 ± 18.7 vs. VPA: $112.0 \pm 15.3 \text{ nmol/min/mg}$). Thus, metformin exerts significant neuroprotective effect in ASD model, modulating behavioral and biochemical changes.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Effect of homeopathic formulation of Arnica montana L in experimental model of rat tendinopathy

Authors:

Renata F.de Lima¹ Daniel B. Nunes¹
Amanda Tissoire Forwille Reis¹ Rivadavia Ramos Neiva Neto² Rafael. C. Carvalho³
Marcelo S. Andrade¹
Joicy Cortez de Sá Sousa² André Luis Lacerda Bachi⁴ João Batista Santos Garcia¹
Maria do Socorro de Sousa Cartágenes¹

Affiliations:

¹ Laboratório Experimental para o Estudo da Dor (LEED) / PPGSAD/ CCBS, Universidade Federal do Maranhão.
² CEUMA- Centro Universitário do Maranhão.
³ Biotério/ CCBS – Universidade Federal do Maranhão.
⁴ Imunologia Pulmonar e do Exercício (IBEPIPE) – UNIFESP.

Keywords: Achilles tendo. Inflammation. Rats.

Abstract:

Introduction: The incidence of tendon pathologies has increased in recent decades, due to the increased sports participation of the population and Arnica montana L. has been used for its anti-inflammatory properties. **Objective:** To evaluate the effect of a homeopathic formulation of Arnica montana L. on tendinopathy in rats. **Method:** Adult Rattus norvegicus (CIAEP: 02.0341.2019) were used, which had tendinopathy induced by intratendinous injection of collagenase, these animals were randomly distributed into 4 groups according to the treatment (n = 5/group; dose mg/kg; oral route): Healthy; CTL (negative control, saline 0.2mL); MLX (Meloxicam; 0.1); A.M.- (Arnica montana L; 50). The evaluations were made by measuring the tendon thickness, analyzing the cytokines IL-10 and IL-1 β , histopathological analysis of the tissue and the proportion of collagen types I and III by Picrosirius Red staining. The results were analyzed using analysis of variance (ANOVA) tests, followed by Turkey's test, with significance set at p < 0.05. **Results:** The extract of A. Montana L. promoted a significant reduction (p < 0.05) in the thickness in millimeters of the lesion in all periods evaluated: 7 days = (0.09); 14 days = (0.13), 21 = (0.03). Histologically, treatment with AM promoted an improvement in the parameters of the arrangement of tendon fibers and in the rounding of the fibroblast nucleus. An increase in the proportion of collagen type I was observed when compared to collagen type

III. Quantitative cytokine prospecting demonstrated that the AM group presented a significant reduction of 102% (p < 0.05) in IL-1 β levels and an increase of 33% (p < 0.05) in IL-10 levels. **Conclusion:** The results denote the anti-inflammatory effect of the A. montana formulation in the model under study, reinforcing its potential role in anti-inflammatory modulation via IL-10.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Physicochemical characterization and evaluation of cytotoxic and hepatoprotective effects of sulfated polysaccharides from *Fucus vesiculosus* and *Spirulina* sp.

Authors:

Vytor Brum Moura¹ Arthur Girardi Carpanez¹ Ana Eliza Andreazzi²
Valquíria Pereira de Medeiros¹

Affiliations:

¹ Department of Biochemistry, Institute of Biological Sciences, Federal University of Juiz de Fora, Juiz de Fora, Brazil.

² Department of Biophysics and Physiology, Institute of Biological Sciences, Federal University of Juiz de Fora, Juiz de Fora, Brazil.

Keywords: Algae. Cytotoxicity. Hepatoprotective effect. Natural products bioprospecting. Sulfated polysaccharides.

Abstract:

Algae are aquatic organisms that represent a rich source of natural, non-animal compounds, such as polyphenols, polysaccharides, fibers, pigments, fatty acids, proteins, minerals, and vitamins. Sulfated polysaccharides (SPs) from macro- and microalgae display high structural complexity, which results in promising biological activities for potential industrial applications. This study aimed to characterize and evaluate the cytotoxic and hepatoprotective effects of SPs from the brown alga *Fucus vesiculosus* and the microalga *Spirulina* sp. The SP fractions (Fv0.5, Fv0.7, SP0.5, SP0.7) were obtained through delipidation, proteolysis, precipitation, and acetone fractionation. Samples were analyzed by agarose gel electrophoresis (PDA buffer), chemical assays, FTIR spectroscopy, and for effects on metabolic activity in tumor cell lines (MCF-7, CACO-2, HEP-G2) and a non-tumor cell line (NIH/3T3) via mitochondrial enzyme activity. Hepatoprotective potential was also assessed in HEP-G2 cells exposed to paracetamol. Chemical assays confirmed absence of protein and phenolic contaminants, 50% total sugars, and ~10% sulfate content. Electrophoresis revealed metachromatic migration bands, confirming sulfate presence, and FTIR spectra showed typical SP bands. Paracetamol reduced HEP-G2 viability by 30–50%. SP fractions were non-toxic to NIH/3T3 and altered mitochondrial activity in tumor lines, suggesting hepatoprotective potential under investigation. The results support further studies on SPs as natural therapeutic agents.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Doxorubicin causes histomorphometric changes and oxidative stress in the parotid glands of rats

Authors:

José Vitor Furuya de Lima¹ Guilherme Eduardo Rocha Silva^{1,3} Ana Clara Emilio Padovezi¹
Renan José Barzotti^{1,2}
Larissa Victorino Sampaio^{1,2} Rayara Nogueira de Freitas^{1,3}
Gabriela Borba Crispim de Carvalho¹ Leonardo dos Anjos Teruel¹
Rafaela Yumi Gregório Fuzishima¹ Antonio Hernandez Chaves Neto^{1,2,3}

Affiliations:

¹ Department of Basic Sciences, São Paulo State University (Unesp), School of Dentistry, Araçatuba. ² Multicenter Graduate Program in Physiological Sciences, São Paulo State University (Unesp), School of Dentistry, Araçatuba.
³ Graduate Program in Sciences, São Paulo State University (Unesp), School of Dentistry, Araçatuba.

Keywords: Doxorubicin. Oxidative stress. Salivary gland.

Abstract:

Doxorubicin hydrochloride (DOX) is a widely used chemotherapeutic agent, but its toxicity is associated with the induction of oxidative stress, a factor involved in salivary gland dysfunction. Therefore, this study evaluated the impact of chronic treatment with DOX on histomorphometry and markers of the redox state of the parotid glands. For this purpose, young Wistar rats (six weeks old) were randomly distributed into three experimental groups (n=12 per group): Control (saline solution), DOX2.5 (2.5 mg/kg) and DOX5.0 (5.0 mg/kg). Administration was performed intraperitoneally, once a week for three weeks. In the fourth week, the animals were euthanized, and the parotid glands were collected for histomorphometric and biochemical analyses (CEUA FOA/UNESP nº 264/2024). For variables with normal distribution, one-way ANOVA was used, followed by the Tukey post hoc test. For non-parametric data, the Kruskal-Wallis test was applied, followed by the Dunn test (p<0.05). Histomorphometry showed a reduction in the area of the acini in the DOX5.0 group compared to the control (p<0.001) and the DOX2.5 group (p<0.01). The area of connective tissue was greater in the DOX5.0 group compared to the other groups (p<0.0001). Total oxidative capacity increased in the DOX5.0 group compared to the control (p<0.01), as did markers of oxidative damage to lipids (p<0.001) and proteins (p<0.001). Total antioxidant capacity did not differ between groups, but uric acid concentrations were higher in the DOX2.5 (p<0.05) and DOX5.0 (p<0.01) groups compared to the control, as was reduced glutathione in the DOX5.0 group (p<0.05). Superoxide dismutase (p<0.05), catalase (p<0.01) and glutathione peroxidase (p<0.05) activities increased in the DOX5.0 group compared to the control. DOX treatment may compromise parotid gland function and oral health due to changes in acinar structures and induction of oxidative damage. (CAPES - 001, FUNDUNESP/Happy Smile Project - Process 3450/2023, 1/2024, ICSB - Proposal No. 18784).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Evaluation of the insecticidal activity of Brazilian pepper tree (*Schinus terebinthifolius* Reddi) fruit extract on the development of *Aedes aegypti* mosquito larvae

Authors:

Luana Celina Coelho Acosta Silva¹ Júlia da Silva Ramos Matos Simone Azevedo Gomes¹
Flávia Borges Mury¹ Raquel de Souza Gestinari¹ Elane da Silva Ribeiro

Affiliations:

¹ Instituto de Biodiversidade e Sustentabilidade (NUPEM/UFRJ).

Keywords: *Aedes aegypti*, *Schinus Terebinthifolius* Reddi and larvicidal action.

Abstract:

The *Aedes* genus comprises insects of the Diptera order, Culicidae family, with over 3200 described species. Among these, the *Aedes aegypti* mosquito is a vector for significant diseases such as dengue, Zika, chikungunya, and yellow fever. *Schinus terebinthifolius* Raddi is an arboreal plant species from the Anacardiaceae family, native to South America. Its fruits, drupe-type, small, reddish, and shiny, are commonly known as pink peppercorns. Plants have their defense mechanism to protect against insects and predators, and therefore, studies on plant extracts represent an alternative to combat disease-carrying vectors. Hence, this study aims to investigate the insecticidal properties of the Brazilian pepper tree (*S. terebinthifolius* Reddi) fruit against *A. aegypti* larvae. To achieve this, Brazilian pepper tree fruits were collected and separated into peel (exocarp) and pulp (mesocarp). The seed (pulp) was extracted in 100 mM sodium phosphate buffer pH 8.0 for 4 hours at 4 °C. Subsequently, the extract was centrifuged, and the supernatant was used for toxicity assays. Third instar larvae (L3) from both laboratory-maintained RED strain mosquitoes and field-collected mosquitoes were used for toxicity assays. The obtained results showed that Brazilian pepper tree extracts at concentrations of 10%, 15%, and 25% were effective in causing the death of these larvae from the first day of the experiment, which lasted for six days. Pupa formation was not observed in any of the treatments using the Brazilian pepper tree extract. Additionally, controls (water and buffer) did not significantly influence larval mortality. These results indicate that the extract from the Brazilian pepper tree fruit has potential in controlling *A. aegypti* mosquito larvae, demonstrating it to be a promising species for further studies.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Dynamics of epididymal protease inhibitor (EPPIN) compartmentalization and colocalization with its binding partner semenogelin-1 in human spermatozoa

Authors:

Beatriz Rezende Santos¹

Noemia Aparecida Partelli Mariani¹ Natália Calixto Miranda Santos¹ Ana Clara Ferrari Themer²

Gisela Alessandra Daroz² Poliana Veneziano Martini¹ Leandro Alves dos Santos³ Luciane

Alarcão Dias-Melicio³ Thiago Afonso Teixeira⁴ Jorge Hallak⁵

Ana Gabriela Pontes Santos² Erick José Ramo Silva¹

Affiliations:

¹ Dpt of Biophysics & Pharmacology, Institute of Biosciences of Botucatu, UNESP, São Paulo, Brazil.

² Dpt of Gynecology & Obstetrics, Botucatu Medical School, UNESP, São Paulo, Brazil.

³ Dpt of Pathology, Botucatu Medical School, UNESP, São Paulo, Brazil.

⁴ Division of Urology, Dpt of Surgery, University Hospital, UNIFAP, Amapá, Brazil.

⁵ Division of Urology, Dpt of Surgery, Hospital das Clínicas, USP, São Paulo, Brazil.

Keywords: Contraceptive Target. Protein expression. Spermatozoa. Sperm function. Male fertility. Reproduction.

Abstract:

EPPIN is a sperm-associated protein that acts as a docking site for the binding of the seminal plasma protein semenogelin-1 (SEMG1), leading to a transient inhibition of sperm motility upon ejaculation. Here, we conducted a comprehensive evaluation of EPPIN expression and its colocalization with SEMG1 in human spermatozoa. Semen was collected from donors (N=3) at the Hospital das Clínicas, Botucatu Medical School (CEP approval: 64342822.9.0000.5411). Spermatozoa were separated by density gradient and processed for either cell fractionation, using TET buffer, followed by Western blot, or immunofluorescence using anti-EPPIN and anti-SEMG1 antibodies. EPPIN distribution was expressed as the percentage of labeled sperm regions. EPPIN/SEMG1 colocalization was assessed in a confocal microscope. Control experiments were performed using either pre-adsorbed or absence of primary antibodies. We confirmed the presence of EPPIN in TET-soluble fraction, containing soluble proteins and membranes, as a ~40 kDa band consistent with dimers. Remarkably, we observed the presence of the same band in the TET-insoluble fraction, indicating that EPPIN is found in intracellular compartments, possibly accessory structures of the flagellum. EPPIN immunostaining was predominant in the post-acrosomal region (67%) and posterior ring (81%) of the sperm head, as well as in the connecting piece (82%), midpiece (71%), and principal piece (55%) of the flagellum. EPPIN/SEMG1 colocalization was observed in all EPPIN-positive immunostained regions. Our results show the association of EPPIN with intracellular compartments in human spermatozoa, providing evidence that its distribution goes beyond the sperm surface. EPPIN interactions with intracellular proteins may provide additional mechanisms for the regulation of sperm motility. EPPIN/SEMG1 colocalization in the flagellum and head supports their relevance for sperm function, contributing to a better understanding of EPPIN function in human spermatozoa.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Cytotoxic effect of plant polysaccharide extracts on pancreatic tumor cells (PANC-1)

Authors:

Maria Gonçalves Pereira¹ Álvaro Xavier Franco² Rafael Aires Lessa¹
Conceição da Silva Martins Rebouças² Denis Francisco Gonçalves de Oliveira² Renata Ferreira de Carvalho Leitão¹ Pedro Marcos Gomes Soares¹

Affiliations:

¹ Universidade Estadual do Ceará, Faculdade de Educação, Ciências e Letras do Sertão Central.

² Universidade Federal do Ceará.

Keywords: Cell death. PANC-1. Pancreatic cancer. Plant polysaccharides.

Abstract:

Polysaccharide extracts (PEs), obtained from five different higher plants, showed effects anti-inflammatory, antithrombotic, antinociceptive, healing, on arthritis, neuroinflammation, pancreatitis and gastritis, and were also chemically analyzed. The aim of this study was to evaluate the cell death effect of these polysaccharide extracts on pancreatic tumor cells (PANC-1). The polysaccharide extracts were obtained by delipidation with methanol and subsequent extraction of the polysaccharides with NaOH, followed by precipitation in ethanol. Cell viability was performed using the MTT method ("3'-[4,5-dimethylthiazol-2yl] 2,5-diphenyltetrazolium bromide) in PANC-1 cell culture (1x10⁴) incubated in the presence of polysaccharide extracts (3.9-1000 µg/mL, serial dilution) at 24 and 48 h. The EPs from the barks of *Libidibia ferrea* and *Ximenia americana* did not show cytotoxic effects. The EP from *Genipa americana* (leaves) reduced cell viability (cytotoxic effect) by 25.8% (500 µg/mL); the EP from *Azadirachta indica* (tegument) reduced it by 27.1% (up to 15.625 µg/mL; 24 h) and by 36.8% to 74% (3.9 to 1000 µg/mL; 48 h); and EP of *Cissus sicyoides* (leaves) reduced by 14.1% to 26.6% (1000 to 15.625 µg/mL; 24 h) and by 32.6% to 79% (3.9 µg/mL to 1000 µg/mL; 48 h) vs negative control. DMSO (positive control) decreased cell viability by 83.3%. It was concluded that the polysaccharide extracts of *A. indica* and *C. sicyoides* demonstrated cytotoxic effects on pancreatic cancer cells-PANC-1, at all concentrations tested, after 24 and 48 h of incubation.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Trans-Anethole Preserves Pulmonary Function and Architecture in Cecal Ligation/Puncture-Induced Sepsis

Authors:

Alice Brito Martins¹

Cléo Vanessa de Queiroz Rolim¹ Francisco Sávio Pitombeira de Lima¹ Heria Bezerra de Menezes¹

Ádila da Cunha Martins¹ Gizele Alves Santos¹

Luana Maria Santos Menezes² João Lucas Nobre da Silva¹ Crystianne Calado Lima¹

Ana Maria Sampaio Assreuy¹ Alana de Freitas Pires^{1,2}

Affiliations:

¹ Universidade estadual do Ceará.

² Centro Universitário Christus.

Keywords: Acute lung injury. Anethole. Lethal sepsis.

Abstract:

Sepsis frequently leads to acute lung injury through systemic inflammation, oxidative stress, and endothelial dysfunction. Male Wistar rats (CEUA: 31032.004852/2024-1) (230-300g) were induced to sepsis by cecal ligation/puncture (18G needle, 10 perforations) (CLP) after anesthesia (xylazine 10mg/kg + ketamine 90mg/kg). SHAM received laparotomy. Trans-anethole (1mg/kg in 1% Tween 80) was administered *p.o.* 1h before surgery. All groups received 2mL 0.9% NaCl (s.c.) and buprenorphine (0.05mg/kg). After 24h, BAL was collected (5mL 1% PBS) for analysis and Lungs were excised: left for micromechanics (2×2×4mm strips, Krebs solution, 0.1-10Hz stress-strain) and oxidative markers; right for histology, assessing alveolar morphometry (Lm, Vv[septo]) and BCI (Weibel's grid). CLP increased leukocytes (8,500±784 vs SHAM 5,167±982/μL) and nitrite (0.39±0.18 vs SHAM 0.18±0.02μM) in BALF,

that were reduced by Trans-anethole (4,800±1,182; 0.14±0.02μM). Sepsis altered the Lung content of protein (2.77±0.19 mg/dL), MDA (206±21.1 μg/mL) and GSH (515.8±42.05μg/mL) compared to SHAM (2.13±0.11; 150.5±3.86; 1,039±32.58). Trans-anethole ameliorated protein (2.26±0.05) and MDA (133.3±11.15), but not GSH. CLP elevated pulmonary tissue resistance (0.1-0.3Hz) and hysteresivity, that were improved by treatment. Histology showed in CLP alveolar collapse (77.59±4.93% vs SHAM 22.41±4.93%), bronchoconstriction (2.94±0.02 vs 2.04±0.10), and enlarged alveoli (268.4±32.92 vs 134.9±11.99μm), reverted by Trans-anethole (161.7±27.19μm). Trans-anethole significantly attenuates sepsis-induced pulmonary pathophysiology by reducing leukocyte infiltration, and nitrosative and oxidative stress, leading to the preservation of lung architecture and function. In conclusion, trans-anethole attenuated sepsis-induced inflammation, oxidative stress and lung damage of acute lung injury induced by sepsis.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Trans-Anethole as a Multi-Target Therapeutic Strategy for Sepsis: Attenuation of Systemic Inflammation and Oxidative Stress

Authors:

Francisco Sávio Pitombeira de Lima¹ Cléo Vanessa de Queiroz Rolim¹ Alice Brito Martins¹
Heria Bezerra de Menezes¹ Ádila da Cunha Martins¹ Gizele Alves Santos¹
Stefanny Ferreira Rocha Silva² Ana Clara Cerás Camurça Vieira² Ana Maria Sampaio Assreuy¹
Alana de Freitas Pires^{1,2}

Affiliations:

¹ Universidade estadual do Ceará.

² Centro Universitário Christus.

Keywords: Anethole. Early sepsis. Organ dysfunction.

Abstract:

Sepsis-induced mortality arises from dysregulated inflammation and multi-organ failure, demanding novel therapeutic strategies. This study demonstrates the multi-target efficacy of trans-anethole (1 mg/kg p.o.) in a blinded, randomized model of early (12-hours) sepsis induced by cecal ligation/puncture (CLP) using male Wistar rats (n=42) following ethical guidelines of animal research (CEUA/UECE: 31032.004838/2024-12). Pre-treatment (1h pre-CLP) significantly attenuated local inflammation (peritoneal leukocytes by 47% (4900 ± 661,5 cells/mL), myeloperoxidase (MPO) activity (57%; 16,75 ± 2,820 U/mL), nitrite (29%; 0,09656 ± 0,005040 μM/mL), MDA (52%; 6,071 ± 0,3336 ηmol/mL), TNF-α (48%; 31,69 ± 4,099 pg/mL), IL-1β (47%; 5093 ± 593,0 pg/mL) and systemic parameters, such as: reduced organ damage markers (creatinine-18% (0,5513 ± 0,009342 mg/dL), CK-MB-49% (1089 ± 102,7 U/L), LDH-51% (663,4 ± 43,72 U/L), AST-34% (253 ± 20,99 U/L), ALT-29% (55.25 ± 2,594 U/L) and mitigated metabolic stress (lactate-56% (26,83 ± 3,798 mg/dL), uric acid-53% (1,821 ± 0,3016 mg/dL) *versus* untreated CLP (all p<0.05). Trans-anethole also enhanced the protective responses lymphocyte elevation (+45%) (2,938 ± 0,1685 cells/mm³), platelet restoration (+51%) (818,3 ± 56,77 cells/mm³), and thrombocyte improvement (+48%) (0,531 ± 0,0610 cells/mm³). Mechanistic analysis revealed three synergistic pathways: neutrophil infiltration suppression via MPO reduction, oxidative stress mitigation via MDA decrease and nitrosative stress control. These findings position trans-anethole as a promising therapeutic candidate for early sepsis intervention, addressing both the inflammatory focus (peritoneal cavity) and systemic consequences (organ dysfunction) via pleiotropic modulation of key pathological cascades.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Evaluation of the toxicity of Aroeira oil (*Schinus terebinthifolius*) and the virulence of the entomopathogenic fungus *Metarhizium anisopliae* in the development of mosquito larvae of the genus *Aedes*

Authors:

Mayara Massena da Silva¹ Laura Tilton de Amorim Coppi¹ Simone Gomes Azevedo¹ Elane da Silva Ribeiro¹

Affiliations:

¹ Instituto de Biodiversidade e Sustentabilidade (NUPEM/UFRJ).

Keywords: *Aedes aegypti*. Aroeira oil. Entomopathogenic fungus. *Metarhizium anisopliae*.

Abstract:

Plants are exposed to various stress factors in the natural environment, such as salinity, water stress, constant threats from predators and pathogens. Therefore, research on substances produced by plants, as well as the use of entomopathogenic fungi, have shown promise for controlling vector insects, such as mosquitoes of the species *Aedes aegypti*. In this sense, the aim of this project was to evaluate the larvicidal action of aroeira oil and the virulence of the fungus species *M. anisopliae* on field mosquito larvae of the genus *Aedes*. For each bioassay, ten larvae in the L3 and L4 development stages were added to 50 mL of aroeira oil solution at concentrations of 0.2%, 0.1%, 0.05%, 0.03%, 0.01%, and 0.0025%; ten larvae were added to 50 mL of solution containing *M. anisopliae* conidia at concentrations of 1x10⁷ conidia/mL and 1x10⁸ conidia/mL, with their respective controls. Larval survival was evaluated daily for 7 days. For the fungus experiments, the LEF 2000 and Esalq 818 strains were used. The results demonstrated that the survival of the larvae was close to the control (91.09%) and the concentration of 0.2% of aroeira oil led to the mortality of 100% of the larvae, already on the first day of treatment, while at concentrations 0.0025% and 0.01%, (98.58%) and (88.89%), respectively. The results from the fungus experiment showed that the Esalq 818 strain had higher lethality at the beginning of treatment until the 2nd day (55 dead larvae - 61,11%), and the LEF 2000 strain, although less lethal in the first two days, demonstrated persistence with mortality of 35 larvae (39,77%) from the third to the fifth day of treatment. The concentration of 1x10⁸ conidia/mL of the fungus showed minimal difference from the concentration of 1x10⁷ conidia/mL. With these results, it is possible to state that at the concentrations tested that were lethal, aroeira oil and the fungus *M. anisopliae* are toxic/virulent for larvae (L2/L3) of *Aedes*.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Metabolites of the endophytic fungus *Ochrocladosporium elatum* protect biomolecules and *Caenorhabditis elegans* from oxidative stress

Authors:

Maria Victória Benites Rodrigues¹ Sarah Lam Orué¹
Natália Guedes Jorge¹ Alécio da Silva Soutilha¹ Alex Santos Oliveira¹ Jaqueline Ferreira Campos¹ Edson Lucas dos Santos¹ Paola dos Santos da Rocha¹ Kely de Picoli Souza¹

Affiliations:

¹ Research Group on Biotechnology and Bioprospecting Applied to Metabolism (GEBBAM), Federal University of Grande Dourados (UFGD), Dourados, Brazil.

Keywords: Antioxidant. Bioprospecting. Endophytes.

Abstract:

Oxidative stress is characterized by an imbalance between the production of reactive oxygen species and the body's antioxidant defenses. In this way, it is associated with the onset and progression of various diseases, such as cardiovascular diseases, diabetes, obesity, and cancer. From this perspective, the prospection of natural metabolites, such as those produced by endophytic fungi, becomes a promising, sustainable, and scalable source of exogenous antioxidants. In this context, this study evaluated the effects of the extract of metabolites from the fungus *Ochrocladosporium elatum* (EFOe) against biomolecule oxidation *in vitro* and oxidative stress in an *in vivo* model. *In vitro*, the protective action of EFOe on DNA was evaluated through oxidation induced by UV and H₂O₂, while its protective effect on bovine serum albumin (BSA) was tested against damage induced by AAPH. *In vivo*, the protective effect of EFOe against oxidative stress was evaluated in *Caenorhabditis elegans* exposed to juglone (250 µM) for 6 hours. Experiments were conducted independently, in triplicate. The results show that, when incubated with EFOe, DNA retained its integrity by 44%, 50%, 60%, 80%, and 90%, and BSA by 54%, 65%, 71%, 83%, and 92%, respectively at concentrations of 25, 50, 100, 250, and 500 µg/mL. In *C. elegans*, while control animals showed viability of 65% (1 hour) and 4% (6 hours), treatment with EFOe maintained animal viability at 69%, 73%, and 78% (1 hour), and 8%, 17%, and 17% (6 hours) at concentrations of 25, 50, and 100 µg/mL, respectively. In summary, the data indicate the antioxidant potential of EFOe, which may contribute to its pharmacological potential in the prevention and treatment of diseases associated with oxidative stress.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Effect of methotrexate conjugated to chitosan nanocomplexes on zymosan-induced arthritis

Authors:

Francisco Glerison da Silva Nascimento¹ Aline Teixeira dos Santos²
Séfora Pontes Balbuena¹ Ana Maria Sampaio Assreuy¹
Judith Pessoa de Andrade Feitosa²

Affiliations:

¹ State University of Ceara (UECE).
² Federal University of Ceara (UFC).

Keywords: Arthritis. Methotrexate. Nanoparticles.

Abstract:

Methotrexate (MTX) has been used as antirheumatic treatment, but in long term and at high doses it causes hepatic and renal adverse effects. To maintain the pharmacological effects on joint inflammation and minimize the adverse effects of MTX administration, this study aimed to evaluate the effect of methotrexate conjugated in chitosan nanocomplexes (MTX-N) in the rat model of zymosan-induced arthritis. Zymosan (500 μ g/25 μ L) or sterile saline (Sham) was injected into the left tibio-tarsal joint of male Wistar rats (180-200 g; CEUA 03610944/2023). The rats were treated intraperitoneally with MTX (40 μ g/kg), MTX-N (40 μ g/kg) 30 minutes before injection of the phlogistic agent zymosan to evaluate joint edema using a digital caliper and joint nociception using a digital analgesimeter. In addition, leukocyte migration (optical microscopy/expressed as number of leukocytes/ml and myeloperoxidase (MPO) activity were quantified in the joint fluid. Statistical differences were analyzed by ANOVA followed by the Bonferroni test. MTX-N reduced joint nociception from the first hour (69.32 vs. zymosan: 55.22 $\pm$ 4 g) to the sixth hour (72.32 vs. zymosan: 21.22 $\pm$ 2 g). MTX-N also reduced joint edema (7.33 $\pm$.1.1 vs. zymosan: 10.22 $\pm$ 1 mm) up to the sixth hour of evaluation (7.8 $\pm$.08 vs. zymosan: 10.8 $\pm$ 1 mm). Joint fluid analysis revealed that MTX-N reduced neutrophil migration (4,333 $\pm$ 200 vs. zymosan: 11,433 $\pm$ 643 cells/ml) and MPO activity (5.46 $\pm$ 1 vs. zymosan: 11.87 $\pm$ 2.6 U/mg). MTX reduced joint nociception (67.83 $\pm$ 21 vs. zymosan: 55.22 $\pm$ 4 g) to the sixth hour (57.00 $\pm$ 14 vs. zymosan: 21.22 $\pm$ 2 g), joint edema (7.25 $\pm$ 0.3 vs. zymosan: 10.22 $\pm$ 1 mm), neutrophil migration (4,058 $\pm$ 277 vs. zymosan: 11,433 $\pm$ 643 cells/ml) and MPO activity (8.48 $\pm$ 2.57 vs. zymosan: 11.87 $\pm$ 2.6 U/mg). It is concluded that methotrexate conjugated in chitosan nanocomplexes has an anti-inflammatory effect on zymosan-induced arthritis, exhibiting effects similar to free methotrexate.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Propolis from *Melipona quadrifasciata anthidioides* and *Scaptotrigona depilis* reduces lipid accumulation in 3T3-F442A cells

Authors:

Isamara Carvalho Ferreira¹ Sarah Lam Orué¹
Maria Victória Benites Rodrigues¹ Pedro Henrique Patrício Barbosa¹ Edson Lucas dos Santos¹
Paola dos Santos da Rocha¹ Kely de Picoli Souza¹ Jaqueline Ferreira Campos¹

Affiliations:

¹ Federal University of Grande Dourados.

Keywords: Bee product. Obesity. Stingless bee.

Abstract:

Obesity is a disease characterized by excess body fat. It is considered an important risk factor for the development of cardiovascular disease and cancer, which are among the leading causes of mortality worldwide. Available treatments are limited and cause side effects, making natural products a promising alternative. Propolis, a product produced by bees through a mixture of plant exudates, mandibular secretions, pollen, and wax, has important biological properties, such as antioxidant and anti-inflammatory activities. Knowing that compounds with anti-obesity potential have already been identified in ethanolic extracts of propolis from *Melipona quadrifasciata anthidioides* (EEP-M) and *Scaptotrigona depilis* (EEP-S), this study aims to evaluate the ability of these extracts to reduce lipid accumulation in a cellular model of 3T3-F442A preadipocytes. The data were expressed as means $\pm$ standard error of the mean. Analysis of variance with a Dunnett's post-test was used to analyze and compare the experimental groups. First, we performed two assays in triplicate to define safe concentrations of the extracts for the cell line. For this purpose, preadipocytes were treated with different concentrations (1, 5, 10, 50, 100, and 500 $\mu\text{g/mL}$) of EEP-M and EEP-S and maintained in incubation for 24 and 48 h. After each respective period, we observed that EEP-M and EEP-S did not promote a reduction in cell viability up to a concentration of 100 $\mu\text{g/mL}$ and 50 $\mu\text{g/mL}$, respectively. Subsequently, three duplicate assays were performed with preadipocytes induced to differentiate and treated with different concentrations (5, 10, and 50 $\mu\text{g/mL}$) of EEP-M and EEP-S. As a result, we observed that EEP-M and EEP-S promoted a reduction in lipid accumulation in adipocytes by $17.4 \pm 0.5\%$ and $10.8 \pm 0.3\%$, respectively, at a concentration of 50 $\mu\text{g/mL}$. The result obtained suggests that the ethanolic extracts of propolis from *M. q. anthidioides* and *S. depilis* have a potential anti-obesity effect.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
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8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: *Erythroxylum anguifugum* and *Erythroxylum pelleterianum* protect against oxidative stress in a *Caenorhabditis elegans* preclinical model

Authors:

Matheus Henrique Franco Alves¹ Emilha Uzum Papaya¹
Igor Victor da Silva¹ Alécio da Silva Soutilha¹ Debora da Silva Baldivia¹ Jaqueline Ferreira Campos² Kely de Picoli Souza² Edson Lucas dos Santos²

Affiliations:

¹ Universidade Federal da Grande Dourados, Faculdade de Ciências Saúde, Dourados, Mato Grosso do Sul.

² Universidade Federal da Grande Dourados, Faculdade de Ciências Biológicas e Ambientais, Dourados, Mato Grosso do Sul.

Keywords: Antioxidants. Cocão. Fruto do pombo. Oxidative stress.

Abstract:

The development of non-communicable chronic diseases (NCDs) is closely associated with redox imbalance, resulting from the accumulation of reactive oxygen species (ROS). Oxidative stress, caused by the overload of endogenous antioxidant defenses, promotes oxidative damage to DNA, lipids, and proteins, contributing to the progression of neurodegenerative and cardiovascular diseases, as well as cancer, which together account for approximately 74% of global mortality. Restoring redox balance presents a promising therapeutic strategy, and the *Caenorhabditis elegans* model, due to its high genetic homology with humans, has been widely used in biological assays. Recognized by the FDA as a preclinical model, *C. elegans* allows for the evaluation of the pharmacological efficacy and toxicity of bioactive compounds. In this study, we evaluated the toxicological safety and antioxidant effects of ethanolic leaf extracts of *Erythroxylum anguifugum* (EAE-A) and *E. pelleterianum* (EPE-A) in *C. elegans*. Nematodes (N2) (10/well) were exposed to the extracts (25–1000 µg/mL in M9 medium), M9 medium, and a solvent control (0.5% DMSO) for 24 and 48 hours at 20 °C. To assess antioxidant protection, L4-stage nematodes were pre-treated with the extracts (50–500 µg/mL) for 1 hour, followed by exposure to juglone (50 µM), a potent inducer of oxidative stress, for 6 hours. The results of this study showed that the extracts did not induce toxicity in the nematodes during the evaluated periods. Additionally, nematodes exposed to both juglone and the extracts maintained approximately 62.69% and 64.52% viability, while those exposed only to M9 medium and juglone showed about 25% viability. The extracts demonstrated toxicological safety and significant antioxidant activity, protecting *C. elegans* against juglone-induced oxidative stress. These findings highlight the therapeutic potential of the extracts against NCDs, reinforcing their relevance to human health.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Tocolytic effect of the aqueous extract of *Lippia origanoides* H.B.K. aerial parts and the action mechanisms involved in different contraction response

Authors:

Anielle Mylena de Medeiros Barbosa¹ Maria Eduarda Ferreira da Silva¹ Jivaldo Gonçalves Ferreira¹

Filipe de Melo Barbosa¹ Rayane da Cruz Albino² Danilo Ribeiro de Oliveira²

Maria da Conceição Correia Silva¹ Alice Valença Araújo¹

Affiliations:

¹ Centro Acadêmico da Vitória (CAV)/Universidade Federal de Pernambuco (UFPE).

² Universidade Federal do Rio de Janeiro.

Keywords: *Lippia origanoides*. Natural products. Primary dysmenorrhea. Uterine contractility.

Abstract:

Primary dysmenorrhea, or menstrual cramps, significantly affects many women, impairing quality of life and often being underestimated by both patients and healthcare providers. Natural products are promising alternatives for managing this condition. *Lippia origanoides* H.B.K. is traditionally used to treat uterine issues, but the pharmacological effects of its aqueous extract —used by quilombola communities in Pará—remains unexplored. This study assessed the tocolytic effect of the aqueous extract from aerial parts of *L. origanoides* (EALO_{AP}). Female Wistar rats (8–12 weeks) received diethylstilbestrol 24 h before euthanasia to induce estrus. Uteri were isolated, placed in Locke Ringer solution and mounted in organ bath at 32 °C. Concentration-effect curves (CEC) for EALO_{AP} were performed in uteri pre-contracted with KCl (60 mmol/L) or Oxytocin (OXY, 3 mmol/L). And CEC for OXY or the muscarinic agonist carbachol (CCh) were constructed in the absence or in the presence of EALO_{AP}. Data were reported as mean ± S.E.M., with significance when $p < 0.05$. The study was approved by CEUA/UFPE (0043/2024). EALO_{AP} (0.1–10,000 µg/mL) relaxed uteri pre-contracted with KCl (Maximum Effect (ME) = $88.1 \pm 2.2\%$, $pEC_{50} = 2.62 \pm 0.03$, $n=5$) and OXY (ME = $119.1 \pm 3.3\%$, $pEC_{50} = 2.72 \pm 0.12$, $n=5$), being more effective when pre-contracted with OXY. EALO_{AP} reduced the contraction induced by CCh (ME = 3.00 ± 0.07 g; $pEC_{50} = 5.41 \pm 0.08$, $n=5$) at the concentrations of 0.1 (ME = $2.76 \pm 0.14\%$, $pEC_{50} = 4.98 \pm 0.05$), 0.3 (ME = 2.26 ± 0.11 g; $pEC_{50} = 4.93 \pm 0.08$), and 1 mg/mL ($pEC_{50} = 4.96 \pm 0.07$, $n=5$). EALO_{AP} also inhibited OXY-induced contractions (ME = 4.03 ± 0.33 g, $n=5$) at the concentrations of 1 (ME = 2.74 ± 0.29 g, $n=5$), 3 (ME = 1.41 ± 0.16 g, $n=5$) and 10 mg/mL (ME = 0.04 ± 0.01 g, $n=5$). These results suggest that EALO_{AP} induces relaxation on isolated rat uterus and acts as muscarinic and oxytocin receptor antagonist. Further studies are needed to confirm these mechanisms and investigate other pathways.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Semenogelin-1 targets the Ca^{2+} channel CatSper to inhibit sperm hyperactivation in mice

Authors:

Noemia Aparecida Partelli Mariani¹ Natália Calixto Miranda Santos¹ Alexandre Dorth de Andrade¹ Tomás J Steeman²
Analia G Novero²
Juliana de Jesus Andrade¹ Beatriz Rezende Santos¹ Giulia Calderaro¹
Ana Clara Ferrari Themer¹ Celio Junior Fernandes¹ Hélio Kushima¹
Mariano Buffone³ Darío Krapf² Polina Lishko⁴
Erick José Ramo Silva¹

Affiliations:

¹ Dpt of Biophysics and Pharmacology, Biosciences Institute of Botucatu, UNESP, São Paulo, Brazil
² Instituto de Biología Molecular y Celular de Rosario (IBR) (CONICET-UNR), Rosario, SF, Argentina
³ Instituto de Biología y Medicina Experimental, Buenos Aires, BA, Argentina
⁴ Dept. of Cell Biology and Physiology, Washington University in St. Louis, School of Medicine, St. Louis, MO, USA

Keywords:

Spermatozoon. Sperm motility. Calcium signaling. Ion channel. Male contraception

Abstract:

Sperm hyperactivation is a high-amplitude motility, enabling sperm to penetrate zona pellucida during fertilization. Among the pathways that trigger hyperactivation, Ca^{2+} channel of sperm (CatSper) activation is the most critical. It has been shown that semenogelin-1 (SEMG1), the major seminal plasma protein, impairs sperm motility by interacting with the sperm protein EPPIN and reducing Ca^{2+} levels. Nevertheless, its effects on hyperactivation and underlying mechanisms remain unclear. Here, we tested whether mouse SEMG1 impairs hyperactivation by targeting CatSper upon EPPIN binding. AlphaScreen was employed to study mouse SEMG1/EPPIN interaction. We used recombinant GST-tagged mouse EPPIN (P22-T134; mEPPIN) and 6xHis-tagged SEMG1 (Q32-G375; full-length mSEMG1 and its truncated constructs: i) Q32-V118 (mSEMG1^{Q32-V118}), ii) R98-G375 (mSEMG1^{R98-G375}), and iii) Y221-G375 (mSEMG1^{Y221-G375}). Computer-assisted sperm analysis and patch clamp were used to evaluate the effects of mSEMG1 constructs on motility and CatSper currents (I_{CatSper}) in mouse spermatozoa (CEUA 5219150420). AlphaScreen revealed the interaction of mEPPIN and mSEMG1, confirming they are interacting partners. Only mSEMG1^{R98-G375} showed similar EPPIN-binding capacity to full-length mSEMG1, indicating it contains the major EPPIN binding site. mSEMG1 inhibited hyperactivation with high potency (IC_{50} 6.8 μM , 95% CI: 1.4-32.9). This effect was not reversed by alkalization with NH_4Cl . Remarkably, mSEMG1, mSEMG1^{R98-G375}, and mSEMG1^{Q32-V118} induced similar inhibition of I_{CatSper} (5 μM : 31%-44%, and 10 μM : 54%-61% inhibition compared to control). The SEMG1-induced inhibition of hyperactivation involves the suppression of I_{CatSper} , mediated by EPPIN-dependent and independent mechanisms. The discovery of novel SEMG1 mechanisms highlights its role in the temporal modulation of sperm function during capacitation and offers a structural basis for developing male contraceptives targeting sperm hyperactivation.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Gastroprotective activity in vivo and in silico of myrtenyl laurate obtained by biotransformation

Authors:

Ana Flávia Seraine Custódio Viana^{1,2,3} Hélio de Barros Fernandes²
Gabriel Henrique Oliveira² Sara Câmara Adolfo Martins²
Paulo Breitner de Melo Araujo^{1,2} Clailson da Silva Pinheiro⁴
Rita de Cássia Meneses Oliveira^{1,2}
Mateusz Kutyla⁵ Marek Stankevič⁶ Mariusz Trytek⁵

Affiliations:

- ¹ Post-Graduate Program of Pharmacology, Federal University of Piauí, Teresina, Piauí, Brazil.
- ² Medicinal Plants Research Center (NPPM), Health Sciences Center, Federal University of Piauí, Teresina, Piauí, Brazil.
- ³ State University of Maranhão, Campus Santa Inês, Maranhão, Brazil.
- ⁴ Laboratory of Functional and Molecular Studies in Physiopharmacology. Postgraduate in the RENORBIO Program, Federal University of Piauí, Teresina, Piauí, Brazil
- ⁵ Department of Industrial and Environmental Microbiology, Faculty of Biology and Biotechnology, Institute of Biological Sciences, Maria Curie-Skłodowska University, Akademicka 19, 20-033, Lublin, Poland.
- ⁶ Department of Organic Chemistry and Crystallochemistry, Faculty of Chemistry, Institute of Chemistry Sciences, Maria Curie-Skłodowska University, Gliniana 33, 20-614, Lublin, Poland.

Keywords: Bioproduct. Docking. Gastroprotection. (-)-Myrtenol.

Abstract:

In recent years, the pharmaceutical industries have recognized biotransformation as an important alternative for drug production. This study aimed to characterize the myrtenyl laurate (ML), derived from the metabolization of (-)-myrtenol by mycelium of *Cladosporium cladosporioides* and to investigate the gastroprotective activity. The structure of ML was confirmed by nuclear magnetic resonance (NMR) and mass spectrometry at Maria Curie-Skłodowska University in Poland. Molecular docking studies of ML were performed using Autodock Vina 1.2.3 (2021) software. In the vivo experiments, female Swiss mice (25 g; n = 6 animals/group) were pretreated orally (p.o.) with vehicle (1% Tween 80 in distilled water); ML (6.26, 12.5 or 25 mg/kg) or pantoprazole (15 mg/kg) an ethanol-induced gastric injury. Histological analysis of the stomachs was carried out to quantify mucus, collagen, and leukocyte count. This protocols were approved by the Ethics Committee on the Use of Animals in Research (approval number 684/21) of the Universidade Federal do Piauí. Results are expressed as mean $\pm$ SEM. Statistical analysis, ANOVA with Tukey's post-test, $p < 0.05$. Molecular docking results demonstrated that ML has binding affinity for the histamine H2 receptor (-5.560 kcal/mol) and the proton pump (-6.897 kcal/mol). In the in vivo study, ML (6.25, 12.5, and 25 mg/kg) demonstrated a reduction in the area of gastric lesions to 16.12 ± 2.4 ; $0.95 \pm 0.3^{**}$ and $0.81 \pm 0.4^{*}$ mm², respectively, when compared to the vehicle group (23.64 ± 2.12 mm²). The pretreatment with ML (12.5 mg/kg) reduced the number of leukocytes ($7.0 \pm 0.5^{*}$ cells) when compared to the vehicle group (23.3 ± 2.4 cells). ML also increased gastric mucus ($15.7 \pm 1.5^{*}$ μ g Alcian Blue/g tissue), when compared to the vehicle group (1.2 ± 0.5 μ g Alcian Blue/g tissue) and increased collagen content ($10.48 \pm 1.60^{*}$ %) relative to the vehicle group ($6.96 \pm 1.8\%$). Therefore, ML appears to have therapeutic potential in the treatment of gastric injury.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Technological prospecting of the medicinal use of *Pectis brevipedunculata*

Authors:

Taciana Gabrielle Pinheiro de Moura Carvalho¹ Rafael Antonio Freire Carvalho¹
Maria do Socorro de Sousa Cartágenes¹ Marcelo Souza de Andrade¹
Mateus Oliveira Viana¹ Rafael França Lima¹
José Renzo Castro Garcês¹
Tereza Cristina Moneiro de Melo Prazeres¹
Michelline Joanatenório Albuquerque Madruga Mesquita¹ Anne Caroline Silva Nogueira da Cruz¹
Rennan Abud Pinheiro Santos ¹

Affiliations:

¹ Universidade Federal do Maranhão.

Keywords: Biotechnological potential. Medicinal plants. *Pectis brevipedunculata*.

Abstract:

Pectis brevipedunculata, a species of the Asteraceae family native to tropical regions of South America, is widely used in Brazilian traditional medicine for its calming and anxiolytic properties. Its essential oil, rich in citral, demonstrates biotechnological potential, including antimicrobial, antifungal, and insecticidal activities. Recent research has explored its integration into nanotechnological systems, with promising applications against leishmaniasis and as a larvicide for *Aedes aegypti*. Despite this promise, scientific literature reveals gaps like the absence of clinical trials, standardized extraction protocols, and the elucidation of molecular mechanisms of its bioactive compounds. This study mapped scientific output and patent records related to *Pectis brevipedunculata* between 2011 and 2025. Searches across databases such as INPI, WIPO, Latipat, SciELO, PubMed, and CAPES revealed only one Brazilian patent focused on the oil's ectoparasitocidal activity and 12 scientific articles primarily addressing its chemical composition and bioactivity. No patents or articles were found for applications in nanotechnology or dermatology, despite these areas being suggested by academic research. Results indicate fragmented academic production, mainly concentrated in Brazil, with limited international collaboration. Furthermore, the absence of studies related to dermatological applications or integrative uses highlights untapped opportunities, particularly in topical formulations to treat skin infections and dermatitis. The therapeutic potential of *Pectis brevipedunculata* requires deeper integration of traditional knowledge and scientific evidence, alongside broader global cooperation. This study underscores the importance of prospecting as a tool for technological innovation, identifying unexplored areas of research and positioning the species as a candidate to address contemporary health challenges.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Biomonitoring of Extracts from the Marine Sponge *Dysidea janiae* with Anti-Inflammatory Potential for the Treatment of Osteoarthritis

Authors:

Beatriz Soares-Silva¹

Cintia Cristina Santi Martignago¹ Dalete Christine da Silva Souza² Mirian Bonifácio¹

Patrícia Lopes Carvalhinho² Laís Caroline Souza-Silva¹ João Henrique Lago²

Ana Claudia Muniz Rennó¹ Alessandra Mussi Ribeiro¹

Affiliations:

¹ Universidade Federal de São Paulo, Santos, Brazil.

² Universidade Federal do ABC, Santo André, Brazil.

Keywords: Osteoarthritis. *Dysidea janiae*. Anti-inflammatory. Antioxidant. Bioprospecting.

Abstract:

Osteoarthritis (OA) is a chronic degenerative joint disease affecting nearly 300 million people globally. Given the lack of therapies capable of halting disease progression. This study aimed to evaluate extracts from the marine sponge *Dysidea janiae*, collected from the Brazilian coast, to identify bioactive compounds with anti-inflammatory and antioxidant properties. After ethanolic/methanolic extraction and chromatographic fractionation, enriched fractions were tested *in vitro* (SISBio nº 28917-1). Protective activity was assessed using a protein denaturation assay; antioxidant capacity by ABTS; IL-6 levels via ELISA in LPS/IFN- γ -stimulated J774 macrophages; and cell viability in Wistar rat chondrocytes. Fraction DJMA4 showed the best biological effects. It significantly protected against protein denaturation at 0.125 mg/mL ($F(8,18)=1312$; $p=0.0464$), outperforming diclofenac. In the ABTS assay, DJMA4 exhibited strong antioxidant activity ($F(7,16)=0.7225$; $p<0.0001$). IL-6 expression was reduced in macrophages [$F(6,17) = 0,9734$; $p = 0,0858$], and DJMA4 preserved chondrocyte viability at 0.05 and 0.1 mg/mL ($p<0.05$), with higher metabolic activity observed at 120h. Spectroscopic analyses (NMR, HRMS and HPLC) identified the major compound in DJMA4 as a phthalic acid derivative, reinforcing its therapeutic relevance. In conclusion, DJMA4 demonstrated potent anti-inflammatory and antioxidant effects with low cytotoxicity, standing out as a strong candidate for OA treatment. *In vivo* studies are in progress for preclinical validation.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Study of the psychopharmacological effects of *Illicium verum* essential oils by inhalation in mice

Authors:

Lívia Carvalho Dantas¹ Felipe Rafael Eugênio¹ Rayane de Souza Del Rei¹
Samantha Costa Amorim Muniz¹ Douglas Siqueira de Almeida Chaves¹ Fábio Fagundes da Rocha¹

Affiliations:

¹ Universidade Federal Rural do Rio de Janeiro.

Keywords: Aromatherapy. Essential oil. *Illicium verum*. Star anise.

Abstract:

Illicium verum (star anise) is a plant commonly used in traditional Chinese medicine. Additionally, narcotic effects, delirium, anesthesia and convulsions have been reported after high-dose administration, indicating compounds acting on the central nervous system (CNS). This study evaluated the effects on the CNS of *Illicium verum* essential oil (OEIV) on mice CNS, after acute treatment using the inhalatory route in a chamber containing 4 cotton balls with OEIV or water on the side walls (CEUA-ICSBS-UFRRJ-07/2023). Animals were divided into 5 groups: Control (CONT) OEIV 25uL, 50 uL and 100uL/cotton ball or Positive Control (Diazepam, DZP or sertraline, SER). Seven minutes after exposure to the inhalation chamber, the behavioral analysis took place. The following tests were carried out: general assessment of CNS effects (GA), open field (OFT), rota-rod (RR), etomidate-induced sleep (EIS), elevated plus maze (EPM) and tail suspension (TST). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. In the GA 0, 30 or 60 minutes after treatment with OEIV100, there was a significant reduction rearing (REA) at t0 (CONT:50 ± 8 REA; OEIV100: 31± 4 REA) and t30 (CONT:29± 3 REA; OEIV100: 21± 3 REA). In the OFT, only the inactivity time increased after OEIV50 (101± 1 s) treatment compared to the CONT (32± 5 s). No significant differences were observed comparing CONT and OEIV. In the EIS or RR, unlike DZP which increased sleep time in the EIS (CONT= 10± 2 min; DZP= 32± 6 min), reduced fall time (CONT= 53 ± 4 s; DZP= 15± 4 s) and increased the number of falls (CONT= 1± 0 falls; DZP= 3± 0 falls) in the RR. A similar profile was found in the TST where only SER reduced the immobility of the animals (CONT= 69 ± 10 s; SER= 29 ± 7 s). There were no differences among the groups in the EPM. It can be concluded from the data obtained that no relevant changes were observed in the CNS induced by OEIV using that methodology and doses tested.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: From *in silico* to *in vivo*: novel PKM2 inhibitor with antineoplastic effect against oral squamous cell carcinoma mitigates tumor growth

Authors:

Lucas Rubini-Dias¹ Vitor Won-Held Rabelo²
Afonso Thales de Sousa Arruda¹ Lucas Nicolau de Queiroz³
Elan Cardozo Paes de Almeida⁴ Paula Alvarez Abreu²
Gabriel Limaverde-Sousa⁵ Bruno Kaufmann Robbs⁴

Affiliations:

- ¹ Programa de Pós-Graduação em Ciências Morfológicas (PCM), Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil.
- ² Instituto de Biodiversidade e Sustentabilidade (NUPEM), Universidade Federal do Rio de Janeiro, Macaé, RJ, Brazil.
- ³ Programa de Pós-Graduação em Ciências Aplicadas a Produtos para a Saúde, Universidade Federal Fluminense, Niterói, RJ, Brazil.
- ⁴ Instituto de Saúde de Nova Friburgo, Departamento de Ciências Básicas, Universidade Federal Fluminense, Nova Friburgo, RJ, Brazil.
- ⁵ Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brazil.

Keywords: Drug repurpose. Glycolysis. Metabolism. Oral squamous cell carcinoma. Pyruvate kinase.

Abstract:

Oral squamous cell carcinoma (OSCC) treatment has several adverse effects. Pyruvate kinase converts phosphoenolpyruvate (PEP) and ADP into pyruvate and ATP in glycolysis, and cells express via alternative splicing either PKM1 or PKM2. PKM2 is overexpressed in proliferating cells, adapting metabolism to provide building blocks or ATP as needed, and its nuclear translocation expresses oncogenes. Thereby, cancer benefits from PKM2, and we aim PKM2 inhibitors that may disrupt cancer cell metabolism as treatment for OSCC. Based on docking, pharmacophoric groups and pharmacokinetics, commercial molecule libraries were screened for ligand candidates. LDH coupled PKM2 reaction was used to measure PKM2 activity and ATP production. MTT assay was used for cytotoxicity *in vitro* against OSCC cell lineages and normal fibroblasts. Video microscopy was used to film cell death morphology. Flow cytometry was used for cell cycle and subG1 analysis. Molecular docking validation was applied for intermolecular interactions mapping. Hemolysis in human blood (CEP#43134721.4.0000.5626) and acute toxicity in C57BL/6 mice (CEUA#2699110419) were tested. Balb/c nude mice were xenotransplanted with OSCC cells. After reaching 300 mm³, 2x tolerated dose was fractioned in 12 doses for treatment (CEUA#6497220421). Screening resulted in 6 molecules, 1 inhibited PKM2 *in vitro* (IC₅₀ 6.3 µM), affecting ATP formation. It showed antineoplastic effect against OSCC cell lineages SCC4, SCC9, SCC25 with average IC₅₀ of 88,46 µM. Videomicroscopy and flow cytometry indicate apoptotic death morphology with high subG1 after 12h. Docking binding pose scored -8.82 kcal/Mol. It was non hemolytic, and LD50 was 250 mg/kg with intestinal moderate damage. 200 mg/kg was tolerated with no deaths. Treated xenotransplanted animals had significantly slower tumor growth, with 35% the final volume. In conclusion, a novel PKM2 inhibitor, with antineoplastic effects *in vitro* and *in vivo* was identified, inducing tumor apoptosis.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Target Fishing based on Phytochemicals with Neuroprotective Potential for Neurodegenerative Diseases

Authors:

Yuri Barboza Gomes¹

Patrícia Santos Carvalhinho-Lopes¹ Beatriz Soares Silva¹

Nayne Isabelli Zugolaro Donzelli¹ Valéria Cunha Moreira¹

Daniela Gonçalves Galasse Rando² Alessandra Mussi Ribeiro¹

Affiliations:

¹ Universidade Federal de São Paulo, Santos, Brazil.

² Universidade Federal de São Paulo, Diadema, Brazil.

Keywords: Bioprospecting. In silico. Molecular modeling. Monoterpenes. Neurodegeneration.

Abstract:

Neurodegenerative diseases (NDs), such as Parkinson's and Alzheimer's, are characterized by the progressive loss of neuronal populations driven by oxidative stress, neuroinflammation, protein and peptide aggregation, and mitochondrial dysfunction. In this context, Brazilian biodiversity represents a rich and still underexplored source of bioactive compounds with pharmacological potential and remarkable chemical-biological diversity for novel therapeutic targets. This study focused on searching for novel drug targets for three phytochemicals isolated from natural products - myrtenol, 1,8-cineole, and limonene - using *in silico* techniques such as target fishing and molecular docking. Computational analysis revealed possible therapeutic targets with crystallized inhibitory ligand, including beta secretase 1 (BACE1), collagenase 3 (MMP13), and cyclin-dependent kinase 2 (CDK2). Among the compounds tested, myrtenol showed stable anchoring through conventional hydrogen bonds and hydrophobic interactions. Notably, it interacted with MMP13, potentially downregulating BACE1 expression in SH-SY5Y cells via translational modulation of the BACE1 active site. Since BACE1 cleaves amyloid precursor protein (APP), initiating the formation of beta-amyloid plaques, this finding highlights myrtenol's possible disease-modifying action. Furthermore, cytotoxicity analysis using MTT assay indicated that Myrtenol is non-toxic to SH-SY5Y cells at concentrations below 62.5 μ M.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Protective Effects of Citronellol on Ethanol-Induced Gastric Lesions: Morphological and Histological Analyses

Authors:

Luciano Rogério Destro Giacóia¹ Isadora Passos Frigero¹
Juan Carlos Ramos de Oliveira¹ Ariane Leite Rozza¹

Affiliations:

¹ Department of Structural and Functional Biology, Institute of Biosciences, São Paulo State University (UNESP), Botucatu, Brazil.

Keywords: Citronellol. Gastroprotection. Gastric ulcer. Stomach lesion.

Abstract:

Gastric ulcer (GU) affects about 10% of adults and can damage tissues from the mucosal to the muscular layer of the stomach. Although conventional treatments, such as proton pump inhibitors, are effective, they may cause adverse effects like nutrient malabsorption and, in the long term, increase the risk of gastric cancer. Citronellol, a monoterpene present in *Cymbopogon* leaves, has anti-inflammatory activity described in other models. This study aimed to evaluate the gastroprotective effect of citronellol in ethanol-induced GU in male Wistar rats. Animals (n=35) were randomly assigned to five groups (negative control, omeprazole 20 mg/kg, citronellol 25, 50, and 100 mg/kg) and treated orally for seven consecutive days. On the seventh day, one hour after treatment, absolute ethanol (5 ml/kg by gavage) was administered to induce GU. After one hour, the animals were euthanized, and their stomachs were collected. The ulcerated area was quantified using ImageJ software, and the stomachs were subjected to histological analyses. Data were analyzed using ANOVA/Tukey or Kruskal-Wallis/Dunn tests ($p < 0.05$). Citronellol prevented the formation of ethanol-induced hemorrhagic streaks in the mucosa, exhibiting a gastroprotective effect at all doses compared to the negative control ($p < 0.001$, $p < 0.05$, and $p < 0.001$), with no difference among the citronellol-treated groups. A histological score (0-12) was established for parameters including cell desquamation, hemorrhage, inflammatory infiltrate, and edema, which were lower in the citronellol-treated groups compared to the vehicle. PAS staining showed maintenance of gastric mucus within gastric glands and on the mucosal surface in the treated groups. There was no difference in body weight or relative weights of spleen, liver, and kidney among groups, indicating absence of toxicity. In conclusion, citronellol shows gastroprotective effect against absolute ethanol-induced gastric lesions. Project approved by the Ethics Committee (CEUA-IBB 5458020623).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Developmental Effects of NSAIDs on Male Rat Offspring Following Intrauterine and Lactational Exposure

Authors:

Luara Magalhães¹

Reggina Lorena Mellado Castro¹ Sara Tawany Caetano dos Santos¹ Bárbara Campos Jorge¹

Julia Stein¹

Lívia Trippe Nagaoka Gonçalves¹ Julia Vitória Francisco de Aquino¹ Giovanna Ferrari Rosalem¹

Arielle Cristina Arena^{1,2}

Affiliations:

¹ Department of Structural and Functional Biology - Morphology Sector; Institute of Biosciences of Botucatu (IBB) – UNESP.

² Center for Toxicological Information and Assistance – CIATOX.

Keywords: Acetaminophen. Aspirin. Nimesulide. Hypothalamic Sexual Differentiation. Male Rats. Non-steroidal Anti-inflammatories.

Abstract:

Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used to manage pain, fever and inflammation, including during pregnancy and lactation. Their effects are mediated by inhibition of cyclooxygenase enzymes (COX-1 and COX-2), reducing prostaglandin E2 (PGE2) production. NSAIDs are also potential endocrine disruptors. PGE2 plays a critical role in nervous system development, particularly in hypothalamic sexual differentiation (HSD), a process highly susceptible to endocrine disruption. Given their placental transfer and possible adverse effects on fetal development, unregulated NSAID use in pregnancy and lactation is concerning. This study aimed to evaluate the impact of intrauterine and lactational NSAID exposure on the early development of male rat offspring. Pregnant rats were divided into 4 groups and exposed orally (gavage): 1. Control (1 mL/kg water); 2. Acetaminophen – Paracetamol - (125 mg/kg); 3. Acetylsalicylic acid – ASA - (6 mg/kg); and 4. Nimesulide (11 mg/kg). Doses were based on therapeutic ranges and adjusted for body surface area in rats. Exposure occurred from gestational day (GD) 15 to lactational day (LD) 22, covering the critical window for male HSD. Male offspring were assessed for body weight and anogenital distance (AGD) on postnatal days (PND) 1, 13, and 22. On PND 22, they were euthanized for hematological and organ weight analyses. Testicular descent and preputial separation were also monitored as indicators of sexual development. Preliminary data show that paracetamol reduced AGD on PNDs 13 and 22, anticipated the day of testicular descent and reduced body weight on this day, besides leading to increased levels of erythrocytes and hematocrit on PND 22. ASA led to increased seminal gland weight on PND 22. Nimesulide caused higher body weight on PND 1 and increased AGD on PNDs 13 and 22. These alterations may indicate a possible androgenic hormonal alteration in the animals, which will be further investigated. (CEUA 7611070225)



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Evaluation of the antiulceraogênico potential of Cannabis sativa-derived Cannabidiol and Cannabinol through *in silico* protein interaction profiling

Authors:

Ceres Lima Batista¹

Caetano Ribeiro Mesquita de Moura Jefferson da Cruz Esteves

Ana Flavia Seraine Custodio Viana² Helio de Barros Fernandes³

Affiliations:

¹ Centro Universitário Facid Wyden.

² Universidade Estadual do Maranhão-Campus Santa Inês.

³ Universidade Federal do Piauí.

Keywords: Cannabidiol. Cannabinol. Gastroprotection. *In silico*. Phytocannabinoids.

Abstract:

Gastric ulcers still represent a relevant clinical challenge due to their high prevalence. Despite therapeutic advances, current treatments have limitations regarding the inflammatory process and ulcer formation. In this context, studies in the field of inflammation and healing report that phytocannabinoids extracted from Cannabis sativa - cannabinol (CBN) and cannabidiol (CBD) - show promising results in this area. Therefore, the aim of this study was to evaluate, using *in silico* models, the antiulcerogenic potential of CBD and CBN against gastric and inflammatory receptors involved in the development of gastric ulcers, as well as to analyze predictive pharmacokinetic and toxicological parameters. The *in silico* evaluation was conducted through molecular docking using the AutoDock Vina software, where the binding affinity of CBD and CBN was assessed against targets obtained from the Protein Data Bank (PDB): H⁺-ATPase (5YLU); cyclooxygenase-1 (COX-1); (3KK6); cyclooxygenase-2 (COX-2); (3LN1); prostaglandin E2 EP3 receptor (6M9T). Pharmacokinetic and toxicological parameters were evaluated using online platforms SwissADME and ProTox-3, respectively. Molecular docking showed the following binding affinity values (in kcal/mol) for the co-crystallized ligand, CBD, and CBN, respectively: H⁺-ATPase (-9.5; -8.0; -9.1); COX-1 (-11.1; -8.5; -8.9); COX-2 (-12.2; -8.9; -9.3); and EP3 (-8.5; -8.7; -8.6). Molecular docking indicated strong binding affinity (≤ -7.0 kcal/mol) for all selected targets, suggesting stable interactions at the binding sites between ligand and receptor. In the Pharmacokinetic evaluations both compounds were found to be considerably lipophilic, with good intestinal absorption and penetration of the blood-brain barrier, and they are substrates that inhibit cytochrome P450 enzymes. No toxicity was parameters studies. for the evaluated parameters. Therefore, in this study, CBD and CBN emerge as potential candidates for investigative studies in the field of gastric healing and inflammation.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Maternal toxicity assessment in Wistar rats exposed to COX-1 and COX-2 inhibitors during gestational and lactational periods and their possible repercussions on female offspring

Authors:

Sara Tawany Caetano dos Santos¹ Reggina Lorena Melado Castro¹ Luara Magalhães¹
Bárbara Campos Jorge¹ Júlia Stein¹
Lívia Trippe Nagaoka¹ Otávio Galassi Vitale¹
Júlia Vitória Francisco de Aquino¹ Giovanna Ferrari Rosalem¹ Arielle Cristina Arena^{1,2}

Affiliations:

¹ Institute of Bioscience of Botucatu – UNESP/Botucatu – São Paulo, Brazil.

² Center for Toxicological Information and Assistance – CIATOX - São Paulo, Brazil.

Keywords: NSAIDs. Gestation. EDCs. Anti-inflammatory.

Abstract:

The use of nonsteroidal anti-inflammatory drugs (NSAIDs) is common due to their wide availability. NSAIDs inhibit cyclooxygenases 1 and 2 (COX-1 and COX-2) enzymes, suppressing prostaglandin synthesis, especially prostaglandin E₂ (PGE₂). While this provides anti-inflammatory, analgesic, and antipyretic effects, reducing PGE₂ may also impair essential functions like vascular homeostasis and fetal development. These drugs cross the placenta and are secreted in breast milk, potentially altering intrauterine and neonatal environments. The impacts of this exposure remain poorly understood. This study aims to investigate the effects of gestational and lactational NSAID exposure on maternal toxicity and early development of female offspring. Pregnant Wistar rats (F0) were exposed, by gavage, to control (water, 1 mL/kg), paracetamol (125 mg/kg), aspirin (6 mg/kg), or nimesulide (11 mg/kg) from gestational day 15 (GD 15) to lactation day 22 (LD 22). F0 females were euthanized at the first estrus after weaning for hematological and organ evaluation. Female offspring were evaluated for initial development (PND 1, 13 and 22), hematological analysis and organ weights at PND 22, and puberty onset. F0 females showed no significant changes in gestational weight gain, body and organ weights. However, hematological alterations were observed: nimesulide caused significant reductions in red blood cells (RBC) and a tendency to a reduction in hematocrit (HCT, $p=0.05$) and to an increase in mean corpuscular hemoglobin (MCH, $p=0.05$) and number of platelets ($p=0.06$); paracetamol significantly increased mean platelet volume (MPV), always compared to control. Female offspring parameters remained unchanged. Data suggest that, although no evident effects on offspring were observed, maternal hematological toxicity indicates possible systemic impairment, with late repercussions yet to be investigated. Financial support: FAPESP (2025/02352-9). Ethics committee: 7611070225 (CEUA).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Cardiovascular effects of (±)-Propranolol, (±)-4-NO₂-propranolol, and (±)-7-NO₂-propranolol in anesthetized rats

Authors:

Raphaella R. G. Cliquet^{1,2} Vivian Fuguera¹ Flaviano Lorenzon¹ Francesco Frecentese³
Giuseppe Caliendo³ Gilberto de Nucci^{1,4}

Affiliations:

¹ Department of Pharmacology, Faculty of Medical Sciences, State University of Campinas (UNICAMP), Campinas, Brazil

² Institute of Biology, State University of Campinas (IB-UNICAMP), Campinas, Brazil

³ Department of Pharmacy, School of Medicine, University of Naples Federico II, Naples, Italy

⁴ Department of Pharmacology, Institute of Biomedical Sciences, University of São Paulo (ICB-USP), São Paulo, Brazil

Keywords: Adrenergic beta-antagonists. Blood pressure. Heart rate.

Abstract:

(±)-Propranolol is a non-selective β -antagonist used in pathologies as hypertension, myocardial infarction, and arrhythmias. This study evaluated the *in vivo* effects of (±)-Propranolol and its synthetic analogues, (±)-4-NO₂-propranolol and (±)-7-NO₂-propranolol, on heart rate (HR) and mean arterial blood pressure (MABP). Protocols were approved by the Ethics Committee for Animal Use of UNICAMP (Protocol No. 6576-1/2024). Adult male Wistar rats (300-400g) were sedated with isoflurane (5%, 1 min), and anesthetized with sodium thiopental (40 mg/kg, i.p.) and ketamine (70 mg/kg, i.p.). Subsequently, the right femoral vessels were cannulated with a PE10 catheter, heparin was administered (600 UI/kg, s.c.), and the artery catheter connected to a pressure transducer (MLT0699 Disposable BP Transducer). After stabilization, the rats received a bolus injection (30-40 μ L) of (±)-Propranolol (0.1 and 100 nmol, 1 and 10 μ mol/kg), (±)-4-NO₂-propranolol (100 nmol, 1 and 10 μ mol/kg), (±)-7-NO₂-propranolol (0.1 and 10 nmol, 1 and 10 μ mol/kg), or the vehicle (saline) and monitored for 1 h. All β -antagonists presented a dose-response negative chronotropic effect. (±)-4-NO₂-propranolol was less potent and efficient than the other two drugs and presented a sympathomimetic reflex in all doses, while (±)-Propranolol and (±)-7-NO₂-propranolol only showed this pattern of returning to baseline in a low dose (100 and 10 nmol/kg, respectively). Higher doses (1 and 10 μ mol/kg) of (±)-Propranolol and (±)-7-NO₂-propranolol were able to induce a sustained reduction of the HR. However, no significant changes were observed in the MABP. Considering the selectivity of (±)-4-NO₂-propranolol to 6-nitrodopamine receptors, the absence of the sympathomimetic reflex in high doses of (±)-Propranolol and (±)-7-NO₂-propranolol indicate the blockage of all β -adrenoceptors and 6-nitrodopamine receptors. Further studies are necessary to evaluate the applications of those nitrated compounds in pathological models.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Modulating Bronchial Vascular Remodeling In An Experimental ACO Overlap Model Through IL-17 Inhibition

Authors:

Leandro do Nascimento CAMARGO^{1,2}
Bruno André RAMPINELLI¹
Tabata Maruyama dos SANTOS^{1,2}
Silvia FUKUZAKI¹
Amanda dos Santos LIGERO^{1,2}
Vitória Helena Kuhn de CAMPOS^{1,2}
Beatriz Mangueira SARAIVA-ROMANHOLO¹
Carla Máximo PRADO⁴
Fernanda Degobbi Tenorio Quirino dos Santos LOPES¹
Edna Aparecida LEICK¹
Renato Fraga RIGHETTI^{1,2}
Iolanda de Fátima Lopes Calvo TIBÉRIO¹

Affiliations:

¹Faculdade de Medicina da Universidade de São Paulo (FMUSP), São Paulo, Brazil, ²Hospital Sírio-Libanês, São Paulo, Brazil; ³Federal University of São Paulo (UNIFESP), São Paulo, Brazil.

Keywords: Anti-IL-17, inflammation, asthma-COPD, Vascular remodeling

Abstract:

Inflammatory mediators and growth factors drive bronchial vascular changes and remodeling, while anti-IL-17 helps control inflammation and remodeling in experimental models. This study investigated the effects of anti-IL-17 on pulmonary vascular remodeling in an experimental ACO model. Fifty-six male BALB/c mice were sensitized with ovalbumin and/or elastase (OVA, PPE, and ACO groups) and treated with or without anti-IL-17. Inflammatory cytokine levels and peribronchial vascular remodeling were assessed. Data were analyzed using ANOVA and the Holm-Sidak test ($p < 0.05$). The study was approved by CAPPesq FMUSP (Process No.1029/2018). In the ACO group, there was a significant increase in IL-1 β (151.7 ± 33.3 vs. 55.7 ± 28.4), IL-4 (3.3 ± 0.8 vs. 1.2 ± 0.2), IL-6 (46.5 ± 5.8 vs. 5.5 ± 2.0), IL-13 (54.6 ± 15.1 vs. 37.6 ± 20.4), and IL-17 (34.2 ± 6.9 vs. 11.8 ± 3.6) pg/mL in bronchoalveolar lavage fluid compared to the SAL group ($p < 0.05$), except for IL-10. There was also an increase in TGF- β (18.4 ± 5.8 vs. 6.5 ± 4.1 pg/mL) and the volume fraction of total collagen fibers (6.3 ± 0.8 vs. 3.7 ± 0.7), type I (14.9 ± 1.7 vs. 1.1 ± 0.3), and type III (2.6 ± 0.9 vs. 0.3 ± 0.1), except for type V, in peribronchial vessels of the ACO group compared to SAL ($p < 0.05$). Treatment with anti-IL-17 in the ACO group led to a significant reduction in: Inflammatory markers in bronchoalveolar lavage fluid: IL-1 β (112.7 ± 10.4 vs. 151.7 ± 33.3), IL-4 (1.37 ± 0.12 vs. 3.3 ± 0.8), IL-6 (28.7 ± 5.8 vs. 46.5 ± 5.8), IL-13 (35.5 ± 9.1 vs. 54.6 ± 15.1), and IL-17 (19.0 ± 2.8 vs. 34.2 ± 6.9) pg/mL, except for IL-10. TGF- β (6.6 ± 1.3 vs. 18.4 ± 5.8 pg/mL), total collagen volume fraction (2.2 ± 0.3 vs. 6.3 ± 0.8), collagen type I (2.5 ± 0.7 vs. 14.9 ± 1.7) and type III (0.5 ± 0.2 vs. 2.6 ± 0.9), except for collagen type V, compared to the ACO group ($p < 0.05$). In this asthma-COPD overlap model, IL-17 inhibition emerges as a promising therapeutic strategy, suggesting its potential to regulate Th2 and Th17 responses and modulate bronchial vascular remodeling pathways.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Malaria-Induced Brain and Kidney Injury: A Novel Approach Using Young BALB/c Mice

Authors:

Maria Fernanda Gouveia¹
Sarah Aparecida dos Santos Alves¹
Lívia Ribeiro Gomes¹
Lavínia Reif Correa de Oliveira¹
Celso Caruso-Neves^{1,2,3}
Ana Acácia de Sá Pinheiro¹

Affiliations:

¹Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil
²Rio de Janeiro Innovation Network in Nanosystems for Health-NanoSAÚDE/FAPERJ, Rio de Janeiro, Brazil
³National Institute of Science and Technology for Regenerative Medicine, Rio de Janeiro, Brazil

Keywords: Cerebral malaria, Malaria-induced acute kidney injury (MAKI), evans blue dye

Abstract:

Cerebral malaria (CM) is a severe complication associated with *Plasmodium falciparum* infection. CM could be accompanied by damage to other organs, including kidneys. Malaria-induced acute kidney injury (MAKI) significantly reduces patient survival. The mechanisms linking CM and MAKI remain unclear. Recent findings suggested that young BALB/c mice infected with *P. berghei* ANKA (*PbA*) develop cerebral alterations, highlighting this model as a potential tool for studying CM-MAKI crosstalk. This work aims to evaluate the dynamic crosstalk of CM and MAKI induced by *PbA* infection. For that, BALB/c mice (3–5 weeks old) were infected with 10⁵ *PbA*-infected erythrocytes (CEUA 032/23) and placed in metabolic cages for urine collection. Brain damage was assessed using Evans blue dye (EB) assay and behavioral changes were evaluated by SHIRPA score. Blood parasitemia increased daily, achieving a maximum of 36% on day 7 post-infection (p.i.). Behavioral changes were observed on infected mice at day 5 p.i., suggesting an established CM. Infection induces brain hypertrophy, as indicated by increased brain-to-body weight ratio, with an average 1.33-fold. We also observed a progressive fold increase of EB extravasation at 5, 6 and 7 days p.i. (2.10, 2.20 and 4-fold, respectively). On survival analysis, infected mice died up to 8 days p.i., corroborating with CM establishment. MAKI was confirmed by urinary flow reduction (1.84-fold) observed at 5 days p.i. Urinary protein excretion revealed molecular weight-dependent alterations, with infected mice displaying increased levels of medium-weight proteins (3-fold) over the 5–7 days p.i. We also observe a tendency of increase in urinary γ GT activity, a marker of tubular injury. These findings allow us to postulate that CM impact on disease prognosis. Further experiments are needed to better clarify MAKI establishment in this model. Our results emphasize the use of young BALB/c mice as a promising model for malaria research.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Expression of COX-2 and IL-6 and inflammatory infiltrate as immunological markers in neuroblastoma

Authors:

Melissa Aparecida dos SANTOS¹
Steffanni Sayala Andrade MARQUES¹
Selene ELIFIO- ESPOSITO¹

Affiliations:

¹ Pontifícia Universidade Católica do Paraná

Keywords: Inflammation. Immune infiltrate. Pediatric tumor. Tumor microenvironment.

Abstract:

Solid tumors generally present an inflamed tumor microenvironment characterized by immune cell infiltration and the release of proinflammatory mediators such as IL-6 and COX-2, which may contribute to tumor progression. The objective of this study was investigate the impact of inflammatory markers in the clinical outcome of patients treated for Neuroblastoma (NB), the most common solid tumor in children, at Hospital Pequeno Príncipe in Curitiba-PR, between 2000 and 2020. Data were collected from medical records of 83 patients. FFPE tumor samples collected at diagnosis (pre-CT) and after chemotherapy (post-CT) were stained for the COX-2 and IL-6 expression by immunohistochemistry (IHC). Statistical analyses were performed using the SSPS package to search for associations between the clinical variables, COX-2 and IL-6 expression, and the presence of immune infiltrate (II) intra or peritumoral. The Research Ethics Committee of PUCPR approved the study (n° 3.573.221 and n° 4.871.938). Most patients (43%) were at high risk. In the IHC morphometric analysis, COX-2 expression was higher in post-CT samples compared with pre-CT, from 2,739.02 to 4,206.48 pixels. IL-6 expression showed a slight increase, from 7,084.95 to 7,444.24 px. In the pre-CT, II were present in 61% of cases, 45% of which were intratumoral. After CT, 52% of samples presented II, 28% of which were intratumoral. The analysis of the association between the II and clinical variables showed significant results for stage ($p = 0.001$), risk ($p = 0.002$), and residual recurrence ($p = 0.007$). COX-2 was associated with the II location ($p = 0.013$) and unfavorable histology ($p = 0.039$) and with the presence of immune cells ($p = 0.001$). IL-6 was associated with bone marrow infiltration and disease extent at diagnosis. The expressions of COX-2 and IL-6 and the presence and localization of inflammatory infiltrates may act as immunological markers associated with tumor progression and poor prognosis in NB.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Monotherapy with 4-bromo-N-(3-nitrophenyl) carbamathiol benzamide during the acute phase of experimental Chagas disease prevents cardiovascular alterations and reduces cardiac lesions in the chronic phase

Authors:

Lucas Felipe DOS SANTOS¹,
Raquel Pires NAKAMA⁷,
Leonardo BERTO-PEREIRA²,
Mateus de Oliveira RODRIGUES-VIANA²
Valéria Ferraz TUTIDA⁶
Douglas MAGALHÃES-SILVA⁶
Lucy Megumi Yamauchi LIONI¹,
Eliandro Reis TAVARES¹,
Maria Isabel LOVO-MARTINS²,
Marli Cardoso MARTINS-PINGE^{2,5},
Fabio Nelson GAVA⁶
Eduardo José de Almeida ARAÚJO^{2,4},
Marcelle de Lima Ferreira BISPO³,
Sueli Fumie Yamada OGATTA¹,
Philenio PINGE-FILHO^{1,2}

Affiliations:

- ¹ Department of Microbiology, State University of Londrina – PR, Brazil
² Department of Immunology, Parasitology, and General Pathology, State University of Londrina, Londrina – PR, Brazil
³ Department of Histology, State University of Londrina, Londrina – PR, Brazil
⁴ Department of Chemistry, State University of Londrina, Londrina – PR, Brazil
⁵ Department of Physiological Sciences, State University of Londrina, Londrina – PR, Brazil
⁶ Department of Veterinary Clinic, State University of Londrina, Londrina – PR, Brazil
⁷ Department of Immunology and Biochemistry, University of São Paulo – PR, Brazil

Keywords: Benzoylthiourea. New molecules. *T. cruzi* infection. Therapeutic alternative.

Abstract:

Introduction: Chagas disease (CD), caused by *Trypanosoma cruzi*, has a treatment gap due to the scarcity of effective drugs in both the acute and chronic phases. Benzoylthioureas, such as 4-bromo-N-(3-nitrophenyl) carbamathiol benzamide (BTU-1p), have demonstrated anti-*T. cruzi* effects *in vitro*. Objective: To evaluate the effects of BTU-1p treatment during the acute phase of CD and investigate its impact on the chronic phase. Methods: BALB/c mice (n = 5-8) were infected intraperitoneally with 500 *T. cruzi* Y strain forms and treated with BTU-1p (12.5 mg/kg) via gavage for 28 days (100 µL/day). The control group received water at the same volume. Parasitemia was monitored on alternate days (day 3 to day 47 post-infection [p.i.]). On days 88 and 89 p.i., electrical activity, blood pressure (BP), and heart rate (HR) were measured. On day 90 p.i., the animals were euthanized, and their hearts were collected for nitric oxide (NO) analysis, parasite load quantification, and histological evaluation (H&E and Picrosirius staining). Statistical significance $p < 0.05$. Protocol CEUA 022.2023. Results: BTU-1p reduced the area under the parasitemia curve (AUC: 186187 vs. 136678 ± 22103), controlled parasitemia on day 7 ($p < 0.05$) during the immunosuppression period, and preserved 90-day survival (100%). Moreover, it maintained BP and HR ($p < 0.05$) without altering cardiac electrical activity ($p < 0.05$). BTU-1p significantly reduced the parasite load in cardiac tissue ($p < 0.001$), indicating a direct effect on the parasite. The treatment also decreased type I collagen deposition ($p < 0.001$) without significantly affecting type III collagen deposition ($p < 0.05$) or the number of infiltrating cells ($p < 0.05$). Additionally, BTU-1p did not alter NO production ($p < 0.05$), suggesting a lower risk of tissue damage. Conclusion: This study provides novel evidence of the effects of BTU-1p in a chronic *T. cruzi* infection model, highlighting its potential as a drug candidate for CD treatment.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Antibiofilm and Antifungal Activities of Tritrpticin (Trp3) Analogs Revealed by Confocal Laser Scanning Microscopy (Clsm) Assays and *In Vivo* Assays Using a *Galleria Mellonella* Model

Authors:

Vitor Martins de ANDRADE¹

Ana HERDADE²

Montserrat HERAS³

Miguel CASTANHO²

Juliana Campos JUNQUEIRA⁴

Katia CONCEIÇÃO¹

Affiliations:

¹Universidade Federal de São Paulo, Laboratório de Bioquímica de Peptídeos, São José dos Campos, São Paulo, Brasil;

²Instituto de Medicina Molecular, Lisboa, Portugal;

³Universitat de Girona, Departament de Química, Girona, Espanha;

⁴Universidade Estadual Paulista, Laboratório de Microbiologia e Imunologia, São José dos Campos, São Paulo, Brasil;

Keywords: 3D culture, confocal microscopy, *Galleria mellonella*, peptide, TRP3.

Abstract:

Antifungal resistance in *Candida spp.* is on the rise, with no corresponding development of new drugs. *C. albicans*, responsible for 80% of candidiasis cases, forms resistant biofilms, hindering infection eradication. The search for effective molecules is urgent. Antimicrobial peptides (AMPs), such as tritrpticin (TRP3), stand out for their broad action against pathogens, including fungi. TRP3, an AMP from the cathelicidin family, exhibits antimicrobial and immunomodulatory actions, noted for its high tryptophan (23.1%) and arginine (30.8%) content. Its nearly symmetrical structure and hydrophobic core, formed by tryptophan residues, favor interaction with cell membranes, leading to membrane disruption and cell death. Biophysical studies show that TRP3 promotes the formation of toroidal pores in different membranes, a mechanism also observed in its analogs. This study investigates the antifungal and antibiofilm action of TRP3 and its analogs (TRP3-NH₂ and TRP3-NH₂-P9A) against *C. albicans*, using confocal microscopy (CLSM) techniques and biophysical analyses, circular dichroism, and zeta potential. Results indicate that TRP3 has a minimum inhibitory concentration (MIC) of 32 µg/ml, while its analogs show 16 µg/ml. Zeta potential assays revealed that the peptides adhere to the fungal membrane, neutralizing its electrical charge and forming pores, suggesting a possible cell death mechanism. CLSM analyses confirmed the efficacy of the analogs against biofilms, eradicating yeasts and hyphae. In tests with the invertebrate model *G. mellonella*, no toxic effects were observed, and there was an increase in the survival of infected animals treated with the peptides. TRP3 and its analogs emerge as promising candidates for the development of new antifungal therapies, highlighting their potential for biotechnological applications in the fight against resistant fungal infections.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Activity of the Bioactive Peptide Ibkt_p-NH₂ on Multispecies Biofilms of *Candida albicans* and *Staphylococcus aureus* and *in vivo* Studies Using *Galleria mellonella* Larvae for the Treatment of Multispecies Infections and Identification of Immunological Alterations

Authors:

Gustavo Matheus LAUREANO²
Katia CONCEIÇÃO¹
Juliana Campos JUNQUEIRA⁶
Montserrat HERAS⁵
Eduard BARDAJÍ⁴
Miguel CASTANHO³
Vitor Martins de ANDRADE^{1,2}

Affiliations:

¹ Universidade Federal de São Paulo, Laboratório de Bioquímica de Peptídeos, São José dos Campos, São Paulo, Brasil;
² Centro Universitário Internacional - UNINTER, São José dos Campos, São Paulo, Brasil;
³ Instituto de Medicina Molecular, Lisboa, Portugal;
⁴ Universitat de Girona, Laboratori d'Innovació en Processos i Productes de Síntesi Orgànica (LIPPSO), Departament de Química, Girona, Spain;
⁵ Universitat de Girona, Departament de Química, Girona, Espanha;
⁶ Universidade Estadual Paulista, Laboratório de Microbiologia e Imunologia, São José dos Campos, São Paulo, Brasil;

Keywords: *Galleria mellonella*, kyotorphin, multispecies biofilms.

Abstract:

Kyotorphin (KTP, L-tyrosyl-L-arginine) is an endogenous dipeptide isolated in 1979 from bovine brain, initially characterized for its analgesic properties. More recent studies have shown that some derivatives of kyotorphin, specifically the Ibuprofen-conjugated derivative (IbKTP-NH₂), exhibit anti-inflammatory and antimicrobial activities. In this study, the derivative IbKTP-NH₂ was evaluated for its antibiofilm activity against bacteria and fungi. Antimicrobial screening of the peptide was performed on *Staphylococcus aureus* (MIC 32 µM) and *Candida albicans* (1000 µM). The direct effect of IbKTP-NH₂ on pre-formed mono and multispecies biofilms was assessed by colony forming unit (CFU) counting. IbKTP-NH₂ significantly reduced the formation of both mono and multispecies biofilms. Filamentation assays demonstrated the potential involvement of the derivative in inhibiting fungal virulence factors, even in co-cultures. Survival after infection was analysed by administering the derivative to *Galleria mellonella* larvae. In the model, a reduction in hemocytes was observed in infected larvae, both individually and in co-infection, while treatment with IbKTP-NH₂ resulted in increased hemocyte counts and improved larval survival. Hydrogen peroxide (H₂O₂) quantification assays revealed the production profile of these molecules during the infectious process, as they are directly related to the larvae's immune response. We suggest that this derivative, in addition to exerting physical activity on membranes, may induce modulation of cellular and humoral immune responses, potentially being useful in the treatment of infections caused by different microorganisms in biofilms.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Hyperglycemia and Endothelin-1 Affect Endothelial Cell Homeostasis by Increasing Glycocalyx Permeability and Degradation In Vitro

Authors:

Carolina de Lima ESQUISATTO¹
Núbia Sabrina MARTINS¹
Vania Luiza Deperon BONATO¹

Affiliations:

¹Depto. de Bioquímica e Imunologia, FMRP-USP.

Keywords: Allergen, Endothelin-1, Endothelial permeability, Syndecan-1, Heparan-sulfate.

Abstract:

Obesity is a risk factor for the exacerbation of asthma, a chronic inflammatory disease that affects the lungs. Increased blood glucose and endothelin-1 (ET-1), a vasoconstrictor molecule, are characteristic of obesity. The endothelial glycocalyx (EG) is composed of a network of proteoglycans, such as syndecan-1 (SDC-1), and glycosaminoglycans, such as heparan sulfate (HS), anchored to the endothelial cell (EC) membrane that performs several functions. Under inflammatory conditions, EG degradation results in increased leukocyte recruitment and increased endothelial permeability (EP). Both asthma and obesity promote EG degradation *in vivo*. However, the factors that lead to this phenomenon are poorly understood. Our objective is to evaluate the role of allergen, hyperglycemia and ET-1 in EG degradation *in vitro*. EC of the EA.hy 926 cell line were stimulated with House Dust Mite (HDM, 25 µg/mL) in normoglycemic (25 mM mannitol) or hyperglycemic (25 mM glucose) medium; or with ET-1 for 6, 12 or 24 hours. The degradation of GE was evaluated by ELISA and PE by transwell assay. EC stimulated with HDM in hyperglycemic medium showed an increase in HS ($p>0.05$), but not in SDC-1, in the culture supernatant after 6 hours of stimulation compared to unstimulated EC. HDM in hyperglycemic medium also promoted an increase in PE compared to EC cultured in normoglycemic medium and not stimulated with HDM. In addition, EC stimulated with ET-1 showed an increase in HS, but not in SDC-1, in the culture supernatant after 6 hours of stimulation compared to unstimulated EC. Taken together, our data suggest that allergen and hyperglycemia exert little influence on EG integrity but directly affect PE. In contrast, ET-1 promotes EG degradation *in vitro*. Therefore, hyperglycemia and ET-1 represent potential triggers of asthma exacerbation in the context of obesity.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Immunoregulation and effects of pre-treatment with aspirin in mice with metabolic syndrome during severe sepsis

Authors:

Leonardo BERTO-PEREIRA¹
Raquel Pires NAKAMA²
Lucas Felipe DOS SANTOS²
Isabella Ramos Trevizani THIHARA³
Guilherme MASSARDI²
Manuela Fancio BOVOLIN²
Gabriela Gonçalves DA SILVA²
Maria Isabel LOVO-MARTINS²
Andressa FREITAS⁴
Marli Cardoso MARTINS-PINGE¹
Phileno PINGE-FILHO²

Affiliations:

¹State University of Londrina; Laboratory of Cardiovascular Physiology and Pharmacology
²State University of Londrina; Laboratory of Experimental Immunopathology
³State University of Londrina; Laboratory of Molecular Biology of Microorganisms
⁴State University of Londrina; Laboratory of Inflammation Pharmacology

Keywords: Cecal ligation and puncture. Obesity. Colony-forming units.

Abstract:

Sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated host response to an infection. The aim of this study was to evaluate the effects of metabolic syndrome (MetS) and aspirin (ASA) pretreatment in a murine model of polymicrobial sepsis, analyzing changes in the peritoneal cavity after sepsis induction by cecal ligation and puncture (CLP). (CEUA: 051.2021) Neonatal Swiss mice were treated from day 1 to day 5 of life with monosodium glutamate (MSG) to induce MetS (4mg/g). The animals were treated with ASA at 40mg/kg, starting on day 60 of life for 15 days. Sepsis was induced by the CLP model after ASA treatment. The animals were euthanized and peritoneal lavage was collected for evaluation of colony-forming units (CFU), leukocyte migration and cytokines: TNF, IFN- γ and IL-6. In another group of animals, survival was evaluated for 168 hours. Differences were considered significant at $p < 0.05$. The presence of CFUs was detected in peritoneal lavage after sepsis, with increased recruitment of lymphocytes ($p \leq 0.01$), macrophages ($p \leq 0.05$) and neutrophils ($p \leq 0.05$), as well as increased concentrations of cytokines such as TNF ($p \leq 0.001$), IFN- γ ($p \leq 0.001$) and IL-6 ($p \leq 0.0001$). MetS and pretreatment with ASA protected against macrophage and lymphocyte recruitment during sepsis. Pretreatment with ASA exerted a negative regulatory effect on the concentration of TNF ($p \leq 0.001$) and IFN- γ . Animals with MetS presented lower concentrations of all cytokines evaluated after sepsis. After sepsis, animals with MetS or treated with ASA also had a higher survival rate compared to septic animals without treatment ($p \leq 0.001$). The results provided a greater understanding of the protective mechanisms of the obesity paradox and pretreatment with ASA associated with sepsis, and may contribute to improving therapeutic protocols considering the metabolic condition of individuals.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Effect of VAcHT reduction on pulmonary alterations induced by exposure to iron particles in an asthma model

Authors:

Tabata Maruyama DOS SANTOS ¹
Renato Fraga RIGHETTI ^{1,2}
Leandro do Nascimento CAMARGO ^{1,2}
Edna Aparecida LEICK ¹
Sílvia FUKUZAKI ^{1,3}
Elaine Cristina de CAMPOS ^{1,2}
Thiago Tafarel GALLI ¹
Beatriz Manguiera SARAIVA-ROMANHOLO ^{1,6}
Carla Máximo PRADO ⁴
Amanda dos Santos LIGERO ^{1,2}
Fernanda Degobbi Tenorio Quirino Dos Santos LOPES ¹
Isabela M BENSENOR ¹
Marcela Souza Lima PAULO ⁵
Paulo A LOTUFO ¹
Iolanda de Fátima Lopes Calvo TIBÉRIO ¹

Affiliations:

- 1- Faculdade de Medicina FMUSP, Universidade de São Paulo, São Paulo, SP, Brazil.
- 2- Hospital Sírio Libanês, São Paulo, SP, Brazil.
- 3- Hospital Alemão Oswaldo Cruz, São Paulo, Brazil.
- 4- Department of Biosciences, Universidade Federal de São Paulo, Santos, Brazil.
- 5- Escola Superior de Ciências da Santa Casa de Misericórdia de Vitória, Vitória, Brazil.
- 6- University City of São Paulo (UNICID), São Paulo, Brazil.

Keywords: Acetylcholine. Asthma. Inflammation. Particulate matter. Pollution.

Abstract:

Pollution is harmful to people with asthma. The effect of cholinergic anti-inflammatory pathways in chronic allergic inflammation associated with pollutants is poorly understood. 108 animals divided into 18 groups (n=6), approval number CEUA:1191/2018. The groups: wide type(WT), genetically modified animals with reduced VAcHT(VAcHTKD) and those sensitized with ovalbumin(VAcHTKDA), exposed to iron in a mining company(Local1) or 3.2 miles away from this company(Local2) for 2 weeks during the summer and winter seasons. It was analyzed to hyperresponsiveness, inflammation, remodeling, responses to oxidative stress and cholinergic system. During the summer, animals WT exposed to Local1 show an increase in hyperresponsivity(%Rrs109.5±15.5, %Raw169.9±32.8) and inflammation(IL17 4.1±0.4) in relation to the animals in the vivarium, while animals exposed to Local2 also show higher levels of IL17(4.1±0.4). During the winter, animals WT reveal that exposure to Local2 increases hyperresponsivity(%Rrs 114.6±19.6) in relation to the animals of the vivarium(29.0±6.4). During the summer, exposure to Local1 increases in %Rrs, Raw and IL5 compared to Local2, while in winter, exposure to Local2 increases IL17 compared to Local1. VAcHTKD animals show an increase in %Rrs, NFκB, IL5 and IL13, but a reduction in α7 in comparison with WT animals. VAcHTKDA animals present an increase in hyperresponsivity, of all inflammatory markers, remodeling and oxidative stress in comparison with animals VAcHTKD. Exposure to Local1 exacerbates hyperresponsiveness, oxidative stress and inflammation in VAcHTKDA animals, while exposure to Local2 increases inflammation, remodeling and oxidative stress. It was concluded that reduced cholinergic signaling amplifies pulmonary inflammation in a model of chronic allergic pulmonary inflammation and when associated with pollution, can aggravate specific responses related to inflammation, oxidative stress and remodeling. Supported:FAPESP(2018-02537-5);LIM20-HCFMUSP.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Silymarin exerts protective effects on arthritis and myositis induced by Chikungunya virus

Authors:

Rafaela Lameira Souza LIMA¹
Ariane Coelho FERRAZ¹
Marília Bueno da Silva MENEGATTO¹
Oluwashola Samuel OLAOLU²
Maria Eduarda Diniz STARLING³
Cintia Lopes de Brito MAGALHÃES^{1,2}

Affiliations:

¹Programa de Pós Graduação em Ciências Biológicas, Universidade Federal de Ouro Preto, Ouro Preto, MG, Brasil

²Programa de Pós Graduação em Biotecnologia, Universidade Federal de Ouro Preto, Ouro Preto, MG, Brasil

³Curso de Medicina, Universidade Federal de Ouro Preto, Ouro Preto, MG, Brasil

Keywords: Alphavirus. Arthritis. Chikungunya. Myositis. Silymarin.

Abstract:

Chikungunya virus (CHIKV) is an Alphavirus that causes severe joint/muscle pain and fever. Our research group is focused on investigating factors contributing to the pathogenesis of some alphaviruses as well as identifying natural substances as a potential treatment. In this sense, silymarin, a complex of antioxidants obtained from *Silybum marianum*, has shown potent antiviral activity against alphaviruses in the literature and studies carried out by our group. Given that joint and muscle pain are prominent symptoms of Chikungunya fever, this study aims to evaluate in an animal model of arthritis and myositis induced by CHIKV whether silymarin can reverse the damage to joints and muscles post-infection. To achieve this, 6-week-old BALB/c mice were infected with CHIKV in the right hind paw pad (10^6 PFU) and the treated groups received 200mg/kg/day of silymarin every 12 hours by gavage (approved by the UFOP Animal Ethics Committee – CEUA 4528291122). Preliminary results from infected animals showed paw edema and inflammation were reduced in animals treated with silymarin. Euthanasia was performed on 7- and 12-days post-infection (dpi), and various organs/tissues including liver, spleen, footpad, quadriceps, tibial, soleus, and extensor digitorum (EDL) muscles were collected. The presence of CHIKV was assessed by qRT-PCR. In the infected group that received silymarin, viral load was reduced in the liver, spleen and footpad at 7dpi, and in the liver, spleen and soleus at 12dpi; additionally, in the quadriceps, tibial, and EDL muscles, no virus was detected in any of infected and treated groups. Histopathology and morphometry were carried out for tissue analysis. Silymarin was able to reduce inflammation in the liver, muscles, joints and medulla of the animals' paws. Thus, these findings support further investigation into the potential benefits of silymarin in treating CHIKV infections.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Albumin Transport Triggers Microglial Activation Before BBB Breakdown in Early Stages of Cerebral Malaria

Authors:

Sarah Aparecida dos Santos Alves¹
Douglas Esteves Teixeira¹
Michele da Silva Siqueira⁴
Raquel Maria Pereira Campos¹
Flavia Carvalho Alcantara Gomes⁴
Pedro Moreno Pimentel-Coelho¹
Celso Caruso-Neves^{1,2,3}
Ana Acacia de Sá Pinheiro¹

Affiliations:

- 1- Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil;
- 2- Rio de Janeiro Innovation Network in Nanosystems for Health-NanoSAÚDE/FAPERJ, Rio de Janeiro, Brazil;
- 3- National Institute of Science and Technology for Regenerative Medicine, Rio de Janeiro, Brazil;
- 4- Institute of Biomedical Sciences, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

Keywords: Cerebral malaria. Endothelial dysfunction. Microglia. Protein endocytosis.

Abstract:

Cerebral malaria (CM) is a severe *P. falciparum* complication that leads to blood-brain barrier (BBB) breakdown. The role of endothelial cells (EC) in protein transport in CM early stages remains poorly understood. Recent findings demonstrated that serum albumin can contribute to cognitive deterioration in neurological disorders. This study aimed to verify the role of brain EC in early CM and the interaction of transported serum proteins with microglial cells. CM was induced in C57BL/6 mice by injecting 106 *P. berghei* ANKA-infected erythrocytes. BBB stability was evaluated 3–5 days post-infection (p.i.). (CEUA 032/23) In vitro assays were performed using mouse microglial cells lineage (BV-2). Evans blue dye assay and endogenous IgG deposition showed BBB breakdown in mice on days 4–5 p.i. However, FITC-labeled albumin accumulated in brain tissue as early as day 3 p.i. which correlated with increased microglial activation revealed by increased IBA-1 marker. Nystatin (8 mg/kg), a caveolae-mediated transport inhibitor, reduced FITC-albumin uptake and microglial activation, improving BBB stability and behavioral outcomes (n=4). Increasing albumin concentrations reduced FITC-albumin endocytosis in BV-2 cells in a dose-dependent manner (n=3). High albumin (HA) concentrations (20 mg/mL) decreased FITC-albumin binding on BV-2, increased IBA-1 expression and induced cell death. BV-2 cells endocytic capacity was also abolished by 25 μ M Pit-Stop 2, a clathrin-mediated endocytosis inhibitor, suggesting a receptor-mediated process. In addition, megalin blockage with 200ug/mL Cilastatin reduced BV-2 cells endocytic capacity. Our results allowed us to postulate that brain EC protein manipulation through a caveolae-dependent manner precedes BBB breakdown in CM and serum albumin can interact with microglial cells promoting activation and cell death, highlighting new insights into CM pathogenesis.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Effects of Anti-IL-33 on Pulmonary Mechanics and Inflammation in an Experimental Model of Asthma Exacerbated by LPS

Authors:

Vitória Helena Kuhn de CAMPOS^{1,2}
Leandro Nascimento CAMARGO^{1,2}
Francine Maria ALMEIDA¹
Amanda dos Santos LIGERO^{1,2}
Gabriela Rodrigues Favilla DE PAULA¹
Beatriz SARAIVA-ROMANHOLO¹
Edna Aparecida LEICK¹
Fernanda DQS LOPES¹
Renato Fraga RIGHETTI^{1,2}
Iolanda Fatima Lopes Calvo TIBÉRIO¹

Affiliations:

¹Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil.

²Hospital Sírio-Libanês, São Paulo, Brazil

Keywords: Anti-il-33. Asthma. Inflammation. Lung mechanics.

Abstract:

Aims: IL-33 is an alarmin involved in the recruitment of inflammatory cells in asthma. Although its inhibition is capable of modulating inflammatory responses and pulmonary remodeling, no studies have addressed the blockade of IL-33 in experimental models of asthma exacerbated by LPS. **Objectives:** To evaluate the effects of anti-IL-33 treatment on pulmonary mechanics and inflammatory pathways in an experimental model of asthma exacerbated by LPS. **Methods:** BALB/c mice were subjected to saline (SAL), OVA (ovalbumin), LPS (lipopolysaccharide), or both OVA and LPS (exacerbated asthma model) for 29 days and were treated or not with anti-IL-33. We evaluated respiratory mechanics and bronchoalveolar lavage fluid (BALF) cells. This study was approved by the Ethics Committee on Animal Use. **Results:** Treatment with anti-IL-33 attenuated the responses of resistance (Rrs-cmH₂O/L/s) and elastance (Ers-ml/cmH₂O) of the respiratory system as follows: OVA+anti-IL-33 (Rrs:0.22±0.09, Ers:0.41±0.04, Gtis:0.29 ±0.05, Htis:0.36±0.03) and OVA+LPS+anti-IL-33 (Rrs:0.26±0.06, Ers:0.4±0.03, Gtis:0.23 ±0.04, Htis:0.39±0.03) compared to OVA (Rrs:1.01±0.25, Ers:0.69±0.19, Gtis:0.9±0.22, Htis:0.74±0.06) and OVA+LPS (Rrs:1.0±1.15, Ers:1.0±0.08, Gtis:0.94±0.14, Htis:0.82±0.05) groups (p<0.05 for all comparisons). There was also a reduction in BALF (10⁴ cells/mL): OVA+anti-IL-33 (total cells 10.6±1.27, eosinophils 1.75±0.46, and macrophages 1.76±0.55) and OVA+LPS+anti-IL-33 (total cells 10.4±1.2, eosinophils 2.4±0.9, and macrophages 7.2 ±0.9) compared to OVA (total cells 20.4±0.4, eosinophils 8.5±1.0, and macrophages 9.1 ±0.6) and OVA+LPS (total cells 27.9±3.6, eosinophils 9.0±1.5, and macrophages 11.6±2.1) groups (p<0.05 for all comparisons). **Conclusions:** Inhibition of IL-33 contributes to inflammation control and improved lung mechanics in a murine model of experimental asthma exacerbated by LPS. **Sponsorship:** FAPESP (2022/01776-1), LIM-20-HC-FMUSP.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Gestational Exposure to Phthalates and Nanoplastics in Rats: Impacts on Gut Microbiota Composition and Intestinal Histology

Authors:

Isabella FÁVARO¹
Natália MAGOSSO¹
Felipe Cristiano BARBOSA¹
Patrick Vieira de SOUZA¹
Mirella Franco MOREIRA¹
Vanessa Aguiar ROCHA¹
Matheus Naia FIORETTO¹
Victória Cristina PINHA¹
Gabriel Aquino de Souza MAIA¹
Wellerson Rodrigo SCARANO¹

Affiliations:

¹Department of Structural and Functional Biology, Institute of Biosciences, São Paulo State University (UNESP), Botucatu, São Paulo, Brazil.

Keywords: Environmental contaminants. Gut microbiota. Nanoplastics. Plasticizers.

Abstract:

Plastic production and pollution increase annually, exposing humans to micro/nanoplastics (MNPs) and contaminants like phthalates, plasticizers that act as endocrine disruptors (EDCs). Recent evidence suggests that MNPs and phthalates can cross biological membranes, compromise intestinal epithelial integrity, and alter microbiota composition, influencing metabolic, immune, and developmental outcomes in gestation. This study was aimed to evaluate the effects of gestational exposure to phthalates and nanoplastics on the composition of the microbiota and histology of colon tissue in pregnant rats. Pregnant rats (CEUA 1174020523) were distributed into 4 groups: C: (control; vehicle); T1: 20µg/kg/day phthalate mixture (PM); T2: nanoplastics (NPs 1mg/kg); T3: 20µg/kg/day PM + NPs (1mg/kg); and orally treated from gestational day 5 (GD5) to GD20. At GD20, 6-8 pregnant rats from each group underwent laparotomy and feces were collected from the cecal region for analysis of the microbiome composition through sequencing and colon region was collected for histological analysis. The statistical test used was ANOVA, followed by Tukey. The microbiome analysis showed an increase in class *Bacilli* ($p=0,02$) and order *Labtobacillales* ($p=0,04$) was observed in T2 and T3 vs. C, while class *Clostridia* ($p=0,04$) decreased in T1 vs. C. Nanoplastics may trigger an inflammatory response favoring *Bacilli* growth while partially preserving *Clostridiales*. A lower Shannon index (diversity and equity of species) was observed in group T1 vs. C ($p=0,04$). Morphometric analysis, through the quantification of colon areas by the *imageJ* program, indicated a reduced muscular layer in T3 ($p=0,03$), suggesting muscle cell degradation and apoptosis due to pollutant exposure. These findings suggest that the effects phthalates on the gut microbiota may be modulated by the presence of nanoplastics, which may lead to alterations in gut integrity and metabolism, influencing critical metabolic functions in the host.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: OM-85 bacterial lysate reduces *P. aeruginosa* susceptibility in cystic fibrosis while exacerbating tissue damage in a preclinical model

Authors:

Evandro Neves SILVA¹
Thiago Caetano Andrade BELO¹
Leonardo Pereira de ARAÚJO¹
Guilherme Landim de REZENDE¹
André Luiz Caliar COSTA¹
Letícia Barbosa SILVA¹
Sávio Rezende SOUZA¹
Elda Gonçalves Souza e LEITE²
Rômulo Dias NOVAES²
Patrícia Paiva CORSETTI¹
Leonardo Augusto de ALMEIDA¹

Affiliations:

¹Laboratory of Molecular Biology of Microorganisms, Federal University of Alfenas (UNIFAL), Alfenas, Minas Gerais, Brazil.

²Department of Structural Biology, Federal University of Alfenas (UNIFAL), Alfenas, Minas Gerais, Brazil.

Keywords: Cystic fibrosis. Microbial lysate. PA14. Respiratory tract infection.

Abstract:

Pseudomonas aeruginosa is a gram-negative bacterium frequently linked to severe infections in cystic fibrosis (CF) patients. It colonizes the lungs, fostering chronic infections that are hard to treat. Over time, these infections cause progressive lung function deterioration, contributing significantly to morbidity and mortality. This study assessed the efficacy of the bacterial lysate OM-85 in a preclinical CF model, evaluating its effects during *P. aeruginosa* infection. C57BL/6 mice (CEUA nº 0032/2024) were induced into a CF model, prophylactically treated with 1mg of OM-85 nasally, and infected with virulent *P. aeruginosa* (PA14). qPCR analysis for bacterial DNA in mouse lungs revealed OM-85-induced microbiota modulation, with decreases in Bacteroidetes and Gammaproteobacteria and an increase in Firmicutes. In the CF model, the drug mainly increased Bacteroidetes and Firmicutes. Upon infection, reduced susceptibility to PA14 was observed, as evidenced by CFU counts. OM-85-treated mice showed elevated IL-17 and IFN- γ production in the lungs, along with reduced IL-10 levels compared to untreated CF mice. Flow cytometry revealed higher influxes of CD11⁺CD86⁺, CD4⁺IFN- γ ⁺, and CD4⁺IL-17⁺ cells in lung homogenates. Consistent with these results, histopathology showed septal thickening, alveolar collapse, and pronounced leukocytic infiltration compared to PBS, OM-85, and CF groups. While hepatic microstructure remained normal in non-infected groups, PA14, CF+PA14, and CF+OM-85+PA14 groups exhibited hepatic parenchyma expansion, sinusoidal capillary collapse, and increased interstitial cellularity. These findings suggest that, despite benefits in reducing infection susceptibility, OM-85 prophylaxis also exacerbates histopathological alterations, underscoring the need for further studies to evaluate its long-term effects.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Rational design of immunogenic peptides for a multiepitope vaccine targeting structural proteins of *Oropouche virus*

Authors:

Letícia Barbosa SILVA¹
Leonardo Pereira de ARAÚJO¹
Evandro Neves SILVA¹
Guilherme Landim de REZENDE¹
Thiago Caetano Andrade BELO¹
Sávio Rezende SOUZA¹
André Luiz Caliari COSTA¹
Patrícia Paiva CORSETTI¹
Leonardo Augusto de ALMEIDA¹

Affiliations:

¹Laboratory of Molecular Biology of Microorganisms, Federal University of Alfenas (UNIFAL), Alfenas, Minas Gerais, Brazil.

Keywords: Epitope. Immunoinformatics. Multivalent. Oropouche. Vaccinology reverse.

Abstract:

The emergence of pandemics raises global concerns about emerging viruses, such as COVID-19, and re-emerging ones, such as Oropouche (OROV). In 2024, Brazil recorded an increase in confirmed OROV cases compared to the previous year. OROV was identified in previously unreported areas, resulting in the first attributed deaths, although cases of miscarriage have been reported since the end of the last century. OROV symptoms are similar to those of Dengue and Zika, leading to underreporting and underestimation of its impact. In the absence of specific treatments, the development of vaccines becomes essential, as vaccination is one of the most effective preventive methods. The objective was to identify immunogenic epitopes in three structural proteins of OROV and construct a multi-epitope vaccine candidate. RefSeq sequences of the nucleocapsid protein and the Gn and Gc glycoproteins were obtained from the National Center for Biotechnology Information Virus and submitted to epitope search in the Immune Epitope Database. Antigenicity, allergenicity, stability, and toxicity analyses were conducted, and the approved epitopes were aligned to the global protein to remove transmembrane regions and N-glycosylation sites. In the end, 13 epitopes were selected and used to construct a multi-epitope vaccine candidate, with beta-defensin and PADRE adjuvants. The protein showed promising results in antigenicity, allergenicity, stability, and solubility. Predictions of humoral and cellular immune responses were performed in C-ImmSim, indicating satisfactory results for 3 doses of the vaccine candidate. 3D modeling of the protein was performed in AlphaFold3, evaluating the molecular docking of the multi-epitope protein with TLR-2, TLR-3, TLR-6, and TLR-8 receptors. Final results indicate an excellent vaccine candidate, but molecular dynamics in silico analyses are needed for structural validation, along with in vitro and in vivo tests to confirm the results.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Conserved immunogenic epitopes in host entry and exit proteins of the reemergent *Monkeypox virus* and the emerging *Alskapox virus*

Authors:

Leonardo Pereira de ARAÚJO¹
Evandro Neves SILVA¹
Guilherme Landim de REZENDE¹
André Luiz Caliar Costa¹
Thiago Caetano Andrade BELO¹
Letícia Barbosa SILVA¹
Sávio Rezende SOUZA¹
Patrícia Paiva CORSETTI¹
Leonardo Augusto de ALMEIDA¹

Affiliations:

¹Laboratory of Molecular Biology of Microorganisms, Federal University of Alfenas (UNIFAL), Alfenas, Minas Gerais, Brazil.

Keywords: Mpox virus, Alskapox virus, Epitope, Conservation.

Abstract:

With the emergence of new pandemics, such as COVID-19, outbreaks of emerging and reemerging viruses have raised global alarm. In June 2022, the World Health Organization declared *Monkeypox Virus* (MPXV), belonging to the *Orthopoxvirus* (OPXV) genus, a global health emergency. In 2023, our research group developed a multi-epitope vaccine based on MPXV entry and exit proteins, with the potential to induce an effective immune response. In 2024, a new virus from the OPXV genus, *Alskapox virus* (AKPV) – recently renamed *Borealpox* – gained attention for causing the first death of an immunocompromised patient. The aim of this study was to identify whether epitopes previously identified by our research group for MPXV are conserved in AKPV, enabling the development of a bivalent vaccine for MPXV and AKPV. Available AKPV genomes were obtained from the NCBI database. Alignments were performed between MPXV epitopes previously identified in AKPV proteins. Epitopes with identified mutations in AKPV proteins were subjected to antigenicity analysis on the Vaxijen server, allergenicity on the AllerCatPro server, and physicochemical properties using the ProtParam tool. The analysis revealed that three of the ten epitopes previously found for MPXV are conserved in AKPV, six have mutations, and one was completely deleted. Of the epitopes with mutations, four present mutations that meet the requirements for antigenicity and structural stability, while two have mutations that make them non-antigenic or unstable. The epitopes conserved between MPXV and AKPV not only provide a basis for the development of a multi-epitope bivalent vaccine but also have significant relevance in the creation of rapid viral identification tests, representing a promising approach for controlling and preventing diseases caused by these emerging and reemerging viruses.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Implications of the Heme Group in the bioenergetics of *Trypanosoma cruzi* isolates from patients with different clinical forms of Chagas disease

Authors:

Bruna Eduarda Siqueira da SILVA¹

Ellen Vieira de LIMA²

Vitor Klipel da Silva BERTOLINI³

Sofia Paola FERNANDEZ¹

Gabriela Silva FUGA¹

Danilo Ciccone MIGUEL³

Fernanda Ramos GADELHA³

Affiliations:

¹ Faculty of Pharmaceutical Sciences, UNICAMP, Campinas, Brazil.

² Faculty of Biological Sciences, PUC-Campinas, Campinas, Brazil.

³ Biology Institute, UNICAMP, Campinas, Brazil.

Keywords: Heme. Mitochondrial bioenergetics. *Trypanosoma cruzi*.

Abstract:

Recognizing the auxotrophic nature of *Trypanosoma cruzi*, the etiological agent of Chagas disease, with respect to heme biosynthesis, this study investigated the impact of exogenous heme availability on the bioenergetic profiles of *T. cruzi* isolates derived from patients (CAAE: 42839615.0.0000.5404). Epimastigote forms (DTU TcII) of three human isolates (I1, I2, and I6) and the reference Y strain were cultured in complete Liver Infusion Tryptose medium under three experimental conditions: heme-supplemented at 20 g/L (Medium A), at 10 g/L (Medium B), and heme-deficient (Medium C). Parasites were adapted to each medium through three successive subcultures initiated at the stationary phase of growth. Intracellular heme concentrations were quantified using the alkaline pyridine method. Hydrogen peroxide (H₂O₂) production was assessed via the Amplex Red assay (Molecular Probes®) and mitochondrial superoxide levels were determined using MitoSOX (Molecular Probes®), both using a Hitachi fluorimeter. Oxygen consumption rates (OCR) were measured using a Hansatech Oxygraph equipped with a Clark electrode (Yellow Springs Instrument Co.). NADPH production was quantified spectrophotometrically (BECKMAN DU640). The results demonstrated that human isolates, but not the Y strain, exhibited significantly elevated intracellular heme concentrations in Medium B compared to the other conditions. Isolates I2 and I6 showed increased H₂O₂ production in Medium A, while Isolate I1 in Medium B. In non-permeabilized cells, the Y strain displayed the highest OCR in Medium B. In digitonin-permeabilized cells, Isolate I1 exhibited an approximate 50% increase in OCR. Notably, Isolate I2 demonstrated markedly reduced NADPH production across all three media conditions, contrasting with the other isolates and the Y strain. These findings suggest that heme availability significantly influences the bioenergetics of *T. cruzi* and that isolate-specific metabolic adaptations are evident.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Detection and identification of microorganisms in ticks of the families Argasidae and Ixodidae from different Brazilian States (Amazonas, Mato Grosso do Sul, and So Paulo) by MALDI-TOF/MS

Authors:

Simone Michaela SIMONS¹
André Gustavo TEMPONE²
Beatriz Alves de ANDRADE²
Dayana Agnes Santos FERREIRA²
Julia Maria Godoi LIMA²
Eder BARBIER³
Hermes Ribeiro LUZ⁴
Marcelo Francisco dos SANTOS¹
Karen da Silva GOMES¹
Darci Moraes BARROS-BATTESTI³

Affiliations:

¹Parasitology Laboratory, Butantan Institute-IBu, SP
²Physiopathology Laboratory, Butantan Institute-IBu, SP
³Department of Pathology, Reproduction and One Health, Paulista State University UNESP, SP
⁴Parasite Control Laboratory, Federal University of Maranhão-UFMA, MA.

Keywords: MALDI-TOF/MS, microorganisms, tick

Abstract:

Ticks are hematophagous ectoparasites of the order Ixodida. Their vectorial capacity makes them one of the most relevant groups in terms of public health and global economic impact, not so much because of the number of species that act as transmitters, but because of the diversity of microorganisms that can be conveyed and/or carried. The aim of this study was to isolate and characterize microorganisms present in the salivary glands and ovaries of ticks collected in the wild. The ticks were collected in 2024. The *Amblyomma sculptum* came from the Parque Ecológico do Tietê, SP (SISBIO 97520). The *A. cajennense* from the municipality of Santa Inês, AM and the *Ornithodoros fonsecai* from the São Miguel cave, Bonito, MS (SisGen A1B5B5C). Extracts from the salivary glands and ovaries were obtained from 10 females and 10 males (only for *A. cajennense*) fasted in a laminar flow using a stereomicroscope. The colonies were isolated using the depletion isolation technique in Petri dishes containing tryptone soy agar medium and incubated in a BOD oven. Ribosomal proteins were extracted from the isolates and identified using mass spectrometry (MALDI-TOF/MS) (Bruker-Daltonics). Twelve isolates were obtained, of which 2 originated from the salivary glands (SG) of *A. sculptum* females (*Microbacterium paraoxydans* and *Gordonia terrae*); 5 from the SG of *O. fonsecai* (*Serratia marcescens*, *Klebsiella oxytoca*, *Streptomyces pluricolineus*, and *S. globisporus*), and none from the ovaries; from *A. cajennense* we obtained 1 isolate from the ovaries (*Pseudomonas aeruginosa*), 1 from the SG of the females (*P. aeruginosa*) and 3 isolates from the SG of the males (*M. paraoxydans* and 2 unidentified isolates). This study shows that pathogenic microorganisms that are difficult to identify, representing a potential risk to public health, can colonize these species. Furthermore, this study is fundamental to understanding the diversity of pathogens associated with ticks.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Purification and Sequencing of a Paralyzing Protein Obtained from the Salivary Glands of the Tick *Ornithodoros brasiliensis* (Acari: Argasidae)

Authors:

Larissa Ayumi KATSUMATA¹
Jose Aparecido Ferreira De LIMA²
Guilherme Rabelo COELHO²
Daniel Carvalho PIMENTA²
Fernando Luiz KAMITANI¹
Karina de Senna VILLAR¹
Maxy Hiro Matsusue UMBELINO¹
Simone Michaela SIMONS¹

Affiliations:

¹Parasitology Laboratory, Butantan Institute, São Paulo, Brazil
²Biochemistry and Biophysics II Laboratory, Butantan Institute, São Paulo, Brazil

Keywords: *Caenorhabditis elegans*. tick. toxicosis. tremor.

Abstract:

Ornithodoros brasiliensis is a tick endemic to Rio Grande do Sul. Its bite is aggressive to humans, causing fever, pain, and intense inflammatory reactions at the site. In dogs, paralysis of the hind limbs followed by death has been reported. The main form of toxicosis caused by tick bites is paralysis, which likely occurs due to the interruption of acetylcholine (ACh) in the presynaptic cleft. Thus, we are working to isolate and identify the native protein compounds with paralyzing activity. The salivary gland extract (SGE) was obtained by macerating the semi-fed tick salivary glands. The protein concentrations of the SGE and fractions were obtained by A280 nm (NanoDrop). The SGE was analyzed by SDS-PAGE 12.5% followed by (RP-HPLC) on a Jupiter C18 column (20A Prominence, Shimadzu Co., Japan). The collected fractions were concentrated in a SpeedVac and resuspended in a diluted PBS solution pH 7.4 (68.7303 g/mol). For the paralyzing activity tests, the samples were added to *Caenorhabditis elegans* in a 48-well plate, and their movement was monitored for up to 24 hours with the aid of an Olympus SZ-STS stereomicroscope. Proteomic studies were conducted by RP-CMS/MS and mass spectrometry using AXIMA Performance MALDI TOF/TOF. 28 chromatographic fractions were obtained and tested in *C. elegans*. Paralyzing activity was detected in fractions 9 and 10. The analysis of these fractions by MALDI TOF/TOF revealed molecules characteristic of neuropeptides and neurotoxins with molecular masses between 6 and 8 kDa. The results indicated that fractions 9 and 10 are associated with paralyzing activity, and the MALDI TOF/TOF profiles revealed molecules with molecular masses consistent with neuropeptides, interestingly suggesting they may belong to the lipocalin family. Future proteomic studies should be conducted in order to obtain the molecules responsible for the observed paralysis. (CEUA No. 4130270323)



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Ivermectin impairs the microbicidal capacity of macrophages in vitro by binding to the TLR-4/MD2 complex, but is beneficial in acute pneumonia in TLR-4 knockout mice

Authors:

Thiago Caetano Andrade BELO¹
Natália Cristina de Melo SANTOS¹
Leonardo Pereira de ARAÚJO¹
Evandro Neves SILVA¹
Ana Sofia Martelli Chaib SALIBA²
Sávio Rezende SOUZA¹
Guilherme Landim de REZENDE¹
André Luiz Caliani COSTA¹
Severino Matias de ALENCAR²
Rômulo Dias NOVAES³
Graziela Domingues de Almeida LIMA³
Flávio Protásio VERAS⁴
Giovane Galdino de SOUZA⁴
Patrícia Paiva CORSETTI¹
Leonardo Augusto de ALMEIDA¹

Affiliations:

¹Laboratory of Molecular Biology of Microorganisms, Federal University of Alfenas (UNIFAL), Alfenas, Minas Gerais, Brazil.

²Department of Agri-food Industry, Food, and Nutrition, 'Luiz de Queiroz' College of Agriculture, University of São Paulo, Piracicaba, São Paulo, Brazil.

³Department of Structural Biology, Federal University of Alfenas (UNIFAL), Alfenas, Minas Gerais, Brazil.

⁴Laboratory of Neuroimmunobiology of Pain, Federal University of Alfenas, Alfenas 37130-001, Brazil.

Keywords: Ivermectin. Macrophages. MD2. *Pseudomonas aeruginosa*. TLR-4.

Abstract:

Toll-like receptor-4 (TLR-4) is a pattern recognition receptor (PRR) widely distributed in macrophages, recognizing pathogen-associated molecular patterns (PAMPs). The MD2 complex integrates with TLR-4, both of which are necessary for the recognition of lipopolysaccharides found in the outer membrane of Gram-negative bacteria, such as *Pseudomonas aeruginosa*. Studies have shown that antiparasitic ivermectin treatment improves the survival of mice after receiving a lethal dose of lipopolysaccharides, although the mechanisms of action in this process remain unclear. Therefore, were conducted in silico studies using molecular docking, and it was found that ivermectin binds to the MD2 complex of TLR-4. To verify whether this binding would interfere with the immunofunctional activity of macrophages, bone marrow-derived macrophages (BMDMs) were stimulated with ivermectin and infected with *Pseudomonas aeruginosa* PA14. It was observed that treatment with ivermectin did not alter cell viability, impaired bacterial clearance, decreased NO and TNF- α secretion. All these results were reversed in vitro when the MD2 complex was inhibited in these BMDMs. To verify the in vivo repercussions, C57BL/6 WT or C57BL/6 TLR-4 KO mice were treated with ivermectin or PBS intraperitoneally for 5 days and infected with PA14, as approved by the Committee on the Ethics of Animal Experiments of the Federal University of Alfenas (CEUA 23/2021). Following treatment, ivermectin decreases the recovery of viable bacteria in the lungs of TLR-4 KO mice. Furthermore, the treatment decreased IL-6 and TNF- α in the lungs of infected mice, with no difference observed in the secretion of NO or IL-10. Thus, it was demonstrated in silico that ivermectin binds to the MD2 portion of the TLR4/MD2 complex, which reduced the microbicidal capacity of macrophages. Despite this, ivermectin treatment showed benefits, especially in TLR-4 KO mice in the recovery of viable bacteria and in the modulation of cytokines.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Histopathological analysis of liver and lung tissues in mice with metabolic syndrome induced to polymicrobial sepsis pretreated with aspirin

Authors:

Manuela Fancio BOVOLIN¹
Raquel Pires NAKAMA¹
Leonardo Berto PEREIRA¹
Maria Isabel Lovo Martins Busch PEREIRA¹
Andressa de Freitas Mendes DIONISIO²
Eduardo José de Almeida ARAÚJO³
Marli Cardoso MATINS-PINGE⁴
Philenio PINGE-FILHO¹

Affiliations:

¹Laboratory of Experimental Immunopathology, State University of Londrina, Londrina-PR
²Laboratory of Inflammation Pharmacology, State University of Londrina, Londrina-PR
³Laboratory of Neurogastroenterology, State University of Londrina, Londrina-PR ⁴Laboratory of Cardiovascular Pathophysiology, State University of Londrina, Londrina-PR

Keywords: Alveolar wall. hepatocyte. obesity. severe sepsis. survival

Abstract:

Metabolic syndrome (MetS) encompasses a set of factors associated with obesity. Another public health problem is sepsis, an organ dysfunction characterized by a systemic inflammatory response to severe local infection. CEUA: 051.2021. The aim of this study was to evaluate the effect of previous treatment with aspirin (ASA) on lung and liver tissue and the survival of animals with or without MetS. MetS was induced in Swiss mice by subcutaneous injections of monosodium glutamate (MSG) (4mg/g), 1st to the 5th day of life. At 60 days, males were treated with ASA (40 mg/Kg) or PBS via gavage for 15 days. After treatment, animals underwent polymicrobial sepsis through cecal ligation and puncture (CLP). After 24 h, lung and liver were collected for optical microscope analysis. Survival was assessed for 7 days. MetS characterization was confirmed through body weight, retroperitoneal and perigonadal fat, Lee index, mean arterial pressure, heart rate, and glycemia. Furthermore, MetS proved protective against sepsis, with an increase in survival rate in animals with MetS (59.09 vs 20.51; $p \leq 0.01$). Pretreatment with ASA was also protective in non-obese animals when compared to untreated controls (50 vs 33.333; $p \leq 0.05$). Sepsis increased the number of pale cells (24.1834 vs 34.6958; $p \leq 0.0001$) and eosinophilic cells (1.83449 vs 6.54504; $p \leq 0.0001$) in liver tissue and decreased alveolar area (64.402 vs 50.146; $p \leq 0.0001$) and increased alveolar wall area in the lungs (35.598 vs 49.854; $p \leq 0.0001$) of control animals when compared to SHAM, but did not alter hepatocyte or lung tissue morphometry in MetS animals. However, pretreatment with ASA in animals with MetS decreased the percentage of cells with nuclei containing 3 or more nucleoli (17.2579 vs 3.55763; $p \leq 0.0001$), indicating lower cellular activity. Thus, further studies are needed to elucidate the underlying mechanisms of MetS and pretreatment with ASA in sepsis.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Selective Cytotoxicity of Novel Ruthenium Metallodrugs Against Leishmania Species Causing Cutaneous Leishmaniasis

Authors:

Kamila Alves FONTOURA¹
Eduardo Matheus Ferreira
AMARAL² Veridiana de Melo
RODRIGUES¹ Renata Santos
RODRIGUES¹ Gustavo Von
POELHSITZ²
Kelly Aparecida Geraldo YONEYAMA¹

Affiliations:

¹Laboratory of Biochemistry and Animal Toxins, Institute of Biotechnology, Federal University of Uberlândia, Minas Gerais, Brazil.

²Institute of Chemistry, Federal University of Uberlândia, Minas Gerais, Brazil.

Keywords: Cutaneous leishmaniasis. Ruthenium complexes. Selective cytotoxicity.

Abstract:

Leishmaniasis is a severe infectious disease with toxic treatments and growing drug resistance. Cutaneous leishmaniasis, caused by *Leishmania amazonensis*, *L. braziliensis*, and *L. guyanensis*, is increasing in Brazil. Due to *Leishmania*'s high proliferation and metabolic similarities to tumor cells, antineoplastic agents may offer therapeutic potential against these parasites. In this context, ruthenium(II)-based metal complexes with established antitumor activity represent a promising avenue for the development of novel antiparasitic therapeutics. In this study, the cytotoxic effects of two novel ruthenium-based metallopharmaceuticals, incorporating β -diketonate and 3-chloropyridine ligands, designated as C1 and C2, were assessed against *L. amazonensis*, *L. braziliensis*, *L. guyanensis*, and RAW 264.7 macrophages. The cytotoxicity assay was conducted using the MTT method, wherein promastigotes were cultured in 96-well plates with Schneider's medium supplemented with 10% fetal bovine serum and exposed to varying concentrations of C1 and C2. Quantitative analysis revealed that C1 exhibited superior efficacy, with IC_{50} values of 8.52 μ M for *L. amazonensis*, 11.35 μ M for *L. braziliensis*, and 13.52 μ M for *L. guyanensis*. In contrast, C2 displayed higher IC_{50} values of 27.10 μ M, 31.89 μ M, and 33.55 μ M for the respective species. In cytotoxicity assessments against macrophages, C1 demonstrated an IC_{50} of 110.90 μ M, while C2 exhibited 89.96 μ M, suggesting minimal cytotoxicity toward non-parasitic host cells. The selectivity index (SI) was calculated to evaluate the therapeutic window of the compounds. C1 demonstrated high selectivity, with SI values of 13.00 for *L. amazonensis*, 9.77 for *L. braziliensis*, and 8.20 for *L. guyanensis*. Conversely, C2 exhibited moderate selectivity, with SI values ranging from 2.68 to 3.31. These findings indicate that C1 has strong anti-*Leishmania* potential, while C2 may require structural modifications to enhance its efficacy.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Chronic supression of beta-adrenergic signaling exaggerates the inflammatory response to systemic LPS in rats

Authors:

Evelyn Igreja Ferreira LEÃO¹
Livia Eduarda Cabrera CORRÊA¹
Tales Ferruccio Vicentini ROSIN¹
Rodrigo Silva de MORAIS¹
Pedro Lourenço KATAYAMA¹

Affiliations:

¹Department of Physiology and Pathology, School of Dentistry, São Paulo State University, Araraquara, São Paulo, Brazil

Keywords: Inflammation. Neuroimmune interactions. Noradrenaline. Beta-adrenergic receptors. Sympathetic nervous system.

Abstract:

The sympathetic nervous system modulates immune function via adrenergic signaling. This study investigated the impact of chronic beta-adrenergic receptor blockade on the acute inflammatory response to lipopolysaccharide (LPS). Male Sprague-Dawley Holtzman rats (~300 g) were treated with a non- selective beta-adrenergic antagonist (Propranolol, 40 mg/kg in drinking water) or control water for 8 weeks. Subsequently, rats received LPS (0.5 mg/kg, i.p.) to induce systemic inflammation or saline control. After 2 hours, plasma was collected via cardiac puncture and analyzed for the levels of TNF-alpha, IL-6, and IL-10 using ELISA. All procedures were ethically approved (Araraquara School of Dentistry, UNESP; 09/2024). Basal plasma levels of TNF-alpha, IL-6, and IL-10 were similar between propranolol-treated and control groups: TNF-alpha (Water + Vehicle: 39.4 ± 32.1 pg/mL vs Propranolol + Vehicle: 70.2 ± 57.2 pg/mL, P>0.05, N=5/group), IL-6 (Water + Vehicle: 522.9 ± 444.9 pg/mL vs Propranolol + Vehicle: 810.7 ± 286.5 pg/mL, P>0.05, N=5/group), IL-10 (Water + Vehicle: 106.0 ± 85.3 pg/mL vs Propranolol + Vehicle: 128.7 ± 103.1 pg/mL, P>0.05, N=5/group). However, in response to LPS, chronic propranolol administration significantly exacerbated the pro-inflammatory cytokine response, as evidenced by higher levels of TNF- alpha (Water + LPS: 1471.9 ± 411.9 pg/mL vs Propranolol + LPS: 4121.4 ± 1397.4 pg/mL, P=0.0002, N=5/group) and IL-6 (Water + LPS: 29393.7 ± 12752.2 pg/mL vs Propranolol + LPS: 79659.6 ± 20022.6 pg/mL, P<0.0001, N=5/group). Surprisingly, propranolol-treated had lower IL-10: IL-10 (Water + LPS: 1212.7 ± 430.4 pg/mL vs Propranolol + LPS: 374.6 ± 198.9 pg/mL, P=0.0004, N=5/ group). These findings suggest prolonged beta-adrenergic blockade exaggerates the acute pro-inflammatory response to LPS while simultaneously impairing the counter-regulatory anti-inflammatory response. Ongoing research aims to elucidate the cellular mechanisms underlying these observations.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Biotechnological Evaluation of BthTX-II for the Control of *Toxoplasma gondii*: A Novel Therapeutic Approach for Congenital Toxoplasmosis

Authors:

Vinícius Queiroz OLIVEIRA^{1,2}
Emanuelle Lorrayne FERREIRA^{1,2}
Guilherme de SOUZA²
Tássia Rafaela COSTA³
Luísa Carregosa SANTOS⁴
Lorena Pinheiro MORAIS¹
Leonardo Alves GARCIA¹
Gabriel de Oliveira SOUZA¹
Bellisa Freitas BARBOSA²
Eloisa Amália Vieira FERRO²
Samuel Cota TEIXEIRA²
Veridiana de Melo Rodrigues ÁVILA¹

Affiliations:

¹Laboratório de Bioquímica e Toxinas Animais, Instituto de Biotecnologia, Universidade Federal de Uberlândia, Uberlândia, MG, Brasil;

²Laboratório de Imunofisiologia da Reprodução, Instituto de Ciências Biomédicas, Universidade Federal de Uberlândia, Uberlândia, MG, Brasil;

³Laboratório de Biotecnologia de Proteínas e Compostos Bioativos (LABIOPROT), Fundação Oswaldo Cruz, FIOCRUZ Rondônia, Universidade Federal de Rondônia (UNIR), Instituto Nacional de Ciência e Tecnologia de Epidemiologia da Amazônia Ocidental, INCT-EPIAMO, RO, Porto Velho, Brasil;

⁴Instituto Multidisciplinar em Saúde, Universidade Federal da Bahia, Vitória da Conquista, BA, Brasil.

Keywords: Toxoplasmose congênita. Corpos lipídicos. Toxina de Serpente.

Abstract:

Congenital toxoplasmosis is a zoonosis caused by the parasite *Toxoplasma gondii*, which affects women during pregnancy and can lead to severe complications for the fetus. The search for new therapeutic approaches reflects the limitations of conventional drugs. Thus, snake toxins present a promising alternative due to the diversity of biomolecules with therapeutic potential. In this study, we aimed to evaluate the effect of BthTX-II, a phospholipase A2 isolated from *Bothrops jararacussu* venom, against *T. gondii*. Cell viability of the BeWo trophoblastic cell line was assessed using the MTT assay. The proliferation, invasion, and reversibility of *T. gondii* were evaluated using the β -Galactosidase assay. Adhesion and Invasion and Reactive Oxygen Species (ROS) were quantified using an immunofluorescence assay. Additionally, lipid droplet (LD) production was quantified using a Nile Red staining assay. BthTX-II exhibited a CC50 of 17.53 $\mu\text{g/mL}$, with significant cytotoxicity observed at concentrations above 6.25 $\mu\text{g/mL}$. Moreover, The IC50 was 20.1 $\mu\text{g/mL}$, with a notable reduction in proliferation observed at concentrations greater than 0.78 $\mu\text{g/mL}$, unlike the pre-treatment of the parasite, which showed no effect on parasite proliferation. Results further indicated parasite recovery to basal levels 24 hours after toxin removal. Additionally, a significant reduction in parasite invasion capacity was observed, accompanied by decreased adhesion and consequently parasite invasion. The toxin does not increase ROS in the infected cell. Treatment with BthTX-II significantly reduced the accumulation of lipid droplets in infected host cells, showing greater effectiveness compared to the conventional treatments with Sulfadiazine and Pyrimethamine. These findings underline the potential of BthTX-II as a promising biotechnological therapeutic option against *T. gondii*.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Cannabidiol reduces nitric oxide and pro-inflammatory cytokine levels in 2-kidney, 1-clip (2K1C) hypertensive rats

Authors:

Mariana Mendonça SOARES¹
Thalita Emanuele Dias Honorato CUNHA¹
Victor Marcelo Peretto de ARAÚJO¹
Davi Azevedo FERREIRA¹
Daniel Wilson A. MAGALHÃES¹
José Rodrigo Nascimento MARTINS¹
Rayanelle T. G. SILVA¹
Claudete da Costa OLIVEIRA²
João Gabriel Gouvêa da SILVA²
Débora Simões de Almeida COLOMBARI³
Josiane Campos CRUZ¹
Sandra Rodrigues MASCARENHAS¹

Affiliations:

¹Centro de Biotecnologia (Cbiotec), Universidade Federal da Paraíba (UFPB);
²APEPI Medicinal Cannabis Research and Patient Support Association, Rio de Janeiro, Brazil;
³Faculdade de Odontologia de Araraquara (FOAr) -UNESP.

Keywords: Inflammation. Immunomodulation. Cannabidiol. 2K1C.

Abstract:

Hypertension is a multifactorial pathophysiology that, if untreated, can lead to cardiovascular, renal, and neurological dysfunctions. Cannabidiol (CBD) is a non-psychoactive phytocannabinoid from the *Cannabis sativa* plant with several reported therapeutic effects. Previously, our group has shown that chronic CBD treatment reduces blood pressure and modulates oxidative stress in the aorta of 2-kidney, 1-clip (2K1C) hypertensive rats. However, its chronic effects on the inflammatory component of hypertension remain poorly understood. Thus, this study aimed to evaluate the immunomodulatory potential of chronic treatment with full-spectrum CBD in the 2K1C rats. For this purpose, male *Wistar* rats (CEUA n°. 2931020424) were subjected to 2K1C surgery to induce hypertension or SHAM surgery (control). After hypertension was established, the animals were treated with CBD (30 mg/kg) or vehicle (olive oil) by intragastric gavage (12/12h for 14 days). These animals were euthanized, and peritoneal macrophages were collected, cultured, and/or stimulated with LPS (1 µg/mL). Subsequently, cell viability analysis was performed by the MTT method, determination of nitric oxide (NO) levels by Griess reagent, and quantifying cytokines by immunoenzymatic assay (ELISA). CBD treatment did not affect macrophage viability but significantly reduced NO production by 34.43%. Furthermore, it also reduced cytokines levels of TNF-α (37.27%) and IL-1β (50.39%). Thus, our preliminary results suggest that chronic CBD treatment exerts immunomodulatory effects in the context of renovascular hypertension rats, which can contribute to the hypotensive CBD effect in this condition. To our knowledge, this is the first study to characterize the immunological impact of CBD in this experimental model. However, further research is warranted to confirm and expand on these findings.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Antibacterial activity of melittin against *Acinetobacter baumannii*, *Enterococcus faecium*, and *Klebsiella pneumoniae*

Authors:

Ana Flávia Marques PEREIRA¹
Elenize Jamas PEREIRA¹
Isabela Denadai MARCIOLA²
Sabrina Santana Toledo ARRUDA²
Ary FERNANDES JÚNIOR²
Rui Seabra FERREIRA JÚNIOR¹

Affiliations:

¹Centro de Estudos de Venenos e Animais Peçonhentos da UNESP (CEVAP – UNESP)
²Universidade Estadual Paulista “Júlio de Mesquita Filho”

Keywords: Nosocomial infections, antimicrobial peptide, natural products, antimicrobial resistance, bacterial resistance

Abstract:

Antimicrobial peptides may represent an alternative strategy to combat antibiotic-resistant bacteria. Melittin, a major component of the *Apis mellifera* bee venom, is an antimicrobial peptide with a broad spectrum of activity. The aim of this study was to evaluate the antibacterial and antibiofilm activities of melittin against *Acinetobacter baumannii*, *Enterococcus faecium*, and *Klebsiella pneumoniae*; to investigate potential synergism between melittin and antibiotics (ampicillin, ciprofloxacin, gentamicin, imipenem, and tetracycline); and to assess the cytolytic activity of melittin in Vero 81 cells. Melittin exhibited antibacterial activity against *A. baumannii*, *E. faecium*, and *K. pneumoniae*, with minimum inhibitory concentration (MIC) values of 8 µg/mL, 4 µg/mL, and 128 µg/mL, respectively. Synergism evaluation showed that for *A. baumannii*, none of the melittin-antibiotic combinations produced a synergistic effect. For *E. faecium*, combinations of melittin with ampicillin, ciprofloxacin, and imipenem showed synergism. In the case of *K. pneumoniae*, all combinations with the tested antibiotics, except for ampicillin, exhibited synergistic effects. Subinhibitory concentrations (75% of the MIC) of gentamicin and tetracycline were most effective in inhibiting biofilm formation by *A. baumannii*. For *E. faecium*, combinations of melittin with ampicillin and ciprofloxacin inhibited biofilm formation by over 80%. In *K. pneumoniae*, melittin combined with ciprofloxacin, gentamicin, and imipenem reduced biofilm formation by more than 60%. Melittin was shown to be safe for Vero 81 cells at concentrations ≤10 µg/mL, maintaining 100% cell viability. The findings of this study may contribute to the identification of novel therapeutic candidates aimed at combating antimicrobial resistance and bacterial biofilm-associated infections.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Temporal dynamics of murine brain B lymphocytes distribution during life

Authors:

Danillo Pereira DANTAS¹
Luciana CONDE-MAIA¹
Raquel Maria PEREIRA CAMPOS¹
André Macedo VALE¹
Pedro Moreno PIMENTEL-COELHO¹

Affiliations:

¹ Instituto de Biofísica Carlos Chagas Filho - Universidade Federal do Rio de Janeiro

Keywords: Brain. B lymphocytes. Chemokines.

Abstract:

The concept of brain immune privilege has been revised considering the findings of the presence of lymphocytes and their possible functions in the brain. The characterization of the lymphopoietic niche in the cerebral meninges has raised interest in the importance of these cells for brain functioning and immunosurveillance. However, little is known about how and when lymphocytes begin to populate the brain and these cells remain in this place, especially in the aging. The aim was to characterize the presence, identify the subpopulations and the location of B cells in the brain along the lifespan. Also characterize the molecules that modulate their presence and activity. C57BL/6 mice P3, P21, 3m and 24m old of both sexes were used. Brains were analyzed by confocal microscopy. Flow cytometry was used to further characterize the lymphocyte's subpopulations. Chemokine and cytokine multiplex kit was used. All experiments approved by CEUA-UFRJ (protocols numbers: 080/17 e 43/20). Antibodies to B220 and IgM were used to identify B cells in different regions of the mouse brain. B cells are present from P3 until adulthood when their numbers decrease, followed by a slight increase in brain aging. Using laminin as a marker of blood vessels, we found B cells mostly associated with the blood vessels and rarely in brain parenchyma. Flow cytometry analysis shows that B-1 and B-2 B lymphocytes increase during life in the brain. Precursor and Immature B lymphocytes are the major phenotypes found in brains in all ages. Pro-inflammatory cytokines TNF- α increase in aging while IL-1 β decreased from P3 to adult. Notably, CXCL13 and CCL5 are upregulated in aging brains. In conclusion, B cell distribution is dynamic during brain development, present in all brain regions and modulated by different cytokines and chemokines especially in aged brain.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Sub-chronic histopathological damage induced by the fungicide carbendazim in *Danio rerio* (Zebrafish)

Authors:

Maysa Zungolo BURRATTINO¹
Amanda de Azevedo VALLE²
Nuha Ahmad DSOUKI²
Rafaella Silva BRITO²
Flavio Kiyoshi TOMINAGA²
Bruno Fiorelini PEREIRA^{1,2}
Fábio KUMMROW¹

Affiliations:

¹ Instituto de Ciências Ambientais, Químicas e Farmacêuticas, Universidade Federal de São Paulo – campus Diadema.
² Laboratory of Molecular and Translational Endocrinology; Department of Medicine, Escola Paulista de Medicina, Universidade Federal de São Paulo.

Keywords: Alterations. Fungicides. Morphometric analysis. Mortality.

Abstract:

Brazil is one of the world's largest consumers of pesticides, which despite contributing to increased agricultural productivity, contaminate ecosystems and pose serious risks to biota. The fungicide carbendazim has been found in freshwater samples in concentrations that are harmful to aquatic life. Ecotoxicological tests are essential to better understand the damage posed by carbendazim to aquatic organisms. Sub-chronic 14-day tests with adult zebrafish were conducted in accordance with OECD-203 protocol. The tests were approved by CEUA (n° 5220291122) and the carbendazim concentrations tested were 25, 50, 250, 500, 1000 and 2000 µg/L, including negative and solvent (DMSO) controls. At the end of the exposure period, the fish were euthanized, and the gills were collected and analyzed to obtain the indexes of pathological changes, aiming to identify the morphological modifications. To assess the normality of the data, the Shapiro-Wilk test was used. Since the data did not present a normal distribution ($p < 0.05$), we used the Kruskal-Wallis test. For multiple comparisons between groups, we used the Dwass-Steel-Critchlow-Fligner test. All tests were performed using the JAMOWI software (version 2.3). In the gills, we observed alterations such as aneurysms, dilation of the apex of the secondary lamella, partial fusion, edema, changes in the curvature of the secondary lamella, proliferation and detachment of the epithelium, damages that characterize circulatory and progressive disorders. The highest rates of tissue alterations were observed at concentrations of 25 and 500 µg/L, with average indices of 11 for both. The concentration of 2000 µg/L caused 100% mortality. The controls no presented statistical differences were presented between tested concentrations, could be by errors in histological preparation, such as the low cutting depth of gills, contributed to the reduction of the sample size. In this sense, the present study considered evaluating only pathologies that were more severe than those evaluated in the control groups. Therefore, it is not possible to attribute the damage observed in the gills to carbendazim. This work was supported by the São Paulo Research Foundation (FAPESP) under Grant [Silva Brito R. (2023/09884-0), Pereira B. F. (2022/03094-5), Valle A. A. (2023/15540-2), Tominaga F. K. (2024/10993-1; 2024/01418-3), and by the National Council for Scientific and Technological Development (CNPq) under Grant [Tominaga F. K. (153725/2024-9), Burattino Z. M. (134999/2024-0)].



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Organ Masses for Mice Dosimetry Models: A Review

Authors:

Christiana da Silva LEITE¹
Juliana Batista SILVA¹
Bruno Melo MENDES¹

Affiliations:

¹Centro de Desenvolvimento da Tecnologia Nuclear (CDTN)

Keywords: Mice organ masses, Monte Carlo, Mice models, Preclinical dosimetry.

Abstract:

In vivo small animal imaging is indispensable for discovering and developing new treatments. Mice are widely used in preclinical research, particularly with nuclear medicine techniques such as positron emission tomography (PET) and single-photon emission computed tomography (SPECT), which enable noninvasive tracking of molecular processes using both short-lived and longer-lived radioisotopes. However, longitudinal studies employing these techniques can result in significant absorbed doses in specific animal organs. This fact could alter gene expression, tumor characteristics, and animal welfare, which could jeopardize the experiment results. Accurate dosimetric modeling is essential to estimate radiation exposure in mice. Computational mice models coupled with Monte Carlo codes for radiation transport increase the accuracy of absorbed dose estimations for organs and tissues. Thus, they can improve reproducibility and avoid biases in preclinical studies. Since the mean absorbed dose in an organ is given by the ratio between the energy deposited and the mass of the respective organ, the knowledge of the organ masses is essential for refine mice dosimetric models and ensure precise radiation dose assessments. This study reviewed around thirty papers using the keywords: MICE ORGAN MASSES, MICE DOSIMETRY, MICE MODEL, and MICE ORGANS. The data were analyzed, refined, and organized into tables, focusing on key factors like gender, strain, and age. Organ masses were added as reported in the original studies. Different literature sources of mass data were identified, including biodistribution studies, mice metabolism investigations, and computational segmentation of frozen organs. Many studies are interconnected, with earlier research laying the groundwork for Monte Carlo simulations. Many papers lack information or provide data for a few organs, highlighting gaps for future research. Addressing these deficiencies would enhance data quality and improve dosimetric modeling.



XXXIX REUNIÃO ANUAL DA FeSB
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Lipid Nanoparticles Conjugated to Docetaxel Combined with Aerobic Exercise Reduce Tumor Burden and Enhance Apoptotic Pathways in Bladder Cancer

Authors:

Victor Rogério Garcia BATISTA¹,
Allice Santos Cruz VERAS¹,
Maria Eduarda de Almeida TAVARES¹,
Rafael Ribeiro CORREIA¹,
Mateus Machado FRIGO²,
Rebeca Vieira e Magalhães RODRIGUES²,
Raul Cavalcante MARANHÃO³
Giovana Rampazzo TEIXEIRA¹.
E-mail: garcia.batista@unesp.br

Affiliations:

¹ Multicenter Graduate Program in Physiological Sciences, São Paulo State University (UNESP), Presidente Prudente, SP, Brazil

² Department of Physical Education, São Paulo State University (UNESP), Presidente Prudente, SP, Brazil

³ Lipid Metabolism Laboratory, Heart Institute (InCor), São Paulo, SP, Brazil.

Keywords: Apoptosis, Bladder, Exercise, Lipid, Nanoparticle.

Abstract:

Bladder cancer (BCa) is a frequent urinary tract neoplasm. The lipid nanoparticle LDE, when conjugated to docetaxel (DTX), shows high tumor specificity with lower systemic toxicity. Aerobic exercise (Ex) can modulate tumor metabolic pathways and enhance targeted therapies. METHODS Bladder cancer was induced with a single 65 mg/kg dose of DMBA at 91 days of age. Animals were divided into five groups (n=10 each): Control, BCa (DMBA), BCa+Ex (DMBA and Ex), BCa+LDE-DTX (DMBA and LDE-DTX at 2 mg/kg in 6 doses), and BCa+LDE-DTX+Ex (DMBA, LDE-DTX, and Ex). CEUA 02/2020. The bladder was analyzed by Western blotting and IHC. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test ($p \leq 0.05$). RESULTS: Weight gain was higher in the BCa group (24.68 ± 18.9) than in the BCa+LDE-DTX+Ex group ($p < 0.05$). Urinary bladder weight in the BCa group (0.46 ± 0.05) was significantly lower in the BCa+Ex (0.29 ± 0.13 , $p < 0.0001$), BCa+LDE-DTX (0.25 ± 0.10 , $p < 0.0006$), and BCa+LDE-DTX+Ex (0.22 ± 0.10 , $p < 0.005$) groups, indicating reduced tumor progression. TGF- β expression, elevated in BCa (5.30 ± 0.57), was reduced in the BCa+Ex (3.27 ± 0.3 , $p < 0.0009$), BCa+LDE-DTX (3.39 ± 0.32 , $p < 0.0007$), and BCa+LDE-DTX+Ex (3.32 ± 0.37 , $p < 0.0011$) groups. BCL-2 levels decreased in BCa+LDE-DTX (0.80 ± 0.06 , $p < 0.003$) and BCa+LDE-DTX+Ex (0.77 ± 0.02 , $p < 0.003$), increasing the BAX/BCL-2 ratio in the treated groups. Caspase-3 levels rose in BCa+LDE-DTX (1.02 ± 0.06 , $p = 0.03$) and BCa+LDE-DTX+Ex (1.02 ± 0.06 , $p = 0.03$) compared to BCa (0.82 ± 0.01). TP53 expression was higher in BCa+LDE-DTX (1.08 ± 0.1 , $p < 0.01$) than in BCa. Finally, p38 expression was elevated in BCa+LDE-DTX+Ex compared to BCa+Ex ($p < 0.02$) and BCa+LDE-DTX ($p < 0.01$). The combination of aerobic exercise and LDE-DTX nanoparticle treatment reduced both overall body and bladder weight, positively modulated proliferation and apoptosis markers, and indicates potential for clinical applications that integrate exercise and nanotechnology-based therapies.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Hydrogel Derived from Bovine Bone Extracellular Matrix Enriched with PRP and Its Influence on Fibroblast Culture

Authors:

Lays Cristine Gonçalves MATTOS¹
Alessandra Ferreira GOMES¹
Wedson Correa dos SANTOS¹
Ezio Henrique Silva GOMES¹
Hellyezer Vilela de MORAIS¹
Breno Valentim NOGUEIRA¹

Affiliations:

¹Universidade Federal do Espírito Santo

Keywords: Biomaterials. Tissue Engineering. Hydrogel. Fibroblasts.

Abstract:

It is estimated that more than 2.2 million bone grafting procedures are performed annually. Therefore, the investigation and development of new bone repair technologies remain a promising research area due to the growing demand. To develop a hydrogel derived from decellularized bovine bone, capable of meeting different grafting needs, by evaluating its influence on cell adhesion, migration, and proliferation processes. Bovine bone was decellularized, ground, and digested to form a collagen-rich hydrogel, which was then enriched with platelet-rich plasma (PRP). L929 fibroblasts were cultured and plated on pure hydrogel and hydrogel supplemented with PRP at concentrations of 10% and 20%. The analysis included phase-contrast and fluorescence microscopy, cytotoxicity assays (MTT) at 3, 6, and 9 days, as well as quality assessments using optical and scanning electron microscopy (SEM). We certify that the proposal is registered with CEUA under No. 48/2018. RESULTS: Preliminary results showed that the hydrogel exhibited biological properties that support cell replication and survival, as demonstrated by MTT assays. Phase-contrast and fluorescence microscopy indicated that the hydrogel promoted cell adhesion and migration compared to untreated cells. The highest cell viability was observed in the hydrogel + 20% PRP treatment, highlighting its importance in cell survival and proliferation. Our preliminary findings demonstrated that the hydrogel exhibited biological properties that enabled cell replication and survival. However, further studies are necessary to improve hydrogel biocompatibility and develop efficient and sustainable production methods.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Evaluation of myelin content by PET with [11C]PIB in an animal model of Down syndrome

Authors:

Fernanda Ayumi Nagay YOSHIHARA¹

Larissa Estessi de SOUZA¹

Carlos Alberto BUCHPIGUEL¹

Lidia Emmanuela Wiazowski SPELTA¹

Daniele de Paula FARIA¹

Affiliations:

¹ Laboratory of Nuclear Medicine (LIM 43), University of São Paulo Medical School.

Keywords: Molecular imaging. Myelin. Animal model. PET. Down syndrome.

Abstract:

Introduction: The myelin sheath is responsible for optimizing the conduction of nerve impulses and plays an important role in cognitive function. In Down syndrome (DS) the formation of this structure is impaired, leading to deficits in white matter development and neurotransmission. This factor is directly linked to the delayed neurodevelopment and cognitive impairments characteristic of these individuals. Therefore, the aim of this study was to monitor changes in myelin content throughout aging in an animal model of DS and to evaluate the role of therapeutic interventions using positron emission tomography (PET) with [11C]PIB. Methods: Trisomic Ts65Dn animals, including males and females were used (CEUA-FMUSP 1292/2019). The animals were divided into three experimental groups: control (aging without intervention; n=6-15); lithium (treatment with 0.25 mg/kg from 2 to 14 months; n=5-15); and exercise (treadmill training 3x/week, from 2 to 20 months; n=4-10). The animals were evaluated at 2 months (before the start of interventions), 5, 8 and 14 months by PET with [11C]PIB and the *standardized uptake value* (SUV) was quantified in the whole brain. Results: Exercise decreased [11C]PIB uptake over time, from 2 months to 5 ($p<0.001$), 8 ($p=0.014$) and 14 months ($p=0.011$). No age-dependent changes in [11C]PIB uptake were observed in the other groups. Furthermore, at 5 months, the radiotracer uptake was higher in the control group compared to the exercise ($p=0.004$) and lithium ($p=0.031$) groups. Conclusion: Physical exercise was associated with lower uptake of [11C]PIB in trisomic animals during aging, while lithium treatment did not cause any changes. Furthermore, the age of 5 months appeared to be more susceptible to variations in myelin content.



XXXIX REUNIÃO ANUAL DA FeSB
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Macrophage Differentiation Induced by Phosphatidylserine / 7 - Ketocholesterol Nanoparticles

Authors:

Gabriela Ruva Fagundes¹
Matheus Henrique Ribeiro¹
Paulo Roberto Costa de Almeida¹
Felipe Janz¹
Larissa Glugoski¹
Luane Bosetto²
Pedro Jeferson Miranda¹
Giovani Marino Favero¹

Affiliations:

¹ State University of Ponta Grossa
² Santa Catarina State University

Keywords: Cancer immunotherapy. Cell culture. Liposome. Macrophage polarization. Phosphatidylserine.

Abstract:

The study investigated macrophage differentiation induced by nanoparticles composed of phosphatidylserine and 7-ketocholesterol, analyzing their implications in immunomodulation. Macrophages play a fundamental role in the immune response and can be polarized into M1 phenotypes, with pro-inflammatory and cytotoxic properties, or M2, with anti-inflammatory and immunoregulatory functions. The differential activation of these cells is crucial for understanding the immunotherapeutic mechanisms. The nanoparticles were synthesized using a combination of phosphatidylserine and 7- ketocholesterol, and were characterized in terms of size and surface charge by zeta potential. The particles presented a diameter in the range of 100 to 200 nm and a negative surface charge, a factor that can influence the interaction with immune cells and cellular uptake. The negative zeta potential suggests colloidal stability of the nanoparticles in aqueous media, contributing to their internalization and influence on cellular processes. Macrophage polarization was assessed by RT-PCR using primers specific for M1 and M2 phenotypic markers. The results indicated a significant increase in the expression of genes associated with the M1 phenotype, such as iNOS, TNF- α , and IL-6, suggesting an inflammatory environment conducive to a pro-inflammatory immune response. This response may be attributed to the presence of 7-ketocholesterol, a compound known to trigger oxidative stress and activation of inflammatory pathways. The cytotoxicity test by the MTT assay revealed a viable reduction in macrophage cells as the concentration of nanoparticles increased. Higher concentrations resulted in significant cytotoxicity, evidencing the dose-dependent effect of the treatment. This response may be related to the induction of cellular stress and activation of apoptotic pathways. The findings of this study highlight the immunomodulatory potential of phosphatidylserine/7-ketocholesterol nanoparticles, favoring macrophage activation for the M1 phenotype. Modulation of the immune response through these nanoparticles may be promising in therapeutic strategies aimed at cancer, suggesting a new approach to immunotherapy in the disease, not applying to tissue damage but a targeted treatment approach.



XXXIX REUNIÃO ANUAL DA FeSB
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Food-derived bioactive compounds and their effect on currently applied chemo and chemo plus immunotherapy regimens for colorectal cancer: an *in vitro* approach

Authors:

Giullia Cavicchioli Barbosa¹ (FAPESP - 2024/09284-6)
Agnes Suemy Varicoda¹ (FAPESP - 2024/06431-8)
Letícia Cardoso Valente¹
Guilherme Ribeiro Romualdo¹ (FAPESP - 2022/06082-8)
Luis Fernando Barbisan²

Affiliations:

¹Experimental Research Unit (UNIPEX), Botucatu Medical School, São Paulo State University (UNESP), Brazil

²Department of Structural and Functional Biology, Botucatu Biosciences Institute, São Paulo State University (UNESP), Brazil

Keywords: Bioactive compounds; Chemotherapy; Chlorogenic acid; Immunotherapy; Colon cancer; Phenethyl isothiocyanate.

Abstract:

Colorectal cancer (CRC) yields a high incidence and poor prognosis, and chemotherapy or chemo and immunotherapy doublets are currently used as treatments, as 5-fluorouracil (5-FU) plus irinotecan (CPT-11) or 5-FU plus bevacizumab (BVZ). However, the outcomes are still unsatisfactory, and in this context, the combination of therapies with food-derived bioactive compounds (BCs) is encouraged. Thus, the objective of this study was to assess whether BCs enhance the antitumor response of current chemotherapy and/or immunotherapy doublets *in vitro*. To this end, a co-culture model of spheroids with the human cell lines HCT-116 (CRC) and CCD18co (fibroblasts) was established (3:1 proportion). The spheroids were individually treated with chemo, immunotherapy or BCs (capsaicin (CPS), chlorogenic acid (CGA), and allyl and phenethyl isothiocyanates (AITC and PEITC)) for 24/48 hours. The viability assay (MTT) was performed to calculate the half-maximal effective concentration (EC₅₀), of each compound (AITC: 7567 μ M $r^2=0.81$; PEITC: 44 μ M $r^2=0.79$; CPS: 393 μ M $r^2=0.92$; CGA: 2081 μ M $r^2=0.83$; 5-FU: 5 μ M $r^2=0.97$; CPT-11: 150 μ M $r^2=0.93$; BVZ: 75 μ M $r^2=0.91$). Subsequently, combinations of BCs with chemo/immunotherapy doublets (5-FU+CPT-11 or 5-FU+BVZ) were tested in spheroid viability using 1/5 and 1/10 of the obtained EC₅₀ values (48h) to reach plausible micromolar physiological concentrations. Data were analyzed by one-way ANOVA and *post hoc* Tukey test ($p<0.05$). AITC was the only BC that promoted the decrease in spheroid viability induced by 5-FU+BVZ (1/5 5-FU+BVZ vs. 1/5 AITC+5-FU+BVZ: 91.9 $\pm$ 7.4 vs. 61.7 $\pm$ 1.5 % of cell viability, $p=0.0001$; 1/10 5-FU+BVZ vs. 1/10 AITC+5-FU+BVZ: 88.7 $\pm$ 3 vs. 94 $\pm$ 0.9 % of cell viability, $p=0.0066$) while the remaining BCs did not show promoting results in both *doublets*. Furthermore, our findings imply that AITC may promote the antitumoral response of chemo and immunotherapy response, while other *in vitro* (invasion, migration and colony formation) and *in vivo* (xenograft) experiments will be performed.



XXXIX REUNIÃO ANUAL DA FeSB
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Implementation of Perinatal Stem Cells Biobank Employing Good Laboratory Practices for Pre-clinical Research in Ophthalmology

Authors:

Helga Caputo Nunes HOLZHAUSEN^{1,2}
Caroline Nascimento BARQUILHA^{1,2}
Mariane Aparecida RISSO^{1,2}
Damiana Pedro de OLIVEIRA²
João Pedro Arantes Aun MIGUEIS²
Ingrid Iara DAMAS¹
Ana Carolina Migliorini FIGUEIRA¹
Mônica ALVES²

Affiliations:

¹ Brazilian Biosciences National Laboratory (LNBio), Brazilian Center for Research in Energy and Materials (CNPEM), Campinas, SP, Brazil.

² Department of Ophthalmology, School of Medical Sciences (FCM), State University of Campinas (UNICAMP), Campinas, SP, Brazil.

Keywords: ocular surface diseases, perinatal mesenchymal stem cells, master cell bank, good laboratory practices, pre-clinical studies.

Abstract: Ocular surface diseases (OSD) are one of the most frequent causes of ophthalmic occurrences, where the conventional treatment is non-curative and presents a significant challenge. The use of perinatal tissues, such as amniotic membrane, was first proposed in 1940 by De Röth, who employed to replace damaged conjunctiva, rather than using oral mucosa membrane. Since then, several techniques and tissue-based products have emerged in this scenario. The objective of this study was to establish a Master Cell Bank under Good Laboratory Practices (GLP) following NIT-DICLA-035 Standards, to obtain cells for pre-clinical trials intended to produce advanced therapy medicinal products for the treatment of OSD. After ethical consent under the approval number CCAE: 58792322.5.0000.5453, internal approval of standard operating protocols and documentation following NIT-DICLA-035, placentas were collected and transported according to regulatory standards, where tissues were separated into chorionic plate, chorionic villus, decidua basalis, amniotic membrane, and umbilical cord and were processed according to three distinct isolation methods: enzymatic digestion, mechanical dissociation and explant culture using xeno-free components, except in enzymatic digestion processing. After 48h and 72h of cell seeding, cell cultures were analyzed for morphology, adherence to the culture plate, presence of colony-forming units, as well as absence of microbiological contamination. Cells from each tissue were isolated, plated, cultured, and frozen for the continuation of the MCB under GLP conditions. These initial results showed the feasibility of the MCB of perinatal stem cells, ready for basic/pre-clinical research, which translates into the importance of biobanks as highly standardized collections, following strict quality control standards, which accelerates the interface between basic research and diagnostic/therapeutic application of the so-called "from bench to bedside".



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Immunogenicity and Standardization of a Method for Quantification of Anti-Cocaine Antibodies Induced by the UFMG-VAC-V4N2 Vaccine in a Non-Human Primate Model

Authors:

Raíssa Lima Gonçalves PEREIRA^{1,2,3},
Sordaini Maria CALIGIORNE^{2,3},
Brian SABATO^{3,4},
Frederico Duarte GARCIA^{1,2,3,4}

Affiliations:

¹ Núcleo de Pesquisa em Vulnerabilidade à Saúde da Universidade Federal de Minas Gerais.

² Departamento de Psiquiatria da Faculdade de Medicina, Universidade Federal de Minas Gerais.

³ Programa de Pós-graduação em Neurociências, Universidade Federal de Minas Gerais

⁴ Programa de Pós-graduação em Medicina Molecular, Universidade Federal de Minas Gerais.

Keywords: Enzyme-Linked Immunosorbent Assay (ELISA) - Anti-cocaine antibodies - Non-human primate

Abstract: Immunotherapy offers a promising strategy to complement traditional pharmacological and behavioral treatments for substance use disorders. The UFMG-VAC-V4N2 (V4N2) vaccine employs a calixarene-based conjugate of cocaine haptens to stimulate anti-cocaine IgG antibody production, preventing cocaine from crossing the blood-brain barrier and reducing its psychoactive effects. This study aimed to standardize an ELISA assay for IgG quantification and evaluate the vaccine's immunogenicity in a preclinical non-human primate model. Five adult marmosets (*Callithrix penicillata*; CEUA 323/2017) received intramuscular immunization with V4N2. ELISA plates coated with either cocaine or GNE-BSA as the solid-phase antigen were assessed for assay sensitivity, linearity, and specificity. Antibody avidity was determined using ammonium thiocyanate dissociation assays. GNE-BSA-coated ELISA demonstrated superior sensitivity and specificity for anti-cocaine IgG detection. Vaccinated marmosets exhibited significantly increased anti-cocaine IgG titers, peaking at the study endpoint. Competition assays confirmed antibody specificity for cocaine and its derivatives, including active metabolites such as cocaethylene. Avidity assays indicated strong antibody binding to cocaine. These results demonstrate that V4N2 induces a robust and specific immune response with a favorable safety profile in *Callithrix penicillata*, supporting its progression toward clinical trials. This study marks a significant step toward innovative immunotherapeutic interventions for cocaine addiction.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Production and characterization of biocuratives derived from human amniotic membrane for clinical application in ophthalmology

Authors:

Caroline Nascimento BARQUILHA^{1,2}
Matheus Schwengber GASPARINI²
Mariane Aparecida RISSO^{1,2}
Helga Caputo Nunes HOLZHAUSEN^{1,2}
Damiana Pedro de OLIVEIRA²
João Pedro Arantes Aun MIGUEIS²
Mariana Miguel de CAMARGO²
Luiz Henrique de Freitas FILHO³
Maria Laura Costa do NASCIMENTO⁴
Ana Carolina Migliorini FIGUEIRA¹
Mônica ALVES²

Affiliations:

¹ Brazilian Biosciences National Laboratory (LNBio), Brazilian Center for Research in Energy and Materials (CNPEM), Campinas, SP, Brazil.

² Department of Ophthalmology, School of Medical Sciences (FCM), State University of Campinas (UNICAMP), Campinas, SP, Brazil.

³ Human Multi-Tissue Bank, Clinics Hospital (HC), State University of Campinas (UNICAMP), Campinas, SP, Brazil.

⁴ Department of Obstetrics and Gynecology, School of Medical Sciences (FCM), University of Campinas (UNICAMP), Campinas, SP, Brazil.

Keywords: Ocular surface diseases. Regenerative medicine. Tissue Engineering. Translational research.

Abstract:

The most severe forms of ocular surface diseases (OSD) still present major treatment challenges. The use of human amniotic membrane (AM) as a biocurative is a promising therapy tool in ophthalmology and regenerative medicine, but there is a gap in the AM processing standardization. Thus, we aimed to systematize the production and thoroughly characterize cryopreserved (C-AM) and freeze-dried AM (FD-AM) under quality standards for clinical applications. AM samples were collected from five human placentas obtained from patients undergoing elective cesarean sections at the University of Campinas maternity hospital (CAISM-UNICAMP, Campinas, SP, Brazil – CAAE 45209521.4.0000.5404). Protocols for decontamination and processing under microbiological control were standardized. The C-AM and FD-AM were then characterized regarding morphology (histological analysis and scanning electron microscopy) and functional properties (transparency, elasticity, transepithelial electrical resistance, biodegradation rates, and growth factor levels by ELISA assay). Microbiological control tests ensured the sterility of the final products. The general ultrastructure and epithelial/stromal tissue layers of AM were preserved after the cryopreservation and freeze-drying processes. Furthermore, the biophysical and mechanical properties, such as transparency, elasticity, and transepithelial electrical resistance, were equally maintained in both groups. The biodegradation test demonstrated that FD-AM was better preserved than C-AM after one month. Additionally, the profile of EGF, FGF2, HGF, PDGF-A/B, TGFβ1, and VEGF levels from protein extract and secretome of C-AM and FD-AM were different. In conclusion, the properties of both biocuratives reinforce their potential as viable therapeutic tools for treating severe forms of OSD. This study contributes to the implementation of standardized processes to ensure the production of safe and highly qualified AM for transplants and clinical interventions.



XXXIX REUNIÃO ANUAL DA FeSB
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Application of Good Manufacturing Practices in Blood Component and Tissue Amniotic Membrane Based Products for Ophthalmology Applications

Authors:

Damiana Pedro de OLIVEIRA^{1,2}
Mariane Aparecida RISSO^{1,2}
Caroline Nascimento BARQUILHA^{1,2}
Helga Caputo Nunes HOLZHAUSEN^{1,2}
Ana Carolina Migliorini FIGUEIRA²
Mônica de Cássia ALVES¹

Affiliations:

¹ School of Medical Sciences (FCM), University of Campinas (UNICAMP), Campinas, SP, Brazil

² Brazilian Biosciences National Laboratory (LNBio), Brazilian Center for Research in Energy and Materials (CNPEM), Campinas, SP, Brazil

Keywords: Autologous serum eye drops, Amniotic membrane for ophthalmology application, Good Manufacturing Practices in Advanced Therapy Products, Quality Control in Bioproducts

Abstract:

The Good Manufacturing Practices (GMP) standards are a set of measures adopted to ensure quality during the handling and manufacturing of products from biological materials. These measures are predicted for any products intended for human use, making them essential in the application of high-quality standards. In practical and mandatory terms, to have a process within the quality standards, following GMP standards, it is crucial to implement the microbiological quality control process. In this context, therapies with bioproducts such as blood components are used in experimental/clinical practice as autologous serum, presenting as biological substitutes for tears, and providing a supply of growth factors, proteins, and immunomodulators. Like blood components, the human amniotic membrane (AM) is already widely used abroad as a commercial biological product for ophthalmological application, with proven therapeutic potential. Thus, the aim of this project was ensuring the quality control of the developed bioproducts. Sterility analyses of autologous serum eye drop samples and AM fragments covered aerobic, anaerobic bacteria and fungi, while the detection of endotoxin was by the LAL assay (Limulus Amebocyte Lysate), and the detection of mycoplasma by polymerase chain reaction (PCR). The results were expressed in absorbance x concentration standard curve graphs for endotoxin, peak melting graphs for mycoplasma, and Colony Forming Unit (CFU) count to estimate the number of viable bacteria and fungi. The process of standardizing the quality of bioproducts from blood components and AM are technically simpler and faster if they are developed, as they can be of minimal manipulation and do not involve modification of genetic material, obeying the respective RDC nº: 504, 55, 34 and 508. Thus, this work contributes to provide high-quality bioproducts for clinical application in ophthalmology.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Mesenchymal Stem Cells from Dental Tissues: A promising Alternative for Regenerative Medicine

Authors:

Julia Klein NAKATANI¹
Giovanna de Araujo Ricardo ROSSANO¹
Anna Beatriz Seara SCHLICKMANN¹
Mateus de Oliveira LISBOA¹
Paulo Roberto Slud BROFMAN¹
Letícia FRACARO¹

Affiliations:

¹ Core for Cell Technology – School of Medicine and Life Sciences – Pontifícia Universidade Católica do Paraná

Keywords: Differentiation. Immunophenotyping. Standardization.

Abstract:

Mesenchymal stem cells (MSCs) are widely studied for their therapeutic potential, including applications in neurodegenerative diseases. Dental pulp stem cells (DPSCs) and apical papilla stem cells (SCAP) are adult cells with self-renewal and differentiation capacities. To ensure the reproducibility of results, the ISCT establishes criteria for the characterization and standardization of MSCs. This study aimed to characterize DPSCs and SCAPs in terms of MSC characteristics and clinical potential. Approved by the PUCPR Research Ethics Committee (6.328.333), DPSCs (n=4) and SCAPs (n=4) were isolated and cultured. Flow cytometry was performed for immunophenotypic characterization, proliferative potential and cellular senescence. The samples were induced to adipogenic and osteogenic differentiation for 21 days and evaluated by staining and microscopy. For the colony-formation assay, cells were cultured, fixed, and stained for counting and colony formation efficiency calculation. Genetic stability was verified by karyotyping using conventional cytogenetics. DPSCs and SCAPs showed fibroblast-like morphology and expressed MSC-specific immunophenotypic markers, with mean proliferation rates of 83.15 ± 4.87 and 88.3 ± 3.67 , respectively, at 24 hours. The percentage of senescent cells was low, with means of 4.7 ± 2.0 (DPSCs) and 7.0 ± 6.27 (SCAPs). In adipogenic differentiation, a few small lipid vacuoles were observed in the sources. In osteogenic differentiation, both showed calcium crystal deposits. Colony-forming capacity revealed no significant difference between sources ($p=0.2171$). All samples exhibited a normal karyotype, indicating genetic stability. This study confirmed that both cells have MSC characteristics, with high proliferation, low senescence, differentiation potential, colony-formation, and genetic stability. The results highlight the importance of rigorous characterization to ensure the reliability of cell therapies and their potential for future research.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Evaluation of platelet-rich plasma adhesion to therapeutic contact lenses

Authors:

Victor de Souza ANTUNES¹
Felipe de Sousa OLIVEIRA¹
Mariane Aparecida RISSO¹
Helga Caputo Nunes HOLZHAUSEN¹
Caroline Nascimento BARQUILHA¹
Damiana Pedro de OLIVEIRA¹
Murilo Godoy Augusto LUIZ¹
Fábio Régis Barreira de FIGUEIRÊDO¹
João Lucas Salge GONÇALVES¹
Pedro Ribeiro ROSA¹
Mônica ALVES¹

Affiliations:

¹Universidade Estadual de Campinas
(UNICAMP)

Keywords: Adhesion. Contact Lenses. Ocular surfaces diseases. Platelets-rich plasma.

Abstract:

Platelet-rich plasma (PRP) gel is a promising treatment for ocular surface diseases due to its regenerative and anti-inflammatory properties, but its topical application faces limitations. This study evaluated different activation methods for PRP gel for ocular application, aiming to determine the most effective strategy for adhesion and stability when combined with a therapeutic contact lens (TCL). Three PRP activation strategies were tested: on an external surface, on the TCL, and directly on the cornea. The study analyzed not only gel adhesion and stability but also its handling feasibility and potential clinical applicability. Gel adhesion and stability were assessed through manipulation tests and exposure to a continuous saline flow. Activation on the external surface proved unfeasible due to gel handling difficulties. Activation on the cornea also showed good adhesion, but activation directly on the TCL demonstrated superior adhesion and resistance to saline flow, making it the most practical and effective approach for clinical application. This method ensures that PRP remains in direct contact with the ocular surface for a prolonged period, potentially enhancing its therapeutic benefits. PRP activation on the TCL is the most suitable method for ensuring adhesion and stability, facilitating its use in ocular treatments. By optimizing this technique, PRP therapy could become a more viable option for treating severe ocular conditions, such as persistent epithelial defects and neurotrophic keratitis. This optimized technique may enhance sustained PRP release and improve therapeutic outcomes in patients with ocular surface diseases. This study was approved by the Comitê de Ética em Pesquisa (CEP) and Comissão Nacional de Ética em Pesquisa (CONEP) in August 2024, with CAE number 75614023.3.0000.5404.



XXXIX REUNIÃO ANUAL DA FeSB
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Gold nanorods functionalized with Angiotensin-(1-7) promote neuroprotection in an in vitro stroke model

Authors:

Aline dos Santos VIEIRA¹
Beatriz Lopes Tecedor BASSI¹
Lucas Alencar SOARES¹
Rosimeire Coura BARCELOS²
Kennedy Batista GONÇALVES²,
Caroline Magalhães JUNQUEIRA³
Lívia Siman GOMES²
Luiz Orlando LADEIRA²
Gisele Eva BRUCH⁴
André Ricardo MASSENSINI¹

Affiliations:

¹Núcleo de Neurociências, NeuroNano, Departamento de Fisiologia e Biofísica, Universidade Federal de Minas Gerais (UFMG)

²Departamento de Física, Universidade Federal de Minas Gerais (UFMG)

³Departamento de Microbiologia, Universidade Federal de Minas Gerais

(UFMG) ⁴Fisiologia, Laboratório de Simulação Realística, Faculdade de Minas (Faminas)

Keywords: Gold nanorod. Neuroprotection. Bioavailability. Brain.

Abstract:

Gold nanorods (RODs) have unique optical/electronic properties and act as efficient nanocarriers. Angiotensin-(1-7) [Ang-(1-7)] shows therapeutic potential, especially for ischemic stroke, but is rapidly degraded in biological media. Its biofunctionalization with RODs may enhance bioavailability. This study evaluated the neuroprotective effects of RODs functionalized with Ang-(1-7) in an *in vitro* stroke model using Oxygen-Glucose Deprivation (OGD). RODs were synthesized, PEGylated, and characterized by UV-Vis (LSPRT/LSPRL), Zeta Potential (ZP), and Ang-(1-7) binding via Nanodrop. Hippocampal slices from 76 male CD1 mice (9–11 weeks, CEUA/UFMG: 269/2020 e 88/2024) were used. Slices were divided into nOGD and OGD groups: nOGD received ACSF with glucose and O₂; OGD received glucose-free ACSF and N₂ (60 min), followed of reperfusion with glucose and O₂ in both groups (120 min). LDH and glutamate release, and caspases 9 and 3 expression were assessed to evaluate viability, excitotoxicity, and apoptosis, respectively. Data were analyzed by two-way ANOVA ($p < 0.05$) with Tukey's post-hoc. RODs measured 24.7×11.1 nm (area: 1250 nm^2). LSPRT/LSPRL λ_{max} (nm): ROD-CTAB: 517/644; ROD: 519/648; ROD+Ang-(1-7): 519/645. Concentrations (nM): 16.6, 9.5, 0.6; ZP (mV): +50.0, +7.7, +31.1, respectively. Ang-(1-7) binding rate was 40%. Lower LDH release was found in ROD+Ang-(1-7) groups at 1.5 nM ($p = 0.001$) and 15 nM ($p = 0.008$) vs. control, and in free Ang-(1-7) ($p = 0.001$). Similarly, glutamate release decreased in ROD+Ang-(1-7) at 1.5 nM ($p = 0.021$) and 15 nM ($p = 0.025$), and in free Ang-(1-7) ($p = 0.019$), with these concentrations being approximately 6,000 and 600 fold lower than that of free Ang-(1-7). Caspase 9 and 3 expression showed no significant changes. Results indicate successful ROD functionalization with Ang-(1-7) and neuroprotection *in vitro*, supporting its use as a delivery strategy *in vivo*. Patent nº: BR102024013958



XXXIX REUNIÃO ANUAL DA FeSB
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Development of an algorithm to design and validate interference RNAs of altered genes in Rett syndrome

Authors:

Beatriz Parreira GOMES¹
Emerson Luis dos SANTOS¹
Sônia Mara da Silva ROCHA¹
Vitor Hugo de Sousa SOARES¹
Laura ZANELATO¹
Helen MIRANDA²
Alysson Muotri³
Roberto Hirochi
HERAI¹

Affiliations:

¹Pontifícia Universidade Católica do Paraná (PUCPR), Laboratory of Bioinformatics e Neurogenetics (LaBiN), Program of Post-Graduation in Health Sciences (PPGCS)
²Case Western Reserve University
³University of California San Diego (UCSD)

Keywords: Amplicon. miRNA. Off-target. TargetFinder.

Abstract:

Rett syndrome is a neurodevelopment condition that is caused by mutations in the MECP2 (methyl-CpG binding protein 2) gene. Rett individuals present several symptoms, including intellectual disability, motor impairment and unilateral movements. Although there are several studies and extensive knowledge regarding genes and dysregulated metabolic pathways in Rett, there is still a relative lack of studies addressing each altered gene individually. Thus, gene silencing techniques using RNA interference (RNAi) can be helpful to establish associations between genotype and phenotype by knocking down protein expression. The RNAi technique is based on a short of approximately 21 length nucleotide amplicon sequence that is designed to target a protein coding gene, leading to the interruption of its translation. However, there is a high chance of non-specific hybridization, with genes other than the intended target, leading to off-target effects, besides other problems related to sequence composition. Thus, this project aimed to develop the TargetFinder tool, an algorithm for the design and validation of RNAi amplicons to treat off-target effects. The implementation of the RNAi amplicon validation rules was carried out using Regular Expressions (regex) in Python and Bash scripting. To identify potential off-targets of a specific amplicon, the script executes the Burrows-Wheeler Aligner (BWA) mapping tool over a unified transcriptome database created by this study. Moreover, TargetFinder performs a compliance check on a provided fasta file to guarantee that the script only provides mappings with high quality and few errors, by applying a scoring threshold defined according to BWA's scoring system. Tests using the created algorithm demonstrated a practical solution to improve RNAi amplicon validation and to minimize off-target effects, contributing to more accurate gene-phenotype analyses and enhancing the specificity of gene silencing experiments in Rett syndrome genes.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Evaluation of oligomycin-preconditioned umbilical cord mesenchymal stromal cells and extracellular vesicles in experimental sepsis

Authors:

Mayck Medeiros Amaral DA SILVA¹
Carla Medeiros SILVA¹
Leticia Rocha Quintino SOUZA²
Adriana Lopes DA SILVA¹
Gabriel de Souza PEDRO¹
Patricia Rieken Macedo ROCCO¹
Rosalia Mendez OTERO²
Pedro Leme SILVA¹
Fernanda Ferreira CRUZ¹

Affiliations:

¹ Laboratory of Pulmonary Investigation, Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil

² Laboratory of Cellular and Molecular Neurobiology, Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil

Keywords: Wharton's Jelly Mesenchymal stromal cells; Sepsis; Acute Respiratory Distress Syndrome; Acute Kidney Injury

Abstract:

Due to high incidence and mortality rates of sepsis and the lack of effective treatment, new therapies are still needed. Wharton's Jelly mesenchymal stromal cells (WJ-MSCs) and their derived extracellular vesicles (EVs) show promising benefits in experimental scenario, but modest results in clinical trials. Preclinical studies in graft-versus-host disease have shown that preconditioning of MSCs with oligomycin improves their immunomodulatory properties, suggesting an innovative approach to enhance their therapeutic potential. We hypothesized that preconditioning of MSCs with oligomycin can induce metabolic changes, alter cellular and their extracellular vesicles (EVs) proteomic profiles, and consequently improve their therapeutic efficacy in sepsis. To investigate this hypothesis, we obtained WJ-MSCs (CAAE:50355521.7.0000.5257), expanded them to passages 3-5 (P3-P5), and stimulated them with 1 μ M Oligomycin for 24 hours (MSC-Omy). Subsequently, cells were cultivated in serum-free medium for 48 hours to obtain the EVs by ultracentrifugation. MSC-Omy exhibit adherence to plastic, expression of CD73 and CD90 (>95%), and lack the expression of CD34 and CD45 (<2%), demonstrating their identity as MSCs. The viability of the cells, evaluated by MTT assay, was significantly higher in MSC-Omy (110.6 $\pm$ 9.6) compared to MSC-N (100 $\pm$ 8.8) (p=0.0284). Oxygraphy demonstrated that MSC-Omy exhibits lower oxygen consumption due to the blockage of ATP synthase by oligomycin. These cells and vesicles will be evaluated by proteomics. Finally, their therapeutic efficacy will be evaluated in a sepsis model in Wistar Rats (CEUA-CCS-UFRJ: 083-24) for lung and kidney function, histoarchitecture and inflammation. We can conclude until now that preconditioning with oligomycin reduces oxidative phosphorylation, did not induce MSCs differentiation and was not toxic for the cells.



XXXIX REUNIÃO ANUAL DA FeSB
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Tracking 50 Years of Knee Osteoarthritis Treatments: A Keyword Analysis Using AI and Constellate.org

Authors:

Douglas Menezes de Souza¹
Kauê Franco Malange³
Catarine Massucato Nishijima²
Carlos Amilcar Parada^{1,2}

Affiliations:

¹ Postgraduate degree in Pharmacology, School of Medical Sciences, University of Campinas (UNICAMP), Campinas, Brazil.
² Department of Structural and Functional Biology, Institute of Biology, University of Campinas, Campinas (UNICAMP), Brazil.
³ Department of Anesthesiology, University of California, La Jolla, San Diego, CA 92037, USA.

Keywords: Knee osteoarthritis. conventional treatment. advanced treatment. keyword trends. artificial intelligence.

Abstract:

The knee osteoarthritis (KOA) is one of the most affected joints resulting in chronic pain. Conventional treatments have palliative effects for reducing pain but not for stopping cartilage disruption. Our study aimed to analyse the use of conventional and advanced OA treatments through keyword trends (1975–2025) via Constellate.org, the use of artificial intelligence to select key OA terms and explore AI-generated visual interpretations of these treatments (ChatGPT and Leonardo.ai). We identified 50 keyword trends related to KOA via Constellate.org and selected 10 by ChatGPT to compare conventional and advanced treatments. This data demonstrated the reduced keyword trends related to conventional therapies (acetaminophen, NSAIDs, opioids) toward advanced approaches, such as macrophage-targeted therapy, PRP, scaffolds, and exosomes. From the 50 keywords, we selected 5 by ChatGPT related to chondrocyte viability and compared their association with treatment types. These terms appeared more frequently in documents linked to advanced treatments, (scaffolds and macrophages) over the past 50 years. However, Constellate.org showed limited keyword data related to side effects, which are mostly associated with conventional treatments. To address this gap, we are conducting a focused analysis using the keywords: osteoarthritis, conventional or advanced treatment, and side effects. ChatGPT's illustration of OA progression showed immune cell infiltration in KOA, leading to cartilage and joint degeneration, supporting the rise of macrophage among the keywords highlighted. The Leonardo.AI could highlight chronic pain- feeling and opioid-related addiction in realistic human illustration. In conclusion, the increasing frequency of KOA keywords related to advanced treatments and chondrocyte viability over the past 50 years suggests growing research interest, especially in the role of macrophages in OA progression, as reflected in our findings and the literature.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Effects of IFN- γ preconditioning of bone marrow-derived mesenchymal stromal cells in a murine model of pulmonary sepsis caused by *Klebsiella pneumoniae*

Authors:

Carla Medeiros da Silva¹
Raquel Ferreira de Magalhaes Sacramento¹
Johnatas Dutra Silva¹
Mayck Medeiros Amaral da Silva¹
Monique Martins Melo¹
Julia Rodrigues Ferraz Silva¹
Christina Maeda Takiya²
Fernanda Ferreira Cruz¹
Marcelo Marcos Morales¹
Pedro Leme Silva¹

Affiliations:

¹ Laboratory of Pulmonary Investigation, Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro.

² Immunopathology Laboratory, Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro.

Keywords: sepsis; cell therapy; mesenchymal stromal cells.

Abstract:

Current sepsis guidelines suggest fluid/antibiotics treatment but lack information about tissue damage prevention. Although mesenchymal stromal cells obtained from bone marrow (BM-MSC)s modulate immune system, limited efficacy is observed due to reduced survival and activity in sepsis microenvironment. On the other hand, IFN- γ pre-conditioning of BM-MSCs may potentiate survival and activity, by TSG-6/IDO mRNA expression, both linked to sepsis pathophysiology. The present study assessed IFN- γ 's pre-conditioning of BM-MSC and its effects on inflammation, damage, and biochemical markers in *klebsiella pneumoniae* sepsis. In vitro, BM-MSCs viability did not modify due to IFN- γ exposure (200 up to 800 ng/mL), while increased TSG-6/IDO mRNA expression ($p < 0.001$). In vivo (CEUA/CSS 042/22), septic mice received naive/pre-conditioned BM-MSCs. Naive/pre-conditioned MSC reduced clinical severity ($p = 0.005$) and mortality ($p = 0.0015$) vs. sepsis. Naive/pre-conditioned MSCs decreased urea ($p < 0.005$) and lung bacterial dissemination ($p < 0.05$). Only naive/200 ng/mL MSCs reduced bacterial load in blood/kidneys. In lungs, both pre-conditioned MSCs reduced alveolar damage ($p = 0.0035/p = 0.0102$, respectively), while only 200 ng/mL MSCs increased lung GlutPeroxidase ($p = 0.0101$)/SOD2 ($p = 0.0004$) and reduced VCAM-1 mRNA expressions in comparison to septic mice. In kidney, inflammation and tubular damage reduced after naive/pre-conditioned MSCs. Both treatments decreased renal TIM-1/IL-18 mRNA and increased GlutPeroxidase [naive ($p = 0.0027$)/200 ng/mL ($p = 0.0492$) MSCs], while only naive/200 ng/mL MSCs increased renal SOD2 mRNA expression ($p < 0.0001$ and $p < 0.0022$). IFN- γ pre-conditioning BM-MSCs improved lung damage, bacterial load, and antioxidants; and renal inflammation/IL-18. However, overall, IFN- γ pre-conditioning (200 up to 800 ng/mL) did not significantly improved outcomes compared with naive MSCs.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Assessment of elastin degradation in a 3D study model of COPD/emphysema

Authors:

Lincoln Gozzi MORO¹,
Ana Paula Barreto de PAIVA¹,
Mayana Cristina da Silva PARDO¹,
Lucas Pires GUARNIER²,
Mauricio Fogaça AZEVEDO³,
Julia Amanda Rodrigues FRACASSO⁴,
Arthur Carmona VILAR⁴,
Isabella da Silva CARVALHO⁴,
Marlon Lemos DIAS⁵,
Mateus Vidigal de CASTRO¹,
João Tadeu RIBEIRO-PAES^{2,4}

Affiliations:

¹ Human Genome and Stem Cell Research Center, Institute of Biosciences, University of São Paulo, São Paulo, SP, Brazil.

² Department of Genetics, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, SP, Brazil.

³ Institute of Biomedical Sciences, University of São Paulo, São Paulo, SP, Brazil.

⁴ Laboratory of Genetic and Cell Therapy, Department of Biotechnology, São Paulo State University, Assis, SP, Brazil.

⁵ Precision Medicine Research Center; Carlos Chagas Filho Biophysics Institute, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

Keywords: COPD. emphysema. elastin. spheroids. western-blot.

Abstract: Chronic Obstructive Pulmonary Disease (COPD) is a heterogeneous pulmonary condition characterized by chronic respiratory symptoms resulting from abnormalities in the airways and/or alveoli, leading to persistent and progressive airflow obstruction of the lungs. Despite significant advances in therapeutic approaches and the continuous incorporation of new drugs into the therapeutic arsenal against COPD, an effective clinical treatment has not yet been achieved. In this context, the development of new *in vitro* models that better recapitulate the lung microenvironment in COPD is essential for elucidating disease pathophysiology and testing novel therapeutic strategies. In this study, a three-dimensional (3D) COPD/emphysema model was established through the differentiation of induced-pluripotent stem cells (iPS). Alveolar spheroids were generated via a four-step protocol: (1) endoderm induction, (2) anterior foregut endoderm specification, (3) differentiation into lung progenitors (NKX2.1⁺ cells), and (4) formation of alveolospheres. To promote 3D growth, NKX2.1⁺ cells were embedded in Matrigel GFR. After 35 d, morphological analysis confirmed the formation of alveolospheres with heterogeneous sizes. To induce emphysematous features, alveolospheres were exposed to porcine pancreatic elastase (PPE, 1 and 10 µg/mL) for 6 h. Western-blot analysis confirmed elastin production by alveolospheres and demonstrated a reduction in elastin band intensity following PPE exposure, indicating an effective elastin degradation by PPE. These findings suggest that iPSC-derived alveolospheres obtained represent a promising *in vitro* platform for investigating COPD/emphysema pathophysiology, especially mechanisms associated with extracellular matrix degradation and alveolar septa destruction — key pathological features and hallmarks of disease progression. Ethical Considerations. The present study was managed according to ethical standards established by Plataforma Brasil (CAAE 34786620.2.0000.5464).



XXXIX REUNIÃO ANUAL DA FeSB
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Modulating effect of systemic laser therapy on inflammation and oxidative stress in a stroke model in mouse

Authors:

Stephanie Oliveira de
ARAUJO^{1,2} Wedson
Correa dos SANTOS ^{1,2}
Alessandra Ferreira
GOMES^{1,2} Ezio Henrique
da Silva GOMES^{1,2} Luis
Miguel Mariani KOCK¹
Barbara POVEGLIANO¹
Lays Cristine Gonçalves
MATTOS¹ Diego França
PEDROSA^{1,3}
Breno Valentim NOGUEIRA^{1,2}

Affiliations:

Federal University of Espírito Santo UFES ¹
Department of Biotechnology UFES ²
Department of Integrated Health Education UFES ³

Keywords: Low intensity laser. Systemic photobiomodulation. Stroke. Inflammation. Oxidative stress.

Abstract:

Stroke is one of the leading causes of morbidity and mortality worldwide. The inflammatory pathway is readily activated in response to cerebral ischemia. In this study, we evaluated the effects of non- intravascular blood laser irradiation (ILIB) therapy in an experimental mouse stroke model. The animals were divided into the following groups: S: SHAM (fictitious surgery), AVE (stroke) and treated with stroke + ILIB: TL (low dose with 3.2 J) and TH (high dose with 6 J). ILIB was performed over the caudal vein/artery transcutaneously. The experimental protocols were approved by CEUA-UFES (No. 14/2022). The motor evaluations of the animals showed improvement in the TH group (S: 214 s $\pm$ 7 s n= 7; AVE: 211 s $\pm$ 11 s n= 10; TL: 216 s $\pm$ 10 s n = 9 and TH: 247 s $\pm$ 6 s n = 10 p<0.05). There was a difference in the macroscopic area of infarction between the TH group and the other groups, where TH had the smallest infarction region (AVE: 43.848 mm² $\pm$ 1.95 mm² n=5/ TL: 31.473 mm² $\pm$ 0.692 mm² n=5 vs TH: 19.864 mm² $\pm$ 2.622 mm² n=5 p<0.05). In addition, we observed significant differences in neutrophil counts in the TH group compared to the other groups (S: 12.5 $\pm$ 2.0 n=9; AVE: 19.7 $\pm$ 8.8 n=10 / TL: 33.0 $\pm$ 10.5 n=10; TH: 60.5 $\pm$ 10.4 n=10 p<0.05). Analyses for inflammatory markers in plasma were performed, and it was observed that the ILIB groups showed a modulation of interleukin production reducing IL-10 levels in the brain compared to S group (S: 24.27 pg/mL $\pm$ 1.08 pg/mL n=6; TL: 22.54 pg/mL $\pm$ 0.98 pg/mL n=6; TH: 21.34 pg/mL $\pm$ 0.36 pg/mL n=6 p<0.05), in addition, IL-6 levels in the brain were lower in the AVE group (S: 4.73 pg/mL $\pm$ 0.11 pg/mL n=6; AVE: 3.97 pg/mL $\pm$ 0.25 pg/mL n=6; TL: 5.46 pg/mL $\pm$ 0.86 pg/mL n=5 and TH: 5.15 pg/mL $\pm$ 0.55 pg/mL n=6 p<0.05). We observed that ILIB treatment attenuated the production of reactive oxygen species in the TH group when compared to the AVE group. This study demonstrated the therapeutic potential of ILIB the inflammatory response.



XXXIX REUNIÃO ANUAL DA FeSB
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Biological Activity of Porcine Placental Extracellular Matrix-Derived Hydrogels: In Vitro Validation and Wound Healing Activity Evaluation

Authors:

Guilherme Gonçalves BARBOSA¹
Bianca Fuzeti CANDIAN¹
Claudio Guilherme de Assis OLIVEIRA¹
Luan Stefani LIMA²
Danielle Kurunzi NUNES¹
Leonardo Consoli SERTÃO¹
Bruna Gomes SILVA¹
Isabela Paulillo D'ONOFRIO¹
João Victor DAMIN¹
Guilherme Henrique Gonçalves de ALMEIDA¹
Douglas Lopes de ALMEIDA¹
Lucas Paulo Jacinto SAAVEDRA¹
Paulo Cezar de Freitas MATHIAS¹
Ana Claudia Oliveira CARREIRA²
Gustavo Henrique Doná Rodrigues ALMEIDA²

Affiliations:

¹Department of Biotechnology, Genetics and Cell Biology, State University of Maringá

²School of Veterinary Medicine and Animal Science, University Of São Paulo

Keywords: Decellularization. Hydrogel. Placenta. Tissue repair.

Abstract:

Hydrogels derived from extracellular matrix (ECM) have emerged as promising biomaterials for cutaneous wound healing. These hydrogels mimic the natural tissue microenvironment, promoting cell migration and proliferation, which are essential for skin regeneration. The ECM, composed of collagen, glycosaminoglycans, and growth factors, provides structural and bioactive support, accelerating the healing process. Studies have demonstrated their efficacy in treating acute and chronic wounds, highlighting their potential to improve skin lesion repair and contribute to advancements in regenerative medicine and wound therapy. Therefore, this study aimed to evaluate the in vitro bioactivity of a hydrogel derived from porcine placental extracellular matrix and its application for cutaneous wound healing in vivo. For this, L929 fibroblast and HaCat keratinocyte cell lines were cultured in the presence of placental ECM hydrogels at a concentration of 1.5 mg/mL for five days, and parameters such as cell viability, adhesion, and migration were evaluated to assess the cytocompatibility and bioactivity of the hydrogels. In vivo, a skin wound model was developed using Wistar rats of 70 days of age (approved CEUA no. 9958021224) and the wound healing was evaluated during 14 days in the presence or absence of the hydrogel. The results showed that both cell types were able to anchor to the hydrogel fibers, allowing for their adhesion. Additionally, the cells remained viable during the analyzed period, and the hydrogel also induced greater cell migration compared to the control group. In vivo, a preliminary macroscopic evaluation demonstrated the wound closure was higher in the treated group compared to the control wounds. In conclusion, these preliminary results suggest that the developed hydrogel is promising for more robust tissue repair assays and, further ahead, as a potential new regenerative approach.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Establishment of a Bioprocess for the Production of a Hydrogel Resulting from the Combination of Alginate and Pequi Oil (*Caryocar brasiliense* Camb.) as a Potential Therapeutic Alternative for Cutaneous Ulcers

Authors:

Mauricio Fogaça AZEVEDO¹
Luís Felipe Antunes PRADO²
Julia Amanda Rodrigues FRACASSO³
Lucas Pires GUARNIER¹
Lincoln Gozzi MORO⁴
Bruno Rian Cintra ALBINO⁵
Arthur Carmona VILLAR²
Alexandre Mendes de ALMEIDA Jr⁵
Matheus Felipe da SILVA⁶
João Tadeu RIBEIRO-PAES²

Affiliations:

¹Department of Genetics, Ribeirão Preto Medical-School, University of São Paulo, Ribeirão Preto, SP, Brazil

²Laboratory of Genetic and Cell Therapy, Department of Biotechnology, Science and Letter School, São Paulo State University, Assis, SP, Brazil

³Department of Biotechnology, School of Sciences and Languages, São Paulo State University (UNESP), Assis 19806-900, Brazil

⁴Human Genome and Stem Cell Research Center, Institute of Biosciences, University of São Paulo, São Paulo, SP, Brazil

⁵Sao Paulo State University (UNESP), School of Sciences, Humanities and Languages, Assis, SP, 19806-900, Brazil

⁶Toxicological Genetics Laboratory, Department of General Biology, State University of Londrina, Londrina, PR, Brazil

Keywords: Cutaneous Ulcers. Three-Dimensional Bioprinting. Regenerative Medicine. 3D Printing. 3D Scanning

Abstract:

Tissue engineering (TE) is an interdisciplinary field that applies principles from engineering and life sciences to develop biological substitutes (biomaterials) capable of restoring, maintaining, or improving tissue function. Among these biomaterials, alginate stands out for its widespread use due to its non-toxic, biocompatible, and non-immunogenic properties. However, no previous reports have described the therapeutic use of alginate in combination with pequi oil (*Caryocar brasiliense* Camb.), a compound known for its bactericidal and anti-inflammatory properties and, therefore, with potential for treating cutaneous ulcers. Based on these premises, we hypothesized that a hydrogel composed of alginate and pequi oil could produce additive or synergistic effects on tissue regeneration. This process could be further optimized through the structuring of biomaterials via 3D printing. In this context, the present study aimed to establish a 3D printing process for the production of an alginate-pequi oil hydrogel as a therapeutic alternative for cutaneous ulcers. To this end, formulations containing different alginate concentrations (4% to 10%) and increasing volumes of pequi oil (1 mL to 10 mL) were prepared and evaluated for their rheological, mechanical, and morphological properties. The 3D bioprinting process was based on virtual anatomical models of ulcers, reconstructed via photogrammetry using a smartphone and additive manufacturing techniques. Based on the results, the formulation containing 7% alginate and 4% pequi oil demonstrated optimized viscosity, structural stability, and print fidelity. In summary, it was possible to establish an efficient and reproducible bioprocess by combining alginate and pequi oil, guided by morphologically defined ulcer models reconstructed from virtual scanning. These findings open new perspectives for personalized regenerative medicine in the treatment of chronic cutaneous ulcers.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Effects of platelet-rich fibrin and platelet-rich plasma on protein expression in experimental arthritis in Wistar rats

Authors:

Mariana Dias¹
Júlia Guedes Marette¹
Letícia Aparecida Brandão¹
Marcelo Augusto Marretto Esquisatto¹
Fernando Russo Costa do Bomfim^{1,2}

Affiliations:

¹Centro Universitário da Fundação Hermínio Ometto
²Sociedade Brasileira de Biociências Nucleares

Keywords: Arthritis, biomaterials, inflammation, tissue repair.

Abstract:

Rheumatoid arthritis is an autoimmune inflammatory disease that affects the synovial joints, resulting in tissue degeneration and impaired joint function. Platelet-rich fibrin (PRF) and platelet-rich plasma (PRP) are autologous biomaterials derived from the patient's own blood and it is rich in growth factors such as vascular growth factor (VGF) and fibroblast growth factor (FGF), which contribute for tissue regeneration. The aim of this study was to evaluate the effects of PRF and PRP in the treatment of experimentally induced arthritis in Wistar rats. After approval by CEUA-FHO (10/2024), twenty-four (n=24) female Wistar rats were assigned into four experimental groups (n=6/group): A, control, saline sterile solution; B, induced arthritis; C, induced arthritis and treatment with PRP; D, arthritis induced and treatment with PRP. Animals received *ad libitum* access to food and water. Arthritis was induced in B, C, and D by intra-articular injection of zymosan (200 µg) after anesthesia with ketamine (0.3 mg/kg) and xylazine (0.1 mg/kg). PRP and PRF were performed after consecutive centrifugation of three another animals' blood with sodium citrate and clot activator, respectively. Three days after induction, animals were euthanized with ketamine (0.9 mg/kg) and xylazine (0.3 mg/kg) followed by cervical dislocation. Joint tissue samples (n=6/group) were collected and processed for immunohistochemical evaluation for the C3 fraction of complement and quantified by the number of positive cells/field in the synovium using the Image J program (NIH). Statistical analysis was performed using ANOVA and Tukey's post-test with GraphPad Prism with significance level of 5% (p<0.05). The results are expressed as mean±SD with significant differences between A (589±54.7) and B (1800±83.6), B and C (600±58.2), and B and D (520.7±48.2) with p=0.0131. Treatments with PRF and PRR are equivalent and act in this way to control the inflammatory process by reducing complement factor C3.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Ultrastructural and Physicochemical Characterization of Extracellular Matrix-Derived Hydrogels from Different Porcine Reproductive Tissues

Authors:

Bianca Fuzeti CANDIAN¹,
Mariana Sversut GIBIN²,
Claudio Guilherme de Assis OLIVEIRA¹,
Guilherme Gonçalves BARBOSA¹,
Danielle Kurunzi NUNES¹,
Leonardo Consoli SERTÃO¹,
Bruna Gomes SILVA³,
Isabela Paulillo D'ONOFRIO³,
João Victor DAMIN¹,
Guilherme Henrique Gonçalves de ALMEIDA¹,
Douglas Lopes de ALMEIDA¹,
Lucas Paulo Jacinto SAAVEDRA¹,
Paulo Cezar de Freitas MATHIAS¹,
Ana Claudia Oliveira CARREIRA³,
Gustavo Henrique Doná Rodrigues ALMEIDA³

Affiliations:

¹Department of Biotechnology, Genetics and Cell Biology, State University of Maringá

²Department of Physics, State University of Maringá

³School of Veterinary Medicine and Animal Science, University Of São Paulo

Keywords: Decellularization. Hydrogel. Reproductive Tissues. Tissue Engineering.

Abstract:

Biomaterials derived from biological matrices have been widely investigated due to their great therapeutic potential in regenerative medicine, since they are able to induce cell proliferation, tissue remodeling, and angiogenesis. Highly vascularized and proliferative tissues, such as the female reproductive tract, present an interesting source to produce acellular matrices that can be used as bioactive hydrogels to induce tissue regeneration. Therefore, this study aimed to characterize the ultrastructural and physicochemical properties of four ECM-based hydrogels derived from decellularized porcine ovaries (Ov), ampullas (Amp), uterus (Ut) and placenta (Pla) to verify their potential applications for tissue repair. For that, decellularized ovarian, ampullary, uterine and placental matrices were sterilized, lyophilized, digested with pepsin and neutralized to produce their respective hydrogels. After that, samples were fixed with 4% paraformaldehyde for scanning electronic microscopy (SEM) analysis. FTIR spectroscopy was used to detect the physicochemical pattern of each hydrogel. SEM analysis demonstrated that all four hydrogels presented fibrillary structures, which were derived from the digested and gelled extracellular matrix. Ov and Ut ECM-derived hydrogels presented a thicker and more robust ultrastructure, which reflected in the gelation pattern and speed. FTIR amide I band deconvolution showed a predominance of α -helix structures in all hydrogels, ranging from 36% in UtECM to 46% in OvECM, indicating a significant helical conformation. β -sheet and β -turn contents varied from 4–7% and 18–22%, respectively, with no significant differences among samples. Random coil structures ranged from 33% in UtECM to 20% in OvECM, suggesting higher structural disorder in UtECM, followed by AmpECM, PlaECM, and OvECM. In summary, the hydrogels presented interesting structural and compositional properties to enhance the investigations for tissue engineering applications.

This research was performed according to institutional ethics committee regulations of the University of São Paulo (CEUAX protocol no. 9963170523).



XXXIX REUNIÃO ANUAL DA FeSB
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Development of the Full-Length IgG Non-Glycosylated Version of the Monoclonal Anti-PD-L1 as an Immunobiological Tool for Prospecting New Antitumor Immunotherapy

Authors:

Marielly Câmara ROCHA^{1,2}
Gabriel Correia LIMA¹
Daniela LUZ¹

Affiliations:

¹ Instituto Butantan
² Universidade de São Paulo

Keywords: Anti-pd-l1. Antitumor immunotherapy. Immune checkpoint inhibitor.

Abstract:

Programmed Death Ligand 1 (PD-L1) is a crucial ligand of the immune checkpoint receptor Programmed Death 1 (PD-1) in the tumor microenvironment. Monoclonal blocking antibodies for this ligand were developed to restore exhausted T cells' ability to eliminate tumors. However, pharmaceutical companies outside Latin America produce most of these commercial antibodies in mammalian cells or hybridomas. The microbial expression system in *Escherichia coli* proposes a faster and lower-cost alternative for producing these molecules. In this scenario and in the context of improving public health and offering national biopharmaceuticals, this project aimed to develop and characterize the full-length IgG non-glycosylated version of the monoclonal anti-PD-L1. The pCOLADuet cIgG aPD-L1 construction was transformed into *E. coli* for expression, followed by the soluble fraction protein A affinity chromatography to anti-PD-L1 purification. The purification yield was 0,114 mg/L, analysis by SDS-PAGE of purified protein exhibited two bands at ~25 kDa and ~50kDa and analysis by Native PAGE of purified protein exhibited a single band at ~150 kDa, suggesting high purity. The antibody functionality was first analyzed by indirect ELISA, confirming binding to the PD-L1 antigen. Functional analysis to evaluate its ability to modulate immune responses is ongoing. Our partial results demonstrate the successful expression and purification of the non-glycosylated IgG anti-PD-L1, if confirmed its ability to modulate immune responses, this molecule raises as a potential antitumor therapeutic tool.



XXXIX REUNIÃO ANUAL DA FeSB
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: In Vitro Biocompatibility and Adhesion of MRC-5 Cells to Chitosan-Based Biomaterials

Authors:

Ana Luíza Hendges DALPIAZ¹
Paula Fernanda Petruf RIBEIRO¹
Fabiane BARCHIKI¹
Paulo Roberto Slud BROFMAN¹
Letícia FRACARO¹

Affiliations:

¹ Pontifícia Universidade Católica do Paraná

Keywords: Apoptosis. Flow cytometry. In vitro model. Viability.

Abstract:

Chitosan has gained prominence in regenerative medicine due to its biocompatible and biodegradable properties, being used in the formulation of hydrogels and films. When combined with crosslinking agents such as glutaraldehyde, it can result in biomaterials with characteristics suitable for supporting cell growth, showing potential for application in medical devices. This study evaluated the cytotoxicity of chitosan-based biomaterials prepared with different concentrations of acetic acid (1M and 1A), aiming to identify the formulation with the best biocompatibility for the MRC-5 cell line. After washing with ultrapure water to remove synthesis residues, conditioned media were prepared with biomaterials 1M and 1A for 48 hours. The MRC-5 cell line was cultured for 6 days in conditioned media derived from both biomaterials. Cell viability (7-AAD) and apoptosis (Annexin-V) were assessed by flow cytometry. To evaluate cell adhesion, cells were cultured on the surface of the biomaterials in non-adherent plates and subsequently stained with DAPI. The results showed that biomaterial 1A significantly reduced cell viability ($p=0.021$) and increased apoptosis ($p=0.013$) compared to the control. Despite these differences, the values remained within acceptable limits for cell culture. DAPI staining confirmed cell adhesion on both biomaterials, supporting previous evidence of chitosan's potential as a platform for cell adhesion. It is concluded that the evaluated biomaterials, especially 1M, exhibit an adequate biocompatibility profile and hold potential for applications in tissue engineering strategies. Additional functional assays are required to confirm the viability and functionality of the cells, aiming at future therapeutic applications.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Influence of Omega-3 Fatty Acid Supplementation on Oxidative Stress, Glycemia, and Lipid Profile in Rats Chronically Treated with Doxorubicin

Authors:

Helena Beatriz Moura MARTINS¹,
Marina Gaiato MONTE¹,
Carolina Rodrigues TONON¹,
Bertha Furlan POLEGATO¹

Affiliations:

¹Faculdade de Medicina de Botucatu, UNESP

Keywords: doxorubicin; oxidative stress; omega-3 fatty acids; dyslipidemia.

Abstract:

Doxorubicin (Dox) is a highly effective chemotherapy drug used to treat various types of cancer; however, it has serious systemic side effects. Omega-3 fatty acids (O3) have lipid-modifying and antioxidant effects. However, their impact on metabolism in animals treated with Dox remains unknown. This study aimed to assess the influence of omega-3 fatty acid supplementation on oxidative stress, lipid profile, and glycemic levels in rats chronically treated with Dox. Male Wistar rats (n=48) were divided into four groups: control, O3, Dox, and Dox + O3. O3 was administered by gavage for six weeks (400 mg/kg/day). Two weeks after initiating O3 supplementation, Dox was administered for four weeks (4 mg/kg, ip, once per week) (CEUA-1428/2023). Lipid profile, glucose levels, and oxidative stress markers were assessed in serum samples. Rats treated with doxorubicin had higher concentrations of total cholesterol (Dox: 302,57±116,44 vs. Non-Dox: 222,61±66,90 mg/dL;p<0.001), triglycerides (Dox: 302,57±116,44 vs. Non-Dox: 222,61±66,90 mg/dL;p=0.005), and glucose (Dox: 157,07±47,02 vs. Non-Dox: 135,02±21,90 mg/dL;p=0.050) compared to those that did not receive Dox. Additionally, rats treated with Dox exhibited higher concentrations of malondialdehyde (Dox: 24,28±19,12 vs. Non-Dox: 12,12±4,66 nmol/mg;p<0.001), carbonylated proteins (Dox: 88,22±33,55 vs. Non-Dox: 64,43±24,73 mol/mg;p=0.022), and superoxide dismutase activity (Dox:53,60±7,16 vs. Non-Dox: 38,11±7,93 U/mg;p<0.001) compared to all animals that did not receive Dox treatment. On the other hand, malondialdehyde (O3: 14,26±4,05 vs. Non-O3: 22,95±20,98 nmol/mg;p=0.004) and carbonylated protein concentrations (O3: 89,49±28,12 vs. Non-O3: 63,07±30,01 moles/mg;p=0.043) were lower in rats treated with O3 compared to those that did not receive O3. In conclusion, Dox increased oxidative stress and impaired the lipid profile, while O3 administration partially attenuated oxidative stress but did not modify lipid alterations.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Effect of whole-body vibration exercise on the hematological profile of diabetic *Wistar* rats: a preliminary study

Authors:

André Luiz Bandeira Dionizio CARDOSO^{1,2}
Yasmin Moura FERNANDES^{1,3}
Matteus de Assis ALVES^{1,2} Eugênio
Fuentes Péres JÚNIOR¹ Ariane da
Silva PIRES¹
Thaís Porto AMADEU^{1,2}
Danúbia da Cunha de SÁ-CAPUTO¹
Mario BERNARDO-FILHO¹

Affiliations:

¹Laboratório de Vibrações Mecânicas e Práticas Integrativas, Departamento de Biofísica e Biometria. Instituto de Biologia Roberto Alcântara Gomes, Policlínica Universitária Piquet Carneiro, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

²Programa de Pós-Graduação em Fisiopatologia Clínica e Experimental, Faculdade de Ciências Médicas, Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro, RJ, Brazil.

³Programa de Pós-Graduação em Ciências Médicas, Faculdade de Ciências Médicas, Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro, RJ, Brazil.

Keywords: Diabetes mellitus, whole-body vibration exercise, hematology, inflammation, platelet function.

Abstract:

Type 1 diabetes mellitus (T1DM) is an autoimmune disease characterized by hyperglycemia, leading to systemic complications, including hematological alterations. Whole-body vibration (WBV) exercise, which subjects are exposed to mechanical vibration (MV) through a vibrating platform, has shown potential benefits in metabolic and physiological parameters. This study evaluated WBV effects on the hematological profile of diabetic *Wistar* rats, focusing on erythrogram, leukogram, and platelet parameters. Ten male *Wistar* rats (250-350g, 2-3 months old) were divided in two groups: diabetic control (DM, n=5) and diabetic treated with WBV (DM+WBV, n=5). T1DM was induced by alloxan (170mg/kg). After 1-week, the diabetes status of the rats was confirmed through the blood collection of the tail tip ($\geq 200\text{mg/dL}$). The DM+WBV underwent 5 WBV sessions per week (MV-50Hz, 0.78mm, at 4 bouts of 30s, with 1-min rest period) for 5-weeks. After treatment, blood samples were collected for hematological analysis, including erythrogram (hemoglobin, hematocrit, red cell distribution width-RDW), leukogram (leukocytes, neutrophils, lymphocytes), and platelet (platelet count, mean platelet volume-MPV). This study was approved by the ethics committee CEUA/UERJ-006/2019. T-test for independent samples was performed using GraphPad Prism 6. Difference was considered at $p\text{-value} \leq 0.05$. Animals treated with WBV did not exhibit significant changes ($p > 0.05$) in erythrogram levels of hemoglobin (12.1 ± 1.1), hematocrit (39.2 ± 1.5), and RDW (15 ± 1.6) concentrations compared to DM ($12.1 \pm 1.1/38.5 \pm 3.5/17.9 \pm 1.6$). Likewise, in leukogram, total leukocytes ($7810 \pm 1180/[DM]5582 \pm 2463$), neutrophils ($3020 \pm 1163/[DM]2449 \pm 1416$), and lymphocytes ($4244 \pm 1075/[DM]2929.4 \pm 1275$) have not changed ($p > 0.05$) after WBV treatment. Also, no significant change was found in platelet such as platelet count ($759 \pm 197.8/[DM]859.6 \pm 324$), and MPV ($6 \pm 0.7/[DM]6 \pm 0$) after WBV. No significant effects were observed from cumulative WBV treatment on hematological markers in diabetic rats. This preliminary study focused exclusively on diabetic animals to investigate the specific impacts of WBV on T1DM-associated hematological disturbances.

Support: CAPES, CNPQ and FAPERJ



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Virulence of Atypical Enteropathogenic Escherichia coli (aEPEC) Strains Isolated From Dogs in an Experimental Model With *Galleria mellonella* Through Intrahemocelic and Oral Infection

Authors:

Yasmim Gomes de MESQUITA¹
Helvécio Cardoso Correa PÓVOA²
André Vitor BARBOSA¹
Andréa Regina de Souza BAPTISTA¹
Ricardo Luiz Dantas MACHADO¹ Simone
Cristina Pereira BRITO¹ Vanilda
Gonçalves Viana KAUFMANN^{1,2}
Aloysio de Mello Figueiredo CERQUEIRA¹

Affiliations:

¹Departamento de Microbiologia e Parasitologia do Instituto Biomédico, Universidade Federal Fluminense (UFF), Niterói, RJ, Brasil.

²Departamento de Microbiologia e Parasitologia Aplicada do Instituto Biomédico, Universidade Federal Fluminense, Nova Friburgo, RJ, Brasil.

Keywords: atypical EPEC. *Galleria mellonella*. Biologic Model. Virulence.

Abstract:

Galleria mellonella larvae are used as an alternative model for evaluating bacterial pathogenicity due to their immune response similar to the mammalian innate response. Its ease of creation and manipulation makes the model a valuable tool in these studies. The zoonotic aEPEC pathway presents the fecal-oral infection route, and adherence to the intestinal mucosa is a relevant step in the infectious process. The objective of this study was to evaluate the virulence of aEPEC strains isolated from dogs and serotypes associated with human infection (strains 21 and 47 – O51:H40 and strain 77 – O117:H40) in an experimental model with *G. mellonella* by means of intrahemocelic (IH) and oral infection (IO). For comparative purposes, a pathogenic aEPEC strain of human origin (H1 – O51:H40) and a non-pathogenic strain (*E. coli* K12 DH5a) were also included. The experiments were carried out in independent triplicates. The strains were cultivated in tryptone soy broth and inoculated at a concentration of 10⁶ CFU/ml (suspension in phosphate buffered saline solution). The results showed that survival after 92 hours was generally lower by the IH route than by the (IO) route. In the IH route, larvae inoculated with strains 21 and 77 showed survival rates of 57.5% and 54%, respectively, and the strains were considered to have moderate virulence (values between 40% and 60%). Infection by IO of these strains showed higher survival percentages (73.3% and 70%, respectively), classifying them as low virulence (values above 60%). The strains tested induced a slight reduction in the hemocyte count after 24 hours of infection ($p > 0.05$) more pronounced in those with higher virulence. The results obtained show that the response of the *G. mellonella* model to infection by aEPEC varies according to the inoculation route. And the evaluation of lower virulence after oral inoculation may be a consequence of important differences between the model employed and the interaction with the mammalian host. Support: CAPES, CNPq, Propi/UFF.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Avaliação de resistência à deltametrina em Linhagens Endogâmicas Recombinantes de *Aedes aegypti*

Authors:

Victor Hugo da Rocha NASCIMENTO¹

Luiz Guilherme Soares da Rocha BAUZER²

Affiliations:

Instituto Oswaldo Cruz - IOC

Keywords: *Aedes aegypti*, Linhagens Endogâmicas Recombinantes, Deltametrina, GWAS

Abstract:

A pesquisa gira em torno do inseto vetor *Aedes aegypti*, que transmite diferentes vírus que causam doenças graves como a dengue, chikungunya, entre outras. Inicialmente, foi feita a coleta de mosquitos de diferentes localidades do Brasil, que foram inter cruzados no laboratório, gerando uma população artificial rica em variabilidade genética. A partir desta população, foram geradas dezenas de Linhagens Endogâmicas Recombinantes (LERs), as quais foram endocruzadas por 20 gerações, até alcançarem a isogenia. As LERs são ferramentas que facilitam o mapeamento genético dos vetores, através da diminuição da variabilidade genética. O objetivo da pesquisa é estudar as bases moleculares de fenótipos ligados a capacidade vetorial, através de Estudos de Associação Genômica Ampla (GWAS, em inglês). O fenótipo escolhido para iniciar os estudos GWAS foi a resistência ao inseticida piretróide deltametrina, amplamente usado para o controle vetorial. Após os vetores chegarem à fase adulta, o processo de avaliação de resistência foi iniciado (Bioensaios). Para cada LER, 150 fêmeas ficam expostas à deltametrina por 1 hora em um tubo com papel impregnado, e depois são transferidas para novos tubos sem os papéis impregnados, onde ficam contidas por 24 horas. Esse período serve para visualizarmos se o mosquito se recuperou da neurotoxina ou se mais mosquitos sucumbiram a ela. Esse processo é repetido mais 3 vezes, assim totalizando 450 fêmeas por linhagem. Quase todas as 60 LERs disponíveis foram expostas a concentração discriminante (CD): 0,03%. As LERs que tiveram mortalidade menor que 98%, estão sendo expostas a cinco vezes a CD (0,15%). As que se mostraram resistentes a esta dose, estão sendo expostas a dez vezes a CD (0,30%). Por enquanto os resultados indicam a existência de 4 LERs susceptíveis, 6 com resistência baixa, 1 com moderada resistência, 2 com alta resistência. 42 LERs estão ainda pendentes.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: The Effects of Moderate Physical Training on Renal Function in Male Rats Treated with Tenofovir Disoproxil Fumarate

Authors:

Juliana Veloso Gusmão SILVA¹
Maria de Fátima Fernandes VATTIMO¹ Mykelly
Gomes ALVES¹
Julia Matheus TSUCHIYA¹
Pedro Henrique Rodrigues dos SANTOS¹

Affiliations:

¹Escola de Enfermagem da Universidade de São Paulo - USP, SP/SP -
Brasil.juliana.veloso.silva@usp.br

Keywords: Acute kidney injury. Exercise. Oxidative stress. Renal function. Tenofovir disoproxil fumarate.

Abstract:

Tenofovir disoproxil fumarate® (TDF), a widely used antiretroviral for HIV and hepatitis B treatment, is renally excreted and can cause kidney damage. Studies report a 10–30% incidence of acute kidney injury (AKI) in patients on TDF therapy. This study investigates whether moderate physical training influences renal function in male rats treated with TDF. Ethics approval: 2100/2024. Adult Wistar rats (n=28) were divided into four groups (n=7/group): Control (C): Received vehicle treatment. TDF: Received TDF (600 mg/kg via gavage for 5 days). Physical Training (PT) group: Underwent swimming training (60 minutes daily, 5 days/week, with 5% body weight attached to the tail) until the 28th day. PT+TDF: Received both TDF treatment and physical training as described. On the 29th day, the animals were euthanized under 5% isoflurane anesthesia, followed by exploratory laparotomy for blood and tissue collection. Results (mean±SD; *p≤0.05, t-test): Renal Function Analyzed: Neutrophil gelatinase-associated-lipocalin (NGAL), Inulin clearance (Inu), NGAL levels (ng/mL): C: (4.7±0.2), TDF: (10.3±4.1*), PT: (5.8±0.3), PT+TDF: (6.6±1.7*). Inulin clearance (mL/min): C (0.8±0.1), TDF: (0.3 ± 0.08*), PT: (0.7 ± 0.11), PT+TDF: (0.4 ± 0.09*). Oxidative Stress Markers: Increased urinary lipid peroxidation (TBARS, nM/g): C: (0.3 ± 0.08), TDF: (0.73 ± 0.2*), PT: (0.2 ± 0.05), PT+TDF: (0.22 ± 0.08)*, Reduced thiol levels (nmol/g of total protein): C: (23.9± 2.08), TDF: (3.56 ± 0.9*), PT: (22.98±6.07), PT+TDF: (27.9±8.01*), Animals subjected to exercise showed improved tubular function PT+TDF (NGAL: 6.6±1.7), and glomerular function PT+TDF (Inu: 0.4±0.09), Oxidative stress showed significant improvement as evidenced by PT+TDF (TBARS: 0.22±0.08), enhanced thiol levels PT+TDF (27.9±8.01). Conclusions: TDF treatment impaired renal function and increased oxidative stress. Moderate physical training showed renoprotective effects, improving tubular function and reducing oxidative damage. Further studies are needed to assess long-term outcomes.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Anti-TSLP Treatment Modulates Pulmonary Response in an Experimental Model of Asthma COPD Overlap

Authors:

Amanda dos Santos LIGERO^{1,2} Leandro
Nascimento CAMARGO^{1,2} Vitória Helena
Kuhn de CAMPOS^{1,2} Francine Maria
ALMEIDA¹
Beatriz Manguiera Saraiva ROMANHOLO¹
Edna Aparecida LEICK¹
Fernanda Degobbi Tenorio Quirino dos Santos LOPES¹ Silvia
FUKUZAKI¹
Tabata Maruyama dos SANTOS^{1,2}
Renato Fraga RIGHETTI^{1,2}
Iolanda Fatima Lopes Calvo TIBÉRIO¹

Affiliations:

¹ Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil ²
Hospital Sírio-Libanês, São Paulo, Brazil

Keywords: TSLP inhibition, airway inflammation, oxidative stress, asthma-COPD overlap syndrome, tissue remodeling.

Abstract:

Asthma-COPD overlap syndrome (ACO) is primarily characterized by airway inflammation and obstruction. However, emerging evidence suggests that peribronchial vascular remodeling and immune dysregulation play crucial roles in its pathophysiology. A deeper understanding of these mechanisms is essential for developing targeted therapeutic strategies. A total of 62 male BALB/c mice were subjected to an ovalbumin-elastase-induced experimental model of ACO and treated with a TSLP inhibitor or left untreated. Key parameters assessed included lung function, inflammatory response, oxidative stress, extracellular matrix remodeling, and molecular signaling pathways. The treated groups received an intranasal administration of anti-TSLP monoclonal antibodies 24 hours before the end of the experimental protocol. The study was approved by the Research Ethics Committee of the HC-FMUSP (research protocol No.1828/2022). ACO+TSLP reduced Rrs compared to ACO ($p<0.05$) and also demonstrated attenuation of Ers, Gtis, Htis, and Raw compared to ACO ($p<0.05$) for all comparisons. ACO+TSLP reduced the total cell count in BALF compared to ACO ($p<0.05$). In terms of inflammation, ACO+TSLP exhibited a reduction in TNF- α , IL-6, and IL-17 compared to the ACO group ($p<0.05$). In the airways, TNF- α and IL-17 were reduced when compared to ACO ($p<0.05$). In the context of tissue remodeling, MMP-9, MMP-12, TIMP-1, and TGF- β significantly decreased in the ACO+TSLP group compared to ACO, both in the airways and parenchyma ($p<0.05$) for all comparisons. Regarding oxidative stress markers, iNOS was significantly reduced in both the parenchyma and airway ($p<0.05$), and iso-PGF-2 α was attenuated in the airway ($p<0.05$), compared to the ACO+TSLP group. No statistically significant differences were found in total collagen in the reference groups. TSLP inhibition contributed to the attenuation of hyperresponsiveness, inflammation, and remodeling in this ACO model. FINANCIAL SUPPORT: FAPESP (2022/01776-1), LIM-20-HCFMUSP.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Sex Differences in Memory Consolidation and Persistence at Different Intensity Stimulus' Levels in Contextual Fear Conditioning

Authors:

Gabriela Jaques SIGARAN¹
Karine Ramires LIMA¹
Gabriela Cristiane MENDES ¹
Giulia Azevedo MARTINEZ¹
Marisele dos Santos SOARES¹
Pamela Billig MELLO-CARPES¹

Affiliations:

¹ Federal University of Pampa

Keywords: Contextual fear conditioning. Estrous cycle. Fear memory. Persistence.

Abstract:

The consolidation and persistence of fear memory can be influenced by biological factors such as sex. This study investigated these differences in rodents subjected to the Contextual Fear Conditioning (CFC) (Ethics Committee protocol 015/2024), using 60 animals: 20 male, 20 female rats without estrous cycle control (NoCycle), 20 female rats with estrous cycle control (Cycle) rats. Rats were divided into groups according to the intensity of CFC stimulus. During the CFC learning session (acquisition), the animals remained in the apparatus for 3 minutes, received a 2-second foot shock (0.3 mA or 0.5 mA), and stayed in the apparatus for additional 30 seconds. Freezing behavior was recorded to assess the association between the context and the aversive stimulus. Memory consolidation was tested 24 hours later, and persistence 15 days later, in a 5 minutes sessions. Statistical analysis was performed using one-way ANOVA for acquisition and two-way ANOVA for testing. During acquisition, there were no significant differences in freezing behavior between the two different stimulus intensities in male rats ($p=0.54$), NoCycle female rats ($p=0.20$), and Cycle female rats ($p=0.71$). In the consolidation phase, males ($p=0.49$), noCycle ($p=0.78$) and Cycle females ($p=0.83$), independent of the cycle phase, consolidated memory for both stimulus intensities. When memory persistence was evaluated, male rats presented decreased fear response at 0.3 mA ($p=0.03$) but memory persistence at 0.5 mA ($p=0.99$). NoCycle female rats presented memory persistence ($p=0.67$), while Cycle female rats, independent of the cycle phase, showed a decrease in freezing behavior at 0.3 mA ($p=0.02$) but showed persistence with 0.5 mA ($p=0.48$), indicating an influence of the estrous cycle monitoring. The results highlight sexual dimorphism in memory persistence, with female rats exhibiting a stronger fear response than male rats at low stimulus intensity. However, estrous cycle monitoring reduced this response.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Osteocyte number density can predict bone maturation

Authors:

João Alberto de Oliveira ALVES ¹
Fabiano Luiz Dulce de OLIVEIRA ^{1,2}
Maiza da Silva PAULA ¹
Beatriz Julião Vieira AARESTRUP ¹
Akinori Cardozo NAGATO ^{1,3}

Affiliations:

¹ Laboratory of Immunopathology and Experimental Pathology, Center for Reproductive Biology - CBR, Federal University of Juiz de Fora (UFJF), Juiz de Fora, Minas Gerais, Brazil.
² Veterinary Institute, Federal Rural University of Rio de Janeiro (UFRRJ), Seropédica, Rio de Janeiro, Brazil. ³ Department of Physiology, Federal University of Juiz de Fora (UFJF), Juiz de Fora, Minas Gerais (MG), Brazil.

Keywords: Animal Models. Cell Count. Diaphyses. Femur. Osteocytes.

Abstract:

Healthy bone is histologically organized in structured patterns that repeat themselves and histomorphometric and stereological bone variables can be used to determine bone integrity and loss, as in the case of fractures, and diseases that modify bone matrix density, such as osteoporosis, rheumatoid arthritis and neoplasms. Experimental models that seek to investigate bone remodeling can benefit from these techniques because knowledge of the density of the number of osteocytes, osteoblasts, osteoclasts, Havers' canals per unit area, for example, can help determine the state of bone maturation. The aim of the study was to compare the density of the number of osteocytes (Nv) per area of healthy bone (BH) and neoformed bone (NB). Photomicrographs of femoral sections from male Wistar rats (*Rattus norvegicus* / 90 days old; 250-300g), stained with hematoxylin and eosin were analyzed using light microscopy. Ten microscopic fields ($\text{mm}^2 \times 10^{-6}$) containing areas of BH and NB after bone defects in the femoral shafts were randomly captured. The study was approved by the Ethics Committee (UFJF-No.009/2015). The areas of bone neoformation were delineated using ImageJ software, and the number of total osteocytes was counted. The normality of the sample was tested using the D'Agostino & Pearson test. Data are expressed as mean $\pm$ standard error. Differences between the groups were tested by unpaired Student's T-test (normal). The significance level was 5% ($p < 0.05$). Osteocyte number density (Nv) was determined by dividing the number of osteocytes occupied by the area of bone tissue. Nv was lower in the BH group ($2,522 \pm 0,1803$) when compared to the NB group ($20,47 \pm 1,896$). The results suggest that the density of the number of osteocytes per bone area varies according to the stage of bone development and that a lower number of osteocytes per area of bone matrix is related to mature bone.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Low-intensity laser therapy has the potential to promote bone neoformation by decreasing the number of osteocytes

Authors:

João Alberto de Oliveira ALVES ¹
Fabiano Luiz Dulce de OLIVEIRA ^{1,2}
Maiza da Silva PAULA ¹
Beatriz Julião Vieira AARESTRUP ¹
Akinori Cardozo NAGATO ^{1,3}

Affiliations:

¹ Laboratory of Immunopathology and Experimental Pathology, Center for Reproductive Biology - CBR, Federal University of Juiz de Fora (UFJF), Juiz de Fora, Minas Gerais, Brazil.

² Veterinary Institute, Federal Rural University of Rio de Janeiro (UFRRJ), Seropédica, Rio de Janeiro, Brazil. ³ Department of Physiology, Federal University of Juiz de Fora (UFJF), Juiz de Fora, Minas Gerais (MG), Brazil.

Keywords: Bone Regeneration. Cell Count. Femur. Low Level Light Therapy. Osteocytes.

Abstract:

Bone development is dependent on nutritional support and oxygen and this is made possible by osteocytes, and maintained by the proportion of osteocytes per area of bone matrix irrigated by them. Newly-formed bones depend on the osteocyte/bone matrix ratio. Research environments are investigating techniques that attempt to modify the bone microenvironment in order to accelerate bone repair, and low-intensity laser therapy (LLLT) has shown promise. It is unclear whether LLLT can modify the density of osteocyte numbers. The aim of the study was to evaluate whether LLLT modifies the density of the number of osteocytes in femoral lesions of Wistar rats after 20" or 90". The study was approved by the Ethics Committee (UFJF-No.009/2015). Male Wistar rats (90 days old; 250-300g) were divided into 'Control' (n=4; no injury), 'Bone injury' (n=4), 'LLLT20' (n=4; LLLT for 20s) and 'LLLT90' (n=4; LLLT for 90s). Bone defects were created on the femoral shaft, exposed or not to LLLT and euthanized

after 7 and 14 days. Histopathological, morphometric and stereological analyses were carried out on ten bone section fields per group (light microscopy), on samples stained with hematoxylin and eosin. The density of the number of osteocytes (Nvcells) was defined by the ratio between Nv and the density of neoformed bone area (mm² x 10⁻⁶) using ImageJ® software. Data were expressed as mean ± standard error followed by Tukey's post hoc test. The significance level was 5% (p<0.05). The group with bone injury (20.47 ± 1.896) presented higher Nvcells when compared to the control (2.522 ± 0.1803; p<0.001). Laser for 20s reduced Nvcells only after 7 days (8.865 ± 4.198; p<0.001), while laser for 90s did not significantly decrease the osteocyte number density (Nvcells) in any period evaluated. The results suggest that laser therapy has the potential to anticipate bone neoformation by decreasing the number of osteocytes, and this was evident after 20 seconds of therapy at 7 days.



XXXIX REUNIÃO ANUAL DA FeSB
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Guide to the husbandry of mice strains at the Instituto de Ciência e Tecnologia em Biomodelos/ Fiocruz

Authors:

Raquel de Oliveira da ROSA^{1,2}
Duanne Alves BELLO²
Jenif Braga de SOUZA²
Thales Xavier de OLIVEIRA²
Thiago dos Santos de ANDRADE²
Isabel Maria Alexandre FREIRE²
Janaína Barcelos Porto FERREIRA²

Affiliations:

¹Mestrado Profissional em Ciência em Animais de Laboratório/ Fundação Oswaldo Cruz– Rio de Janeiro, Brasil

²Instituto de Ciência e Tecnologia em Biomodelos/ Fundação Oswaldo Cruz– Rio de Janeiro, Brasil

Keywords: Biomodels. Cryopreservation. Management. SPF Mice.

Abstract:

The mice *Mus musculus* is the most used animal model in scientific research due to its genetic proximity to humans and high adaptability. At the Instituto de Ciência e Tecnologia em Biomodelos (ICTB/Fiocruz), 26 strains of mice are maintained in a specific-pathogen-free (SPF) facility, ensuring the preservation and controlled breeding of the animals. Currently, there is no specific guide regarding the breeding and maintenance of these strains at ICTB, which motivated this study. The objective is to develop a technical guide that compiles the specificities of breeding and management, along with genetic information and key research lines associated with the mice strains. The guide is being developed within the SPF mice breeding colony of the Serviço de Criação de Roedores e Lagomorfos (SCRL) in collaboration with the Serviço de Biotecnologia e Desenvolvimento Animal (SBDA), targeting researchers and technicians from Fiocruz and similar institutions. Data collection was conducted from June to December 2024, following an inclusion order that prioritized strains that were already cryopreserved, those in the process of cryopreservation, and finally, the most used in research. The information was obtained from the genealogical records of the area and through a literature review of the original repositories. To date, detailed information of nine strains has been cataloged: Balb/c Scid, BKS db, C3H/HEJ, Cav-1, D011-10, iNOS, NOD J, Nod Scid, and Nude. This information includes conditions of the micro and macroenvironment, genetic classification, zootechnical data, husbandry peculiarities, and cryopreservation, as well as the main research lines utilizing these strains. The guide will serve as a resource to enhance biorepository management, ensure the reproducibility of experiments, and facilitate access to information regarding each strain, thereby contributing to the quality of research conducted at Fiocruz and partner institutions.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Determination of the Ammonia Concentration and Change Frequency of the B6.Cg-Tg(K18-Ace2)2prlmn/J Strain at the Institute of Biomodels Science and Technology

Authors:

Yasmin Travassos de SOUZA^{1,2}
Aline da Cruz REPOLEZ¹
Davi Monteiro dos SANTOS¹
Duanne Alves BELLO¹
Monique Grazielle Oliveira PAPA¹
Rodrigo Silva PEREIRA¹
Isabel Maria Alexandre FREIRE¹
Jenif Braga de SOUZA^{1,3}
Mariana Boechat de ABREU^{1,2}

Affiliations:

¹Instituto de Ciência e Tecnologia em Biomodelos Fundação
Oswaldo Cruz/FIOCRUZ – Rio de Janeiro, Brasil
²Instituto de Biofísica Carlos Chagas Filho, UFRJ, Rio de Janeiro
³Universidade do Estado do Rio de Janeiro - UERJ, Brasil

Thematic axis: Laboratory Animal Sciences and Alternative Models

Keywords: Ammonia. B6.Cg-Tg(K18-ACE2)2PrImn/J. Bedding. Mouse. Welfare.

Abstract:

The bedding used in mouse breeding plays a fundamental role in ammonia absorption, nest formation, and thermal regulation. Respiratory tissues are particularly sensitive to ammonia, and increased exposure can lead to inflammation. However, there are no established guidelines for ammonia exposure limits in rodents. This study aimed to compare three different bedding substrates for B6.Cg-Tg(K18-ACE2)2PrImn/J mice in terms of absorption capacity. Maintenance boxes were used with 7 animals per box, and the tested substrates were *Pinus elliottii* wood shavings, Aspen wood shavings, and *Pinus elliottii* flakes. A sample size of 10 boxes per experimental group was determined. Ammonia measurements were taken every two days for five weeks using the SP2ND NH₃ device (Senko, Brazil), which was placed inside the cages for six minutes. Cage replacement occurred when the ammonia concentration reached the cutoff point of 130 ppm. Data were analyzed using ANOVA and Bonferroni posthoc tests, expressed as mean $\pm$ standard deviation; $p \leq 0.05$. Ammonia concentrations in cages with *Pinus* wood shavings were higher between days 7 and 8 after bedding replacement (169.0 ± 24.16) compared to Aspen (41.26 ± 13.05) and flakes (35.83 ± 38.82) ($p \leq 0.03$). Based on the cutoff point, *Pinus* wood shavings had a replacement interval of 7.47 ± 3.54 days, shorter than the 16.97 ± 6.23 days observed with *Pinus* flakes ($p = 0.006$). Aspen showed an intermediate result, with 12.7 ± 8.09 days. Thus, there is greater potential for optimizing animal facility management at SCRL/Fiocruz, ensuring improved animal welfare when using flake bedding compared to wood shavings. As a future perspective, data on the respiratory physiology of animals under different conditions will be evaluated. Ethics Committee Approval: CEUA: LW-54.19



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Effects of swimming and resistance training on the diaphragm of male and female dystrophic mice

Authors:

Tábata Gabriely Pereira Barbosa de SOUZA¹

Luana Lima Rocha da SILVA¹

Túlio de Almeida HERMES¹

Affiliations:

¹ Departamento de Anatomia, Universidade Federal de Alfenas-MG

Keywords: Muscular dystrophy. Swimming. Muscle regeneration. Diaphragm.

Abstract:

Duchenne muscular dystrophy (DMD) causes progressive muscle degeneration. While swimming shows therapeutic potential through muscle regeneration and anti-inflammatory effects, high-intensity exercise may worsen damage in dystrophic muscle. Using mdx mice (DMD model), we compared moderate swimming (30min/session, 4x/week for 4 weeks) versus high-intensity climbing (6 sessions, 50% body weight) in six groups: (mdxM/F) sedentary males/females, (mdxSM/F) swimming males/females, (mdxRM/F) resistance (climbing) males/females. Muscle strength was assessed before and after the experimental protocol, and blood samples were taken to quantify creatine kinase (CK), a marker of muscle damage. The diaphragm muscle was analyzed histologically to quantify central nuclei and areas of inflammation using hematoxylin and eosin (HE) staining. The results demonstrated that resistance training promoted greater muscle strength gain (mdxRM: 2748.25±623.31 vs. mdxRF: 1453.05±813.54) compared to swimming (mdxSM: 1813.28±4905.57 vs. mdxSF: 1797.09±2744.20). However, this gain was accompanied by higher creatine kinase levels (mdxRM: 623.31±2748.25 vs. mdxRF: 813.54±3238.00), indicating greater muscle damage. Swimming showed distinct effects: in males, a greater number of central nuclei was observed (16.39±0.50 vs 12.75±0.30 in resistance-trained) while in females there was smaller inflammation area (8.60±1.80 vs. 12.12±1.20 in resistance-trained). These results suggest that both swimming and resistance exercise promote milder damage in female mdx mice than in male mdx mice. Future research should investigate molecular mechanisms behind these gender-divergent responses to optimize clinical applications. The study was approved by the Animal Use Ethics Committee (CEUA) of UNIFAL-MG (protocol no. 0023/2024).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Effects of the ketogenic diet on gut microbiota of rats with cerebral palsy

Authors:

Nathalia Caroline de Oliveira MELO¹
Vitória Felício SOUTO²
Jakssuel Sebastian Dantas ALVES¹
Gisélia de Santana MUNIZ¹

Affiliations:

¹Graduate Program in Nutrition, Federal University of Pernambuco.

Keywords: Gut bacteria. Ketogenic diet. Neuroinflammatory Disease.

Abstract:

Cerebral palsy (CP) is the main cause of chronic motor dysfunction in childhood, resulting in motor deficits, changes in muscle plasticity and central nervous system impairment, and the ketogenic diet (KD) has been studied as a possible therapeutic approach. This study investigated the influence of KD on the gut microbiota of rats submitted to an experimental model of CP. With the approval of Ethics Committee for use of animals of the Federal University of Pernambuco (protocol 100/2021), 60 Wistar rats were used, divided into control group (C, n=15), ketogenic group (K, n=15), cerebral palsy group (CP, n=15) and cerebral palsy group with ketogenic diet (CPK, n=15). At birth CP was induced by perinatal anoxia and sensorimotor restriction, and from weaning to 65 days of life the animals received control diet (11% lipid, 64% carbohydrates, 25% protein and 3,4kcal/g) or ketogenic (71% lipid, 10% carbohydrates, 19% protein and 5,4kcal/g). During euthanasia, a fecal sample was collected from the ileocecal region, which was submitted to 16S sequencing (V3-V4 region) for evaluation of its composition. The results were analyzed in Microbiome Analyst®. KD was able to modulate the microbiota of CP carriers by reducing the abundance of Proteobacteria phylum (C:13.9 2.9, K:0.6 0.7, CP:43.1 4.9, CPK:0.4 0.2, p=0.03), Enterobacteriaceae family (C:0.1 0.0, K:0.1 0.0, CP:70.4 1.0, CPK:0.3 0.2, p=0.01) and *Escherichia-Shigella* genus (C:13.9 2.9, K:0.6 0.7, CP:43.1 4.9, CPK:0.4 0.2, p=0.03), at the same time it favored the increase of the Firmicutes phylum (C:79.5 4.3, K:94.9 1.4, CP:51.3 8.1, CPK:93.1 7.0, p=0.02), of the Ruminococcaceae family (C:1.9 0.8, K:0.6 0.7, CP:1.9 0.6, CPK:10.3 3.2, p=0.00) and the *Rombutsia* genus (C:4.8 0.2, K:8.0 0.5, CP:1.2 0.1, CPK:10.9 0.6, p=0.02). In this context, the consumption of KD since childhood was able to modulate the gut microbiota in CP carriers, especially by favoring the reduction of bacteria associated with dysbiosis and neuroinflammation.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Weight and metabolic outcomes of light-phase time-restricted feeding combined with western-style diet in Wistar rats

Authors:

Vitória Felício SOUTO²
João Carlos Fonseca da SILVA¹
Fernando Wesley Cavalcanti de ARAÚJO¹
Jakeline Olindina Francelino¹
Nathalia Caroline de Oliveira MELO¹
Elizabeth do NASCIMENTO¹

Affiliations:

¹Graduate Program in Nutrition, Federal University of Pernambuco.

Keywords: Chrononutrition. Intermittent fasting. Obesity. Western diet.

Abstract:

Time-restricted feeding (TRF) is an intermittent fasting strategy that limits food intake to an 8–12-hour window within 24 hours. When this window is misaligned with the circadian rhythm, chronodisruption may occur, leading to physiometabolic dysregulation. This study aimed to evaluate the weight and metabolic effects of feeding time inversion associated with a Western-style diet in female Wistar rats. With approval from the Ethics Committee of the Federal University of Pernambuco (protocol 0017/2023), 24 rats were initially divided into a control group (C, n = 7) and an obese group (O, n = 17). Obesity was induced using a diet containing 39.7% carbohydrates (52.31% from simple sugars), 24.5% protein, and 35.8% lipids (4.6 Kcal/g). The obese group was then subdivided into an *ad libitum* obese group (OA, n = 8) and a restricted obese group (OR, n = 9), followed by weight and metabolic analysis. TRF consisted of 12 h fasting during the dark phase and 12 h feeding during the light phase. Parameters analyzed included absolute weight gain, total caloric and macronutrient intake, plasma glucose, and total cholesterol. Data were tested for normality (Shapiro–Wilk), followed by one-way repeated measures ANOVA and Tukey's post hoc test. Western diet consumption led to weight gain in OA, while TRE reduced this in OR ($F(2, 20) = 12.80, p = 0.0003$), associated with reduced energy intake in OR ($F_{\text{total}}(2, 20) = 0.9082, p = 0.0005$). Macronutrient intake was higher for lipids ($F_{\text{lipids}}(2, 19) = 3.003, p < 0.0001$), then carbohydrates ($F_{\text{carbohydrates}}(2, 19) = 146.6, p < 0.0001$), and protein ($F_{\text{protein}}(2, 19) = 1.053, p < 0.0001$). Plasma glucose increased in OR ($F_{\text{glucose}}(2, 20) = 5.838, p = 0.0101$), with no significant change in cholesterol ($F_{\text{cholesterol}}(2, 20) = 5.184, p = 0.0153$). Thus, misaligned feeding impairs glycemic control, even with weight loss.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Physicochemical characterization of two types of collagen and their use in the construction of 3D skin-equivalent models

Authors:

Leonardo Francisco
DIEL¹ Luiza Meurer
BRAND¹ Lisiane
Bernardi²
Marcelo Lazzaron LAMERS².

Affiliations:

¹Basic Research Center in Dentistry, School of Dentistry, Federal University of Rio Grande of Sul, Porto Alegre, Brazil.

²Department of Morphological Sciences, Institute of Basic Health Sciences, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil.

Keywords: 3D culture, organotypic, skin model, collagen, extracellular matrix.

Abstract:

Cell culture serves as a pivotal methodology for elucidating various cellular processes and the mechanisms underlying disease pathogenesis. Recent trends underscore the necessity of developing models that closely mimic the physiological characteristics of living tissues. Accordingly, there is a concerted effort to establish three-dimensional (3D) models that integrate diverse cell types and components of the cellular microenvironment. Hydrogels play a critical role in the fabrication of these models due to their biomimetic properties and the ability to create a viscoelastic environment conducive to cellular maintenance and development. We conducted a comprehensive physicochemical characterization of collagen extracted from rat tail and porcine tendon, utilizing this collagen as a hydrogel for the construction of an organotypic skin model. Furthermore, we investigated the influence of extracellular matrix (ECM) components, fibronectin and laminin on the structural and functional properties of the model. Our findings revealed physicochemical similarities between the collagens derived from both sources however, the use of porcine collagen resulted in a model characterized by a less permeable epithelium and diminished expression of the aquaporin gene. This outcome was correlated with a more porous matrix comprising smaller and thinner collagen fibers, alongside enhanced fibroblast activity, evidenced by increased expression levels of elastin and fibronectin. Moreover, the introduction of ECM components contributed to the development of a thicker and more mature epithelium, characterized by heightened expression of epithelial markers, including keratin 10, aquaporin, and filaggrin, in conjunction with augmented fibroblast activity. The utilization of porcine tendons for the extraction of type I collagen demonstrated its viability and additionally, the integration of ECM components significantly enhanced the robustness of the model.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Establishment and characterization of a 3D hepatic steatosis spheroid model for MASLD studies and preliminary phytotherapeutic screening with *Pereskia grandifolia* extract

Authors:

Lucas Pires GUARNIER¹
Cibele Ferreira PIMENTEL²
Julia Helena Oliveira DE BARROS²
Érica Almeida AMORIN²
Gabriella Oliveira Alves Moreira DE CARVALHO²
Lincoln Gozzi MORO³
Julia Amanda Rodrigues FRACASSO⁴
Mauricio Fogaça AZEVEDO¹
Mateus Vidigal DE CASTRO³
Regina Coeli dos Santos GOLDEBERG²
Francislaine Aparecida dos Reis
LIVERO⁵ Marlon Lemos DIAS²
João Tadeu RIBEIRO-PAES⁶

Affiliations:

¹Department of Genetics, Ribeirão Preto Medical-School, University of São Paulo, Ribeirão Preto, SP, Brazil ²Precision Medicine Research Center; Carlos Chagas Filho Biophysics Institute, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil. ³Human Genome and Stem Cell Research Center, Institute of Biosciences, University of São Paulo, São Paulo, SP, Brazil. ⁴Department of Biotechnology, School of Sciences and Languages, São Paulo State University (UNESP), Assis 19806-900, Brazil ⁵Laboratory of Cardiometabolic Pharmacology, Federal University of Paraná, Curitiba, PR, Brazil ⁶Laboratory of Genetic and Cell Therapy, Department of Biotechnology, Science and Letter School, São Paulo State University, Assis, SP, Brazil

Keywords: Drug screening. Liver. MAFLD. Spheroids. Therapeutics.

Abstract:

Metabolic dysfunction-associated steatotic liver disease (MASLD) is a highly prevalent condition associated with obesity, insulin resistance, and chronic hepatic inflammation. The development of in vitro models that reproduce key features of the hepatic microenvironment is essential for studying disease mechanisms and evaluating therapeutic strategies. In this study, a three-dimensional hepatic steatosis model was standardized using co-culture spheroids composed of 10⁴ HepG2 and LX2 cells (n=48), generated in concave-bottom 96-well plates coated with 2% agarose. After formation, steatosis was induced by exposure to 2 mM oleic acid conjugated with 10% BSA for 48 h. Morphological characterization included volumetric analysis ($V = 4/3\pi R^3$), confirming homogeneous spheroid formation and sphericity values close to 1. Oil Red O staining, performed in spheroids and monolayer cultures, confirmed peripheral lipid accumulation. Spheroids were fixed for immunofluorescence and histological analysis (H&E). Steatotic spheroids (SS) exhibited positive staining for α -SMA and Col1a2, markers associated with LX2 hepatic stellate cell activation into a myofibroblastic phenotype, along with reduced E-cadherin, indicating fibrogenesis and epithelial disorganization. H&E analysis revealed ballooning and intracellular lipid accumulation, consistent with advanced steatosis. To evaluate the model's potential for compound screening, SS were treated for 48 h with three concentrations (40, 80, and 100 μ g/mL)



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

of *Pereskia grandifolia* extract. Treated spheroids exhibited weaker immunostaining for α -SMA and Col1 α 2 at all concentrations compared to untreated SS. Additionally, CYP3A4 immunostaining, weakly detected in SS, was more evident in treated and control spheroids, suggesting a partial reversion of the steatotic phenotype. These results support the utility of this 3D model for MASLD research and phytotherapeutic screening.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Therapeutic Potential of Ora-Pro-Nóbis (*Pereskia aculeata*) Extract Supplementation in Ischemia/Reperfusion-Induced Muscle Injury

Authors:

Bianca Miranda VITAL¹
Wagner CORSINI¹,
Luis Felipe CUNHA¹
Marcos ZAN¹
Túlio de Almeida Hermes¹.

Affiliations:

¹Federal University of Alfenas, Anatomy Department – UNIFAL – MG.

Keywords: Ischemia and reperfusion. Muscle injury. Skeletal muscle. *Pereskia aculeata*

Abstract:

The ischemia-reperfusion injury (I/R injury) can be caused by compression or crushing, the application of tourniquets, orthopedic procedures, transplants, among others. There is also an accumulation of free radicals and reactive oxygen species (ROS), which can cause damage to skeletal muscle tissue and apoptosis. The hypoxia caused by ischemia prevents ATP production, and the cell fails to generate sufficient levels of antioxidant agents to maintain balance. Reperfusion further induces the inflammatory process and oxidative stress, worsening the injury. Six animals per group were used (CEUA no. 0039/2023) to investigate whether treatment with the hydroalcoholic extract from the leaf and stem of the Latin American plant

P. aculeata reduces inflammation and has antioxidant effects on I/R injury. The biochemical markers ALT, AST, CK, and urea were analyzed from the serum. Histological analyses showed that the leaf extract did not cause toxicity; however, they indicated a reduction in the mesangial area of the kidneys and disorganization of the hepatic parenchyma of the stem extract.. The analysis of the gastrocnemius and soleus muscles showed an increase in damaged fibers in the injured groups. Regarding treatments with dipyrone and the stem extract, the leaf extract proved to be more beneficial in repairing damaged muscle fibers and the mesangial area. CK levels ranged from 266.23±210.58 to 3352.56±3055.53 U/L, with changes up to +469.05% and -54.80%. ALT ranged from 27.37±6.48 to 73.33±34.92 U/L (+105.83% to -23.17%) and AST from 23.49±19.39 to 232.70±138.43 U/L (+502.62%). Damaged fibers in the gastrocnemius reached 75.72±3.98% (+623.36%) and in the soleus 72.02±6.96% (+2898.33%). Our current results suggest that *P. aculeata* specifically improves muscle dysfunction caused by IR, showing a distinct effect compared to the stem extract.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Technological development of a purified, high-fat, pelletized experimental diet for laboratory rodents

Authors:

Letícia Maria Paula da SILVA¹
Julia Bôro de LIMA¹
Camila Venturini Ayres CUNHA¹
Leandro Kansuke OHAROMARI¹
Susana Castelo Branco Ramos NAKANDAKARI²
Adelino Sanchez Ramos da SILVA¹
Eduardo Rochete ROPELLE¹
José Rodrigo PAULI¹
Maria Cristina Chiarinelli Nucci MASCARENHAS¹
Chiu Chih MING¹
Dennys Esper Corrêa CINTRA¹

Affiliations:

¹ Universidade Estadual de Campinas - Unicamp ²
Yale

Keywords: AIN-93. High-fat diet. Mice. Obesity. Pellet.

Abstract:

High-fat diets are widely used in the development of experimental models of obesity and related comorbidities. However, their high fat content results in a pasty, overly soft texture, which may compromise the experiment by interfering with food intake, animal behavior, shelf life, cage hygiene, and diet integrity. This study aimed to improve the texture of the traditional high-fat diet to produce durable and resistant pellets that promote gnawing, as the diet is intended for rodents. Fifteen new formulations were developed, following American Institute of Nutrition standards, varying amounts of solids, fat, temperature, processing, and a structuring agent developed in our lab. These were analyzed for consistency, snap force, water activity, and oil exudation. The three most prominent were tested in an animal model approved by the Ethics Committee (CEUA 6XXX-1/2023), using four-week-old male Swiss mice fed experimental diets for 8 weeks to assess obesogenic efficiency compared to the softened high-fat diet and a normocaloric commercial control. Groups: normocaloric diet (A), soft diet 1 (B), new soft diet 2 (C), new pelletized diets 3 (D) and 4 (E). Statistical analysis: ANOVA and Tukey's test ($p \leq 0.05$). Material choice and processing affected pellet hardness, with granulation and ingredient order having more impact than dextrinized starch or cellulose content. All diets showed low water activity. Pelletized diets (D/E) had low oil exudation, with minimal fat loss. Animals fed new formulations showed greater weight gain, waist circumference, and fat mass versus groups A and B. The results suggest successful obesity induction, improved handling, reduced contamination diet, lower loss of caloric density, and greater intake accuracy. Biomolecular tests are needed to confirm metabolic success.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Bacteriological profile of mice and rats in Brazilian animal facilities

Authors:

Jhenifer Alves de CAMARGO¹
Júlia Zago CASTELLI¹
Daniel GIANFRANCISCO¹
Bruna Rafaella Zanardi PALERMO¹
Joana Leticia Alves LACERDA¹
Daniele Masselli Rodrigues DEMOLIN¹

Affiliations:

¹ Centro Multidisciplinar para Investigação Biológica na Área de Ciência em Animais de Laboratório - CEMIB/UNICAMP -São Paulo.

Keywords: Health monitoring, Bioethics, Bacteria, Agents, Brazil.

Abstract:

The presence of pathogenic and opportunistic bacteria in laboratory rodents poses a significant threat to the scientific validity of biomedical experiments, introducing an uncontrolled variable that may affect experimental outcomes. This study aimed to assess the incidence and prevalence of these microorganisms in rodents housed at 17 Brazilian animal facilities, monitored in 2024 by the Animal Health Quality Control Laboratory (LCQSA) at the Multidisciplinary Center for Biological Research in Laboratory Animal Science (CEMIB) at UNICAMP. A total of 282 animals and biological samples were analyzed using microbiological culture and polymerase chain reaction (PCR). The most prevalent bacteria detected were *Helicobacter spp.* (44.7%), *Rodentibacter spp.* (23.7%), *Staphylococcus aureus* (17.4%), and *Klebsiella pneumoniae* (8.3%). Other relevant isolates included *Pseudomonas aeruginosa*, *Streptobacillus moniliformis*, and *Filobacterium rodentium*. The bacteriological profiling revealed a high frequency of opportunistic and pathogenic agents, many of which are highlighted in FELASA recommendations, suggesting the use of animals with inadequate sanitary status in scientific experiments, compromising their reproducibility. These findings underscore the urgent need for standardized sanitary monitoring and strict bioexclusion practices in animal facilities to ensure experimental reliability and compliance with ethical standards. This study was approved by the Ethics Committee on Animal Use of UNICAMP (CEUA No. 6224-1/2023), in accordance with current guidelines for laboratory animal research.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Tocilizumab, a monoclonal antibody that may prevent acute kidney injury

Authors:

Julia Matheus TSUCHIYA¹
Juliana Veloso Gusmão SILVA¹
Mykelly Gomes ALVES¹
Pedro Henrique Rodrigues dos SANTOS¹
Eloiza de Oliveira SILVA¹
Maria de Fátima Fernandes VÁTIMO¹

Affiliations:

¹Escola de Enfermagem da Universidade de São Paulo – USP, SP/SP –
Brasil.juliana.veloso.silva@usp.br

Keywords: Acute kidney injury; Monoclonal antibody and Ischemia

Abstract:

According to Kidney Disease: Improving Global Outcomes (KDIGO, 2012), during hospitalization, 1 in 5 adults (21.6%) and 1 in 3 children (33.7%) developed acute kidney injury (AKI), caused by renal insults that lead to oxidative stress. In this context, Tocilizumab is a monoclonal antibody that inhibits interleukin-6, initially used for rheumatoid arthritis treatment and later tested for COVID-19 therapy. Objective: To evaluate the effect of Tocilizumab on renal function and the oxidative profile of rats with ischemic AKI (iAKI). Methodology: This is an experimental study with a quantitative approach, using adult male Wistar rats divided into 4 groups (N=7). Groups: Control (SHAM); TCZ (Tocilizumab; 4mg/kg, i.p.; single dose); Ischemia and Reperfusion (I/R; clamping of renal pedicles); and TCZ+I/R (Tocilizumab treatment followed by ischemia). Renal function was assessed through serum and urinary creatinine, as well as the oxidative profile. This study was approved by the Animal Ethics Committee (CEUA), protocol no. 2014/2023. Results are presented as (mean ± SD; *p ≤ 0.0001, t-test). The findings showed statistically significant differences according to these tests. Analyses showed a significant reduction in serum creatinine levels and increased inulin clearance in the TCZ+I/R group compared to the I/R group. Regarding the oxidative profile, assessed by FOX and TBARS methods, an increase in urinary peroxides was observed in the TCZ group compared to the SHAM group, while a significant reduction was noted in the TCZ+I/R group compared to the I/R group. The urinary nitrate test revealed lower expression in the TCZ+I/R group. Similarly, the thiol test demonstrated reduced antioxidant activity in the TCZ+I/R group compared to the treated control groups. Conclusion: Tocilizumab confirmed a renoprotective effect, improving renal function and reducing oxidative activity, presenting itself as a potential adjuvant in conditions of renal ischemia risk.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Evaluation of chemotherapy response in precursor B-cell acute lymphoblastic leukemias (B-ALL) with different transcriptional profiles in the II7rCPT in vivo model

Authors:

Mariana Dias¹
Mayra Gabriella Simplício Bomfim²
Juliana Ronchi Corrêa³
José Andres Yunes³

Affiliations:

¹Centro Universitário da Fundação Hermínio Ometto
²Universidade Estadual de Campinas
³Centro Infantil Boldrini

Keywords: Interleukin-7 receptor. Precursor B-cell acute lymphoblastic leucemia. Transcription profile. Treatment resistance.

Abstract:

Acute Lymphoblastic Leukemia (ALL) is the most common childhood cancer, characterized by the uncontrolled proliferation of immature hematopoietic cells. Mutations in the interleukin-7 receptor (IL7R) are observed in both T- and B-cell leukemias, making it a relevant target for investigation. This study evaluated the chemotherapy response of precursor B-cell leukemias with different transcriptional profiles in the II7rCPT in vivo model. Leukemic cells from the II7rCPT model, classified into different transcriptional profiles (Vav_32 and CD19_39) were used. Cells were thawed, recovered, and intravenously injected into C57BL/6 mice, following protocol CEUA 0002/2019. The animals were monitored weekly through blood counts to track disease progression. After leukemia was established, the mice were euthanized in accordance with ethical guidelines, and the spleens were collected for leukemic cell isolation. Samples were subjected to MTT cell viability assay to assess sensitivity to chemotherapeutic agents Vincristine (VINC), Dexamethasone (DEXA), and Methotrexate (MTX). Absorbance was measured using the Synergy H1 Hybrid Reader, allowing calculation of IC50 values. Vav_32 cells were resistant to MTX, while CD19_39 cells were sensitive to the drug. Both responded similarly to DEXA and VINC, with low IC50 values, suggesting sensitivity to the treatment. Sensitivity to MTX varied among leukemia profiles, possibly reflecting differences in proliferation rates. Based on the drug's mechanism of action, we infer that Vav_32 leukemic cells undergo fewer cell divisions, while CD19_39 cells display a more proliferative profile, which may influence treatment response.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: It's not rocket science: talking to society about aging factors on social media

Authors:

Gisele DE SOUSA¹

Sheila S. ANDRADE² Alessandra V. S. FARIA³

Affiliations:

¹Método Once, Ribeirão Preto, São Paulo, Brazil

²Department of Biochemistry and Tissue Biology, Institute of Biology, Universidade Estadual de Campinas (UNICAMP), Campinas, São Paulo, Brazil

Keywords: Biology. Science outreach. Storytelling.

Abstract:

In today's digital world, misinformation spreads rapidly, highlighting the need for scientific marketing to amplify expert voices. To bridge academia and society, an Instagram post was created to promote the article "Decoding the impact of ageing and environmental stressors on skin cell communication." This study explores how pollution-induced molecular damage disrupts skin cell processes, leading to premature aging and chronic inflammation. The post was created with the use of related images and explanatory text in the caption presenting concepts about the cellular and molecular biology of the skin in a didactic way for the public to understand and within marketing strategies with the goal to set it apart from so many contents on the media. The post begins with the headline "Proteção sem complicação, o segredo da pele saudável", as a strategy to attract the attention of readers. In addition, the caption also had a warning: "você está ficando velha!". After these calls to action, the informative text followed by presenting concepts discussed in the article that was promoted. The results were very successful, since the profile on which the campaign was promoted has 664 followers, achieving 1088 views and 100 interactions after just 24 hours of posting. The engagement rate of 15.06%, representing the percentage of the account's total followers who interacted with it. This is considered an excellent rate, above the general average in many industries (between 1% and 5% on networks like Instagram, depending on the niche and follower base). Reaching a total of 48.54% among people who viewed the post. This indicator is relevant for assessing the quality of content, especially in marketing strategies aimed at well-defined target audiences, considering that values above 20% are generally excellent. Based on the marketing strategies used to produce the content, we were able to validate our model, published in De Sousa et al, 2024. Furthermore, the success of the publication demonstrates that it is possible to share scientific impact beyond the lab, thus, communicating science to society, with scientific evidence, and the effectiveness of this communication depends on the researcher knowing: the dynamics of their target audience and the channel of powerful communication.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Neuroscience in Teacher Training: Enhancing Pedagogical Innovation for Adolescent Education

Authors:

Ana Luiza Trombini TADIELO¹

Bruna Tarasuk Trein CRESPO¹

Pâmela Billig MELLO-CARPES¹

Affiliations:

¹ Federal University of Pampa, Uruguaiana, RS, Brazil

Keywords: Neuroscience; Teacher Training; Executive Functions; Neuroeducation; Pedagogical Innovation.

Abstract:

Understanding neuroscience can improve the educational strategies choice by aligning teaching practices with students' cognitive and emotional development. This study evaluates the impact of a continuing professional development course in neuroscience for schoolteachers, focusing on adolescent learning. The course aimed to enhance teachers' knowledge of executive functions, neuroplasticity, memory, and emotional regulation, fostering their application in pedagogical innovation. The program involved 40 participants selected from 71 applicants, with 26 completing the training. Teachers' neuroscience knowledge was assessed before and after the course (Ethics Committee 83043524.9.0000.5323). The results indicated a significant knowledge improvement ($p = 0.0391$, Wilcoxon test), with correct answers increasing from 50% to 80.76% for executive functions and from 52.5% to 88.46% for short-term memory comprehension. Understanding of neuroplasticity also improved (72.5% to 96.15%), reinforcing the importance of pedagogical choices in shaping students' neural development. The program also strengthened teachers' comprehension of emotional processing, with correct responses increasing from 17.5% to 88.46%. However, the inhibitory control knowledge showed a decline (52.5% to 38.46%), highlighting an area for further training. A qualitative analysis of teachers' reflections after the course revealed three core themes: (1) Pedagogical adaptation, emphasizing the need for personalized teaching strategies; (2) Balancing tradition and innovation, integrating neuroscience insights with existing educational frameworks; (3) Active participation, reinforcing student-centered learning as crucial for knowledge retention. Neuroscience-based teacher training enhances conceptual understanding, pedagogical application, and the creation of effective, emotionally safe learning environments, warranting further research on long-term impacts. Financial support: CNPq. Unipampa.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: The Impact of Neuroscience Outreach through Neuroblitzes in Primary Schools in Uruguaiana/RS

Authors:

Tamires dos Santos PAIVA¹
Pamella Andrea Pinto LARANJA²
Luciano Silva JUNIO³
Ana Luiza Trombini TADIELO⁴
Pâmela Billig MELLO CARPES

Affiliations:

Universidade Federal do Pampa

Keywords: Neuroscience, Education, Pedagogical Innovation.

Abstract:

Neuroscience has been increasingly integrated into education, expanding the understanding of the brain and contributing to more effective teaching and learning. The POPNEURO Outreach Program promotes the popularization of neuroscience and combats educational neuromyths. This study evaluated the impact of Neuroblitzes, scientific outreach activities conducted in public schools in Uruguaiana/RS, on students' knowledge and content retention (CEP 3.058.377). The activities took place over eight weeks, and featuring interactive and dynamic lessons on brain function. The topics covered included: (1) Who can be a scientist?; (2) Introduction to the nervous system; (3) Neurons and synapses; (4) Memory and learning; (5) Brain health; (6) Nervous system and senses; (7) Technology and the brain; and (8) Emotions. Each session followed a structured format: (1) pre-questionnaire; (2) lecture-style class; (3) practical activity; and (4) post-questionnaire. The initiative involved 76 primary school students. The average score in the pre-questionnaires was 53.7% (SD = 17.0), increasing to 59.0% (SD = 19.3) in the post-questionnaire ($p = 0.00299$, Wilcoxon test). For illustration, in lesson 4, the neuroplasticity understanding increased from 48.6% ($n = 37$) to 83.2% ($n = 63$). In lesson 5, knowledge about the relationship between sleep and cognition improved from 55.2% ($n = 42$) to 91.4% ($n = 69$). Content retention also improved, with the average self-perceived retention increasing from 45.8% to 72.3%. The results indicate that the interactive approach of Neuroblitzes contributed to knowledge consolidation and the correction of neuromyths, strengthening the role of scientific outreach in primary education. Neuroblitzes have proven to be an effective strategy for the popularization of neuroscience in education, promoting learning. The theoretical-practical methodology enhanced knowledge retention and student engagement, reinforcing the importance of scientific outreach in schools.

Financial support: PROEC/Unipampa. AGIPAMPA/Unipampa. CNPq.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: DoHAD Bulletin: a bulletin for the promotion of science and culture

Authors:

Bárbara Alves MARIZ¹
Otávio Augusto Silva COSTA¹ Maria Clara Rocha Vaz de MELO¹ Fernanda Júlia Rosa SILVA¹
Ana Clara Ribeiro Rezende VILELA¹ Iago Emanuel Fernandes METZKER¹ Luys Antônio
Vasconcelos CAETANO¹ Rafael de Souza LOPES¹
Luana Lohane Figueiredo de AGUILAR¹ Fernanda Castro SOARES¹
Júlia Tanure NERY¹ Ágatha Fraga FERREIRA¹ Fernando FELICIONI¹

Affiliations:

¹School of Medicine, Atenas University Centre, Prefeito Alberto Moura, 6000, 35701-383. Sete Lagoas, MG, Brazil.

Keywords: Culture. Scientific diffusion. DOHaD. Education. Nobel prize.

Abstract:

PISA (Programme for International Student Assessment) metrics highlight weaknesses in Brazil's basic education. In 2018, the average reading score was 413 points, and 66% of students did not read texts longer than 10 pages. In 2022, the country scored 410 points and ranked 62nd in science, reflecting challenges in higher education due to a limited academic and cultural repertoire. Undergraduate programs should serve as strategic spaces to bridge educational gaps. Believing that reading, cinema, and historical reflection enrich academic training, this study presents the methodology of the DOHaD (Developmental Origins of Health and Disease) Bulletin, guided by the motto of promoting science and culture. Each edition follows the theme of the Nobel Prize in Physiology or Medicine, inspired by all awards since the first in 1901. The advising professor serves as editor, while 12 rotating students are responsible for the sections: Editorial, which introduces the edition, with its student serving as the co-editor of the Bulletin; DOHaD, discussing a postnatal condition with prenatal origins; Nobel Prize, presenting the laureate and their research; Specialist, where a professor analyzes the awarded discovery's current implications; and Cultural, recommending a film or documentary. So far, 16 editions have been produced, 14 covering the Nobel Prizes from 1901 to 1914, each with three pages, and two special editions: one on the 2024 Nobel Prize and another on death and mourning, contextualizing World War I. The bulletin is distributed in print, displayed on institutional panels, and hosted on Google Sites, reaching 839 students. It is also promoted via Instagram, with 108 followers. Over 100 editions remain to be produced. Quantitative and qualitative assessments will be conducted to evaluate its impact on students' academic and cultural development.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Engaging Cell Biology Students through Interactive and Contextualized Online Resources

Authors:

Isaias Cavalcante de OLIVEIRA¹

Isabela Souza Xavier da SILVA²

Fábio SIVIERO³

Affiliations:

¹ Instituto de Biociências (IB - USP)

² Escola de Comunicações e Artes (ECA - USP)

³ Instituto de Ciências Biomédicas (ICB - USP)

Keywords: Cell Biology. Education. Educational Technology. Interactive Learning. Scientific Literacy.

Abstract:

Cell Biology is introduced as early as high school and continues through higher education, forming a cornerstone of the life and health sciences. However, the highly descriptive nature of the subject, combined with varied teaching methods, can diminish student engagement and lead to uneven comprehension, especially at the undergraduate level. The Interactive Atlas of Cell Biology (ATIN, <https://atin.icb.usp.br/>) addresses these challenges by contextualizing cellular concepts within real-world physiological and health scenarios, thereby emphasizing their relevance. ATIN's regularly updated materials, grounded in classic academic texts and peer-reviewed research, are presented in accessible language. Organized into thematic areas — ultrastructure, biological processes, clinical correlations, and exercises— the platform supports both guided exploration and self-directed learning. Students benefit from infographics, images, interactive H5P activities, and downloadable print materials designed to foster inclusivity and assist those with limited internet access. Instructors can adopt ATIN as a course complementary reference or foundation for active learning activities, given its alignment with the usual courses programs. As a free-access platform, ATIN attracts approximately six thousand monthly visits, with 91% originating from Brazil and 6% from other Portuguese-speaking regions, underscoring the importance of assessing user comprehension and providing inclusive, adaptable content. This steady growth affirms ATIN's effectiveness as a dynamic, engaging tool for deepening cell biology understanding and enhancing scientific literacy—without sacrificing scientific rigor.

Acknowledgments: PRG-USP, ICB-USP.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: VHISTO: A mobile and web application for histology learning in undergraduate education

Authors:

Bruna ROMANA-SOUZA¹
Adilmar Coelho DANTAS²
Mário João JUNIOR³
Marcelo Zanchetta do NASCIMENTO²

Affiliations:

¹ Department of Histology and Embryology, Rio de Janeiro State University
² College of Computer Science, Federal University of Uberlândia
³ School of Medicine, Rio de Janeiro State University

Keywords: histology education, virtual microscopy, undergraduate education

Abstract:

Virtual microscopy (VM) is a technology for showing microscope slides using computers. In Brazil, the implementation of VM is based on the analysis of digitalized glass slides using a specific software in a laboratory. However, students cannot access the digital material outside the university, complicating the revision of the slides at home. Therefore, our group developed a new multiplatform mobile and web application aimed to the virtual study of histological slides, called VHISTO. This study evaluated the effectiveness of the VHISTO on undergraduate students' performance and satisfaction. Between 2023 and 2024, nursing students (n=168) who took the histology course were included in the study and signed the consent (CAAE: 69684217.7.0000.5259). The participants were invited to answer a 26-question survey, previously validated (Cronbach's alpha = 0.65), which verified student's perceptions, such as handling, suitability, learning effectiveness and academic performance. In all semesters, there was a higher number of female (n=136) than male (n=32) (Z score = 11.35, p<0.0001) with an average of 22.2±5.2-year-old (Kruskal-Wallis test, p=0.0033). All participants had at least one mobile device (such as smartphone or tablet) and one type of internet connection (such as wireless or mobile) to access the application. The majority of students reported that they used the application to revise the slides once a week for 5-30 minutes at home or university. The subjective impressions of the students showed that the application was easy to use and had images with outstanding quality. In addition, the application helped them to revise the slides and to study for histology tests. Nonetheless, the participants also described that the application did not increase their interest for histology and some students said that they prefer to use the traditional microscopy. However, the use of application interfered in the rate of students' approval in the histology course. Using students' score tests, it was observed that higher numbers of accesses to application were related to higher scores in the histology tests (Cohen's $d = 1.13$). In conclusion, the VHISTO is a suitable device for histology learning that has a good acceptability of the students and contributes to undergraduate education. Financial support: FAPERJ



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Empowering Undergraduates to Confront Cancer Misinformation: An Interdisciplinary Project-Based Educommunication Approach

Authors:

Fábio SIVIERO¹ Agnaldo ARROIO²
André Chaves de Melo SILVA³ Claudemir Edson VIANA³
Fernando Rodrigues de Moraes ABDULKADER¹ Mariana Kiomy OSAKO⁴
Roger CHAMMAS⁵
Guilherme Andrade MARSON⁶

Affiliations:

¹Instituto de Ciências Biomédicas - USP,
²Faculdade de Educação - USP,
³Escola De Comunicações e Artes - USP, ⁴Faculdade de Medicina de Ribeirão Preto - USP,
⁵Faculdade de Medicina - USP,
⁶Instituto de Química - USP.

Keywords: Cancer Misinformation. Health Communication. Higher Education. Precision Oncology. Project-Based Approach.

Abstract:

Fighting misinformation is a major challenge in higher education, especially concerning cancer prevention and treatment. We developed an innovative online synchronous course (PRG0033-“We Need to Talk About Cancer: Interdisciplinary Projects to Combat Misinformation and Prejudice”) for 60 undergraduates from diverse fields at the Universidade de São Paulo. Using a project-based approach, teams of two to four students produced short, targeted videos to debunk common cancer-related misconceptions. The first half of the course was dedicated to theoretical foundations on oncology, scientific communication strategies, and media production techniques. Specialists from Comprehensive Center for Precision Oncology (C2PO) and six PhD students acting as teaching assistants offered collaborative support, and students also benefited from discussions with the staff of other successful misinformation-combat initiatives (NoX, Oncociência e Imprensa Jovem). Participants selected specific audiences and topics, including HPV and cancer, palliative care, natural vs. synthetic products, sugar consumption, electromagnetic radiation, diets, misinformation, prostate cancer, and e-cigarettes. The resulting videos are available at <https://tinyurl.com/C2POPGR0033>. Selected results from a structured questionnaire assessing students’ perceptions indicate that: 76% of the 29 undergraduates that completed the course reported newly developed interests in Science and Health, 97% adopted a more critical stance toward cancer-related information, and 90% felt more confident to communicate scientific knowledge or confront misinformation. Further stages in this project may include evaluating the perception of lay audiences regarding misinformation on cancer. The findings regarding learning objectives suggest that interdisciplinary, collaborative learning effectively enhances students’ ability to promote reliable health information. CAAE: 78308724.0.0000.5467. Acknowledgement: PRG-USP, C2PO.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Biomedical Action Project: new playful methodologies in the awakening of future scientists

Authors:

Julia Guedes MARETTI ^{1,1}
Letícia Cristina GONÇALVES ^{1,2}
Carlos Roberto Escrivão GRIGNOLI ^{1,3}
Cristina FRANCHINI ^{1,4}

Affiliations:

^{1,1} Discente do curso de Biomedicina do Centro Universitário da Fundação Hermínio Ometto-FHO, Araras/ SP.
^{1,2} Docente do curso de Biomedicina do Centro Universitário da Fundação Hermínio Ometto-FHO, Araras/ SP.
^{1,3} Docente e coordenador curso de Biomedicina do Centro Universitário da Fundação Hermínio Ometto- FHO, Araras/ SP.
^{1,4} Coordenadora da Pró-reitoria de comunidade e extensão do Centro Universitário da Fundação Hermínio Ometto- FHO, Araras/ SP.

Keywords: Biomedicine. Educational games. Playful learning. Scientific skills. University extension.

Abstract:

Biomedicine is essential for health promotion, yet its presence in basic education remains limited. To address this gap and enhance academic training, the Biomedical Action Project employs active methodologies with a playful approach, making learning more dynamic and accessible. The initiative incorporates educational games developed by students, such as Sangue Certo, #Partiu Jornada da Urinálise, Micro em Ação, and Toxicological Mystery, disseminating biomedical knowledge to children and adolescents. The project encompasses five thematic areas—blood, urinary, microbiological, toxicology, and integrative and complementary health practices (PICS)—distinguishing itself through the collective creation of educational materials and the direct application of activities in public and private schools. Furthermore, a new theme is developed each semester, fostering knowledge diversification and broadening students' perspectives on Biomedicine. Weekly meetings prepare biomedical students for school interactions, enhancing their communication, teaching, and scientific skills. The impacts are significant: students strengthen essential competencies such as scientific communication, professional conduct, and creativity while gaining practical teaching experience. For basic education students, educational games make learning more engaging, facilitate the assimilation of biomedical concepts, and spark interest in science. As a result, the school environment transforms into a space for experimentation and knowledge-building, bridging academic content with students' realities. Thus, Biomedical Action Project is not just an extension initiative but an innovative educational model, fostering the connection between university and community, encouraging critical thinking, and contributing to the development of future scientists and informed citizens aware of Biomedicine's importance.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: From the Brain to the Classroom: Neuroeducation and Pedagogical Innovation in Teacher Training

Authors:

Pâmella Andréa Machado da Silva Laranja PINTO¹

Ana Luiza Trombini TADIELO²

Tamires dos Santos PAIVA³

Pâmela Billig Mello CARPES

Affiliations:

¹Universidade Federal do Pampa ²Universidade Federal do Pampa ³Universidade Federal do Pampa; Universidade Federal do Pampa

Keywords: Neuroscience; Teacher Training; Executive Functions; Neuroeducation; Pedagogical Innovation.

Abstract:

The application of neuroscience in education can enhance teaching and learning. The POPNEURO Program integrates neuroscience and biosensors into teacher training, promoting innovative pedagogical practices based on scientific evidence. This study reports on the actions and outcomes of the 13th edition of the CNAE, analyzing its impact on the training of future teachers and their understanding of neuroscience concepts applied to pedagogical practice, with emphasis on biosensors as educational tools (Ethics Committee 83043524.9.0000.5323). The methodology involved active training of undergraduate students through a five-week in-person course, structured in modules on neuroscience, neuroplasticity, learning, and pedagogical innovation, using biosensors and the Lt platform. Initially enrolling 40 participants, the course was completed by 26, who answered initial, pre-, and post-course questionnaires. Discursive Textual Analysis (DTA) revealed significant improvement in conceptual understanding. Pre-course responses reflected a more intuitive view of neuroeducation, while post-course responses showed more structured reasoning, incorporating scientific knowledge on memory, attention, emotions, and evidence-based strategies such as spaced repetition, motivational environments, and sensor-based feedback. Statistical analysis using a paired t-test indicated a significant increase in performance: estimated pre-course accuracy was 83.0% (22.4 ± 14.2), rising to 93.1% post-course (25.1 ± 6.1), with a statistically significant difference ($t(7) = -2$).



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
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Title: Medical students' perception of the time spent on social media and its consequences on study time

Authors:

Cesar Cortez GUIMARÃES¹
José Guilherme Prado KATAOKA¹
Camila Linhares Taxini PASSOS¹
Luís Henrique MONTREZOR¹

Affiliations:

¹ Universidade de Araraquara - UNIARA

Keywords: Anxiety. Engagement. Perception of learning. Social media.

Abstract:

Information and Communication Technologies are tools used in the teaching-learning process. It is essential for teachers to know how and when to use such strategies and to take advantage of students' affinity and skills in using certain technologies. The objective of this study was to analyze medical students' perceptions regarding the time spent on social media and its consequences on study time. The study was approved by the Research Ethics Committee (CAAE 40019820.9.0000.5383). A total of 37 students participated in the study (14 men [37.83%] and 23 women [62.17%]), with an average age of 20 years, from the second and third semesters of the course. Voluntarily and anonymously, students answered an online questionnaire via Google Forms, which included questions about their behavior regarding social media usage. The results show that 97.3% of students own smartphones, which they received between the ages of 8 and 12. Additionally, 97.3% of students have between 2 and 4 social media profiles, which they created between the ages of 12 and 15. Only one student does not have a smartphone or social media profile. The most frequently used social media platforms are Instagram (35 students, 94.59%), TikTok (20 students, 50.05%), and WhatsApp (17 students, 45.94%). The perception of the time students spend on social media ranges from 2 hours (7 students, 18.91%) to 4 hours (10 students, 27.02%). A total of 32 students (86.48%) reported using social media for entertainment, interaction with peers, and obtaining information. However, 34 students (91.89%) stated that the time spent on social media causes anxiety due to not studying, a sense of procrastination, and difficulty concentrating on their studies. The results suggest that students use social media via smartphones for entertainment, interactions, and information. However, paradoxically, they perceive that the time spent on social media fragments their concentration, generates feelings of anxiety, and procrastination.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Obesity and endocrine disruptors: an e-book approach for teaching science in high school

Authors:

Jhonathan Marques DE-FILIPPI¹

Anatalia Kutianski Gonzalez

VIEIRA¹

Elizabeth Teixeira de Souza¹

Bruna Gonçalves¹

Affiliations:

¹ Universidade do Estado do Rio de Janeiro

Keywords: *E-book. Endocrine disruptors. Obesity.*

Abstract:

Obesity is a growing global concern, with projections from the World Obesity Atlas indicating a significant increase by 2035. In Brazil, an estimated one-third of children and adolescents will be affected. Beyond well-known factors such as poor diet and physical inactivity, recent evidence highlights the role of endocrine disruptors—chemical substances found in plastics, cosmetics, and canned foods—which make exposure control particularly challenging. Scientific education, supported by digital tools, plays a key role in raising awareness among young people about these issues. In response to this need, a digital e-book was developed to promote scientific literacy and foster interdisciplinary discussions on obesity and its associated factors. Created using Canva, the material comprises over 120 pages, featuring accessible texts, AI-generated illustrations, stock images, and various visual elements available on the platform. The content introduces the endocrine system, its main organs and structures, and explores its relationship with obesity and the influence of endocrine disruptors. It also presents didactic resources and complementary topics that support educational activities. The e-book was designed in accordance with the Brazilian National Common Curricular Base (BNCC), which defines core learning objectives across all stages of basic education, and with Digital Information and Communication Technologies (TDIC), referring to digital tools integrated into teaching practices. Aimed at high school students and teachers, the material serves as a versatile educational resource that can be incorporated into lesson plans or used for autonomous learning. It may contribute to the development of scientific thinking and a deeper understanding of the complex relationship between human health and environmental exposures.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Autonomy and satisfaction in human physiology: a study on student experiences in Brazil

Authors:

Fernando Sérgio OLIVEIRA¹

Maria Flávia Marques RIBEIRO²

Gislaine Alves de OLIVEIRA³

Bruno Dutra ARBO⁴

Affiliations:

¹ Postgraduate Program in Biological Sciences: Physiology – ICBS – UFRGS, Porto Alegre, RS

² Department of Physiology – ICBS – UFRGS, Porto Alegre, RS

³ Ceará State University, Quixeramobim, CE; ⁴ Department of Pharmacology – UFRGS, Porto Alegre, RS

Keywords: Autonomy. Human Physiology. Satisfaction. Students.

Abstract:

Human Physiology education is crucial in health science curricula as it promotes understanding of the body's complex functions. Although autonomy and satisfaction are essential for student learning, literature reveals a significant gap regarding students' autonomy and satisfaction in studying Physiology. Therefore, this study aimed to assess students' perceptions of autonomy and satisfaction in Human Physiology. An online questionnaire with 25 items was applied to 999 undergraduate students from public and private institutions who had coursed the subject. Key topics included students' views on their study autonomy and satisfaction with the discipline. Results showed that 33.1% of students reported good or excellent autonomy, while 41.4% rated their autonomy as poor or very poor. Furthermore, 60% stated they could not study without teacher assistance. Student satisfaction was nearly split, with 43.3% expressing satisfaction and 45.1% dissatisfaction. A closer analysis indicated that medical students, younger students, and those from federal institutions perceived greater autonomy compared to students from other courses, those over 30, and those from private institutions (76%, 40%, and 34.1% reporting good or excellent autonomy vs. 28.1%, 18.2%, and 18%, respectively). A similar pattern was observed for satisfaction: medical, younger, and public institution students scored higher than their counterparts did (80.3%, 51.2%, and 48.1% vs. 51.1%, 27.9%, and 21.2%). Statistical analysis showed a significant positive correlation between autonomy and satisfaction (Spearman $r = 0.574$; $P < 0.0001$). These findings suggest that students with greater autonomy exhibit higher satisfaction in the studying Human Physiology and underscore the need for educational strategies that enhance the development of autonomy. UFRGS Research Ethics Committee (CEP - UFRGS). Certificate of Ethical Appreciation Presentation (CAAE) – 65438122.1.0000.5347.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Recording and blocking of electric signal in annelids – a valuable teaching tool in physiology and pharmacology

Authors:

Sabrina Santos DAMIÃO¹ Roberto Queiroga LAUTNER²

Affiliations:

¹ Federal University of Juiz de Fora ² Federal University of Juiz de Fora

Keywords: Annelids. biophysics. pharmacology. physiology

Abstract:

The genesis of action potentials (AP) is an unequivocal manifestation of biophysics. A relatively simple way to obtain AP recordings is to use microelectrodes in annelids. This is possible due to the rudimentary nervous system of these invertebrates, which allows the detection of a voltage at the microelectrode terminals. Local anesthetic (LA) is capable of blocking sodium channels, preventing the genesis and conduction of the electrical signal. We have developed a teaching practice aimed at undergraduate and graduate courses in the biomedical areas. Methodology: the annelid was immersed in Ringer's solution with 5% ethanol for 5 minutes, then placed on an EVA plate and 3 electrodes were attached. After equilibration, it was subjected to a mechanical stimulus with a blunt-tipped needle. The electrical signal (millivolts) generated was recorded in PowerLab transducer and converted to a percentage value (control). The animal was then anesthetized with 0.3ml of lidocaine (10 mg/ml) and stimulated at minutes 1, 2, 3, 4, 5, 10, 15, 20, 25, 30 and 40. The electrical signals generated after each stimulus were recorded and the percentage values obtained. Results: A robust electrical signal was observed immediately after the control stimulus. In the 1st and 2nd minutes after the administration of LA, no reduction in the electrical signal was observed. From the 3rd minute onwards, there was a reduction (~10%). The decrease continued until the 10th minute, when the greatest reduction in the electrical signal was observed (~90%), remaining stable until the 20th minute, when the electrical signal was gradually reestablished. At 40 minutes, the electrical signal was similar to the control. Conclusion: The use of annelids in teaching integrates biophysics, physiology and pharmacology, allowing a theoretical-practical approach. The experiment highlighted the pharmacodynamic and pharmacokinetic aspects of LA, favoring interdisciplinary understanding.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Effect of Innovative Educational Strategies on Learning About Cells

Authors:

Karina R. Casale¹,
Camila Carvalho¹,
Julia F.A. Oliveira¹,
Laura T.C. Zégio¹,
Nikoly M.S. Brito¹,
Wesley M.S.
Soares¹, Amandha
C.S. Souza¹, Dianne
A. S. Munizz,
Fernanda K. Marcondes¹

Affiliations:

¹ Departamento de Biociências, Faculdade de Odontologia de Piracicaba – UNICAMP, Piracicaba – SP;

² Escola Estadual Professor José Martins de Toledo, Piracicaba – SP

Thematic axis: Education and Dissemination of Knowledge in Biomedical Sciences

Keywords: Basic education. Cell structure. Teaching materials

Abstract:

Attention difficulty and low student learning are major challenges in basic education in Brazil. In this context, active learning methodologies offer a promising approach as they increase interest and understanding of the studied topics. This study aimed to analyze the effect of educational materials on learning about cell structure, assessed through cognitive gain [(post-test – pre-test) / (10 – pre-test)]. Student perceptions of the didactic resources used were also analyzed. A total of 26 ninth-grade students from a public school in São Paulo (SP) participated in this research, upon signing the assent and informed consent forms, after approval by the institutional ethics committee. In lesson 1, the class teacher administered a pre-test and gave a theoretical lecture on eukaryotic and prokaryotic cells. The theoretical explanation combined the use of a blackboard, slides, and 3D models of animal and plant eukaryotic cells as well as prokaryotic cells, with movable organelles. The theoretical explanation continued in lessons 2 and 3, also including the use of cards with images of cellular structures in augmented reality, via an app, with students divided into groups. In lessons 4 and 5, a memory game with images and information about cell types and components was also used. In lesson 6, a post-test was applied, and each student rated, from 0 (did not help) to 10 (helped a lot), the contribution of the educational materials to their learning. The mean post-test (6.44 ± 1.07) was significantly higher than the pre-test (3.48 ± 1.37 , $p < 0.05$, paired Student's *t*-test). The cognitive gain was classified as moderate (0.45 ± 0.16), and the average rating for the contribution of the educational materials to learning was 8.15 ± 1.64 . These results indicate that the teaching strategies used have the potential to contribute to learning about cell structure.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Medical students' perception of Team-Based Learning (TBL) as a collaborative learning tool in Human Physiology

Authors:

Francisco Fábio Bezerra de OLIVEIRA^{1,2}

Maria Misrelma Moura BESSA²

Affiliations:

¹ Universidade Regional do Cariri

² Faculdade Paraíso Araripina

Keywords: Active learning. Collaboration. Human Physiology. Medical education. Team-Based Learning.

Abstract:

Team-Based Learning (TBL) is an active methodology that enhances the retention of curricular content in a dynamic and objective manner, while also encouraging collaboration among students an essential skill in medical education. This study aimed to investigate the perception of undergraduate medical students regarding TBL as a collaborative learning tool in the discipline of Human Physiology. Participants were second-semester students from the Medical School of Faculdade Paraíso Araripina (FAP-Araripina), enrolled in the curricular unit of Biological Functions, between March 2024 and March 2025. Students received preparatory study guides, completed individual tests in the classroom, and were then organized into small groups to discuss the questions and build a consensual group response. At the end of the sessions, they answered a questionnaire with four items assessing various aspects of the methodology. The study was approved by the Research Ethics Committee of Centro Universitário de Juazeiro do Norte (CEP-UNIJUAZEIRO), under opinion number 6.319.172. Results showed that 98.5% of participants approved the use of TBL; 92.6% reported improved understanding of the content; 96.2% stated that group discussions significantly contributed to their learning; and 70.6% affirmed that their doubts were clarified during the activity. Additionally, a significant improvement in group performance was observed when compared to individual performance ($p < 0.01$). It is concluded that TBL fosters student engagement, autonomy, and learning, promoting a more active and collaborative approach. The methodology proved effective in consolidating knowledge in Human Physiology and should be encouraged as a teaching strategy in medical education.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Toxicology in high school textbooks

Authors:

Lucas Michel Pereira MARTINS¹
Karolina Lima QUEIROZ¹
Samantha Crisóstomo RODRIGUES¹
Leticia Priscilla ARANTES¹

Affiliations:

¹Universidade do Estado de Minas Gerais – Unidade Passos

Thematic axis: Education and Dissemination of Knowledge in Biomedical Sciences

Keywords: Biology Education. Chemistry Education. National Common Core Curriculum. National Textbook Program. Natural Sciences.

Abstract:

The daily contact with various substances and the risk of poisoning highlight the importance of disseminating knowledge in Toxicology and its close relationship with the skills that should be developed in schools. This study aims to investigate how Toxicology is presented in High School textbooks of Natural Sciences approved by the 2021 National Textbook Program (currently in use). The adopted research method was an exploratory bibliographic analysis with a qualitative approach. To date, 3 out of 7 collections have been analyzed: *Ciências da Natureza* – Lopes & Rosso (Editora Moderna), *Multiversos - Ciências da Natureza* (FTD), and *Diálogo – Ciências da Natureza e suas Tecnologias* (Moderna), each consisting of 6 books. Biology, Chemistry, and Physics are treated in an interdisciplinary manner and are well contextualized, with relevant and up-to-date examples from life routine. Significant discussions were identified especially about Environmental Toxicology (pollutants and environmental impacts), Social Toxicology (legal and illegal drugs), and Occupational Toxicology (use of personal protective equipment). However, several gaps were detected. Topics associated with the workplace environment lacked examples of toxin exposure that are often overlooked. None of the collections included examples of poisonous animals and plants or provided guidance on care and first aid. Furthermore, toxicity was only associated with the risk of death, without exemplifying signs of intoxication. It was not mentioned that some toxicants could be beneficial in certain situations, such as insecticides and medicines derived from toxins. Although the skills related to Toxicology proposed by the National Common Core Curriculum were addressed in the High School textbooks, many topics could be better explored to promote and maintain health in the community.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Science Laboratories in High School: Advances and Setbacks Throughout the 2000

Authors:

Dryelle Rodrigues de OLIVEIRA¹
Gustavo Diniz de MESQUITA-TAVEIRA¹
Juliana CAMACHO-PEREIRA¹

Affiliations:

Universidade Federal do Rio de Janeiro¹

Keywords: School Census, School Infrastructure, School Science Laboratory

Abstract:

Scientific education is essential for promoting scientific literacy and developing critical thinking. School science laboratories play a fundamental role in strengthening this learning process. Between 2000 and 2023, education investments fluctuated due to changes in federal administration, reflecting different government priorities over the years. This study investigates the distribution of science laboratories in Brazilian high schools during this period, based on data from the School Census, which includes the total number of schools and the number of institutions equipped with this resource. The results show that between 2000 and 2016, there was continuous growth in the number of schools with laboratories, increasing from 4,678 to 8,562—an 83% rise. This progress reflected policies aimed at expanding educational infrastructure. However, from 2016 onward, a significant decline was observed, coinciding with a period of political instability and reduced education investments, which directly affected school infrastructure. The sharpest drop occurred in 2018, when the number of schools with laboratories fell to 7,362—a 14% reduction compared to 2016. In 2022, there was another decline of 5.1%, resulting in 8,300 schools. In 2023, we observed an increase of 5.6%, totaling 8,768 schools. Furthermore, additional research is needed to determine whether schools with laboratories effectively use these facilities in teaching.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Take a look: Scientific dissemination about eye health and research in vision science

Authors:

Lucas Kunio HIGUTO¹
Eduardo Melani ROCHA¹
Luccas Castilho Toledo De Moura LACERDA²
João Victor de Oliveira BARCALA²
Olívia Maria pissoloto LONETTO²

Affiliations:

¹Faculdade de Medicina de Ribeirão Preto – USP
² Colégio estadual Cid de Oliveira Leite

Keywords: book. Internet. scientific dissemination. eye Health. eye disease

Abstract:

The research area utilizes conventional dissemination channels such as radio, TV, and digital platforms to share established and proven knowledge, technologies, and disease prevention methods with the public. Rádio USP, maintained by the University of São Paulo, has been running a science communication segment on visual health called "Fique de Olho" since 2017, covering topics related to vision and eye health weekly. The population plays a fundamental role in amplifying knowledge and sustaining educational and research institutions, which ultimately support organizations dedicated to knowledge production and service provision. Among these services is the scientific dissemination of health-related topics, such as climate and environmental disorders. The objective is to bring relevant topics and technologies into discussions with students and to compile bibliographic material from the "Fique de Olho" segment for the publication of a book. This work is a science communication initiative and does not involve human research interventions. The study involved weekly meetings to discuss topics, bibliographic research, media application testing, and monitoring of community engagement. The preliminary discussion of topics with reliable sources, the production process, and the follow-up on audience response through meetings and digital tools will enhance the "Fique de Olho" segment and serve as a learning and academic enrichment opportunity for the students involved. Previously broadcasted radio segments were selected, transcribed using an appropriate application, and categorized by specific topics. Over six months, biweekly discussions were held to determine relevant subjects for the radio program, fostering students' engagement in both science communication and academic activities. Additionally, through digital applications, previous segments were reviewed and transcribed, leading to the organization of a book aimed at promoting eye health awareness among the general public.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: SintomaBot: an AI-powered virtual tutor for enhancing physiology-based clinical reasoning in health science education

Authors:

Camila Soares BEZERRA¹
Bruno de Peghin CARRAL¹
Nathalia Aparecida Zanata SOARES³
Alecia de Jesus ANDRADE¹
Camilo LELLIS-SANTOS¹

Affiliations:

¹ Department of Biological Sciences, Universidade Federal de São Paulo, campus Diadema.

Thematic axis: Education and Knowledge Dissemination in Biomedical Sciences.

Keywords: Active learning. Artificial Intelligence in Education. Clinical Reasoning. Health Science Education. Virtual Tutor.

Abstract:

The development of clinical reasoning relies on a strong understanding of health sciences, yet conventional teaching methods often provide direct answers without fostering critical thinking. This study, inspired by the rise of AI in education, aims to create a virtual tutor to assist students in developing diagnostic reasoning, with a focus on physiology. The study adopts a qualitative research and development approach. The chatbot was built using the Gemini AI framework and evaluated by STEM students using a 10-point Likert scale, with 10 as the highest score. The study was approved by the UNIFESP Research Ethics Committee (CAAE: 14020419.0.0000.5505). Named SintomaBot, the virtual tutor was developed in JavaScript and TypeScript. Unlike traditional chatbots with predefined responses, SintomaBot analyzes student-generated questions and offers feedback. Its key feature is AI's ability to assess the relevance of questions related to physiology cases, promoting further inquiry and reflection, thereby enhancing diagnostic questioning skills. SintomaBot is physiology-centered, making it suitable for any student enrolled in a physiology course, including those in exact sciences. It adapts to the user's knowledge level by integrating physiology concepts into problem-based learning (PBL). Pedagogical evaluations showed high ratings for its didactic effectiveness (9.0 ± 0.8), ability to overcome learning challenges (9.2 ± 0.8), and perceived learning enhancement (8.8 ± 0.8). However, interacting with the chatbot required dual cognitive effort to understand scientific concepts (5.6 ± 4.3). Results suggest that SintomaBot effectively stimulates clinical reasoning development. Further studies are needed to assess its effectiveness in clinical contexts or for learning physiology through clinical reasoning. Its implementation offers significant potential for training future healthcare professionals.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Use of Active methodologies to encourage the participation of caregivers of People with disabilities in the collaborative construction of knowledge

Authors:

Luciano Osio RAMOS¹
Alethéa Gatto BARSCHAK²
Sofia Castanheira PAIS³,
Lucila Ludmila Paula GUTIERREZ⁴

Affiliations:

- ¹ Universidade Federal de Ciências da Saúde de Porto Alegre
- ² Universidade Federal de Ciências da Saúde de Porto Alegre
- ³ Universidade do Porto
- ⁴ Universidade Federal de Ciências da Saúde de Porto Alegre

Keywords: Active methodologies. Health education. Informal caregivers for people with disabilities.

Abstract:

The use of active methodologies (AM) aimed at health education for caregivers of people with disabilities (PwD) indicates an effective strategy to encourage participation and joint construction of knowledge. This paper aims to report on the use of AM as a driver of the participation of caregivers of PwD in the collaborative construction of knowledge, with an emphasis on health education. The university extension project "Supporting and Educating Families of People with Disabilities" in partnership with the postgraduate program Teaching in Health, both from the Federal University of Health Sciences of Porto Alegre (UFCSPA), takes place together with a partner institution located in Porto Alegre/RS, which provides free health care for PwD from low-income families. This initiative is part of a research project approved by UFCSPA (CAAE 63424222.8.0000.5345). The team is composed of pharmacists and pharmacy, biomedicine, and psychology students. They work on health education issues with 15 mothers who care for PwD in the waiting room of the aforementioned institution, every 15 days, with 1 hour of activities using MA (brainstorming, workshops, board games, myth and truth boxes, and discussion groups). These activities covered a variety of topics, such as depression, STI prevention, and medication disposal, among others. Through a guiding question posed to the team ("How do you think the AMs used influence the activities we carry out?"), we sought the impressions of the working group formed by extension workers and researchers about 20 interventions carried out between May 2023 and September 2024. As a result, we collected reports such as: "I think AMs are very useful for opening a light and at the same time enriching conversation with the exchange of experiences", "Mothers feel included and therefore feel comfortable participating", and "The discussion circles enhance participation since they bring examples and share their experiences with other caregivers". Based on the perception of the working group, it is believed that the use of AM enhances the involvement of caregivers of PwD and provides a space for sharing previous knowledge and, consequently, for building new knowledge. In addition, it was noted that AMs played a significant role in the field of education.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Gamification as a strategy for teaching Viral Hepatitis to families of People with Disabilities

Authors:

Luciano Osio RAMOS¹
Alethéa Gatto BARSCHAK²
Sofia Castanheira PAIS³,
Lucila Ludmila Paula GUTIERREZ⁴

Affiliations:

- ¹ Universidade Federal de Ciências da Saúde de Porto Alegre
- ² Universidade Federal de Ciências da Saúde de Porto Alegre
- ³ Universidade do Porto
- ⁴ Universidade Federal de Ciências da Saúde de Porto Alegre

Keywords: Health education. Informal caregivers of people with disabilities. Popular education. Viral Hepatitis.

Abstract: The extension project "Supporting and Educating Families of People with Disabilities (PwD)" from the Federal University of Health Sciences of Porto Alegre (UFCSPA) provides health education to informal caregiver families of PwD who are awaiting free care for their dependents at Educandário São João Batista (Porto Alegre/RS). The objective of this work was to report an experience on the dissemination of knowledge about Viral Hepatitis (VH) through gamification, based on the popular health education model, which seeks to promote collective knowledge-building and encourage health promotion and disease prevention. This initiative is part of a research project approved by UFCSPA (CAAE 63424222.8.0000.5345). The activity happened in June 2023, where knowledge was collectively built among seven extensionists and the informal caregivers through a discussion circle, followed by the application of a board game. Ten informal caregivers of PwD were divided into pairs and questioned about the topic. When a pair answered correctly, they get the chance to roll the dice and move their game piece along the board path according to the number obtained. With each dice roll, concepts, treatments, and prevention strategies related to VH were discussed. This process continued until one of the pairs reached the end of the board, winning the game. It was observed that the game fostered engagement and motivation among participants. Some of the statements collected include: "I will get the vaccine because I don't know if I've taken it", "I won't lend my nail clippers anymore, now that I know alcohol doesn't kill HV" and "I will take my family to the health center". The results demonstrate that the participants became agents of change, actively seeking medical care and spreading health knowledge within their families and communities.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Teaching of Anatomy through the extension project Laboratório Didático de Anatomia (PEAnato): implications of inequality between public and private institutions

Authors:

Nicolli Fernandes MUNIZ¹
Anne Beatriz de SOUZA¹
Leticia Gebra TARDIVO¹
Izabelle de Souza SANTOS¹
Carolina Cadamuro MENARDI¹
Sergio PEREIRA¹

Affiliations:

¹ São Paulo State University (UNESP), School of Sciences (FC), Bauru, São Paulo, Brazil.

Thematic axis: Education and Dissemination of Knowledge in Biomedical Sciences.

Keywords: Education. Human Anatomy. University Extension.

Abstract:

The University Extension Project *Laboratório Didático de Anatomia* (PEAnato) has the main purpose of promoting Human Anatomy education for students from public and private schools by applying this knowledge through cadaveric specimens. Teachers can access the calendar and schedule visits, offering students a unique learning experience about the human body. During visits to the anatomy laboratory, cadaveric specimens are distributed on six tables throughout the lab, each representing a human body system – skeletal, muscular, circulatory, urinary, digestive and respiratory, genital, and nervous systems. Before entering the lab, the project coordinator emphasizes the importance of respecting cadaveric specimens and encourages visitors to ask questions during their visit. Visitors are provided with gloves so they can handle the material during explanations by monitors, integrating practice with theory. In 2023 and 2024, PEAnato received 53 visits, serving 1,760 people – 884 from private schools and 876 from public schools. Most students were from middle school (58.7%), while 41.3% were from high school. These results show that PEAnato welcomed a similar number of students from both school types. However, private schools more easily schedule last-minute visits, whereas public schools must plan well in advance due to transportation needs. This highlights regional inequalities between public and private schools and underscores a lack of state support for extracurricular experiences in public education.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Can active methodologies like scavenger hunt be interesting in learning physiology?

Authors:

Eduardo Benfatto BERNA¹ Emilly Brandão SCHUCK¹ Yasmin da Silva RODRIGUES¹ Marilene PORAWSKI¹
Lucila Ludmila Paula GUTIERREZ¹

Affiliations:

¹ Universidade Federal de Ciências da Saúde de Porto Alegre

Keywords: Active methodologies, Endocrine System, teaching-learning process.

Abstract:

Active methodologies are important to contemplate student learning, helping in the development of facilitating skills in the teaching-learning process. Thus, the "Endocrine System Scavenger Hunt" was developed and the objective of this study was to evaluate student self-perception of learning through the use of this methodology. The methodology is divided into four stages that encourage the study of hormones and endocrine glands. Students who took the Physiology discipline of the Pharmacy course at the Federal University of Health Sciences of Porto Alegre (n = 33) in 2023 and who signed the Free and Informed Consent Form were included (CAAE 53558718800005345). After classes on this system and as a review before the evaluation, the scavenger hunt was applied by the teacher and the students were divided into groups to participate in four activities, namely: "Hormone Slap"; the "Anatomy Hunt" with Balloons, the "Rounds of Cork marking" with questions and answers, and the "Pass the Endocrine System Line". Each question was discussed together with the class (immediate feedback). At the end of the semester, the students answered a Likert-type questionnaire with an open-ended question (justify yourself), highlighting that 78.8% of the students agreed/totally agreed that the scavenger hunt helped them review the content previously studied. When asked if the methodology motivated them to learn, 72.7% of the students agreed or totally agreed. In the open-ended question, the students were asked to justify their answers, obtaining statements such as "It encouraged physical activities, in addition to helping them understand the content at the same time" and "It's cool to learn with methods other than the traditional ones". The results suggest that the activity can be an efficient tool for teaching physiology, enabling group interaction and review of concepts.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Scientific dissemination and education about blood donation for deaf people

Authors:

Gabriela de Mello COLOMBO¹,
Luiza Maria de Oliveira Braga SILVEIRA¹, Alethéa Gatto BARSCHAK¹,
Lucila Ludmila Paula GUTIERREZ¹

Affiliations:

¹ Universidade Federal de Ciências da Saúde de Porto Alegre

Keywords: blood donation, scientific dissemination, People with disability.

Abstract:

There is a lack of scientific literature on the deaf population and blood donation. The aim of this study was to identify whether educational videos facilitate deaf people's access to knowledge about blood donation. After approval by CEP-UFCSPA (CAAE 64402922.3.0000.5345), 3 videos on blood donation were developed in Libras, with subtitles and audio in Portuguese. The topics covered were: definition of blood donation and requirements for donation, donation procedure and the destination of donated bags. The videos had an average duration of 1 minute and 40 seconds. Invitations to participate in the research were sent by e-mail to 20 higher education institutions in Rio Grande do Sul and so they could contact their deaf communities. After signed the informed consent form, deaf individuals answered a pre-test questionnaire, watched the videos and answered a post-test. Eight valid responses were obtained. The McNemar test and paired t-test were performed to assess the statistical relevance of the intervention. The analysis was performed individually, comparing the pre- and post-test statements that were comparable in relation to the content. It was observed that only one question presented $p < 0.05$. In the others, there was no statistical difference due to the high number of correct answers before the intervention. The qualitative evaluation of the videos, performed with "Yes" or "No" questions, verified the relevance of the videos. The interviewees felt a desire to donate blood after watching the videos and stated that the videos helped them to learn about the subject and that the content covered was important. It was possible to verify that educational videos about blood donation were effective in creating a space for dialogue and awareness among the deaf population and that they can function as relevant Educational Technologies for attracting donors, in addition to scientific dissemination on the subject.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Assessment of emotional activation levels of university students in different periods of the semester

Authors:

Letícia Corrêa VAZ ¹ Mauren Assis de SOUZA¹

Affiliations:

¹ Universidade Federal do Pampa – Campus Uruguaiana

Keywords: Emotional state. Feelings. Salivary cortisol. Stress. University students.

Abstract:

The emotional state helps guide our decisions and behaviors, significantly affecting our experience and interaction with the environment. The present research aimed to evaluate the levels of emotional activation of university students at different times during the semester. It was approved by the ethics committee of Unipampa under number 53789221.1.0000.5323. For this purpose, the Positive and Negative Affect Schedule (PANAS) and salivette tubes were used for subsequent analysis of salivary cortisol. Three collections were conducted with the same participants at the beginning, middle, and end of the semester, where all 47 responded to the PANAS, and the 11 participants who were in their first year of undergraduate studies additionally used the salivette tubes. For statistical analysis, the Shapiro-Wilk and Wilcoxon tests were used. As a result, it was possible to verify that students showed greater perceptions of positive feelings at the beginning of the semester compared to the end of the semester, such as determination ($Z = -2.646$, $p = 0.008$), interest ($Z = -2.629$, $p = 0.009$), and inspiration ($Z = -2.043$, $p = 0.041$). Regarding negative feelings, a significant increase was observed when comparing the beginning and the end of the semester in feelings such as guilt ($Z = -2.827$, $p = 0.005$), irritation ($Z = -2.042$, $p = 0.041$), and annoyance ($Z = -2.682$, $p = 0.007$). Additionally, through the analysis of salivary cortisol, a significant increase in salivary cortisol was observed when comparing the beginning and the middle of the semester ($Z = -2.040$, $p = 0.043$), but not when comparing the middle and the end of the semester ($Z = -0.712$, $p = 0.477$). Considering this, we can conclude that throughout the semester, the participating students experienced a decrease in their positive feelings and an increase in negative feelings, which is reinforced by the rise in salivary cortisol, indicating an increase in the students' stress levels.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: The impact of ALDH2 polymorphism on endothelial function in young healthy East Asians

Authors:

Karoline Correia de MEDEIROS¹
Beatrice LIOY² Tiago PEÇANHA³ Rehan JUNEJO²
Guilherme G ARTIOLI¹

Affiliations:

¹Departamento de Anatomia, Instituto de Ciências Biomédicas, Universidade de São Paulo, Brazil

² Department of Life Sciences, Manchester Metropolitan University, UK

³ Institute of Sport, Manchester Metropolitan University, UK

Keywords: Aldh2. Endothelial function. Fmd. Polymorphism.

Abstract:

Aldehyde dehydrogenase 2 (ALDH2) is a major alcohol-metabolizing enzyme involved in the detoxification of reactive aldehydes formed during lipid peroxidation. ALDH2 is encoded by the ALDH2 gene, where a common polymorphism (rs671, or *2 allele) leads to lowered enzyme activity, impaired aldehyde detoxification and sustained oxidative stress. The rs671 polymorphism is prevalent in East Asian populations, where 30-50% of the individuals carry the polymorphic allele. The *2 allele has been associated with increased risk for cardiovascular diseases, but the underpinning mechanisms are still uncertain. We investigated the impact of the rs671 polymorphism of the ALDH2 gene on the endothelial function. The study was approved by the Ethics Committee of the Manchester Metropolitan University. Twenty-one healthy non-smoking East Asians (19 Chinese, 1 Korean, 1 Japanese; age=24±4 y; BMI=22.3±2.6 kg•m², systolic blood pressure= 109.5±10.7 mmHg; diastolic blood pressure=72.7±8.7 mmHg) took part in the study. They were genotyped for the rs671 polymorphism using real-time PCR from mouthwash samples and underwent an assessment of the brachial artery endothelial function using the flow-mediated dilation technique (FMD) following 5 minutes of forearm occlusion and reperfusion. The participants were then grouped as carriers (n=9) or non-carriers (n=11) of the *2 allele. Differences between groups were analysed using unpaired t-tests. Absolute FMD was marginally lower in carriers (0.26±0.09 mm) vs. non-carriers (0.32±0.06 mm) (p=0.082). Likewise, FMD% was marginally lower in carriers of the *2 allele (8.07±3.13%) compared to non-carriers (10.2±1.9%) (p=0.079). These data indicate that sustained aldehyde accumulation may have detrimental effects to the endothelium and may lead to endothelial dysfunction in the long-term. In young, healthy individuals, the impact of the polymorphism appears to be discrete, but this might become more pronounced during ageing or under pathogenic processes.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Spatial abilities modulate students' performance in interpreting cladistic graphical representations: a 3D-printed cladogram as a haptic educational tool

Authors:

Fernando DAROS¹ Michelle Manfrini MORAIS¹ Cibele BRAGAGNOLO¹ Ilana FICHBERG¹
Camilo LELLIS-SANTOS¹

Affiliations:

¹Universidade Federal de São Paulo

Keywords: Active learning. biology education. cladogram interpretation. haptic learning. spatial ability

Abstract:

Biology education emphasizes conceptual learning, yet some topics, such as cladistics and phylogenetic systematics, require spatial reasoning to interpret graphical representations. Research suggests that spatial ability varies among students, potentially influencing their understanding of cladistic principles. However, traditional instructional resources often lack haptic tools that facilitate concept manipulation. This quasi - experimental study investigated whether a 3D-printed cladogram (3DC) could enhance learning outcomes for students with low spatial ability (Low-SA). Undergraduate STEM students (N=97) were randomly assigned to either a paper-based or a haptic-enhanced cladogram reading activity. Spatial ability was assessed via a mental rotation test, learning outcomes were analyzed by pre- and post-tests, and cognitive load and affective responses were measured through Likert-scale questions. The Unifesp Ethics Committee approved this study under the registration number CAAE: 67363323.1.0000.5505. Results showed that Low-SA students performed worse on recall and transfer questions than high-spatial ability students (50.42 ± 19.6 vs. 62.60 ± 19.3 , $p=0.003$). However, exposure to the 3DC significantly improved learning gains in Low-SA students compared to the traditional activity (0.48 ± 0.2 vs. 0.25 ± 0.3 , $p=0.014$). Concept visualization also improved with 3DC manipulation (3.38 ± 1.5 vs. 5.17 ± 0.9 , $p=0.01$), while intrinsic cognitive load decreased (4.00 ± 1.3 vs. 2.33 ± 1.2 , $p=0.009$). Regardless of spatial ability, 3DC exposure reduced extraneous cognitive load, increased motivation, and enhanced positive affective responses. These findings suggest that interpreting cladograms is not solely a biological reasoning task but also involves spatial cognition. Haptic tools like the 3D-printed cladogram can support Low-SA students, improving their conceptual understanding and engagement in learning.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Tiktok and epilepsy disorder: a cross-sectional study of social media content quality

Authors:

Giovanna Bruno BORGES¹

Anna Carolina Tavares de OLIVEIRA^{1,2} Victor Rodrigues SANTOS^{1,2}

Affiliations:

¹ Department of Morphology, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte

² Postgraduate Program of Neurosciences, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte

Keywords: Epilepsy. TikTok. Social media.

Abstract:

Introduction: TikTok, reaches millions of users worldwide. It hosts a variety of content, including chronic diseases such as epilepsy. Epilepsy is characterized by a persistent predisposition to generate epileptic seizures, along with cognitive, psychological and social consequences. In the digital age, many users turn to platforms like TikTok to share experiences and disseminate information about epilepsy and seizures. However, the accuracy of this information is often questionable. Objective: This work evaluates the quality and reliability of information about epilepsy shared on TikTok. Methods: Two evaluators selected 600 videos, 300 each, on TikTok by searching for "epilepsy" in Portuguese and followed the sequence suggested by the algorithm. Videos underwent a double-blind evaluation and was discarded if it met any of the following criteria: non-Portuguese language, unrelated, content unavailable or duplicate. The remaining videos were categorized as: Informative, Personal Experiences, Memes, Misleading, or Harmful. The Patient Education Materials Assessment Tool for Audiovisual Materials criteria and Content tables were used to assess the overall quality, understandability, actionability, stigma topics and overall content approached by Informative and Misleading videos. Results: From the 600 videos, 229 were categorized as Informative (93), Personal Experiences (83), Misleading (35), Memes (9) and Harmful (9). Out of these categories, a significant statistical difference was found in views, shares and saves ($p < 0.05$) and in the quality assessment, between Informative (0.47 ± 0.01) and Misleading (0.29 ± 0.02) videos ($p < 0.0001$). Content classification showed a prevalence of definition (43), symptomatic (42) content in Informative videos, as opposed to symptomatic (29) and definition (17) content in Misleading videos. Conclusion: Although TikTok serves its purpose as a platform for entertainment and sharing of experiences, it cannot be considered a reliable platform for the research of information regarding epilepsy.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Understanding Obesity Through Podcasts: Neuroendocrine Regulation of Appetite and New Therapeutic Strategies

Authors:

Isabella Wakim FERLA¹ Gabriela Takahashi COELHO¹ Lício Augusto VELLOSO² Letícia S. PIRES²

Affiliations:

¹ Pontifícia Universitária Católica de Campinas

² Obesity and Comorbidities Research Center, Universidade Estadual de Campinas

Keywords: Appetite; brain; energy expenditure; GLP-1; obesity.

Abstract:

This science communication project was developed as part of the Scientific Initiation Program in Scientific Journalism, selected in the "Comunicar Ciência" public call by the São Paulo Research Foundation (FAPESP) in partnership with Channel Futura of the Roberto Marinho Foundation. The objective was to produce an informative and accessible podcast series on obesity, combining rigorous scientific content with strategies of clear communication. Titled "Peso na Consciência" (Weight on Conscience), the series is composed of three episodes that explore obesity from different perspectives: neuroendocrine regulation of appetite, advances in pharmacological treatment, and the recognition of obesity as a global epidemic and chronic disease. The episodes were developed by medical students based on scientific literature, clinical lectures, and interviews with specialists. All scripts were written in lay language, aiming to inform and engage the general public. The project contributes to scientific literacy and to reducing stigma around obesity, presenting evidence-based approaches in a dynamic and empathetic format. The podcast is still ongoing, with three additional episodes in development, and will be made publicly available on Spotify to expand reach and impact.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Scientific Journalism Research Project: Energy balance in the context of body mass regulation and its impact on the development of obesity

Authors:

Maria Júlia Bernáth PICCOLOTTO¹, Isabela Zullo de BIASI¹,
Letícia da Silva PIRES², Licio Augusto VELLOSO²

Affiliations:

¹Faculdade de Medicina da Pontifícia Universidade Católica de Campinas,
²Obesity and Comorbidities Research Center, Universidade Estadual de Campinas

Keywords: Energy balance. Health. Lifestyle. Obesity prevention. Scientific journalism.

Abstract:

Based on the theme of energy balance, a scientific journalism research project was created by developing three reports exploring body mass regulation and obesity from different perspectives: its contributing factors and strategies to combat this disease. The initiative was selected by the "Communicating Science" project promoted by the São Paulo Research Foundation (FAPESP) in partnership with Canal Futura of the Roberto Marinho Foundation. This scientific project was developed based on 6 reports covering the following topics: the impact of energy balance on body weight; lifestyle and genetics related to obesity; new research on combating obesity; the role of the brain in energy balance; the contribution of the NHLH2 protein to obesity treatments; and new perspectives on obesity treatment. In order to bring scientific evidence, alongside with a literature review on the subject, interviews were conducted with specialists in endocrinology, nutrition and physical education. These interviews and the research revealed the complexity of obesity, highlighting the interaction between genetic, behavioral and environmental factors. Additionally, they outlined the new strategies to treat this disease, which include lifestyle changes and pharmacological interventions. The main reason behind these reports is to spread scientific knowledge in a simple and accessible manner so that the population can understand it. To achieve this, lay language was used, as well as interviews with qualified professionals. Visual resources such as images, graphs and tables were also used to simplify comprehension. In conclusion, this project establishes that tackling obesity requires an integrated approach, considering physical exercise, diet and, in some cases, medication. Obesity is a relevant and growing issue affecting the entire world and therefore essential to be addressed in order to prevent and combat it.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Contribution of formative assessments to study routines in university: student perception

Authors:

Ana Luisa BETIOLLI¹

Maria Antonia Ramos de AZEVEDO² Fernanda Klein MARCONDES¹

Affiliations:

¹ Departamento de Biociências, Faculdade de Odontologia de Piracicaba – UNICAMP, Piracicaba – SP;

² Departamento de Educação, Instituto de Biociências, Universidade Estadual Paulista, Rio Claro, Brasil

Keywords: higher education, formative assessment, physiology

Abstract:

In Brazil, incoming university students often retain the habit, developed in basic education, of studying only before summative assessments at the end of curricular units or academic terms. This approach can hinder meaningful learning and make it difficult for students to adapt to university life. In this context, formative assessments play a crucial role in the teaching-learning process. This study assessed students' perceptions of how learning assessment strategies contribute to structuring their study routines in higher education. Approved by the institutional ethics committee, the study involved 32 students enrolled in a Human Physiology course during the second semester of a Dentistry program. Formative assessments were implemented with grading based on participation or correct answers. These assessments were conducted both in groups — through in-class question discussions, educational games, and written problem-solving exercises outside of class — and individually, via interactive Lt-Kuracloud platform, with explanatory texts and exercises with immediate feedback. At the end of the semester, students rated the usefulness of these strategies in managing their study time and routine on a scale from 0 (not helpful) to 10 (very helpful), via online form, selecting up to three justifications. The average scores were 9.44 ± 0.91 for participation-based grading and 9.44 ± 0.8 for formative assessments. Key justifications included: participation-based grading increased motivation and reinforced learning; the online platform supplemented the professor's explanations and reinforced learning; formative assessments decreased procrastination, supported content review, exam preparation, and the development of a consistent study routine. These findings suggest that, from students' perspectives, formative assessments enhance time management, promote effective study habits, and facilitate the transition to university learning.



XXXIX REUNIÃO ANUAL DA FeSB
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Active Methodology in the Teaching of Human Physiology: An Experience Report

Authors:

Evellyn Caroline Mota Padilha de OLIVEIRA^{1,2} Guilherme Luiz Ferreira da Silva ARRUDA^{1,2} Paulo Macêdo MATTOS¹

Maria Alice de Albuquerque RODRIGUES¹ Aline de Freitas BRITO ^{1,2}

Affiliations:

¹ Escola Superior de Educação Física, Universidade de Pernambuco, Recife-PE.

² Programa Associado de Pós-Graduação em Educação Física UPE/UFPB, Universidade de Pernambuco, Recife-PE

Keywords: Engagement. Human Physiology. Membrane Transport. Sustainable. Models. Active. Methodologies.

Abstract:

Most students eventually succumb to distractions—particularly due to the constant use of electronic devices—compromising both engagement and content absorption. In light of this challenge, active methodologies can emerge as an effective strategy. This study aims to report on the experience of applying active methodologies through sustainable models in the teaching of membrane transport, evaluating their impact on student engagement and comprehension in Human Physiology. The activity was conducted on March 12 with second-year students from the Physical Education course. Prior to the practical session, a theoretical lecture on the topic was delivered, along with an explanatory guide on the activity. On the day of the application, a pre-model questionnaire was administered to assess the students' prior knowledge. Subsequently, students were divided into groups and directed to their respective models, with a rotation system established to allow every student to interact with all models. At the end of the session, students returned to the classroom and completed a post-model questionnaire to evaluate the methodology's impact on their understanding of the subject. Analysis of the responses revealed that 65.9% of the students found the model activity useful for reinforcing previously learned concepts, while 95.1% expressed interest in participating in similar activities in the future. Moreover, 52% stated that the activity was essential for clarifying doubts and deepening their understanding of the content. Thus, it can be concluded that the application of active methodologies in the teaching of human physiology positively impacted both student engagement and comprehension.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: The Myth of High-Load Training for Strength Gains: A Research-Based Approach to Teaching Muscle Physiology

Authors:

Maria Alice de Albuquerque RODRIGUES¹ Paulo Macêdo MATTOS¹
Evellyn Caroline Mota Padilha de OLIVEIRA¹ Aline de Freitas BRITO^{1,2}
Érica Maria GRANJEIRO^{1,2}

Affiliations:

¹ Higher School of Physical Education, University of Pernambuco, Recife-PE.

² Associated Postgraduate Program in Physical Education UPE/UFPB, University of Pernambuco, Recife-PE.

² Department of Biological Sciences, State University of Feira de Santana (UEFS), Feira de Santana-BA, Brazil.

Keywords: Research-Based Teaching, Physical Education, Human Physiology.

Abstract:

Human Physiology is of fundamental importance for the work of health professionals, especially for Physical Education teachers. One of the major professional challenges for graduates in Physical Education is the widespread presence of myths among their trainees. The myth that only high loads promote strength gains is widely disseminated, especially in gyms. The aim of this study was to highlight the importance of Research-Based Teaching (RBT) for learning Human Physiology in the undergraduate Physical Education program, especially for demystifying myths. This study was approved by the Research Ethics Committee under approval number 1.607.562. The RBT methodology was applied in the Human Physiology course taught to 12 undergraduate Physical Education students at the University of Pernambuco. In the classroom, students were encouraged to answer the following guiding question: "Is it necessary to lift maximum loads to gain strength?" To answer this question, students were divided into groups (3 to 4 people each) and were instructed to carry out the following didactic stages: 1) selection of the myth; 2) literature review of scientific articles to determine the loads and types of exercises; 3) implementation of a practical activity in the laboratory; 4) data collection, including the subjective perception of effort; 5) analysis of muscle fatigue and variables such as number of repetitions and rest time. The results of the research conducted by the students showed that pyramid training promoted greater muscle fatigue and progressive adaptation, especially in sedentary individuals, efficiently recruiting muscle fibers without the need for maximum loads. Thus, RBT fostered the development of investigative skills and helped students elucidate myths about the skeletal muscular system, promoting a broader and more scientific understanding of strength training, which is essential for the education of Physical Education professionals.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Reels as a Didactic Tool for Young Learners: An Experience Report from Faciologia

Authors:

Evellyn Caroline Mota Padilha de OLIVEIRA^{1,2} Camyla Nascimento de Sant'Anna ^{1,2}

Paulo Macêdo MATTOS¹

Maria Alice de Albuquerque RODRIGUES¹ Aline de Freitas BRITO^{1,2}

Affiliations:

¹ School of Physical Education, University of Pernambuco, Recife-PE.

² Joint Graduate Program in Physical Education UPE/UFPB, University of Pernambuco, Recife-PE.

Keywords: Digital content. Engagement. Immediate feedback. Human physiology. Reels.

Abstract:

With the advancement of digital media, the consumption of short videos, such as Reels, has significantly increased. However, this trend also presents challenges, such as low engagement among young learners when it comes to education, especially in complex subjects like Human Physiology. In response, the Faciologia extension project aims to innovate physiology teaching by developing Reels that combine science with everyday content in an accessible and engaging way, making learning more dynamic and attractive. The process begins with selecting physiological topics that have direct connections to young people's daily lives. For example, the digestive system can be explained in relation to alcohol consumption, making the content more relatable to viewers. Once the topic is defined, other essential elements are refined. The script is designed to be clear and dynamic. A charismatic and didactic presenter is chosen to connect with the audience. Technical quality is prioritized: clear audio, well-framed video, legible subtitles, well-executed filming, short duration, and an agile pace to maintain viewer attention. Instagram analytics show promising results: out of 18.3 thousand views, 52.8% came from Reels. Additionally, the audience is predominantly young, with 37.2% of views coming from the 22-34 age group and 26.9% from the 18-24 age group, reinforcing the relevance of the content for this demographic. Based on these findings, we conclude that using Reels as an educational tool has proven highly effective in teaching human physiology to young learners by combining a development methodology that prioritizes engaging content, dynamic scripts, and high technical quality with the interactive format of Reels.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Can The Methodology Motivate Tutoring? A Practical Strategy For Teaching Human Physiology: An Experience Report

Authors:

Evellyn Caroline Mota Padilha de Oliveira Paulo Macêdo
Maria Alice de Albuquerque Aline de Freitas

Keywords: Engagement. Immediate Feedback. Google Forms. Human Physiology. Monitoring.

Abstract:

Teaching interventions through monitoring emerge as a complementary strategy to conventional teaching; however, the way in which they are conducted directly influences their success. During our experiences in monitoring sessions for teaching human physiology—initially based on a dialogical expository methodology—we identified a recurring problem: low student interaction and engagement. To overcome this limitation, we implemented Google Forms as an alternative tool to stimulate student participation. The implementation was carried out during a Human Physiology monitoring session with second-year students from the Physical Education course, where a questionnaire developed by the course instructor—based on a prior directed study and featuring an immediate feedback system—was employed. In this setup, whenever a student answered a question—regardless of whether the response was correct—the correct answer was immediately displayed along with an explanation drawn from reference textbooks in Human Physiology. During the online monitoring session, students accessed the form and submitted their responses, and in cases of doubt, the monitors were available synchronously to provide clarifications. This experience revealed that the platform enabled rapid feedback and motivated student participation, particularly among those who are reluctant to speak in public. Furthermore, direct access via mobile devices made the experience more practical and aligned with the digital habits of the current generation, thereby enhancing learning retention. Although no formal quantitative analysis was conducted, the overall class perception indicated that the methodology was well received and facilitated the assimilation of Human Physiology content. In conclusion, the application of an online form with an immediate feedback system proved effective in engaging and facilitating student participation.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: The Impact of Active Methodologies in Health Education: An Experience Report of the Faciologia Extension Project

Authors:

Evellyn Caroline Mota Padilha de OLIVEIRA^{1,2} Emanuelle Souza Aguiar PIMENTA^{1,2}
Pedro Guilherme Santos CAVALCANTI^{1,2} Gabriel Huggins Uchoa de MIRANDA^{1,2} Paulo Macêdo MATTOS¹
Maria Alice de Albuquerque RODRIGUES¹ Aline de Freitas BRITO^{1,2}

Affiliations:

¹ School of Physical Education, University of Pernambuco, Recife-PE.

² Joint Graduate Program in Physical Education UPE/UFPB, University of Pernambuco, Recife-PE.

Keywords: Bitmojis. Carousels. Mind Maps. Memes. Reels.

Abstract:

With the 2020 pandemic, the spread of fake news intensified misinformation and negatively impacted public health. In this context, Faciologia emerged as an extension project that promotes health education through accessible scientific content, linking human physiology to everyday topics. The primary objective of the project is to render scientific knowledge both comprehensible and engaging by employing active methodologies to broaden its reach. The strategies follow a hierarchy of impact, with Reels as the principal tool, followed by memes, carousels, mind maps, and bitmojis. The Faciologia methodology begins by selecting a topic relevant to the target audience, followed by choosing the optimal format for its dissemination. For example, memes are used for simple and direct concepts by leveraging humor and irony, while Reels enable more detailed and dynamic presentations through audiovisual resources. Mind maps assist in organizing knowledge by facilitating conceptual connections, carousels structure information in sequential steps, and bitmojis make the content more interactive—especially for young audiences. Data from Faciologia's Instagram indicate 18.3 thousand views, with 52.8% attributed to Reels. Regarding content impact, 30% are Reels, 22.73% are memes, 27.27% are carousels, 8.18% are mind maps, 4.55% are conceptual maps, 3.64% are bitmojis, and 1.82% are live sessions, with video lessons also representing 1.82%. These figures demonstrate the effectiveness of active methodologies in disseminating scientific knowledge. The results indicate that the appropriate selection of approach directly influences audience reach and engagement, reinforcing the importance of innovative strategies in health education.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: "Oncologia Descomplicada: Scientific Communication as a Tool for Healthy Education and Social Transformation"

Authors:

Nicolle de Souza RODRIGUES¹,
Maria Eduarda de Oliveira MARQUES¹, Carlos Antônio de Artagão Silvério SOUZA¹, Gabriel Pereira RIBEIRO¹,
Julianna Ramos dos Reis Tavares FREIRE¹ Giovana de Souza Ferro BARBOSA¹
Ana Julia Borba de OLIVEIRA² Ricardo de Mattos SANTA-RITA^{1,2} Raquel de Souza GESTINARI^{1,3}
Daiana Vieira LOPES^{1,2}

Affiliations:

- ¹ Medical Science Institute, Federal University of Rio de Janeiro (UFRJ), Macaé, RJ
² Integrated Morphology Laboratory, Biodiversity and Sustainability Institute, Federal University of Rio de Janeiro (NUPEM/UFRJ), Macaé, RJ
³ Integrated Laboratory of Translational Biosciences, Biodiversity and Sustainability Institute, Federal University of Rio de Janeiro (NUPEM/UFRJ), Macaé, RJ

Keywords: Cancer. Digital platforms. Education. Health. Prevention.

Abstract:

Cancer is one of the leading causes of morbidity and mortality worldwide, resulting from the interaction between genetic, environmental, and behavioral factors. Despite scientific and therapeutic advancements, misinformation remains a major challenge for public health. In this context, health education is essential to empower the population to make informed decisions about their health. With this goal in mind, the "Oncologia Descomplicada" extension initiative was created—an initiative for scientific outreach aimed at disseminating accessible information about cancer. Using digital platforms, the project translates complex scientific content into simplified language, making it easier for the general public to understand. Educational materials are produced on risk factors, prevention, diagnosis, treatment, and the quality of life of oncology patients. These materials are made available on the @onco_descomplicada Instagram profile, expanding the reach of information. Since its inception, the project has published over 40 pieces of content through both feed posts and stories, reaching more than 2,000 views and approximately 700 followers. Active engagement demonstrates the initiative's relevance in promoting oncology knowledge. The project also holds virtual and in-person meetings, such as discussion groups and workshops. In addition to combating misinformation, the project contributes to population empowerment by encouraging healthy habits, recognizing warning signs, and seeking qualified medical care. The dissemination of accessible scientific information is crucial for dispelling myths, combating stigmas, and raising awareness about cancer. Thus, health education becomes an indispensable tool for transforming society's relationship with oncology, generating positive impacts on prevention, early diagnosis, and treatment of the disease.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Learning the effects of intravenous solutions on cell volume in a lecture combined with an educational game

Authors:

Lucas Adam Prado JULIÃO¹ Fernanda Klein MARCONDES¹

Affiliations:

¹ Departamento de Biociências, Faculdade de Odontologia de Piracicaba – UNICAMP, Piracicaba – SP;

Keywords: higher education, game-based learning, tonicity

Abstract:

Game-based learning facilitates the application of knowledge and helps students identify both well-understood and unclear content through problem-solving, while fostering curiosity and interest. In health sciences, mastering the concepts of tonicity and osmolarity is crucial for understanding how intravenous solutions affect cell volume. In a previous study conducted by our research group, an educational game was considered useful for understanding these topics, according to undergraduate health science students. This study aimed to investigate whether students' perceptions regarding the educational game's contribution to their understanding of these topics can be empirically supported through learning assessment outcomes. Following approval from the institutional Ethics Committee, 61 Dentistry students enrolled in a Physiology course participated. A pre-test was conducted before class. The professor gave an interactive lecture, alternating theoretical explanations with slides and the use of the educational game. This game involves analyzing images of test tubes containing blood mixed with different solutions after centrifugation. Working in groups, students were instructed to compare the volume of the precipitate in the tubes and complete tables using provided cards, which indicated the direction of water flow across the red blood cell membrane and the classification of the solutions in terms of osmolarity and tonicity. In the subsequent class, a post-test was administered, comprising questions equivalent—but not identical—to those presented in the pre-test. The mean post-test (8.47 ± 1.99) was significantly higher than the pre-test (4.06 ± 1.72 , $p < 0.05$, paired Student's *t*-test). Cognitive gain was calculated using the formula: $[(\text{post-test} - \text{pre-test}) / (10 - \text{pre-test})]$, and was classified as high (0.76 ± 0.04). This result indicates that the educational game promoted student learning about tonicity, osmolarity and the effects of intravenous solutions.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Use of the Kahoot Platform in Reducing Anxiety and Autonomic Regulation in Academic Assessments: A Pilot Study

Authors:

Paulo Macêdo MATTOS¹

Rodrigo Montenegro WANDERLEY^{1,2} Laila Barbosa de SANTANA^{1,2} Fabiano Ferreira de LIMA^{1,2}
Evellyn Caroline Mota Padilha de OLIVEIRA¹ Maria Alice de Albuquerque RODRIGUES¹ Aline de Freitas BRITO^{1,2}

Affiliations:

¹ Higher School of Physical Education, University of Pernambuco, Recife-PE.

² Associated Postgraduate Program in Physical Education UPE/UFPB, University of Pernambuco, Recife-PE.

Keywords: Anxiety. Formative Assessments. Active Methodology. Heart Rate Variability.

Abstract:

The school and academic community faces great psychological stress during formative assessments, which is evidenced by increased sympathetic nervous system activity and decreased heart rate variability (HRV). As a strategy to reduce anxiety levels before assessments, the use of active methodologies, such as "Quizzes" via Kahoot, is proposed. With the approval of the Research Ethics Committee (CEP) number 46710821.8.0000.5192, a test was carried out in the second semester of 2023 with 17 students, 11 men and 6 women with an average age of 20.7 years, from the bachelor's degree class in Physical Education at the Higher School of Physical Education. HRV was assessed with a Polar H10 belt and ELITE HRV application, recording 10 minutes of RR intervals. Anxiety was measured by the Beck Anxiety Scale, with high consistency ($\alpha = 0.92$). After this initial measurement, the students answered a quiz via the Kahoot platform and their HRV and anxiety were successively analyzed again. The results showed a decrease in the Standard Deviation of RR Intervals (SDNN) from 37.2 ms to 30.5 ms, in the Root Mean Square Difference (RMSSD) from 19.5 ms to 15.8 ms and in the Percentage of RR Intervals with a Difference Greater than 50 ms (pNN50), which fell from 1.55% to 0.85%. These results indicate a still significant sympathetic activation, possibly less intense than in traditional methods, suggesting that this approach may have a less impact on autonomic regulation. Preliminary data showed that 47.06% had mild anxiety and 29.41% had severe anxiety. However, after the quiz, the proportion of students classified with moderate anxiety dropped to 23.53%. While initial findings point to potential benefits of more dynamic assessment strategies like Kahoot, it is important to note that this is a preliminary study with a small sample size. These results provide a starting point for further research into how flexible assessment approaches may positively impact student well-being during assessment processes.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Multisensory Approach to DNA Structure and Extraction: An Inclusive Educational Strategy

Authors:

¹Milena Andreotti MINETTO

²Pedro Victor CARVALHO

²Beatriz Rocha Ilidio RODRIGUES ²Lorena Maria Mendes da SILVA ²Gabriel CORDEIRO

²Michelle Gomes da SILVA

³Fernanda Ramos GADELHA

Affiliations:

¹Programa de Pós-Graduação Em Genética e Biologia Molecular, Instituto de Biologia, UNICAMP, Campinas, SP, Brazil;

²Programa de Pós-Graduação em Biologia Molecular e Morfofuncional, Instituto de Biologia, UNICAMP, Campinas, SP, Brazil;

³Departamento de Bioquímica e Biologia Tecidual, Instituto de Biologia, UNICAMP, Campinas, SP, Brazil

Keywords: Active learning. Inclusive Education. Molecular biology. Teaching tools.

Abstract:

Teaching the structure and function of DNA remains a core challenge in biology education, particularly when accessibility is limited by a reliance on visual models. This study presents a multisensory, hands-on approach to teaching molecular biology concepts, integrating tactile, auditory, and olfactory stimuli to enhance student engagement and comprehension. A 3D DNA model was developed using different materials to differentiate molecular components through texture and form, facilitating an inclusive learning experience. Additionally, a guided DNA extraction activity allowed students to explore genetic material using sensory cues beyond sight. The methodology was designed to foster active participation, encourage collaborative learning, and promote accessibility in science education. Preliminary observations suggest that this approach not only benefits students with visual impairments but also enhances overall understanding by reinforcing abstract molecular concepts through experiential learning. This work highlights the importance of universal design in education, demonstrating that inclusive strategies can enrich the learning experience for all students.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Nexus: A Gamified Approach to Teaching Cell Biology

Authors:

¹Beatriz Rocha Ilidio RODRIGUES ¹Lorena Maria Mendes da SILVA ²Milena Andreotti MINETTO
¹Pedro Victor CARVALHO ¹Gabriel CORDEIRO
¹Michelle Gomes da SILVA
³Fernanda Ramos GADELHA

Affiliations:

¹Programa de Pós-Graduação em Biologia Molecular e Morfofuncional, Instituto de Biologia, UNICAMP, Campinas, SP, Brazil;
²Programa de Pós-Graduação Em Genética e Biologia Molecular, Instituto de Biologia, UNICAMP, Campinas, SP, Brazil;
³Departamento de Bioquímica e Biologia Tecidual, Instituto de Biologia, UNICAMP, Campinas, SP, Brazil

Keywords: Active learning. Cell biology. Gamification. Teaching tools.

Abstract:

Gamification has emerged as a powerful pedagogical strategy to bridge theory and practice, particularly in subjects like Cell Biology, where abstract and microscopic concepts challenge student comprehension. The pedagogical challenges inherent to teaching Cell Biology, characterized by its abstract concepts, microscopic phenomena, and extensive nomenclature, often result in superficial comprehension and limited knowledge retention when employing traditional teaching methodologies. To mitigate these challenges, the present study developed "Nexus," an educational card game designed to be integrated within a structured teaching sequence for cellular and molecular biology. This resource is intended primarily for secondary education but possesses adaptability for undergraduate disciplines. First, didactic sessions are taught focusing on cellular architecture and organelle functions. Subsequently, students play the "Nexus" game that involves the strategic matching of cards representing cellular components and their respective functions. This game was developed to increase student interest and engagement, driven by competitive dynamics centered on the subject matter. Furthermore, it also aims at establishing a positive learning environment characterized by active student participation in discussions about Cell Biology. "Nexus" enhances conceptual understanding and aligns with active learning methodologies by positioning students as central participants in the educational process. The integration of educational tools such as "Nexus" into curricula can effectively transform conventional pedagogical methods into more interactive and meaningful educational experiences, while concurrently cultivating student curiosity and critical thinking proficiencies.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Educational impact of *Ciência com Letícia* channel: viewer demands in biomedical sciences content

Authors:

Letícia Dalla Vechia HENSCHÉL¹

Affiliations:

¹Federal University of Paraná

Keywords: Biomedical sciences. Online videos. Scientific communication.

Abstract:

Ciência com Letícia (CcL) is a YouTube channel focused on Biomedical Sciences education, targeting undergraduate students in health-related fields. It covers topics such as Cell and Molecular Biology, Physiology, and Neuroscience in a simplified and didactic manner. The ten most viewed videos on the CcL channel were analyzed, considering the period from the first publication (02/01/2021) to 04/05/2025. Data were stratified by topic, number of views, user retention time (URT), and types of comments. The most viewed topics were: Homeostasis and Body Fluids (4 videos), Endocrinology and Metabolism (3 videos), Biomedicine (2 videos), and Cell Biology (1 video). The channel's most viewed video (9,107 views) explains the difference between cytoplasm and cytosol. It had the second highest URT (76%) and was highlighted in the comments for its clarity and objectivity. The video with the highest URT (88.8%) covered the pathophysiology of Polycystic Ovary Syndrome, ranking tenth in views. Interestingly, both videos with the highest URT were between 2 and 3 minutes long. The second most viewed video (8,456 views) differentiated osmolarity, osmolality, osmosis, and osmotic pressure. Comments emphasized its clarity, objectivity, teaching approach, and content simplification. In conclusion, there is a high demand among students for basic Physiology and Cell Biology content, as well as for short videos that communicate complex subjects in a clear, simplified, and didactic way.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: The surface-area-to-volume-ratio approach with jelly cubes: innovations on a protocol to address interdisciplinary studies for basic and higher education

Authors:

Paulo Fernando Guedes Pereira MONTENEGRO¹ Yen Galdino de PAIVA¹
Kléber da Silva VIEIRA¹

Affiliations:

¹Universidade Federal da Paraíba

Keywords: Jelly. Diffusion. Physiology. Teaching.

Abstract:

As the size of an object increases, its volume increases at a greater rate than does the surface area. This surface area to volume ratio has a direct impact on energy and substance movement across cell membranes and the integument, and is a core concept in physiology, present as part of educational curriculum. Many secondary and primary schools, as well as higher education institutions in North America have adopted laboratory classes to address this topic, using agar gel cubes with different sizes, impregnated with a pH indicator substance (phenolphthalein). The cubes are then immersed in an acid solution, which diffuses into the cubes, changing their color gradually. We have designed a modified protocol using cheaper materials, such as red cabbage anthocyanins, colorless unflavored jelly, and vinegar. Other innovations on the original protocol include 1) preparation of jelly objects with the same volume, but different shapes compared to the cubes, which provides the opportunity to address the effect of object shape on diffusion; 2) vinegar solutions at different temperatures, to address the effect of this parameter on diffusion rates. We have used this modified protocol in animal physiology classes for Biology students at Universidade Federal da Paraíba, and the students have actively engaged in the practical activity, reaching satisfactory conclusions on the effect of size and shape on diffusion process. This modified protocol may also be used in secondary and primary schools in an interdisciplinary approach, linking different concepts such as surface area to volume ratio, pH, diffusion, as well as graph construction. It may also be an opportunity for the students to be familiar with the scientific method. The modified protocol is an effective alternative to the traditional approach addressing surface area to volume ratio, since it has a lower cost and enables measurements of different parameters affecting diffusion rates.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Brazilian Brain Bee for Undergraduates as a Platform for Disciplinary Integration: A Historical and Educational Analysis (2018–2024)

Authors:

Gustavo Diniz-Taveira¹ Danielle Carvalho Gonçalves¹ Fayla Souza da Silva¹
Gláucio Aranha¹
Aliny Carvalho Dematté¹ João Vitor Galo Esteves¹ Alfred Sholl-Franco¹

Affiliations:

¹Núcleo de Divulgação Científica e Ensino de Neurociências, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro NuDCEN, IBCCF, UFRJ Avenida Carlos Chagas Filho, 373, bloco G, Sala G0-015, Ilha do Fundão, Cidade Universitária, Rio de Janeiro, RJ 21.941-902, Brasil

Laboratório de Vídeo Educativo, Instituto NUTES, Universidade Federal do Rio de Janeiro LVE, NUTES, UFRJ Avenida Carlos Chagas Filho, 373, bloco A, Subsolo, Ilha do Fundão, Cidade Universitária, Rio de Janeiro, RJ 21.941-902, Brasil

Organização Ciências e Cognição OCC Avenida Juscelino Kubitschek, 627, Centro, Joinville, SC 89.201-100, Brasil

Keywords: Knowledge olympiads; higher education; neuroscience competition

Abstract:

The Brazilian Brain Bee for Undergraduates (OBNG), launched in 2018, is a national neuroscience competition aimed at undergraduate students, coordinated by the non-profit organization Ciências e Cognição in partnership with the Núcleo de Divulgação Científica e Ensino de Neurociências (NuDCEN), of the Carlos Chagas Filho Institute of Biophysics at the Federal University of Rio de Janeiro (UFRJ). Its mission is to promote neuroscience education, foster interdisciplinary engagement, and encourage early academic involvement. This historiographic and educational analysis draws from publicly available documentation on the official OBNG website (<https://obng.cienciasecognicao.org>), including participation reports, structural descriptions, and annual summaries. The competition follows a three-phase format: (1) a multiple-choice quiz on foundational neuroscience; (2) an advanced theoretical exam in neuroanatomy, physiology, and clinical neuroscience; and (3) a final live question round before a panel of experts. Initially held in person, OBNG has operated entirely online since 2020, significantly expanding its national accessibility. Participation grew from 20 students in 2018 to 302 in 2024. Milestones include a 111% increase in 2020 (57 participants) after going online, a drop in 2021 (30, -47%), and a 310% growth in 2023 (123 participants) following integration with the Brazilian Physiology Olympiad (OBFis; ([h https://obfis.cienciasecognicao.org](https://obfis.cienciasecognicao.org))). The 2024 edition recorded a 145% increase. Across its six editions, students from at least ten undergraduate programs—including Medicine, Biomedicine, Nursing, Psychology, Pharmacy, Nutrition, Biotechnology, Dentistry, Physical Education, and Biological Sciences—have participated. OBNG has emerged as a consolidated platform for transdisciplinary integration, bridging health and life sciences. Its inclusive structure, national reach, and open-access resources contribute to equitable science education. Future studies should examine its long-term academic and professional impact.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Brazilian Brain Bee Participant Profile in 2024: Female Predominance and Sociodemographic Diversity

Authors:

Gustavo Diniz-Taveira¹ Fayla Souza da Silva¹
Danielle Carvalho Gonçalves¹ Gláucio Aranha¹
Aliny Carvalho Dematté¹ João Vitor Galo Esteves¹ Alfred Sholl-Franco¹

Affiliations:

¹Núcleo de Divulgação Científica e Ensino de Neurociências, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro NuDCEN, IBCCF, UFRJ Avenida Carlos Chagas Filho, 373, bloco G, Sala G0-015/046, Ilha do Fundão, Cidade Universitária, Rio de Janeiro, RJ 21.941-902, Brasil

Laboratório de Vídeo Educativo, Instituto NUTES, Universidade Federal do Rio de Janeiro LVE, NUTES, UFRJ Avenida Carlos Chagas Filho, 373, bloco A, Subsolo, Ilha do Fundão, Cidade Universitária, Rio de Janeiro, RJ 21.941-902, Brasil

Organização Ciências e Cognição OCC Avenida Juscelino Kubitschek, 627, Centro, Joinville, SC 89.201-100, Brasil

Keywords: Knowledge Olympiads; Education; Neuroscience; Diversity

Abstract:

The Brazilian Brain Bee (OBN), affiliated with the International Brain Bee (IBB), has been held in Brazil since 2013 as a hybrid-format neuroscience competition for high school students. To enhance accessibility and diversity, OBN expanded its website, developed a Candidate Portal (PC) with study resources, launched the Local Decentralized Neuroscience Olympiad (OLDN) for students without local committees, established a public school quota system, and introduced affirmative actions promoting gender and racial inclusion. The national competition includes multiple-choice exams in morphology, clinical, and fundamental neuroscience, followed by a "Live Questions" stage featuring open-ended responses. Participant data were accessed via the OBN portal based on registration information. 2024 OBN reached 2,185 participants—a 49% increase from 2023. Cisgender women accounted for 62.3% (1,362), nearly double that of cisgender men (31.4% or 687). Additionally, two transgender men, one transgender woman, four intersex individuals, 119 identifying as "other" (5.4%), and 1 participant who did not disclose gender were recorded. Racial self-identification revealed that 61.4% were white (1,341), 5.4% East Asian (119), 0.4% Indigenous (8), 23.8% mixed-race (520), and 5.5% Black (120); 6.4% (139) opted not to declare. Participants represented all Brazilian regions: Southeast (79.4%), North (7.8%), South (7.2%), Northeast (5.0%), and Central-West (0.6%). Regarding family income, 30.1% reported earnings above three minimum wages, 19.9% between two and three, 16.8% between one and two, and 11.5% below one minimum wage. The notable rise in female participation reflects the impact of OBN's affirmative strategies, contributing to broader representation and inclusion in neuroscience. The initiative continues democratizing knowledge and inspiring students across Brazil to explore neuroscience.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Analysis of life habits of basic school students and development of a teaching resource for teaching physiology and health

Authors:

Layla Saluane Barbosa dos SANTOS¹ Clarissa Silva SAMPAIO¹
Érica Maria GRANJEIRO¹

Affiliations:

¹State University of Feira de Santana (UEFS)

Keywords: : Health education, human physiology, schoolchildren

Abstract:

Introduction: Science teaching, especially Physiology Teaching in Basic Education, can stimulate curiosity, clarify doubts and act in disease prevention and health promotion. Objectives: This study aims to evaluate the profile of lifestyle habits of students in a Public School in the State of Bahia and describe the production of teaching material to promote the health of schoolchildren. Methods: The project was approved by the Research Ethics Committee (CEP) of the State University of Feira de Santana (UEFS) under number 4 4991115.3.0000.0053. The work was divided into two stages. Stage 1: Application of an online form to assess the Lifestyle and Risk Factors for Cardiovascular Diseases of elementary school students. Stage 2: development of a digital game with data from the Food Guide for the Brazilian Population of the Ministry of Health. Results: Of the 73 students, 54.8% were female, 23.3% considered themselves overweight, and 52.1% considered their level of physical activity to be "Not Very Active". The majority reported never having measured their blood pressure (61.6%), heart rate (78.1%), and waist circumference (82.2%) and hip circumference (91.8%). Approximately 50% reported not having routine exams, 46.6% reported not controlling what and how much they eat, and 16.4% reported having some health problem. The educational game developed, called Nutritious Fun, addresses topics such as natural foods, processed foods, and good eating habits. Conclusion: The data suggest an increase in the incidence of overweight and obesity, low levels of physical activity, inadequate dietary control, and poor self-care among students. The inclusion of an educational game about healthy eating in Physiology Education has the potential to improve self-care and promote healthy habits.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Perception of undergraduate students about the use of biosensors associated with an online teaching platform in human physiology teaching

Authors:

Larissa de Almeida DIAS¹ Marisele dos Santos SOARES¹
Anna Cecília Perretto Vieira de SOUZA¹ Nelson Gustavo Novais MARINHO¹ Pâmela Billig MELLO-CARPES¹

Affiliations:

¹Physiology Research Group, Federal University of Pampa, Uruguaiana, Brazil.

Keywords:

Pedagogical innovation. Active learning. Teaching physiology. Educational technology.

Abstract:

The physiology education is constantly evolving, but the use of the traditional teaching model still predominates. Innovative strategies, such as combining practical classes and active methods, can enhance learning. This study, approved by the Ethics Committee (6.585.144), analyzed students' perceptions about the use of biosensors associated with an educational online platform (Lt/ADInstruments) to collect real biological data in Human Physiology practical classes. Students from the Physical Therapy and Nursing courses from the Federal University of Pampa were divided into groups for laboratory activities, using biosensors integrated into the Lt platform to facilitate data acquisition and analysis during experimental classes, including electromyography, electroencephalography, others. At the end of the semester, a perception questionnaire, which was answered by 96 from the 126 students enrolled. Among them, 75% (n=72) strongly agreed that the biosensors and the platform were essential for understanding the content, 19,79% (n=19) partially agreed, 4,17% (n=4) were neutral, and 1,04% (n=1) partially disagreed. Regarding the impact of the use of biosensors on their performance in Physiology evaluations, 52,08% (n=50) strongly agreed that its use impacts positively, 33,33% (n=32) partially agreed, 12,50% (n=12) were neutral, and 2,08% (n=2) partially disagreed. In addition, 81.25% (n=78) considered the biosensors valuable for Physiology teaching, while 72,92% (n=70) stated that the use of sensors improved their interest in scientific research. Considering an scale from 0 to 10, 51,04% (n=49) attributed a score of 10 for the use of biosensors in Physiology teaching, 23,96% (n=23) a score of 9, 12,50% (n=12) a score of 8, 7,29% (n=7) a score of 7, 3,13% (n=3) a score of 6 and 1,04% (n=1) gave a score of 5 or 3. The results suggest that biosensors and the Lt platform favor the Physiology teaching-learning process and scientific understanding.

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XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: XVI Brain Awareness Week in Rio de Janeiro: Science, Evolution, and Diversity in Interactive and Intergenerational Practices

Authors:

Alfred Sholl-Franco^{1,3}

Gláucio Aranha^{2,3}

Affiliations:

¹Núcleo de Divulgação Científica e Ensino de Neurociências, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro NuDCEN, IBCCF, UFRJ Avenida Carlos Chagas Filho, 373, bloco G, Sala G0-015, Ilha do Fundão, Cidade Universitária, Rio de Janeiro, RJ 21.941-902, Brasil

²Laboratório de Vídeo Educativo, Instituto NUTES, Universidade Federal do Rio de Janeiro LVE, NUTES, UFRJ Avenida Carlos Chagas Filho, 373, bloco A, Subsolo, Ilha do Fundão, Cidade Universitária, Rio de Janeiro, RJ 21.941-902, Brasil

³Organização Ciências e Cognição OCC Avenida Juscelino Kubitschek, 627, Centro, Joinville, SC 89.201-100, Brasil

Keywords: Brain literacy. Education. Inclusion. Neuroscience. Science outreach.

Abstract:

The XVI Brain Awareness Week (BAW) in Rio de Janeiro (2025), themed “Evolution and Diversity”, is the longest-standing continuous BAW initiative in Brazil. Coordinated by Núcleo de Divulgação Científica e Ensino de Neurociências (NuDCEN/IBCCF/UFRJ, Ciências e Cognição (OCC), and Espaço Ciência Viva (ECV), the program extended beyond the traditional BAW calendar, integrating preparation, outreach, and community engagement over five weeks. The cycle began on February 22 with the Scientific Mediation Training Course, which certified 94 volunteers to facilitate public interaction with neuroscience content. On March 11 and 18, the VisiLab initiative welcomed students from two public schools—Gaspar Vianna and Carmela Dutra—to the UFRJ Neuroscience Museum (<http://museudeneurociencias.org>), in partnership with the Espaço Memorial Carlos Chagas Filho (EMCCF/IBCCF/UFRJ), strengthening the link between schools and university. On March 13, Brain Day at School engaged 200 children and educators at Anísio Teixeira Municipal School (Niterói/RJ), in workshops covering comparative neuroanatomy, EEG, prosthetics, emotional prosody, AI and cognition games, and motor coordination and learning activities. The initiative culminated on March 29 with Brain Saturday at ECV, offering hands-on learning to families and the public. With live streams, certified academic credits, and broad dissemination (@semanadocerebrorj), the event promoted early neuroscience engagement and mutual exchange between educational institutions. The BAW marks the start of a year-long program, which continues through neuroscience summer and winter courses, teacher training programs, and weekly VisiLab sessions at the Neuroscience Museum (IBCCF/UFRJ and OCC)—consolidating a permanent platform for inclusive scientific literacy. By expanding BAW’s temporal and institutional scope, the XVI edition deepened public inclusion and fostered long-term scientific literacy through participatory, interdisciplinary practices.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Impact of Sleep Quality on Academic Performance in Children With and Without Learning Difficulties

Authors:

Alfred SHOLL-FRANCO^{1,2,3}
Fabrício Bruno CARDOSO^{1,2,3}
Aliny Carvalho DEMATTÉ^{1,2,3}
João Vítor Galo ESTEVES^{1,2,3}
Luana PINHEL¹
Washington Adolfo BATTISTA¹
Filipe M. BONONE¹
Vitor da Silva LOUREIRO¹

Affiliations:

¹Núcleo de Divulgação Científica e Ensino de Neurociências, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro (NuDCEN, IBCCF, UFRJ). Avenida Carlos Chagas Filho, 373, bloco G, Sala G0-015, Ilha do Fundão, Cidade Universitária, Rio de Janeiro, RJ 21.941-902, Brasil

²Laboratório de Inovações Educacionais e Estudos Neuropsicopedagógicos (LIEENP), Faculdade CENSUPEG, Avenida Juscelino Kubitschek, 627, Centro, Joinville, SC 89.201-100, Brasil

³Organização Ciências e Cognição OCC Avenida Juscelino Kubitschek, 627, Centro, Joinville, SC 89.201-100, Brasil

Keywords: Academic performance. Children. Learning difficulties. Learning. Sleep.

Abstract:

Learning difficulties (LD) are a significant barrier to children's academic success, and sleep quality (SLQ) may modulate this relationship. This study assessed the association between SLQ and academic performance (AP) in 1,501 Brazilian schoolchildren aged 8 to 10, including 810 with LD and 691 without LD. Inclusion criteria ensured cognitive, behavioral, and sensory integrity. AP was measured using a randomized 30-question test based on the SAEB item bank, and SLQ was assessed through a 22-item Likert scale covering sleep routines and daytime functioning. Results revealed that children with LD had significantly lower AP scores (mean = 11.80) and higher SLQ scores (mean = 51.66), reflecting poorer sleep quality, compared to children without LD (AP mean = 18.84; SLQ mean = 30.18). The difference in SLQ between groups was statistically significant ($p < 0.001$) and explained 26.02% of the variance. Furthermore, Pearson's correlation showed a strong negative association between SLQ and AP ($r = -0.646$; $p < 0.01$), indicating that poorer sleep quality was consistently linked to reduced academic performance. These findings emphasize the relevance of sleep as a modifiable factor affecting school achievement, particularly among children with LD. Promoting regular sleep routines and healthy sleep habits may mitigate some of the learning challenges faced by this population. We recommend integrated educational and health-based interventions, including sleep hygiene education, individualized learning support, and family engagement, to optimize cognitive performance and overall well-being in school-aged children.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Boosting Executive Functions Through Rubik's Cube Training: A Neuropsychopedagogical Approach in Early Childhood Education

Authors:

Fabício Bruno CARDOSO^{1,2}

Érica Garcia Silveira GONÇALVES³ Aliny Carvalho DEMATTÉ^{1,2}

João Vítor Galo ESTEVES^{1,2} Washington Adolfo BATTISTA¹ Filipe M. BONONE¹

Alfred SHOLL-FRANCO^{1,2}

Affiliations:

¹ Núcleo de Divulgação Científica e Ensino de Neurociências, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro NuDCEN, IBCCF, UFRJ Avenida Carlos Chagas Filho, 373, bloco G, Sala G0-015, Ilha do Fundão, Cidade Universitária, Rio de Janeiro, RJ 21.941-902, Brasil

²Laboratório de Inovações Educacionais e Estudos Neuropsicopedagógicos (LIEENP), Faculdade CENSUPEG, Avenida Juscelino Kubitschek, 627, Centro, Joinville, SC 89.201-100, Brasil

³ Secretaria Municipal de Educação de Brusque-SC, Praça das Bandeiras, 77, Centro, Brusque, SC 88350-051, Brasil

Keywords: Executive Functions. Neuropsychopedagogical Intervention. Electroencephalography.

Abstract:

Academic learning difficulties are frequently associated with deficits in executive functions (EFs), particularly in working memory, inhibitory control, and cognitive flexibility. Among the cognitive processes linked to EFs, rapid automatized naming (RAN) stands out as a key indicator of lexical access efficiency and processing speed, both crucial for reading fluency. This study investigated the effects of a Neuropsychopedagogical Intervention (NPpI) on RAN performance and neural efficiency in children aged 7 to 9 years. Twenty participants were randomly assigned to an experimental group (EG; $n = 10$), which received the intervention, and a control group (CG; $n = 10$), which received no additional training. The intervention aimed to stimulate cognitive mechanisms underlying lexical retrieval and processing efficiency through structured activities. All assessments were conducted post-intervention. Behavioral performance was evaluated using the RAN test, measuring naming speed and accuracy. Electrophysiological data were collected via EEG, focusing on the Theta/Alpha Ratio (TAR) at parietal electrodes PZ, P3, and P4. The EG showed faster naming times ($M = 35.18s$; $SD = 6.82$) and fewer errors ($M = 1.30$; $SD = 0.95$) compared to the CG ($M = 40.19s$; $SD = 6.03$; $M = 2.40$; $SD = 1.07$). EEG analysis revealed significantly lower TAR values in the EG at PZ ($M = 1.88$; $SD = 0.69$ vs. CG: $M = 2.73$; $SD = 0.77$), P3 ($M = 1.69$; $SD = 0.44$ vs. CG: $M = 2.95$; $SD = 0.92$), and P4 ($M = 1.62$; $SD = 0.32$ vs. CG: $M = 3.13$; $SD = 0.77$), all with $p <$

0.01. These findings indicate enhanced neural efficiency following NPpI. Overall, the results support the effectiveness of structured neuropsychopedagogical interventions in improving automatized naming and modulating cortical activity. Future research should explore long-term outcomes, applicability to diverse populations, and broader cognitive impacts to optimize learning enhancement strategies.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

**Title: Proposal
digital game for**

**for a
learning respiratory physiology**

Authors:

Efeh Victório Monteiro CREMPE¹
Joao Vitor FONSECA²
Josimary Moraes Vasconcelos OLIVEIRA¹
Paulo Alexandre BRESSAN² Roseli SONCINI¹
Ricardo Murilo Pereira EMÍDIO¹

Affiliations:

¹Federal University of Alfenas, Graduate Program in Physiological Sciences.
²Federal University of Alfenas, Institute of exact sciences

Keywords: Active strategies. Educational games. Respiratory physiology.

Abstract:

Schools and universities are rethinking their teaching methodologies and practices in order to meet the demands of students and companies, which require professionals who are better trained and adaptable to the rapid changes in the market. The use of educational games is a strategy that has been increasingly used in education, with the aim of making the learning process more dynamic and attractive for students, and can help to arouse interest in the content studied. In the current post-pandemic scenario, it has been observed that many digital technology resources can help in the teaching-learning process. In view of the above, this study aims to create an educational digital game to assist in classes aimed at teaching respiratory physiology at undergraduate level. It is important to note that the production of educational software is a complex, time-consuming process due to a combination of technical, creative and organizational factors. To this end, some important aspects have already been defined: it will be developed for personal computer (PC) and mobile electronic devices, it will be produced in the Unity 6 game engine and will adopt a two-dimensional (2D) structure. The storyline was conceived around a nanorobot as the main character, who will receive information about physiology and face challenges presented by a scientist throughout various stages. The artwork for these characters has already been created in the Aseprite program, using inspiration generated by the Copilot AI. In addition, phase selection screens and initial screens were developed. So far, the project has made significant progress in the initial design and development of the basic mechanics, laying a solid foundation for the game's continued production.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Obesity, genetics and the sweetener saccharin: use of e-books as a didactic resource in context of DOHaD theory

Authors:

Gabriel Herniman BASTOS¹
Bruna Gonçalves da SILVA²
Carolina Tavares SCHUMANN³
Waldiney Cavalcante de MELLO³
Tiago Savignon Cardoso MACHADO³
Anatalia Kutianski Gonzalez VIEIRA³

Affiliations:

¹Universidade do Estado do Rio de Janeiro UERJ - RJ ²Centro Universitário Gama e Souza UNIGAMA - RJ
³Instituto de Aplicação Fernando Rodrigues da Silveira CAP-UERJ - RJ

Keywords: Biology teaching. Sweeteners. Obesity. E-book. DOHaD.

Abstract:

The impossibility of holding in-person classes during pandemic led to the emergence of new educational resources to support students during the educational crisis that arose during this period. Among the innovations that emerged were E-books. This material enabled access to knowledge through digital platforms, contributing to the continuity of the educational process. This resource is used in the scientific field to provide accurate content to different population segments, promoting access to information in society in a more accessible way in terms of language, thus facilitating understanding by laypeople. The aim is to produce E-books on topics related to Biology education and DOHaD. One E-book addresses genetics topics for high school and early higher education, such as Mendelian laws and the chromosomal basis of inheritance. Through the use of graphics and exercise lists, the material allows for a comprehensive and didactic approach. Considering the increased consumption of hypercaloric and ultra-processed foods by various groups, the emergence of substances capable of sweetening foods without interfering with daily caloric intake has been presented as a solution. The consumption of these substances, such as saccharin, is associated with the development of several physiological changes. For this reason, the other E-book was based on a research project developed by some of the authors of this work, focusing on the effects of overfeeding during lactation and the consumption of the sweetener saccharin on the cardiovascular physiology of adult male mice. This was developed to provide scientific analysis content on obesity, sweeteners, and cardiovascular physiology, using adapted terminology and introductory content to ensure full understanding of the research by the general public and classroom. So, the creation of E-books is an excellent way to provide accurate and didactic scientific knowledge, as it aligns with the technological reality of today's society.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Knowledge of Human Physiology among Physical Education Students: Perception and Applicability in the School Context

Authors:

Andrelize Ferreira CÔRREA¹ Letícia Corrêa VAZ¹
Mauren Assis de SOUZA¹ Lidiane DAL BOSCO¹

Affiliations:

¹Universidade Federal do Pampa

Keywords: Human physiology. Physical education. Students' perception. Teaching program.

Abstract:

The undergraduate Physical Education (PE) teaching program is designed to prepare educators to promote physical activity through sport and to raise students' awareness of the importance of physical activity for health. This study aimed to assess the knowledge of basic human physiology among graduates of the PE teaching program and to evaluate their perceived readiness to apply this knowledge in a school setting. The study was approved by the institutional ethics committee (protocol number 5.971.316). A total of 33 graduating students participated. Data were collected using a previously validated online questionnaire, and statistical analyses included the Wilcoxon and Chi-square tests. The results showed a higher number of correct than incorrect answers in the knowledge test ($Z=-3.121$, $p=0.002$). Regarding the self-perceived mastery of physiology content acquired during the program, most participants reported a neutral attitude ($\chi^2=15.636$, $p=0.004$). More than half of the students considered it relevant to include physiology topics in school physical education ($\chi^2=16.242$, $p=0.003$), and a significant proportion acknowledged the applicability of this content in their teaching practice ($\chi^2=13.818$, $p=0.008$). However, when asked about their willingness to develop a lesson focusing on physiology, no significant response was found ($\chi^2=6.848$, $p=0.144$). Based on these findings, we conclude that students have consolidated some basic physiology concepts and recognize their relevance to school physical education. However, it remains unclear how prepared they feel to deliver physiology-based lessons. Although most participants recognized its applicability and feasibility, many still expressed uncertainty about their mastery and ability to implement such content in the classroom. Further research is needed to explore effective strategies for integrating physiology into PE curricula in ways that enhance both content mastery and teaching competence among future educators.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: From health basic sciences experiments to data analysis: an interdisciplinary proposal for scientific initiation in high school conducted by M.Sc. and Ph.D. students as a part of “Anísio Project”

Authors:

Thiago G. HECK^{1,2,3,4}
Diovana G. de BATISTA^{1,2,3,4}
Samara C. KNEBEL^{1,3}
Bruno, B. BONIATI^{2,4}
Kelvin J. ROBE^{2,4}
Dhileane A. R. QUIXABEIRA^{2,4}
Luis A. P. HERNANDEZ^{2,4}
Laura S. CORREA^{1,3}
Lucas M. SULZBACHER^{1,3}
Fernando J. GONZALEZ⁵

Affiliations:

¹Grupo de Pesquisa em Fisiologia (GPef), Universidade Regional do Noroeste do Estado do Rio Grande do Sul (UNIJUÍ), Ijuí, RS, Brasil.
²Grupo de Pesquisa em Computação Aplicada (GCA), Universidade Regional do Noroeste do Estado do Rio Grande do Sul (UNIJUÍ), Ijuí, RS, Brasil.
³Programa de Pós-Graduação em Atenção Integral à Saúde (PPGAIS), Universidade Regional do Noroeste do Estado do Rio Grande do Sul (UNIJUÍ), Ijuí, RS, Brasil.
⁴Programa de Pós-Graduação em Modelagem Matemática e Computacional (PPGMMC), Universidade Regional do Noroeste do Estado do Rio Grande do Sul (UNIJUÍ), Ijuí, RS, Brasil.
⁵Programa de Pós-Graduação em Educação nas Ciências (PPGEC), Universidade Regional do Noroeste do Estado do Rio Grande do Sul (UNIJUÍ), Ijuí, RS, Brasil.

Keywords: Community Engagement, Science Education, Active Learning

Abstract

The “Anísio Project” is a community outreach project of UNIJUÍ, which involves six graduate programs. This proposal is inspired by the legacy of the vision of Anísio Teixeira, who stood for constructivist schools, with the students developing an active and transformative role in society. First, all master and doctorate graduate students (GRADS) completed an interdisciplinary discipline (30h) about University Extension, which was taught by professors from the programs. This formation includes scientific thinking, extension, and social and educational perspectives when the university is close to the community. GRADS needed to propose an immersive experience for first-year high school students (HIGHS) from public schools at the university, investigating scientific issues together with GRADS. Herein, we will present the activities conducted together by the three GRADS from Integral Health Care (IHC) and five from the Mathematical and Computational Modeling (MCM) Graduate Programs. IHC and MCM GRADS organized short expositions of their projects and elaborated real experiments to be executed by the HIGHS. During the first visit to the schools, GRADS presented all the experiment opportunities for the HIGHS chosen. Thus, 120 HIGHS visited 13 science and technology expositions (~20min) organized by GRADS in laboratories. Half of the students were divided into ten research groups after lunch (prepared by the gastronomy course students). Each group (named by a Brazilian Physiologist) had 3-10 HIGHS as researchers, a GRAD as advisor, and an undergraduate student as assistant (called chaperones!). HIGHS executed experiments to answer physiological questions, and in this processes, learn about scientific method, research questions, variables, unit measurements, conducting experiments, collect and analyze data. After that, on the 2nd visit to the school, GRADS taught HIGH how to write an abstract about their research. It was a transformative experience for all subjects involved. Support: CAPES-PROEXT-PG 2023



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Influence of Instagram on the level of physical exercise of followers

Authors:

Bárbara Elisiário OLIVEIRA¹ Renata Andrade ÁVILA¹ Ester de Souza INOCÊNCIO¹ Leonardo DOS SANTOS¹

Affiliations:

¹Postgraduate Program in Physiological Sciences - UFES, Vitória/ ES

Keywords: Instagram. Physical exercise. Scientific divulgation.

Abstract:

The @cafe.comciencia profile is an initiative aimed at popularizing science through posts about physiology and health on Instagram. Among the topics covered, physical activity stands out, having been addressed in 18 posts, including scientific articles, topics suggested by followers, and the workout routines of researchers from the PPGCF-UFES. Based on the audience's interest in content related to physical activity, this study evaluates the influence of such content on followers' motivation to engage in physical activity, using the engagement rate ($E = \text{interactions} \times 100 / \text{reach}$) and poll responses via Instagram Stories. When asked whether seeing other people exercising motivated them (7 responses), participants answered: "It motivates me a lot" (43%) and "It motivates me a little" (57%). In response to the question "Does consuming content about physical exercise motivate you?" (10 responses), 70% said "It really motivates me" and 30% said "It motivates me a little." For the question "Does seeing scientists exercising motivate you?" (12 responses), 92% responded "It really motivates me," and 8% said "It motivates me a little." The engagement analysis showed that the post with the highest engagement was a carousel explaining the relationship between physical exercise, female hormones, and the cardiovascular system, with an engagement rate of $E = 22.63\%$, followed by a carousel post on the benefits of calisthenics ($E = 18.92\%$) and another on the difference between physical activity and exercise ($E = 12.32\%$). Given the engagement with posts about physical activity and the poll responses, it can be concluded that exposure to this type of content serves as a motivator for practicing physical activity, highlighting the importance of science divulgation in public decision-making.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: "What Hormone Is This?": A Didactic Game to Support Learning of Endocrine Physiology in Health Education

Authors:

Camilly Bandeira AMARAL¹ Eduarda Esquelbeck DIAS¹ Isleide Dias THOMÉ¹ Fabiana Luca ALVES¹

Affiliations:

¹ Unifacear

Keywords: Active learning. Didactic game. Endocrine system. Health education.

Abstract:

Human Physiology is a fundamental subject in several health-related undergraduate programs. However, the limited number of class hours, the extensive content, and the complexity of physiological concepts often present challenges to student learning. In this context, active learning methodologies have gained prominence for encouraging student engagement, autonomy, and critical thinking. Among these, didactic games stand out for making content exploration more interactive and dynamic. They are useful tools for reviewing content, supporting formative assessment, and addressing diverse learning needs. When well-planned and aligned with curricular goals, educational games can help transform traditional classroom settings into more participatory and student-centered learning environments. This study aimed to develop a didactic game focused on the Endocrine System to enhance the teaching and learning of Human Physiology. The game, titled *"What Hormone Is This?"*, challenges players to guess a secret hormone chosen by their opponent, based on clues related to its physiological mechanisms. Each player receives six wooden plaques with hormone names and six cards containing five descriptive hints. A correct guess eliminates the opponent's plaque; an incorrect guess flips the player's own plaque. The winner is the one who eliminates the most opponent plaques. The game was subjected to a pilot test during the Unifacear Career Fair, and the participants reported that the material contributed to both the review of previously learned hormones and the acquisition of new hormonal concepts. This game is a playful and effective educational resource that can foster more meaningful learning experiences in Endocrine Physiology, while making classes more interactive and engaging. Their use can stimulate not only cognitive development but also social and emotional engagement, reinforcing their value in contemporary educational settings.



XXXIX REUNIÃO ANUAL DA FeSB
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Teaching cell biology through augmented reality in high school: an experience report

Authors:

Gabriel VICTORIANO¹ Carlos Henrique CONTE¹ Milena ALVES¹
Fernanda Klein MARCONDES²

Affiliations:

¹ Escola Estadual José Nicolau Piráguine, Diretoria de Ensino de Jaú

² Departamento de Biociências, Faculdade de Odontologia de Piracicaba – UNICAMP, Piracicaba – SP

Keywords: Basic Education. Technology. Educational tool.

Abstract:

The lack of student interest and their perception that studying biological processes is limited to memorizing terms and concepts are major challenges for Biology education in high school in Brazil. Considering the interaction of younger generations with technology, augmented reality can be a useful educational tool for teaching Biology in the classroom. The objective of this experience report is to describe the use of augmented reality to promote learning on the topic of "Cell Organelles," applied to two first-year high school classes at a public school in São Paulo in 2024. The teacher gave a theoretical lesson on prokaryotic and eukaryotic cells and their organelles, using slides provided by the São Paulo State Department of Education. In the following class, one week later, students were divided into groups and received cards with images of cells and cell organelles. They were instructed to point their cell phone cameras at these images, viewing them in augmented reality through the Educards (WeduFAST) app. A total of 38 students participated in these activities, demonstrating interest and interacting with classmates and the teacher, making comments and asking questions that connected the characteristics of the cells and organelles to the details they observed in the augmented reality images. The teacher noted that students who typically struggled showed enthusiasm when recognizing the organelles in these images and identifying their functions. In spontaneous remarks, some students said that it was very different to view the cells and organelles in augmented reality compared to the drawings on the slides. This experience indicates the potential for using augmented reality in Biology education, although further studies are needed to assess whether the use of augmented reality can make the content more comprehensible and the learning process more engaging. In addition, this experience highlights an educational use of mobile phones in the classroom.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Tutoring In Basic Disciplines In Undergraduate Courses In The Health Field: An Experience Report

Authors:

Tainá Aparecida Haubert^{1,2} Mauren Assis de Souza¹ Patrick da Silveira Gonçalves² Liciane Fernandes Medeiros²

Affiliations:

¹ Universidade Federal do Pampa - Campus Uruguiana - RS ² Universidade La Salle - Campus Canoas - RS

Keywords: Academic Monitoring. Active Methodologies. Graduation.

Abstract:

Academic tutoring complements students' education and helps tutors develop their teaching skills. The tutor's goal is to act as a facilitator without replacing the role of the teacher responsible for the course. In this sense, the tutoring program can be applied to various disciplines, but it is worth highlighting introductory courses in health education, which are often considered complex by students. This descriptive study reports the experiences of a physical education student as an academic tutor from 2021 to 2024. The courses involved were Molecular Biology and Biochemistry, Kinesiology and Biomechanics, Exercise Physiology, Human Physiology, and Human Morphology, offered both in person and remotely. Initially, student participation in tutoring was low, possibly due to a lack of awareness about the program and shyness. To encourage engagement, strategies such as group meetings were implemented, yielding positive results. Additionally, active methodologies were incorporated, including clinical case discussions and educational games. Despite the challenges, the strategies adopted by the tutor were successful, and students reported satisfaction with the experience. Academic tutoring provided tutors with the opportunity to enhance their teaching skills and gain a deeper understanding of the course content. The main challenges were low student participation and the development of effective teaching materials, both of which were gradually overcome. Through active methodologies, students were able to clarify doubts, deepen their knowledge, and apply theory to practice. This study underscores the academic significance of tutoring and recommends further research to assess its impact.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Perception of sus users about epidemiology and risk factors of human papillomavirus in São Félix do Xingu, PA, Brazil

Authors:

Ana Beatriz da CONCEIÇÃO^{1*} Anna Beatriz Silva e SILVA^{1*} Aline Andrade MOURÃO¹

Affiliations:

¹Grupo de Estudos e Pesquisas em Fisiologia e Saúde na Amazônia (GEPFSA). Instituto de Estudos do Xingu. Universidade Federal do Sul e Sudeste do Pará. São Félix do Xingu, Pará, Brasil.

Keywords: Health education, Epidemiology, Risk factors, HPV.

Abstract:

Human Papillomavirus (HPV) is a sexually transmitted infection (STI) with a high global prevalence and is associated with various diseases, including cervical cancer and other genital and oral lesions. Given its significant public health relevance, this study aimed to analyze the perceptions of users of the Brazilian Unified Health System (SUS) regarding the epidemiology and main risk factors associated with HPV in the municipality of São Félix do Xingu, in the state of Pará, Brazil, specifically in the Monte Negro residential area. The study combined a literature review with data collection through semi-structured questionnaires containing both open- and closed-ended questions. All participants signed the Free and Informed Consent Form and the final project is still waiting for process in Committee of Ethics in Human Research. The results indicate a low level of knowledge about STIs, HPV, and associated risk factors among participants. Approximately 65.5% were unaware of what STIs are, and 44.8% did not recognize HPV as a virus. A significant portion of respondents demonstrated misunderstandings regarding modes of transmission and symptoms of HPV. Although participants expressed a generally positive perception of SUS and public awareness campaigns, the findings underscore a clear need to strengthen health education initiatives. High rates of adherence to HPV vaccination were observed, which represents a positive outcome. However, the lack of awareness regarding the recommended age for vaccination suggests persistent misconceptions among the population. Underreporting of HPV and STI cases in São Félix do Xingu further highlights challenges related to access to healthcare services and the collection of accurate epidemiological data. Social vulnerability and geographic isolation were identified as contributing factors to low vaccine coverage and underreporting. The study emphasizes the urgent need for more effective educational campaigns, particularly those promoting condom use and raising awareness about HPV vaccination. Enhancing communication and health education is essential to improving vaccine uptake and supporting public health efforts in the region. FINANCIAL SUPPORT: GEPFSA/Unifesspa, Secretaria de Saúde do Município de São Félix do Xingu, PA (SEMSA-SFX)



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Epidemiological Situation of Visceral Leishmaniasis in São Félix do Xingu-PA, Brazil in the last five years (2020-2024)

Authors:

Geane Borges dos SANTOS^{1*} Anna Beatriz Silva e SILVA¹ Aline Andrade MOURÃO¹

Affiliations:

¹Grupo de Estudos e Pesquisas em Fisiologia e Saúde na Amazônia (GEPFSA). Instituto de Estudos do Xingu. Universidade Federal do Sul e Sudeste do Pará. São Félix do Xingu, Pará, Brasil.

Keywords: Public health, Visceral Leishmaniasis, Amazônia Paraense, Epidemiology.

Abstract:

Visceral leishmaniasis (VL) is a chronic zoonosis caused by the parasite *Leishmania chagasi* and transmitted by the phlebotomine vector *Lutzomyia longipalpis*. VL is a parasitology of public health relevance, which can affect especially the most socioeconomically vulnerable population and those living in areas with poor basic sanitation and little infrastructure. Epidemiological surveillance in the state of Pará has recorded 3,811 cases of VL, among which 198 deaths were recorded (2010-2024). However, scarce research combined with underreporting of cases in municipalities with large territorial extensions, such as São Félix do Xingu, PA, has been a problem in terms of planning prevention actions and treatment guidelines for those infected. The study sought to analyze the prevalence of VL in São Félix do Xingu, PA between 2020-2024, identifying associated factors and the most affected groups over this period. An epidemiological study was carried out based on VL notifications from the Municipal Health Department, available in the DATASUS System. The data analyses were quantitative and expressed as percentage (%). In the last five years, 10 cases of VL were registered in the São Félix of Xingu. Compared to the total number of cases of the cutaneous disease, which registered 224 cases in the same period of time, and also considering the data from the state of Pará, there is a possibility of underreporting of registered cases. The cases were reported by neighborhoods of the city, towns and indigenous villages. In the years 2020-2021, 60% of the cases were registered, 6 notifications. In 2022, no cases were registered and between 2023-2024, 4 cases were registered, representing 40% of the notifications in the five-year period. The next steps of the research include the application of semi-structured questionnaires, aiming to identify the level of knowledge and profile of the population that uses the SUS regarding the risk factors, prevention and treatment of VL. FINANCIAL SUPPORT: GEPFSA/Unifesspa; Secretaria de Saúde de São Félix do Xingu-PA. Sems S.F.X.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Health Under the Microbial Lens: Unveiling Invisible Threats in the School Routine

Authors:

Jennifer Rayane Veríssimo de OLIVEIRA¹, Maria Rita Fagundes VARGAS¹
Guilherme Inocência MATOS¹ Odemar Cardoso SILVA¹

Affiliations:

¹ Centro Federal de Educação Tecnológica Celso Suckow da Fonseca (CEFET/ RJ), Maracanã, Rio de Janeiro, Brasil

Keywords: Bacteria. Pathogens. Public health. School hygiene.

Abstract:

School environments often experience high circulation of individuals from various regions within the same city, which can facilitate the spread of different pathogens. In this context, the present study pursued to raise awareness within the community of the Federal Center for Technological Education Celso Suckow da Fonseca (CEFET/RJ), in Rio de Janeiro, regarding the possible presence of potentially pathogenic bacteria within school facilities. To this aim, samples were collected from various internal points on campus, such as bathroom faucets, elevator buttons, and common study and leisure areas. The investigation focused on detecting enterobacteria and coliforms, groups considered relevant for public health surveillance according to the scientific literature. The samples were cultured in selective and differential media, allowing for the isolation of bacterial colonies based on morphological characteristics. Identification was performed through bacterioscopic analysis using Gram staining and specific biochemical tests. Eight distinct morphotypes were isolated, of which four were Gram-negative bacilli and three of them tested positive in the catalase assay. No coliforms were detected, as evaluated using specific media designed for their identification. The results were presented during academic exhibitions within the institution, encouraging discussions about microbiological risks in school settings and highlighting the importance of both individual and collective hygiene practices. Preliminary findings indicate the need for further analysis, as well as additional sampling, to achieve more accurate bacteria characterization in different areas of the campus.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Bringing rare diseases to light: the importance of awareness and disclosure

Authors:

Luiza de Assis Carvalho ARAÚJO¹ Luciana dos Santos VIANA¹ Laura Carneiro da SILVA¹
Maria Gabriela Lemos Alves de CARVALHO¹ Sarah FURLANETTO¹
Maíra Marcelino SILVA¹
Jordan Ribeiro dos Santos SOUZA¹ Daiana Viera LOPES²
Flávia Borges MURY²
Raquel de Souza GESTINARI²

Affiliations:

¹ Instituto de Ciências Médicas (CMM)/ Universidade Federal do Rio de Janeiro

² Instituto de Biodiversidade e Sustentabilidade (NUPEM/CCS)/ Universidade Federal do Rio de Janeiro

Keywords: Genetics. Rare diseases. Scientific dissemination. Social network.

Abstract:

Rare diseases are characterized by their low frequency, affecting approximately 65 people in every 100,000 individuals. Currently, more than 8,000 rare diseases are known to exist in the world, with diverse etiologies, including genetic, infectious, and immunological factors. The challenges associated with this rarity, such as lack of knowledge and scarcity of specific information, in addition to their chronic, degenerative, and potentially lethal nature, have made rare diseases a public health priority. Despite affecting millions of people globally, awareness and knowledge about these conditions are still limited, both in society and among health professionals. In this context, the *@geneticarara.ufrj* page on Instagram® was created to disseminate scientific information on the subject using clear and accessible language. To date, publications have been produced explaining rare diseases and the primary rights of people affected by these conditions, including access to medicines provided by public institutions. In the six months since its creation, the page has gained more than 200 followers, and the six posts published have reached around 2,000 views in the last 90 days. The tool has proven to be an effective mechanism for disseminating knowledge and to constituting a welcoming environment where people affected by rare diseases can feel more understood and informed about their condition.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Visuomotor Impairments and Reading Difficulties in Schoolchildren: Unveiling a Hidden Educational Crisis

Authors:

Aliny Carvalho Dematte¹ João Vitor Galo Esteves¹ Fabrício Bruno Cardoso² Filipe Meneguelli Bonone² Alfred Sholl-Franco¹

Affiliations:

¹ Universidade Federal do Rio de Janeiro ² Faculdade CENSUPEG

Keywords: Education.reading-skills.visuo-motor

Abstract:

Visuomotor skills are fundamental to reading and writing development. Deficits in these domains can significantly impair academic performance, yet the lack of systematic screening in schools contributes to underdiagnosis. This study applied screening tools to identify visuomotor difficulties and analyze their correlation with reading performance in children aged 7 to 9 (CAAE nº24822819.2.0000.5263). A total of 1,532 students from public schools in Joinville, Blumenau, and Brusque (SC, Brazil) were assessed using the Visuomotor Screening Scale for Schoolchildren (VSSS), the Reading and Writing Scale (RWS), and a Reading Comprehension Test (RCT). Results showed that on the VSSS, 85.02% of 7-year-olds scored below the age-expected range (typical group: 22.32 ± 4.42 ; atypical: 50.85 ± 11.7). Among 8-year-olds, 83% were below expected levels (typical: 22.13 ± 4.55 ; atypical: 50.35 ± 12.06), and among 9-year-olds, 84% scored below expected (typical: 20.6 ± 3.1 ; atypical: 48.4 ± 12.5). In the RWS, 65.86% of 7-year-olds scored below expected (typical: 34.76 ± 5.46 ; atypical: 55.92 ± 8.17). For 8-year-olds, 96.25% underperformed (typical: 22.05 ± 4.03 ; atypical: 50.54 ± 11.37), and among 9-year-olds, 97.5% fell below the expected range (typical: 19.92 ± 3.47 ; atypical: 48.72 ± 11.81). In the RCT, 76.84% of 7-year-olds underperformed (typical: 84.08 ± 7.46 ; atypical: 48.99 ± 8.55); for 8-year-olds, 74.95% (typical: 83.22 ± 7.57 ; atypical: 48.84 ± 8.29); and for 9-year-olds, 83.39% (typical: 87.37 ± 4.6 ; atypical: 51.55 ± 11.24). A strong correlation was observed between the VSSS and the RWS and RCT performance, suggesting a relationship between impaired reading skills and visuomotor deficits. Nine-year-olds presented the lowest scores across all measures, possibly reflecting the delayed effects of the COVID-19 pandemic on literacy. These findings reveal a high prevalence of undetected visuomotor difficulties and emphasize systematic school-based screening to assess their educational impact.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: The Impact of Neuroscience Outreach through Neuroblitzes in Primary Schools in Uruguaiana/RS

Authors:

Tamires dos Santos PAIVA¹ Pamella Andrea Pinto LARANJA¹ Luciano Silva JUNIO¹
Ana Luiza Trombini TADIELO¹ Pâmela Billig MELLO CARPES¹

Affiliations:

¹Universidade Federal do Pampa

Keywords: Neuroscience, Education, Pedagogical Innovation.

Abstract:

Neuroscience has been increasingly integrated into education, expanding the understanding of the brain and improving teaching and learning practices. The POPNEURO Outreach Program promotes neuroscience popularization and combats educational neuromyths. This study evaluated the impact of Neuroblitzes—scientific outreach actions held in public schools in Uruguaiana/RS—on students' knowledge and content retention (CEP 3.058.377). The activities occurred over eight weeks with interactive lessons on brain function. Topics included: (1) Who can be a scientist?; (2) Introduction to the nervous system; (3) Neurons and synapses; (4) Memory and learning; (5) Brain health; (6) Nervous system and senses; (7) Technology and the brain; and (8) Emotions. Each session followed the same structure: pre-questionnaire, lecture, hands-on activity, and post-questionnaire. Attendance varied, so the analysis focused on the impact of individual lessons. Lesson 3 ("Neurons and Synapses") had 88 participants. Students had a mean of 3.30 correct answers (SD = 0.46) in the pre-test and 3.13 (SD = 0.33) in the post-test. Despite the slight average drop, the Wilcoxon signed-rank test showed a statistically significant difference ($p = 0.0001$), indicating changes in students' knowledge and response patterns. Correct answers in question 2 (neuron structure) rose from 62.5% ($n = 55$) to 85.2% ($n = 75$), and in question 4 (a false statement on speed), correct rejections increased from 77.3% ($n = 68$) to 92.0% ($n = 81$). These findings suggest that the Neuroblitzes' interactive format supported knowledge consolidation and neuromyth correction. This theoretical-practical approach also enhanced student engagement and reinforced the importance of science communication as a strategy to promote meaningful learning in primary education. Moreover, the initiative fosters critical thinking, inclusion, and scientific citizenship among children in public education settings.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Impact of Using a 3D Model of Cell Membrane Proteins Combined with an Online Platform in Human Physiology Education

Authors:

Karine Ramires LIMA¹

Nelson Gustavo Novais MARINHO¹ Giulia Azevedo MARTINEZ¹

Anna Cecília Perretto Vieira de SOUZA¹ Larissa de Almeida DIAS¹

Cláudio Felipe Kolling da ROCHA² Ana Carolina de Souza da ROSA¹ Pâmela Billig MELLO-CARPES¹

Affiliations:

¹ Physiology Research Group, Federal University of Pampa, Uruguai, Brazil.

² Laboratory of Physiology and Biophysics, Feevale University, Novo Hamburgo, Brazil.

Keywords: Active learning. Physiology teaching. Three-dimensional models. Membrane proteins. Educational technology.

Abstract:

Human physiology is essential in health education, but its abstract nature makes assimilating microscopic concepts challenging. Traditional methods are often ineffective, requiring dynamic educational approaches to improve the learning process. In this study, approved by the Research Ethics Committee of the Federal University of Pampa (protocol 3091629), we analyzed the impact of the use of a 3D cell membrane model (M3D) associated with an online platform on cellular physiology comprehension. A total of 43 Nursing and Physiotherapy students enrolled in the Human Physiology course participated. In a practical class, students used the M3D with the online platform Lt/AdInstruments, which provided assembly instructions and reflective questions. Learning and perception were assessed through a pre-test and post-test with four questions: two on self-perception and two on knowledge. Shapiro-Wilk and Wilcoxon tests analyzed self-perception, while the Chi-square test evaluated performance. In the pre-test, 34.88% (n=15) confidently differentiated membrane proteins, 58.14% (n=25) had difficulty, and 6.98% (n=3) could not. After the activity, 86.05% (n=37) felt confident, while 13.95% (n=6) still had difficulties but felt capable ($p < 0.0001$). Regarding Na⁺/K⁺ATPase function, 9.30% (n=4) responded confidently in the pre-test, 48.84% (n=21) with difficulty, and 41.86% (n=18) could not answer. Afterward, 74.42% (n=32) became confident, 23.56% (n=10) had difficulties, and 2.33% (n=1) could not answer ($p < 0.0001$). No statistical differences were found in test performance. For protein types, 58.14% answered correctly in the pre-test and 46.51% in the post-test ($p = 0.2804$). For active transport, correct answers dropped from 25.58% to 16.28% ($p = 0.2890$). Although M3D increased students' confidence in their content knowledge, its impact on test performance was not significant, highlighting the importance of investigating the direct effect on learning and not just the perception of learning.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Effects of a Cardiac Cycle Puzzle on Learning and Perceptions of Veterinary Medicine Students

Authors:

Daniella Viveiros MEIRELLES¹ Fabrício Luciani VALENTE¹

Affiliations:

¹ Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brasil

Keywords: Educational game. Learning. Physiology. Teaching. Veterinary Medicine.

Abstract:

The aim of this study was to evaluate the effect of a puzzle about the Cardiac Cycle on the learning and perception of students of the Veterinary Histophysiology discipline of the undergraduate course in Veterinary Medicine at the Federal University of Viçosa (UFV). The research was previously approved by the Ethics Committee for Research with Human Beings of UFV under opinion number 6.174.507. First, the students attended two theoretical classes of 1 hour and 40 minutes on Cardiovascular Physiology, which addressed histological, anatomical and physiological aspects, including the phases of the Cardiac Cycle in domestic animals. Subsequently, in a practical class, a class of 17 students answered an exercise with eight multiple-choice questions about the Cardiac Cycle and were then randomly divided into four groups to carry out the activity with the puzzle about the Cardiac Cycle. During the experience with the educational game, students described the phases of the Cardiac Cycle, associating them with their physiological events, exchanging knowledge among themselves and with the teacher. After solving the puzzle, students repeated the same assessment exercise. Short-term learning was assessed by errors and correct answers before and after the educational game, using the Wilcoxon Test (significance level of 5%), which revealed a significant increase in correct answers ($V = 9$, $p = 0.01027$), with the median increasing from 4 (pre-educational game) to 6 (post-intervention). To assess student perception, a Likert scale question was used (ranging from 1- I totally disagree to 5- I totally agree), with a predominance of responses "4 - I agree" ($n = 12$), median and mode equal to 4, mean of 3.88 and standard deviation of 0.7, demonstrating a mostly positive perception of the pedagogical intervention. Despite the small group evaluated, it can be said that the puzzle contributed to student learning about the Cardiac Cycle and can be used as a complementary teaching resource. Acknowledgements: Capes, CNPq, Fapemig.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Exploring gustatory and olfactory perception and their contributions to methodological advances in teaching in public schools in Macaé

Authors:

Victoria Navartovicis MARTINS^{1,2} Aurenita Émile Sá MIRANDA^{1,2} Maria Luiza Vieira Silva e PAULA^{1,2}

Maria Gabriela da Silva Oliveira GABRIEL^{1,2} Kailany Neto CALINÇANI^{1,2}

Celina Garcia FONSECA¹ Cintia Monteiro de BARROS^{1,2} Elane da Silva RIBEIRO^{1,2} Flávia Borges MURY ^{1,2}

Affiliations:

¹ Universidade Federal do Rio de Janeiro

² Instituto de Biodiversidade e Sustentabilidade

Keywords: Anatomy, Extension, Education

Abstract:

Teaching human anatomy is essential for future health professionals, providing essential knowledge for their training. Technological advances, with printed books and teaching materials, have contributed to the improvement of learning techniques, but access to and assimilation of this knowledge are still limited. Research indicates difficulties in memorizing and understanding anatomical structures and functions, which requires the use of varied teaching methodologies. Outreach projects in the area of anatomy promote education about the human body for the general public, strengthening the relationship between academic institutions and the community, and facilitating access to updated information and practical learning. The project "Human Body in Action: practicing to better understand it" carries out educational activities, such as investigating taste perception through experimentation with different foods. This dynamic relates the human senses to the nervous system, assessing knowledge about olfactory and taste structures. The activity begins with an introduction and questions to assess participants' prior knowledge, followed by an explanation using anatomical models, highlighting the regions responsible for smell and taste, and how the brain processes this sensory information. Then, practical experiments are carried out with foods representing the five flavors. After the dynamic, the same questions are discussed with the aim of making a new observation about the evolution of learning. Finally, projects like this one aim to integrate undergraduate courses into public and private schools in Macaé, disseminating knowledge to children and young people, in addition to bringing them closer to the academic environment and the possibilities it offers.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Accessibility and usability in the development of technological solutions for the SUS

Authors:

João Gabriel Pereira LOPES¹ Aline da Silva ALVES² Rosane Abdala LINS²

Affiliations:

¹Colégio Pedro II – Campus Duque de Caxias ²Instituto de Comunicação e Informação Científica e Tecnológica em Saúde – Fiocruz

Keywords: Accessibility. Inclusion. Public health. Technology. Usability

Abstract: This work aims to develop accessible and usable digital solutions aimed at strengthening the Public Brazilian Health system (SUS), with a focus on including people with disabilities in access to public health information and services. The proposal integrates knowledge of computer science, inclusive design and public policies, combining technical and social aspects through an interdisciplinary approach. The methodology is based on a bibliographic survey of accessibility guidelines (WCAG 2.3 and eMAG), mapping of barriers in digital health platforms and the development of responsive prototypes with an emphasis on usability. The tools used include HTML, CSS, SQLite3, React, Express.js and Git. The study is based on the recognition that universal digital access is fundamental to promoting equity in health, especially among historically marginalized groups. As a result, accessible prototypes of web pages will be developed that contribute to broadening the reach of public health actions and services, based on principles of accessibility, responsiveness and simplified language. As well as furthering students' technical training, the initiative provides practical support for public institutions to improve their digital strategies. The project reinforces the importance of combining technology, science, and social justice to tackle inequalities in the field of health and information.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: Carrapatos e febre maculosa: jogo educativo como uma proposta de instrumento pedagógico de ensino

Authors:

Betina Cordeiro MONTEIRO^{1,2}
Marinete AMORIM²
Karla BITENCOURTH²

Affiliations:

¹Instituto de Aplicação Fernando Rodrigues da Silveira (CAp/UERJ)
²Programa de Vocação Científica (PROVOC) da FIOCRUZ- RJ
³Laboratório de Carrapatos e outros Artrópodes Ápteros (LAC/IOC/FIOCRUZ-RJ)

Keywords: Atividade Lúdica. Doença associada ao carrapato. Promoção da Saúde.

Abstract:

Os carrapatos são artrópodes da ordem Ixodida, que em um ou mais estádios do seu ciclo de vida são ectoparasitos hematófagos obrigatórios de vertebrados terrestres. No Brasil a principal zoonose associada aos carrapatos é a febre maculosa, doença infecciosa febril aguda com curso variável. Entretanto, a biologia e a importância do ixodídeo como agente causador de doenças são pouco conhecidas pela população. A orientação e a prevenção são as ferramentas mais eficientes e para isso são necessárias medidas educativas. Os jogos educativos são estratégias lúdicas, prazerosas e participativas utilizadas para o processo de ensino-aprendizagem, levando o educando a uma maior apropriação dos conhecimentos envolvidos e a atuar como agente multiplicador de conhecimento. O objetivo é desenvolver um jogo educativo desplugado, composto por tabuleiro e cartas, como estratégia metodológica para ensino sobre carrapatos e a febre maculosa para alunos do ensino fundamental II (6º ao 9º ano). O jogo simulará uma viagem de férias, em família, a um hotel fazenda em área endêmica para febre maculosa brasileira. O tabuleiro, composto por 45 casas divididas em quatro blocos, simulará diferentes locais e situações em que o adolescente deve tomar decisões para estar protegido dos riscos associados aos carrapatos. A cada rodada, o participante irá retirar uma carta, de acordo com a casa do tabuleiro em que está, e deverá definir o que fazer para evitar o parasitismo e desenvolver a doença. As cartas trarão informações gerais sobre os carrapatos (ciclo de vida, alimentação e transmissão de patógenos) e a febre maculosa (a doença, cenários de transmissão/infecção e bioagentes, prevenção, diagnóstico e tratamento). Ganha o jogo quem primeiro concluir todas as etapas e chegar ao fim do tabuleiro. Dessa forma, o jogo é uma estratégia para capacitar adolescentes a identificar cenários de risco para febre maculosa, formas de prevenção e conduta adequada diante de casos potenciais da doença.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Título: Tratamento documental em projetos de pesquisa: gestão de documentos e gestão de acervos para preservação, acesso e difusão

Autores:

Cristiane D'Ávila Lyra ALMEIDA¹ Renata Lourenço Mendes KESSLER² Amanda Marcello da Silva COSTA³

Afiliações:

¹Casa de Oswaldo Cruz (COC/Fiocruz) ²Casa de Oswaldo Cruz (COC/Fiocruz)

³ Escola Politécnica Joaquim Venâncio (EPSJV/ Fiocruz)

Palavras-chaves: gestão documental; preservação e difusão de arquivos; favelas e periferias, ciência

Resumo:

O gerenciamento de documentos é essencial para preservar e democratizar o conhecimento e a memória. Sob essa ótica, a presente proposta se configura como subprojeto da pesquisa intitulada "Geração cidadã de dados: cartografia dos coletivos de comunicação comunitária para promoção da saúde", coordenada por pesquisadores da Casa de Oswaldo Cruz (COC/Fiocruz), da Cooperação Social da Presidência da Fiocruz e do Instituto de Comunicação e Informação Científica e Tecnológica em Saúde (ICICT/Fiocruz), com apoio do Dicionário de Favelas Marielle Franco, e desenvolvida em articulação com comunicadores comunitários dos bairros Maré, Manguinhos, Alemão e Jacarezinho. O objetivo deste subprojeto é realizar a gestão dos documentos nato-digitais e dos representantes digitais dos gêneros sonoro, audiovisual, textual e iconográfico resultantes do projeto de pesquisa acima referido desde sua produção, como forma de aprimorar a manutenção do contexto original dos documentos e possibilitar sua preservação e acesso a longo prazo. Essa iniciativa está em conformidade com a Política de Preservação e Gestão de Acervos Culturais das Ciências da Saúde (2013), da Casa de Oswaldo Cruz/Fiocruz, com o Programa de Preservação Digital de Acervos da Fiocruz (2020) e com os protocolos estabelecidos pelo Sistema de Gestão de Documentos e Arquivos da Fiocruz (Sigda). Trata-se de uma iniciativa inovadora no campo da gestão documental, visto que propõe a organização da produção documental da pesquisa em curso, considerando os possíveis usos futuros dessa documentação. Tais atividades, além de concretizarem as ações previstas no projeto, evidenciam a imprescindibilidade da preservação no âmbito científico. A produção da ciência não se limita à realização de pesquisas, mas se concretiza também por meio dos registros que as documentam. Assim, torna-se indispensável a gestão adequada desses produtos, pois não há ciência plena sem a organização, preservação e acesso aos seus próprios vestígios documentais.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

8 A 11 DE JULHO 2025
UNICAMP, CAMPINAS/SP

Title: O movimento e gênero musical BRock e sua importância durante a ditadura militar: Possibilidades por meio da CienciArte e Pesquisa Baseada em Artes

Authors:

Beatriz Pinheiro TORQUATO¹

Márcio Luiz Braga Corrêa de MELLO²

Adrielle Macêdo Fernandes da SILVA³

Nathalia Sena Sassone PERRONE⁴

Affiliations:

¹ Instituto Oswaldo Cruz/IOC/Fiocruz; Laboratório de Inovações em Terapias, Ensino e Bioprodutos (LITEB/IOC); Escola Politécnica de Saúde Joaquim Venâncio/PROVOC; Colégio Pedro II - Humaitá II

² Instituto Oswaldo Cruz/ IOC/Fiocruz; Laboratório de Inovações em Terapias, Ensino e Bioprodutos (LITEB/IOC)

³ Instituto Oswaldo Cruz/IOC-Fiocruz; Programa de Pós-Graduação em Ensino em Biociências e Saúde- PGEBS; Laboratório de Inovações em Terapias, Ensino e Bioprodutos (LITEB/IOC)

⁴ Universidade Federal do Estado do Rio de Janeiro - RJ; Instituto Oswaldo Cruz/IOC/Fiocruz; Laboratório de Inovações em Terapias, Ensino e Bioprodutos (LITEB/IOC)

Keywords: BRock. Cienciarte. Música. Pesquisa baseada em artes. **Abstract:**

A investigação enfatiza metodologias inovadoras ainda pouco utilizadas em nosso país: A abordagem CienciArte e a Pesquisa Baseada em Artes (PBA). Assim, o subprojeto pretende analisar expressões artísticas, tendo como ênfase movimentos sociais e manifestações culturais que usufruem da escrita criativa como meio de perpetuação de identidade e cultura. Utiliza-se como foco de estudos para a pesquisa, o BRock, um movimento musical e social que se posicionou contra a ditadura civil-militar no Brasil (1964-1985). O objetivo da pesquisa inclui investigar as contribuições da música em movimentos sociais, especialmente as do BRock no período da Ditadura Militar. Portanto, realizamos aprofundamento bibliográfico nas principais temáticas de pesquisa: analisar o lado lírico do movimento BRock e construir atividades para uma oficina multi-artística. Para tal, fizemos um levantamento bibliográfico com leitura e fichamento de artigos. Além disso, a metodologia envolve dois pilares principais, a abordagem CienciArte, que resume o conceito de produzir ciência a partir da arte, e a PBA que busca fundar uma nova maneira de pesquisar e educar, através das artes, incluindo a expressão por meio de letras e melodias. A pesquisa a qual este projeto se insere foi submetida ao Comitê de Ética da Fundação Oswaldo Cruz/IOC e foi aprovada em 26/11/2024. Até o momento, é possível dizer que o BRock carrega consigo um significado cultural e político. Ademais, compreendemos que movimentos musicais transcendem o método de estruturar uma música, podendo atingir sociedades e ser capaz de mudar completamente a maneira de viver de um indivíduo. Também vemos que é possível construir um paralelo entre a opressão civil-militar que nosso país sofreu entre as décadas de 60 e 80 com a opressão social que o capitalismo exerce sobre a sociedade brasileira contemporânea, visando elaborar atividades artísticas e dinâmicas utilizando as metodologias do estudo e o conhecimento adquirido a partir das leituras e debates sobre o tema.



XXXIX REUNIÃO ANUAL DA FeSB
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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UNICAMP, CAMPINAS/SP

Title: Microperimetry as a valuable tool for assessing changes in visual function following voretigene neparvovec-rzyl gene therapy

Authors:

Caio Henrique Marques Texeira¹
Mariana Matioli da Palma¹
Huber Martins Vasconcelos Junior¹
Juliana Maria Ferraz Sallum¹

Affiliations:

¹Universidade Federal de São Paulo, São Paulo, SP, Brazil

Keywords: fixation; functional outcome; microperimetry; retinal sensitivity; *voretigene neparvovec-rzyl*

Abstract:

Voretigene Neparvovec-rzyl (VN) is the first gene therapy approved for treating Inherited Retinal Dystrophies (IRD) caused by biallelic pathogenic mutations in the *RPE65* gene. Early symptoms of *RPE65*-mediated IRD include low vision at birth or early childhood, nystagmus, difficulty seeing in low light, visual field loss, and decreased light sensitivity. Microperimetry (MP), a psychophysical technique for evaluating retinal sensitivity and fixation in the macula, is valuable for assessing functional vision but was not included in the pivotal studies for VN. This study investigates visual function changes after VN therapy using MP measurements. Five patients (ten eyes) with biallelic pathogenic *RPE65* mutations underwent MP testing before and after VN gene therapy. MP parameters, including mean sensitivity (MS), fixation loss, and fixation stability indices, were compared pre- and post-gene therapy. Best-Corrected Visual Acuity (BCVA) was also compared with MP measurements. Statistical analyses ($p < 0.05$) were performed. This study was approved by the Ethics Research Committee (approval number 0387/2023) of the Universidade Federal de São Paulo. Results showed improvement in all MP measurements post-gene therapy. Statistically significant increases were found in retinal sensitivity, especially in MS ($p = 0.013$) and dimmer stimulus perception ($p = 0.002$). Age was strongly correlated with MS improvement ($\rho = -0.911$; $p < 0.001$), with younger patients showing greater gains. No difference was observed in BCVA after therapy. To our knowledge, this is the first study to explore VN gene therapy visual outcomes using MP and to present results for two new *RPE65* variants - c.985delT (p.Cys329Alafs*15) and c.1004A>T (p.Glu335Val) - not previously described. This study highlights that MP offers valuable insights beyond visual acuity and other tests used in pivotal studies with VN, providing more accurate endpoints for assessing retinal function and therapy outcomes in *RPE65*-IRD patients.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Insulin nanoemulsion eye drops for treatment of dry eyes in Sjögren's Disease: A randomized clinical trial Phase I/II

Authors:

Diego Rocha GUTIERREZ¹

Eduardo Melani ROCHA²

Affiliations:

Faculdade de Medicina de Ribeirão Preto- USP

Keywords: Insulin, cornea, nanoemulsion

Abstract:

Background: Dry eye disease (DED) is a cardinal manifestation of patients with Sjogren's disease (SjD), often refractory to conventional treatment with lubricant eye drops. In animal models, insulin applied to the ocular surface stimulates exocrine secretion and cornea wound healing. Furthermore, nanoemulsions can solve the technological challenge of long-lasting persistence and penetration into corneal and conjunctival barriers. This study aims to investigate whether insulin nanoemulsion (INS) is safe and would improve signs and symptoms of DED in SjD. Methodology: Thirty-two patients with SjD were recruited and randomly allocated, double- masked, into placebo (PCB) or insulin (at 20 UI/mL) groups. Subjective data using the Ocular Surface Disease Index - OSDI questionnaire, and objective data, such as tear film breakup time (TBUT), keratitis fluorescein score (CF), conjunctival lissamine green staining (LG), and Schirmer test (ST) were measured at 3 visits over 4-weeks follow-up period, applying the eyedrops provided 3 times a day. Results: Twenty-three patients participated in the 3 visits and completed the study (n INS = 10, PCB = 13). There were no reports of adverse effects that prevented using the eyedrops. No statistically significant difference in intraocular pressure was observed between the groups ($p > 0.05$). Local and systemic side effects were evaluated, with visual blurring being the only side effect that differed between the groups ($p < 0.05$), predominantly in the INS group, mild in intensity, and self-limited to the moment following application. Both groups observed improvement in pre- and post-treatment symptoms through OSDI analysis ($p < 0.05$). However, no statistically significant results were observed between insulin eye drops and placebo. Conclusions: The use of nanoemulsions containing insulin eye drops is safe in patients with SjD. A longer follow-up and different doses may be necessary to assess its therapeutic potential better.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Salivary and urinary concentrations of metals in Sjögren's Disease

Authors:

Ana Luisa da Cunha ALMEIDA¹,
Lina Prado BAFFA¹,
Vera Sathie IWAMI,
Eduardo Melani ROCHA¹

Affiliations:

¹Ribeirão Preto Medical School – University of São Paulo

Keywords: Dry eye. Metal exposure. Sjögren Disease

Abstract:

Introduction: As known, environmental factors are associated with dry eye disease and may play a part in Sjögren's disease (SjD) pathophysiology. This work aims to identify a potential correlation between metals exposition and clinical manifestations of SjD. For that matter, it compares 29 metals in urine and saliva samples of SjD and healthy control patients. Materials and Methods: The volunteers were 57 women with SjD and 21 age and sex-matched controls. The Ocular Surface Disease Index (OSDI) was applied to translate the intensity of the dry-eye symptoms on a scale from 0 to 100. To measure the secretion levels of these patients, the Schirmer Test and the whole saliva flow were evaluated. Blood and urine samples were collected and analyzed. The research had the approval of the Institutional Review Board. Results: The clinical tests showed that the values were lower in SjD patients in comparison with the control group ($p < 0.05$). The analysis of urine and saliva samples revealed higher levels of some metals in patients with SjD ($p < 0.05$). There were 16 metals found elevated in the saliva and 9 in the urine. 6 of them being elevated in both samples: Al, Hg, Mn, Mo, Sb and U. The correlation was positive between OSDI score and copper levels in the saliva samples ($p < 0.0002$). Conclusions: The present work reveals that metals in the saliva and urine of SjD patients are higher than in controls. These observations can be interpreted as a result of the lower flow of these secretions, as expected in the disease. However, this alone would not explain the variation between the metals studied. That way, it suggests that environmental contaminants play a part in the pathophysiology of SjD, leading to the necessity of further investigation.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Clinical study of nanoemulsion eye drops containing insulin for the treatment of ocular damage due to dry eye diseases

Authors:

Vera Sathie Borges IWAMI¹ Mateus Maia MARZOLA¹ Beatriz Carneiro CINTRA Ana Luisa ALMEIDA Eduardo Melani ROCHA¹

Affiliations:

¹Ribeirão Preto Medical School – University of São Paulo

Keywords: Dry eye. Sjögren Disease.

Abstract:

This work aims to analyze a potential therapeutic value of eye drops containing insulin nanoemulsion (INS) 20 UI/ml for treating dry eye symptoms in patients with Sjögren's disease (SjD). Current treatments, such as lubricants and corticosteroids, often have limited effectiveness. The hypothesis is that insulin nanoemulsions could be a safe and effective topical alternative. A case-control study was conducted with women diagnosed with SjD per ACR-EULAR 2016 criteria. Participants were divided into two groups: INS (n=10) and placebo (PCB; n=13), applying one drop in each eye three times a day for three weeks. Evaluations were performed at baseline (t1), week two (t2), and week four (t3), using the Ocular Surface Disease Index (OSDI), Tear Film Break-Up Time (TFBUT), Corneal Fluorescein Staining (CF), Lissamine Green Conjunctival Staining, and the Schirmer Test (ST). All 23 participants (average age 62±8.75) completed the study. OSDI scores at t3 were similar [INS: 28.01; PCB: 25; p>0.05], as were other tests: TFBUT [INS: 6.4; PCB: 4.66], CF staining [INS: 4.5; PCB: 6.0], Lissamine staining [INS: 4.5; PCB: 5.0], and ST [INS: 7.8; PCB: 9.54], all with p>0.05. Adherence analysis via bottle weight confirmed average use of three drops per eye daily. Losses to follow-up, due to external factors, were similar across groups. Insulin eye drops at the studied concentration appear safe, with no signs of ocular damage or adverse effects. However, no significant symptom improvement was observed, suggesting the need for further research. Challenges included limited sample size, short follow-up (30 days), and adherence issues. Future studies should explore different concentrations, longer monitoring, and application in other ocular conditions.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Inhibition of FAAH, the anandamide-degrading enzyme, provides neuroprotection in photoreceptor degeneration in rd10 mice

Authors:

Maria Clara da Silva de JESUS¹
Camila Feitosa MAGALHÃES^{1, 2}
Hilda PETRS-SILVA³,
Lucianne FRAGEL-MADEIRA¹

Affiliations:

¹Department of Neurobiology, Institute of Biology, Fluminense Federal University, Niterói, RJ, Brazil.

²Instituto Estadual de Hematologia Arthur de Siqueira Cavalcanti (HEMORIO), Rio de Janeiro, RJ, Brazil.

³Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

Keywords: Endocannabinoid system. Murine model. Retina. Retinitis pigmentosa.

Abstract:

Retinitis pigmentosa (RP) is an inherited neurodegenerative disorder marked by progressive retinal photoreceptor loss, leading to vision impairment. We used the PDE6 β rd10/rd10 (rd10) mouse model, where a PDE6 β subunit mutation causes photoreceptor degeneration. The endocannabinoid system has neuromodulatory roles in retinal protection and visual processing. Our previous findings showed that daily intraperitoneal URB597 injection, a fatty acid amide hydrolase (FAAH) inhibitor, increased peripheral photoreceptor survival while sparing the central retina. However, systemic effects highlight the advantages of intravitreal administration. This study evaluated FAAH blockade via intravitreal injection for neuroprotection in rd10 mice. Approved by CEUA-UFF (#1464280219), rd10 mice at postnatal day 18 (P18) received 1 μ l intravitreal of PBS 0.01M/DMSO 0.01% or URB597 (30nM, 100nM). Eyes were collected 24h later for histological analysis. Recoverin-positive photoreceptors were quantified, photoreceptor rows were measured via H&E staining, and apoptosis was assessed by TUNEL. Assays were performed in ≥ 4 experiments; data are mean $\pm$ SEM followed by one-way ANOVA. Results showed increased recoverin-positive cells in total retina at 100nM (CTR=100.0 $\pm$ 5.28; 30nM=106.9 $\pm$ 4.76; 100nM=115.2 $\pm$ 4.37, $p=0.0560$). The central region had significant increase (CTR=100.0 $\pm$ 5.29; 30nM=118.0 $\pm$ 8.04, $p=0.0900$; 100nM=120.4 $\pm$ 5.35, $p=0.0498$), but not in the periphery (CTR=100.0 $\pm$ 5.27; 30nM=104.6 $\pm$ 5.36; 100nM=112.4 $\pm$ 5.56, $p=0.2051$). Photoreceptor rows tended to an increase in 100nM (CTR=7.97 $\pm$ 0.59; 100nM=8.56 $\pm$ 0.40) in all retina. TUNEL assay reduced apoptosis in total retina at 100nM (CTR=41.07 $\pm$ 6.67; 30nM=35.97 $\pm$ 5.47; 100nM=13.88 $\pm$ 3.72, $p=0.0032$), central (CTR=41.66 $\pm$ 9.51; 30nM=27.94 $\pm$ 9.10; 100nM=12.04 $\pm$ 3.38, $p=0.0251$) and peripheral retina (CTR=41.42 $\pm$ 6.15; 30nM=47.20 $\pm$ 8.12; 100nM=18.18 $\pm$ 5.12, $p=0.0366$). These findings showed that intravitreal FAAH inhibition with URB597 provides neuroprotection on photoreceptor degeneration in a murine RP model. Financial Support by CAPES, CNPq, FAPERJ, and UFF.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Cannabidiol administration mitigates photoreceptor degeneration in rd10 mice, a model of retinitis pigmentosa

Authors:

Greysa Saraí BARRIOS LEÓN¹
María Fernanda DOS SANTOS FINOCHIO¹
Hilda PETRS-SILVA²
Lucianne FRAGEL-MADEIRA¹

Affiliations:

¹Department of Neurobiology, Institute of Biology, Fluminense Federal University, Niterói, RJ, Brazil.

²Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

Keywords: Retinitis pigmentosa, Cannabidiol, Neuroprotection.

Abstract:

Retinitis pigmentosa (RP) is a hereditary neurodegenerative disease characterized by progressive photoreceptor loss, leading to vision impairment and blindness. Currently, there is no cure, and available treatments only manage symptoms. Given the urgent need for neuroprotective strategies, this study evaluated the effects of cannabidiol (CBD) on RP progression using the Pde6 $\beta^{rd10/rd10}$ (rd10) murine model, a well-established system for retinal degeneration research. Phytocannabinoids like CBD interact with the endocannabinoid system, modulating inflammation, oxidative stress, and apoptosis—key mechanisms in neurodegenerative diseases. This project was approved by the Ethics Committee on Animal Use at Universidade Federal Fluminense (CEUA-UFF, approval number 9492100724). At postnatal day 18 (P18), when starts photoreceptor degeneration at the periphery of the retina in rd10 mice, animals were anesthetized and received intravitreal injections of 1 μ L of CBD (30 μ M or 100 μ M, diluted in 1% DMSO+PBS 0.01 M) or vehicle solution. After 24 hours, eyes were collected for histological analysis using immunofluorescence for recoverin (a photoreceptor marker) and hematoxylin-eosin (HE) staining to assess retinal morphology. Results showed a significant increase in recoverin-positive photoreceptors in 100 μ M CBD-treated group compared to controls, both centrally (control: 282.5 $\pm$ 24.79; CBD 100 μ M: 453.3 $\pm$ 55.57, $p=0.0492$, $n=3$) and peripherally (control: 301.5 $\pm$ 67.89; CBD 100 μ M: 453.7 $\pm$ 38.17, $p=0.0185$). HE staining revealed no significant differences in thickness when analyzing the entire retina. However, at 100 μ m intervals analyses, significant differences emerged between 100 μ M CBD-treated and control groups ($p=0.0153$ at 100 μ m from the optic nerve; $p=0.006$ in the peripheral retina). These findings suggest that CBD exerts neuroprotective effects on rd10 mouse retinas. Further studies with larger samples and extended follow-ups are needed to confirm these results.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Inhibitor of anandamide-degrading enzyme (FAAH) preserved photoreceptors via CB1 and CB2 receptors in rd10 mice

Authors:

Aline Estacio Ribeiro de MATTOS¹
Camila Feitosa Magalhães²
Hilda PETRS-SILVA³
Lucianne FRAGEL-MADEIRA¹

Affiliations:

¹Department of Neurobiology, Institute of Biology, Fluminense Federal University, Niterói, RJ, Brazil.

²Instituto Estadual de Hematologia Arthur de Siqueira Cavalcanti (HEMORIO), Rio de Janeiro, RJ, Brazil.

³Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

Keywords: Endocannabinoids. Neuroprotection. Retinitis pigmentosa.

Abstract:

Retinitis pigmentosa (RP) leads to progressive loss of retinal photoreceptor cells and eventual vision loss. This study investigated whether CB1 or CB2 cannabinoid receptors mediate photoreceptor neuroprotection after daily intraperitoneal (IP) injections of URB597, a FAAH inhibitor, in a murine model of RP. Approved by the Animal Research Committee (CEUA-UFF 1464280219), Pde6 β rd10/rd10 (rd10) mice received IP injections from P14 to P18 with CB1 (SR141716) or CB2 (AM630) antagonists (1 mg/kg) alone or combined 30 min later with URB597 (0.3 mg/kg, FAAH inhibitor). Control animals (CTR) received 0.1% DMSO in 0.01 M PBS. Retinas were collected 24 h later for immunostaining for Recoverin (photoreceptors) and GFAP (reactive gliosis). The number of photoreceptor cells was not altered throughout the retina after treatments with antagonists or URB597 (CTR: 229.1 $\pm$ 10.54; URB597: 271.9 $\pm$ 15.94; SR141617: 211.4 $\pm$ 20.35; SR141617+URB597: 233.2 $\pm$ 29.41; AM630: 210.3 $\pm$ 18.02; AM630+URB597: 219.6 $\pm$ 24.23). However, in peripheral retina, URB597 increased photoreceptor survival, an effect blocked by both CB1 or CB2 antagonists (CTR: 243.9 $\pm$ 13.45 vs. URB597: 319.1 $\pm$ 21.05; p = 0.0072; SR141617+URB597: 218.0 $\pm$ 10.76 p = 0.0061; AM630+URB597: 222.7 $\pm$ 11.48; p = 0.0044). In central retina no significant changes were observed (CTR: 214.2 $\pm$ 11.65; URB597: 224.3 $\pm$ 12.31; SR141617: 207.8 $\pm$ 20.38; SR141617+URB597: 228.3 $\pm$ 31.71; AM630: 207.3 $\pm$ 18.88; AM630+URB597: 213.6 $\pm$ 26.25). GFAP expression also did not show changes in any of the retinal areas (CTR Peripheral: 63.88 $\pm$ 10.80; URB597: 60.59 $\pm$ 11.94; SR141617: 79.40 $\pm$ 31.51; SR141617+URB597: 59.73 $\pm$ 15.13; AM630: 58.52 $\pm$ 17.71; AM630+URB597: 67.64 $\pm$ 23.83; CTR Central: 60.53 $\pm$ 9.830; 61.33 $\pm$ 11.33; AM630: 60.54 $\pm$ 14.44; AM630+URB597: 60.24 $\pm$ 15.17). These findings indicate that the endocannabinoid system plays a neuroprotective role in a murine model of RP and underscore its potential as a target for therapeutic strategies in retinal degeneration. Financial support: CAPES, CNPq, FAPERJ and UFF.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Enhanced P2Y₁ receptor gene expression and its blockade increased photoreceptor degeneration in a murine model of retinitis pigmentosa

Authors:

Douglas Penaforte CRUZ¹,
Heitor Roque CRUZ¹
Jean Bezerra SILVA²
Alexander Henning ULRICH²
Lucianne FRAGEL-MADEIRA¹

Affiliations:

¹Department of Neurobiology, Institute of Biology, Fluminense Federal University

²Department of Biochemistry, Institute of Chemistry, University of São Paulo (IQ-USP)

Keywords: Neurodegeneration. Purinergic Receptor. Purinergic Signaling. Retinal disease.

Abstract:

Retinitis pigmentosa (RP) is a genetic disease leading to progressive vision loss. Although treatments have been explored, long-term results are limited. This study investigates the potential of the P2Y₁R, a purinergic metabotropic receptor, as a therapeutic target for RP, given its role in neurodegenerative diseases. The study was approved by Committee on Animal Research (CEUA-UFF nº 2311120422). C57black/6 (Bl6) and PDE6 β ^{rd10/rd10} (Rd10) mice at key ages for photoreceptor degeneration (P13, P19, P25) were anesthetized, and retinal samples were collected for RT-qPCR. P2Y₁R expression was higher at P19 in Rd10 when compared to Bl6 (Bl6: P13= 1.036 $\pm$ 0.21; P19= 1.002 $\pm$ 0.05; P25= 1.035 $\pm$ 0.19; Rd10: P13= 0.8603 $\pm$ 0.06; P19= 1.540 $\pm$ 0.16 p= 0.0328; P25= 0.6827 $\pm$ 0.24 a.u; n= 3). An acute intravitreal injection of MRS2179 (P2Y₁R antagonist) at 10, 50, or 100 μ M in Rd10 mice at P18 reduced photoreceptor cell number 24 hours later, with the 50 μ M concentration specifically decreasing recoverin immunostaining in central retina (Control: 297.3 $\pm$ 20.87; MRS 10 μ M: 252.1 $\pm$ 23.27; MRS 50 μ M: 219.1 $\pm$ 15.00 p=0.0398; MRS 100 μ M: 249.1 $\pm$ 27.31 recoverin+ cells; n= 5). Accordingly, the photoreceptor layer thickness was decreased with MRS2179 50 μ M in the center of retina (Control: 26.98 $\pm$ 1.095; MRS 10 μ M: 24.24 $\pm$ 1.808; MRS 50 μ M: 20.52 $\pm$ 1.680 p=0.0079; MRS 100 μ M: 21.34 $\pm$ 1.290 μ m; n= 8). After treatment, GFAP reactivity showed no difference between groups (control: 53.88 $\pm$ 15.39, MRS 10 μ M= 60.44 $\pm$ 6.84, MRS 50 μ M= 56.37 $\pm$ 6.56; MRS 100 μ M= 67.31 $\pm$ 3.97 GFAP intensity, n= 3). Finally, the TUNEL assay for analysis of apoptotic cells showed an increased cell death 24 hours after treatment with MRS2179 100 μ M (Control: 21.56 $\pm$ 4.302; MRS 50 μ M: 31.76 $\pm$ 4.203; MRS 100 μ M: 39.19 $\pm$ 3.944 TUNEL+ cells; p=0.0079, n= 4). These findings exert that P2Y₁R is crucial for photoreceptor survival at early stages of degeneration in RP murine model, particularly in the central retina. Financial Support: CAPES, CNPq, FAPERJ and UFF.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: FAAH inhibition as a potential therapy to alleviate oxidative stress in a murine model of retinitis pigmentosa

Authors:

Millena Costa CRISÓSTOMO¹
Kaylane da Silva FERREIRA¹
Rafael de Freitas AZEVEDO-REPOSSI^{1,2}
Camilla Feitosa MAGALHÃES^{1,3}
Hilda PETRS-SILVA²
Lucianne FRAGEL-MADEIRA¹

Affiliations:

¹Department of Neurobiology, Institute of Biology, Fluminense Federal University, Niterói, RJ, Brazil.

²Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

³Instituto Estadual de Hematologia Arthur de Siqueira Cavalcanti (HEMORIO), Rio de Janeiro, RJ, Brazil.

Keywords: FAAH. Microglia. Neuroprotection. Oxidative Stress. Retinitis Pigmentosa.

Abstract:

Retinitis Pigmentosa (RP) is a hereditary neurodegenerative disease characterized by the progressive loss of photoreceptors, leading to blindness. Still incurable, RP involves multiple factors, including microglial activation and an imbalance in the production of reactive oxygen species (ROS). Previous results from our group showed that *in vivo* inhibition of FAAH, an enzyme that degrades anandamide, provided 43% neuroprotection of photoreceptors. This study evaluates the effect of FAAH inhibition on reducing the inflammatory profile in the PDE6 β ^{rd10/rd10} (rd10) murine model of RP and was approved by the Animal Use Ethics Committee (CEUA-UFF 1464280219). FAAH inhibition was induced by URB597 through daily intraperitoneal (IP) injections (0.3 mg/kg, P14–P18/P24) or intravitreal (IV) injections (30/100 nM, P18). Controls received 0.01% DMSO in 0.01M PBS. After 24 hours, retinas were collected for analysis of activated microglia (CD68) using western blotting or incubated with H2DCFDA (50 μ M, 37°C, 1h) for ROS quantification. The analyses were conducted with at least 3 independent experiments. Data are presented as mean $\pm$ standard error of the mean. URB597 IP administration reduced ROS levels by 32% (CTR = 772.7 $\pm$ 98.23; URB = 526.4 $\pm$ 85.32; p = 0.0373). However, at P25, no significant difference was observed (CTR = 156 $\pm$ 28.05; URB = 202.1 $\pm$ 22.61; p = 0.1126). The analysis of microglial activation after IP administration, based on CD68 expression, showed no differences between the observed groups (CTR = 100 $\pm$ 26.38; URB = 85.25 $\pm$ 27.82; p = 0.3601). IV injections of 30 and 100 nM showed a reduction in ROS levels of 50.1% and 56%, respectively (CTR = 1356 $\pm$ 61.08; 30nM = 675.7 $\pm$ 227.4; p = 0.0771; 100nM = 598.7 $\pm$ 230; p = 0.0531 vs CTR). These findings indicate that FAAH inhibition has a neuroprotective effect in the rd10 murine model by reducing oxidative stress in the early stages and potentially slowing disease progression.



XXXIX REUNIÃO ANUAL DA FeSBE
LX CONGRESSO ANUAL DA SBFis
XVI CONGRESSO DA SBBN
XXIII REUNIÃO ANUAL DA BRAVO

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Title: Sex Influence on Retinal Degeneration in the RD10 Model of Retinitis Pigmentosa

Authors:

Kaylane da Silva FERREIRA¹
Millena Costa CRISÓSTOMO¹
Hilda PETRS-SILVA²
Lucianne FRAGEL-MADEIRA¹

Affiliations:

¹Department of Neurobiology, Institute of Biology, Fluminense Federal University, Niterói, RJ, Brazil.

²Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

Keywords: Photoreceptors. RD10. Retinal Degeneration. Retinitis Pigmentosa. Sex Differences.

Abstract:

Retinitis pigmentosa (RP) is a degenerative disease affecting retinal photoreceptors, leading to progressive vision loss. The PDE6b^{rd10/rd10} (rd10) mouse model mimics RP in humans. Sex differences influence the progression of various diseases and their responses to treatment, but remain poorly explored in RP. Investigating these differences in the rd10 murine model is crucial for advancing personalized therapies. This study analyzed sex-related differences in retinal degeneration at postnatal days 18 and 19 (P18 and P19), critical stages of photoreceptor loss. Approved by the Animal Research Ethics Committee (CEUA-UFF 1464280219), analyses were conducted with 4 males and 5 females at P18 and with 2 males and 3 females at P19. Mice were euthanized with an overdose of isoflurane, and their eyes were processed for recoverin immunohistochemistry, a photoreceptor marker. Data are presented as mean $\pm$ standard error of the mean and were analyzed using an unpaired t-test. At P18, females had 15.4% fewer photoreceptors than males in total retina (Male= 614.8 $\pm$ 33.44; Female= 519.9 $\pm$ 10.18; p = 0.0099). A possible reduction of about 19% was observed in central retina of females (Male= 568.0 $\pm$ 54.93; Female= 458.0 $\pm$ 34.76; p = 0.0605), while differences in the periphery were less pronounced (Male= 620.7 $\pm$ 77.22; Female= 554.7 $\pm$ 20.37; p = 0.1664). At P19, no significant sex differences were found in total (Male= 427.4 $\pm$ 29.10; Female= 395.0 $\pm$ 54.00; p = 0.3436), central (Male= 361.5 $\pm$ 37.50; Female= 351.2 $\pm$ 68.09; p = 0.4589), or peripheral retina (Male= 503.5 $\pm$ 67.50; Female= 528.3 $\pm$ 87.83; p = 0.4271). These findings suggest that females may be more vulnerable to retinal degeneration, particularly at P18. Further research is needed to understand sex-related differences in RP progression.



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Title: Effect of varespladib on the reversal of crotoxin-induced neuromuscular blockade

Authors:

Elisa Santiago PEREIRA¹

Leticia Santos BUENO¹

Paula Ladeira ORTOLANI²

Consuelo Latorre Fortes DIAS²

Walter Luís Garrido CAVALCANTE¹

Affiliations:

¹Departamento de Farmacologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais (UFMG), Brasil.

²Centro de Pesquisa e Desenvolvimento, Fundação Ezequiel Dias (FUNED), Brasil.

Keywords: Crotoxin. *Crotalus durissus terrificus*. Neuromuscular Junction. Varespladib. Venom

Abstract:

Snakebite envenomation is a neglected disease that primarily affects economically vulnerable populations. Despite the efficacy of antivenom, its limited availability and adverse reactions highlight the need for complementary treatments. This study evaluated varespladib, a synthetic molecule, in reversing the neuromuscular blockade induced by crotoxin (CTX, from *Crotalus durissus terrificus* venom) in mouse phrenic nerve-diaphragm preparations (CEUA 25/2021). The preparations were maintained in Ringer's solution (carbogen, 35°C). Myographic recordings used indirect electrical stimulation (0.5 ms, 0.2 Hz, 3 V). Three protocols were conducted: (a) CTX (5 µg/mL) followed by varespladib (5 µg/mL) at 10, 30, or 60 min (n=4-7); (b) CTX with washing at 30 or 60 min (n=5-8); (c) CTX, washing (30/60 min), followed by varespladib (n=3-7). Morphological analysis assessed tissue integrity and myonecrosis index (n=4-5). The data (mean) were analyzed by Student's t-test and ANOVA (p<0.05). CTX caused an irreversible blockade, promoting 96% inhibition of twitches at 180 min (p<0.01 vs. control). Varespladib prevented the blockade when administered at 10 minutes after CTX (p>0.05 vs. control), showing partial effects at 30 and 60 minutes with 42% and 33% inhibition, respectively (p<0.01 vs. control). Washing performed 30 minutes after CTX exposure prevented blockade progression, maintaining 50% contractile activity, while at 60 minutes the blockade was irreversible (p<0.01 vs. control). Varespladib administered after washing promoted the reverse of blockade (100% recovery) at both 30 and 60 minutes (p>0.05 vs. control). Therefore, Varespladib prevented and reversed CTX-induced blockade without causing direct muscular effects, demonstrating its potential as a complementary or first-aid therapy in snakebite envenomations.



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Title: Omega-3 and prednisolone therapy enhances the efficiency of limb movements during gait

Authors:

Vitória Luíza Alves de Souza Rios¹
Bruno dos Santos Ricardi¹
Joyce Santana Rizzi¹
Lorraine Silva Requena¹
Maria Eduarda Oliveira Matheus¹
Laísa Taís Cabral Rodrigues Xavier²
Mirela Barros Dias²
Cintia Yuri Matsumura²
Renato Ferretti²

Affiliations:

¹ Universidade Estadual Júlio de Mesquita Filho

² Departamento Biologia Estrutural e Funcional, Setor de Fisiologia, IBB, UNESP Botucatu

Keywords: Gait analysis, omega 3, prednisolone, mdx mice

Abstract:

Dystrophin (DYS) deficiency increases membrane permeability, leading to calcium influx and myonecrosis, resulting in muscle weakness and mortality. Prednisolone (P) is used to alleviate symptoms in mdx mice. Omega-3 (O3) improves myonecrosis and muscle regeneration in mdx mice, showing potential for therapy. Gait analysis using CatWalk in mice with muscular disorders is valuable for accurate assessment of motor function, aiding in understanding disease progression and evaluating potential therapeutic interventions. However, the combined effects of P and O3 on mdx mice locomotion remain unclear. We examined P (5mg/kg/day) and O3 (300mg/kg/day) alone or combined for 15 days in mdx mice, assessing body weight (BW), CK levels, muscle mass (MM), and gait parameters. Data were analyzed using ANOVA/Kruskal-Wallis tests ($p > 0.05$). No isolated O3 exposure, either alone or combined, has shown any difference in BW, CK, and MM. Histopathologically, the O3 and P treatments decreased necrosis and inflammation. No treatments affected the general gait parameters, including speed, cadence, stride length, and base of support. However, P+O3 therapy demonstrated a reduction in swing time concurrent with an increase in swing speed, paw print area, and stance time. Thus, isolated O3 and P spare muscle fibers in the absence of dystrophin, and the combination may enhance the efficiency of limb movements during gait. Financial support from FAPESP (2022/15510-3; 2023/04100-1), PIBIC (9594), and CAPES (88887.915060/2023-00; 88887.948761/2024-00). Protocol number CEUA 1349260423 (ID 000584).