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Book of Abstracts

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- 398 Effect of temperature on acute sublethal contamination by atmospheric particulate matter in Nile tilapia (*Oreochromis niloticus*)
- 399 Toxicological evaluation of subchronic oxandrolone administration in female rats undergoing strength training
- 400 Effects of cyanobacteria *Arthrospira* (*Spirulina*) *platensis* (AP) on liver tumor evolution in a diethylnitrosamine induced mice model
- 401 Effect of temperature on histopathological damages caused by Setttable Atmospheric Particulate Matter (SePM) in tilapia, *Oreochromis niloticus*
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- 405 Histopathological markers in gills of juvenile shrimp (*Macrobrachium rosenbergii*) after contamination by settleable atmospheric particulate matter
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- 418 Chronic exposure to metalliferous atmospheric particulate matter induces changes in gills, liver and kidneys of *Oreochromis niloticus*
- 420 1,2,3-Triazoles as inhibitors of proteolytic, coagulant and phospholipase activities caused by *B. jararaca* and *B. neuwiedi* snake venom
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Title	Metabolic rate of the red-winged Tinamou, <i>Rhynchotus rufescens</i> (Aves: Paleognathae: Tinamiformes)
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Session	Biologia Ambiental, Evolução e Biologia Comparada

Abstract and Keywords	<p>In order to maintain their high metabolic demands, birds have evolved cardiorespiratory adaptations to enhance oxygen uptake and transport, while heart size has been regarded as one of the main limiters for sustained aerobic activities such as flight. Among the palaeognathae birds, the Tinamidae are the only ones capable of flying, even though they have, relatively, the smallest heart among all groups of living birds. In addition, some recent studies have proposed that their small heart and limited flight capabilities represent the ancestral condition of all birds. The present research project aims to analyze resting metabolism of the Tinamidae species <i>Rhynchotus rufescens</i>. To do so, 12 individuals previously approved by the ethics committee (CEUA: 22.1.255.59.2; SISBIO: 65000-4), were submitted to flow-through respirometry, in order to determine their O₂ consumption ($\dot{V}O_2$), CO₂ production ($\dot{V}CO_2$), and respiratory quotient. Preliminary results found a $\dot{V}O_2$ of 13.57 ± 2.65 mL/min.Kg, a $\dot{V}CO_2$ of 9.78 ± 1.89 mL/min.Kg, and a respiratory quotient of 0.73 ± 0.10. Comparing the measured $\dot{V}O_2$ with the expected one when using the allometric equation $\dot{V}O_2$ (mL/min) = $15.13 \times Mb^{0.68}$, where Mb stands for body mass, a significant difference was found (mean difference: 3.25 ± 1.06; t: -3.05; p < 0.05). The same difference was found when the $\dot{V}O_2$ of <i>R. rufescens</i> were compared to other Tinamidae, such as <i>Nothoprocta ornata</i> (t: -5.24; df: 11; p < 0.05) and <i>N. perdicaria</i> (t: -3.00; df: 11; p < 0.05), and the Red Junglefowl, <i>Gallus gallus</i> (t: -7.26; df: 11; p < 0.05). These preliminary results indicate that the <i>R. rufescens</i> may have a $\dot{V}O_2$ significantly smaller than the one expected for a bird of its size. In light of these findings, further analysis of the subsequent steps in the oxygen cascade could provide valuable insights into the underlying mechanisms and address the questions surrounding the cardiorespiratory system of the Tinamidae.</p> <p>Keywords: Birds, Respirometry, Tinamidae</p>
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Title	Comparison of exploratory behavior of four terrestrial isopods (<i>Armadillidium vulgare</i>, <i>Cubaris murina</i>, <i>Porcellionides pruinosus</i>, and <i>Atlantoscia floridana</i>) in an open-field
Authors	João Balieiro Floridi Silvio Morato
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Session	1 - Biologia Ambiental, Evolução e Biologia Comparada

Abstract and Keywords	<p>A recent study showed the exploratory behavior of the terrestrial isopods to be similar to that of rats. It would be interesting to know whether other species also behave similarly. The objective of this project is to compare the exploratory behavior of four species of isopods submitted to an open-field. The species are: <i>Armadillidium vulgare</i>, <i>Cubaris murina</i>, <i>Porcellionides pruinosus</i> and <i>Atlantoscia floridana</i>.</p> <p>Fifteen adults of each species were used. The animals were kept in polypropylene boxes (40x25x7 cm) with a substrate consisting humus and leaf litter. Pieces of tree bark were placed in each box for the animals to hide under. The boxes were kept in a room with a window to the outside, allowing natural light/dark cycle. The temperature was maintained between 24° and 27° C. The substrate was moistened daily with a sprayer. The subjects were fed fish food.</p> <p>The subjects were submitted to a square open field (10x10x5 cm) made of black Plexiglass, with white sulphite paper (75 g/m²) as floor. Each subject was gently placed for 60 seconds inside a PVC tube (3x2.2 cm) placed in the center of the open field. After this time, the tube was removed, and the behavior recorded for 10 minutes. All sessions were recorded with a webcam placed 30 cm above the apparatus and connected to a circular fluorescent lamp. For the analysis, the image in the monitor of the floor of the open-field was divided into 25 2-cm squares with the OBS-Studio software. The videos were analyzed by a trained observer using the X-PloRat software. The data were analyzed using a One-way Analysis of Variance.</p> <p>Statistics showed the subjects of all four species spent more time in the corners than along the walls and the center. It also showed that the species <i>A. floridana</i> and <i>P. pruinosus</i> exhibited a significant higher total crossing of the squares and higher frequency of crossings in the central squares.</p> <p>Keywords: Exploratory behavior, terrestrial isopods, open-field, <i>Armadillidium vulgare</i>, <i>Cubaris murina</i>, <i>Porcellionides pruinosus</i>, <i>Atlantoscia floridana</i>.</p>
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Title	Diazepam alters exploratory behavior of woodlice (<i>Cubaris murina</i>)
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Session	1 - Biologia Ambiental, Evolução e Biologia Comparada

Abstract and Keywords	<p>In a recent study, the exploratory behavior of the terrestrial isopods (<i>Armadillidium vulgare</i>) and rat (<i>rattus norvegicus</i>) males was compared in analogous setups. Since the results were very similar, suggesting analogous drug effects.</p> <p>240 Adult isopods (<i>Cubaris murina</i>) were used. The animals were kept in polypropylene boxes (40x25x7 cm), with a substrate consisting of humus, leaf litter and two wooden blocks (10x5x1.5 cm) where isopods could hide. The subjects were fed fish food. The substrate was moistened daily with a sprayer. The room was kept on a natural light cycle with temperature kept between 24° and 27° C. The subjects were submitted to the moist/dry test, an equivalent of the light/dark test for rats. Diazepam (Valium, Roche, Brazil) was used in a 2.0 mg/ml solution and administered with a micropipette in volumes of 25, 50, and 75 µl, applied to the dorsal area. For each volume tested, a control subject (receiving the same volume of water) and tested. The data were recorded with a webcam and then analyzed using X-Plor software.</p> <p>Kruskal-Wallis one way analysis of variance on ranks showed differences in the frequency of crossings ($H_{[3]} = 99,017$, $P = <0,001$) and in the time spent in the dry section ($H_{[3]} = 70,632$, $P = <0,001$). The multiple comparisons test of Dunn showed that, except for the 25 µl volume, the other two volumes produced significantly smaller frequencies ($P < 0,05$) than the control. It also showed that subjects treated with diazepam, in all volumes, spent significantly more time than controls ($P < 0,05$).</p> <p>The results subjects exhibited suggest diazepam impairs motor control, since it caused a decrease in the frequency of crossings. Diazepam also significantly increased the time spent in the dry section, suggesting a decrease in defense behavior, or perhaps in fear or anxiety-like behavior. The same effect obtained with rats that increase the time spent in the light section.</p> <p>Keywords: Exploratory behavior, diazepam, motricity, <i>Cubaris murina</i>.</p>
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Title	Comparative study: Pole test
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Session	1 - Biologia Ambiental, Evolução e Biologia Comparada

Abstract and Keywords	<p>A recent study showed similarities in the exploratory behavior of rats and woodlice submitted to analog devices (open field, Light-dark and Moist-dry tests). Since the results were very similar, we raised the hypothesis that woodlice could display similar behaviors in other devices. To test this hypothesis we used the pole test and compared the descend time of mice and woodlice.</p> <p>15 Adult isopods (<i>Cubaris murina</i>) and 12 adult mice (<i>Mus musculus</i>) were used. The woodlice were kept in polypropylene boxes (40x25x7 cm), with a substrate of white sand and two wooden blocks (10x5x1.5 cm) under which the isopods could hide. The subjects were fed fish food. The substrate was moistened daily with a sprayer. The room was kept on a natural light cycle with temperature kept between 24° and 27° C. Mice were housed five per cage under conditions of constant temperature (21–23 °C) and maintained on a 12:12-h light/dark cycle with food and water available ad libitum.</p> <p>The subjects were placed head up on top of a bar (20-cm wooden pole 1 cm in diameter for the mice, 12-cm threaded bar 0,5 cm in diameter for the woodlice). We measured the time the subjects took to turn head down and the time to descend the pole. The subjects were submitted to three trials.</p> <p>The results showed that the mice had an average time of 2,42s to turn head down in the first trial, 2,42s in the second trial and 1,83s in the third. Isopods took 9,27s in the first trial, 5,00s in the second and 3,80s in the third. The mice had an average time of 13,17s to descend in the first trial, 9,50s in the second trial and 9,08s in the third. Isopods took 15,20 s in the first trial, 11,40s in the second and 11,07s in the third.</p> <p>The results suggest the pole test, which evaluates motor coordination could be used with isopods as well, since descent time were very similar, in spite of the turning time being much higher for the woodlice. Further studies are needed to validate the isopods as a model for the pole test device.</p> <p>Keywords: Pole test; Descent time; Turning time; <i>Cubaris murina</i>; <i>Mus musculus</i>.</p>
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Title	6-nitrodopamine-induced contractions on <i>Crotalus durissus</i> mesenteric artery in vitro
Authors	Mayara dos Santos Ribeiro ¹ , Gilberto De Nucci ² , Renato Filogônio ² , Cleo Alcântara Costa Leite ³ , Tobias Wang ⁴ , Mariana Gonçalves de Oliveira ¹
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Session	1 - Biologia Ambiental, Evolução e Biologia Comparada

Abstract and Keywords	<p>6-Nitrodopamine (6-ND) has recently been described as a major modulator of cardiovascular function. Earlier studies on the physiological role of 6-ND have focused on responses in isolated aortic rings from corn snakes and rattlesnakes; therefore, we aimed to assess how 6-ND affects resistance vessels in vitro. Experimental protocols were approved by the Ethics Committee for Animal Use of UNICAMP (5266-1/2019). The second branches of the mesenteric arteries from South American rattlesnakes (<i>Crotalus durissus</i>) were dissected and mounted on a wire myograph containing Krebs-Henseleit's solution (37 °C; 95%:5%, O₂:CO₂). The viability of each vessel was tested by (KCl 120 mM), and the concentration-response curves for 6-ND, dopamine, noradrenaline, and adrenaline were obtained. At the end of the concentration- response curve, the alpha-adrenoceptor antagonist phentolamine (10 µM) was added to the organ bath. In a separate set of experiments, vessels were pre-incubated with 6-ND (10 nM) for 30 min, then a concentration-response curve to noradrenaline was constructed. All four catecholamines induced constriction of the mesenteric vessels (n=4 each), and the maximal contractile response induced by adrenaline was the highest. Vasoconstriction induced by all four catecholamines was significantly attenuated by phentolamine. Interestingly, pre- incubation with 6-ND led to a significant increase in the maximal contractile response to noradrenaline (10.94 ± 1.44 mN, n=4) compared to control curves (5.81 ± 3.96 mN, n=4), with no changes in the noradrenaline potency (pEC₅₀). Our results showed that while 6-ND- induced contractions in the mesenteric artery were smaller than those induced by noradrenaline and adrenaline, it exhibited notable synergism with classical catecholamines as a contractile agent, a phenomenon absent with dopamine, noradrenaline, or adrenaline. Future investigations should provide deeper insights into the physiological role of 6-ND in this vascular context.</p> <p>Keywords: nitrocatecholamine, cardiovascular, blood pressure, blood vessel, reptile.</p>
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Title	Acute toxicity and avoidance in <i>Eisenia fetida</i> after exposure to commercial estrogens: variations of the standard method and inferences on susceptibility
Authors	Arthur Dartagnan Chaves dos Santos ¹ Guilherme Ventura Caleffi ² Antonio Carlos Alves Maranhão ² Wilias Silva Santos Greison ³
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Session	1 Biologia Ambiental, evolução e biologia comparada

Abstract, Ethics Committee Number*, and Keywords	<p>We aimed to assess both lethal and sublethal acute toxicity and its impact on escape behavior in <i>Eisenia fetida</i> worms. Eighty test animals were selected from a commercial cluster and distributed equally in 8 compartments (n=10) in a 9cm glass aquarium with fertilized soil. Three dilutions of cyproterone acetate 2mg and ethinylestradiol 0,035mg were prepared: from a 1M TRIS stock solution, from 100% glycerin, and TAE buffer solution. Similarly, 1ml of enantate of noretisterone 25mg and estradiol valerate was added in equal solvents. 100ml of each dilution was dispensed into each compartment and controls for glycerin and TRIS were established. In addition, one positive control with barium chloride and sulfonic acid (1:1) 100g, and a negative control without intervention were established. The assay was conducted without feeding and after 48 hours the results were revealed. Data were analyzed by mean comparison. The negative control compartment was valid; no dead worms were found. Positive control evidenced 24 dead individuals and 7 living ones. TRIS control evidenced a 50% lethal effect (Ec50%), although in dilutions 80% in the norethisterone compartment and 90% in the cyproterone cluster; all worms in reveal were alive. Glycerin solvent as control presented a 30% lethal effect and, respectively, 90% of escape behavior associated with the toxicity of norethisterone and 40% in cyproterone. TAE buffer with cyproterone behaved as Ec50%. 21 migrants were found dead in negative control. We inferred lower escape behavior in TRIS solvent (85% vitality, p <0,05) and lower toxicity in cyproterone associated with ethinylestradiol. Worms susceptible to hormones migrated to positive control and non-susceptible ones were held in their original cluster alive for the duration of the experiment.</p> <p>Keywords: Ecotoxicology; Commercial estrogens; Hormone metabolism; Escape behavior</p>
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Title	Effects of temperature fluctuations on oxygen-carrying capacity, stress and immunity in Nile tilapia (<i>Oreochromis niloticus</i>)
Authors	Mariana Tardio Depintor Michelly Pereira Soares Renato Filogonio Israel Luz Cardoso Cléo Alcântara Costa Leite
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Session	Environmental Biology, Evolution and Comparative Biology

Abstract, Ethics Committee Number*, and Keywords	<p>The progression of temperature (T) alteration in waterbodies impacts physiological responses of aquatic organisms, affecting their survival and all functional processes. We analyzed the impact of varying T (around acclimation reference ~25°C) on several functional indexes of Nile tilapia, <i>Oreochromis niloticus</i>. We analyzed blood samples to evaluate oxygen-carrying capacity of the blood (O₂CC), innate immune activity and stress signaling after 96h in each T (15, 20, 25 and 30°C, CEUA 8147160123). There were 100% survival after experimental protocols. T change affected the support for O₂CC, as evidenced by the decline in hematocrit at 15°C (22.6, 29.4, 30.8, 28.0%, related to 15, 20, 25, 30°C); and mean corpuscular volume (90.0, 135.3, 130.1, 119.3 fL); and the compensatory increase in mean corpuscular hemoglobin concentration (48.2, 33.0, 36.9, 35.6 g dL⁻¹). Hemoglobin, erythrocytes and mean corpuscular hemoglobin remained unaltered. T distancing from acclimation point caused a reduction in total plasma protein (3.8, 4.2, 5.1, 4.4 mg dL⁻¹) and leukocyte respiratory activity (0.3, 0.2, 0.3, 0.2 DO); and an increase in lysozyme (28.9, 28.1, 21.9, 29.5 ng dL⁻¹) and leukocyte counting (119.1, 93.2, 70.0, 110.4 x10³ μL⁻¹). Thrombocyte counting was reduced in fish exposed to 15 and 30°C (72.6, 84.1, 86.8, 44.6 x10³ μL⁻¹). Exposure to 15, 20 and 30°C increased plasmatic levels of cortisol (30.3, 28.9, 16.7, 33.5 ng mL⁻¹). Therefore, while T reduction reflects in lower aerobic capacity and immune activity, any acute change in T were associated with stress response. These changes denote general reduction of fish aerobic capacity, immune response and energy allocation, which may impact environmental use and ecological interactions. These findings emphasize the necessity of proper protocols to assess risk related to the effects of climate change.</p> <p>Keywords: Temperature, Environmental risks, Physiological responses, Health status</p>
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Title	Evaluation of the role of redox metabolism in the tolerance of tardigrades to anhydrobiosis
Authors	Felipe Diego Medeiros ¹ Daniel C. Moreira ² Carlos André Ornelas Ricart ¹ Marcelo Hermes-Lima ¹
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Session	01 - Biologia Ambiental, Evolução e Biologia Comparada

Abstract, Ethics Committee Number*, and Keywords	<p>Tardigrades are highly resilient microinvertebrates that endure extreme environments through cryptobiosis. Biochemical and physiological adaptations are crucial for their survival amidst the environmental stresses they face. This study aims to comprehend the adaptive mechanisms of these organisms, especially in response to desiccation, emphasizing the potential essential role of endogenous antioxidants in surviving, recovering from anhydrobiosis, and subsequently returning to normal levels of metabolic activity. Our investigation involves the use of two inhibitors - aminotriazole (ATZ) and buthionine sulfoximine (BSO) - targeting the antioxidant activity/capacity of tardigrades from the genera <i>Paramacrobiotus</i> and <i>Milnesiumm</i> collected at the Darcy Ribeiro campus of the University of Brasília. The animals are subjected to preincubation for 24 hours in different concentrations of enzymatic inhibitors, then are induced into anhydrobiosis. They remain in this anhydrobiotic state for 24 hours and are subsequently rehydrated. The project is in an experimental phase, but previous results indicated that pre-incubation with ATZ (1 mM) causes tardigrade death after 24 hours of anhydrobiosis, with no observed effect in the control group. Currently, we are determining the activity of antioxidant enzymes catalase and glutathione S-transferase, as well as measuring levels of lipid peroxidation and carbonylated proteins. The study highlights the importance of enzymes and antioxidants for the survival of these animals after desiccation, indicating that anhydrobiosis requires strict control of ROS production. Tardigrades employ primary strategies to deal with the danger of oxygen toxicity, exhibiting an increasing efficiency of antioxidant defenses and metabolic control of energy production and consumption processes. The work provides evidence that antioxidant defenses, such as ROS-scavenging enzymes and other molecules, represent a key group of molecules necessary for desiccation tolerance and redox balance. Research licensed by ICMBio and SisBio (permit number 90279-1).</p> <p>Keywords: tardigrades; redox metabolism; anhydrobiosis.</p>
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Title	Effects of prednisolone therapy on locomotion and pulmonary ventilation in dystrophin-deficient mice
Authors	Joyce Santana Rizzi Lorraine Silva Requena Bruno dos Santos Ricardi Vitória Luíza Alves dos Santos Rios Maria Eduarda Oliveira Matheus Laisa Taís Cabral Rodrigues Xavier Mirela Barros Dias Cintia Yuri Matsumura Renato Ferretti
Affiliations	Laboratory of Muscle Biology (LMBio), Department of Structural and Functional Biology, Sao Paulo State University (UNESP), Botucatu, Brazil
Session	02 - Anatomia

Abstract, Ethics Committee Number*, and Keywords	<p>The absence of dystrophin (DYS) leads to muscle degeneration, inflammation, fat replacement, and calcium influx which culminates in muscle weakness, impaired mobility, respiratory failure, and premature mortality in patients with Duchenne muscular dystrophy (DMD). The <i>mdx</i> mouse serves as a model for studying the absence of DYS. Despite the lack of a cure, prednisolone (P) is commonly used to alleviate DMD symptoms in <i>mdx</i> mice. However, the effects of P on pulmonary function, locomotion, biochemical, calcium level, and histopathology in <i>mdx</i> mice remain unknown. In this study, we evaluated the effects of P therapy (5mg/kg/day for 15 days) in <i>mdxP</i> using various assessments including plethysmography for pulmonary ventilation function, CatWalk for gait parameters, HE staining for histopathological analysis, and western blot for calcium-handling protein levels. Data underwent normality testing (ANOVA/Kruskall-Wallis, $p \leq 0.05$), with $p \leq 0.05$. CEUA 1349260423. Our study revealed abnormalities in gait and pulmonary ventilation parameters in dystrophic mice (<i>mdxOG</i>) compared with wild-type mice. We observed reductions in stance phase, swing, and speed between limbs in <i>mdx</i> mice. P treatment reduced steps per second while increasing the base of support between the caudal paws and prolonging the swing phase time. Additionally, P increased tidal volume and minute ventilation compared to <i>mdxOG</i>. Furthermore, progression of necrosis and inflammation was reduced in the <i>mdxP</i>, indicating a positive effect of P. Calsequestrin levels were increased in P treatment. Overall, treatment with P for 15 days demonstrated positive effects on locomotion and lung ventilation in <i>mdx</i> mice, likely attributable to its reduction of necrosis and inflammation, as well as its improvement of muscle fiber calcium-handling and pathophysiology in the absence of dystrophin. Financial support from FAPESP (2022/15510-3; 2023/04100-1), PIBIC (9594), and CAPES (88887.915060/2023-00; 88887.948761/2024-00).</p> <p>Keywords: Gait analysis, Pulmonary ventilation, <i>mdx</i> mice, Duchenne muscular dystrophy, Prednisolone treatment.</p>
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Title	Curcumin therapy improves pulmonary ventilation in <i>mdx</i> mice
Authors	Bruno dos Santos Ricardi Joyce Santana Rizzi Lorraine Silva Requena Vitória Luíza Alves dos Santos Rios Maria Eduarda Oliveira Matheus Laísa Taís Cabral Rodrigues Xavier Mirela Barros Dias Cintia Yuri Matsumura Renato Ferretti
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Session	02-Anatomia

Abstract, Ethics Committee Number*, and Keywords	<p>The deficiency of dystrophin (DYS) leads to heightened membrane permeability, causing calcium influx, which triggers mitochondrial dysfunction that culminates in myonecrosis. The DYS loss initiates a chain reaction of myonecrosis, and inflammation, contributing to muscle weakness, loss of mobility, respiratory failure, and premature mortality. Despite the absence of a definitive cure, prednisolone (P), is administered to alleviate DMD symptoms. Flavonoids, such as curcumin (C) and Epigallocatechin gallate (EGCG), have shown promise in studies for improving myonecrosis. Drug study assesses unknown effects on pulmonary function, locomotion, biochemistry, and histopathology in <i>mdx</i> mice. We evaluated 15 days of P therapy (5mg/kg/day), C (1mg/kg/day), and EGCG (5mg/kg/day) in <i>mdx</i> mice. The body weight (BW), body length (BL), CK, calcium levels, muscle mass (MM) were examined. Plethysmograph was performed to evaluate pulmonary ventilation function, CatWalk for gait parameters, and HE for histopathological analysis. Data underwent normality testing (ANOVA/Kruskall-Wallis, $p \leq 0.05$), with $p \leq 0.05$. CEUA: 1349260423. The therapies affected the BW, gait and pulmonary parameters. The BL, MM, CK and calcium levels were unaltered by any treatment. Adipose tissue, central and peripheral nuclei were similar to OG. However, the P therapy decreases necrosis, inflammation, and gait parameters (cadence, stand time, swing speed, stride length and width). The C treatment increases tidal volume and pulmonary ventilation compared to OG. The EGCG therapy decreases CK, however, the tidal volume and pulmonary ventilation were decreased compared to C. The P therapy has positive effects on muscle phenotype, and it improves gait parameters. However, the C therapy improved respiratory function over EGCG treatment. Thus, multidrug therapy may be interesting to restore phenotype and function of muscle fibers in the absence of dystrophin. Financial support from FAPESP (2022/15510-3), PIBIC, and CAPES.</p> <p>Keywords: Gait analysis, Pulmonary ventilation, <i>mdx</i> mice, flavonoids therapy</p>
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Title	Gait variations in C57 black mouse strains
Authors	Lorraine Silva Requena Joyce Santana Rizzi Bruno dos Santos Ricardi Vitória Luíza Alves dos Santos Rios Maria Eduarda Oliveira Matheus Felipe Cantore Tiburcio Cintia Yuri Matsumura Renato Ferretti
Affiliations	Laboratory of Muscle Biology (LMBio), Department of Structural and Functional Biology, São Paulo State University (UNESP), Botucatu, Brazil
Session	02 - Anatomia

Abstract, Ethics Committee Number*, and Keywords	<p>Gait analysis is crucial for motor function in rodents, influenced by age and genetic background. The CatWalk system allows the measurement of gait parameters. Differences between the C57BL/6 (BL6) and C57BL/10 (BL10) mouse strains impact gait characteristics, with BL6 generally outperforming BL10 in cognitive and motor tests. Despite similarities in strength, BL10 mice may exhibit coordination deficiencies compared to BL6 and cross-tension gait analysis are lacking. The aim was to provide gait reference data for preclinical studies. We analyzed gait parameters and patterns using CatWalk between BL10 and BL6 mice across ages (30, 45, 90, and 180 days). CEUA 3584280621. ANOVA tests were performed considering $P < 0.05$. Body weight and body length showed increments in both strains, with BL10 exhibiting smaller increments but similar correlations compared to BL6. Body surface analysis revealed differences between strains at different ages. Gait analysis highlighted differences in general parameters such as running duration, number of steps, cadence, and speed, especially at 90 days. The BL10 showed less variability in the base of support and printing position, indicating different gait patterns. The step cycle, standing time, and swing time showed differences between deformations, especially at different ages. Printing parameters showed increases in BL10 mice compared to BL6. The correlation between imprint area and intensity differed in BL10 exhibiting a distinct pattern across ages. Gait analysis revealed differences in general, spatial, and temporal parameters between strains at different ages. These results underscore variations in biometric parameters and gait patterns between BL10 and BL6 mice across different ages, providing valuable insights into the locomotor behavior of these strains for preclinical investigations. Financial support from FAPESP (2022/15510-3; 2023/04100-1), PIBIC (9594) and CAPES (88887.915060/2023-00; 88887.948761/2024-00).</p> <p>Keywords: Gait analysis, C57 mice strains, locomotor behavior, preclinical investigations.</p>
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Title	The effect of vitamin E supplementation on gait parameters in mdx mice
Authors	Maria Eduarda Oliveira Matheus Lorraine Silva Requena Joyce Santana Rizzi Vitória Luíza Alves dos Santos Rios Bruno dos Santos Ricardi Cintia Yuri Matsumura Renato Ferretti
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Session	02 - Anatomia

Abstract, Ethics Committee Number*, and Keywords	<p>Dystrophin (DYS) deficiency elevates membrane permeability, prompting calcium influx and myonecrosis, culminating in muscle weakness and mortality. Vitamin E (VitE) has been investigated for its potential therapeutic effects in MDX mice due to its antioxidant properties. Oxidative stress plays a role in the pathogenesis of DMD, and antioxidants like vitamin E may help mitigate this stress and reduce muscle damage. 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 VitE on mdx mice locomotion remain unclear. We examined VitE (5mg/kg/day) for 15 days in mdx mice, assessing body weight (BW), body length (BL), muscle mass (MM), and gait parameters. Data were analyzed using ANOVA/Kruskall-Wallis tests ($p \leq 0.05$). CEUA 1349260423. The VitE treatment has shown any difference in BW, BL, and MM. Histopathologically, the VitE treatment decreased necrosis and inflammation. VitE therapy demonstrated a reduction in swing time with similar in swing speed, and stance time. Thus, VitE protects muscle fibers in the absence of dystrophin, and it may enhance the efficiency of limb movements during gait. Financial support from PIBIC (9594), FAPESP (2022/15510-3; 2023/04100-1), and CAPES (88887.915060/2023-00; 88887.948761/2024-00).</p> <p>Keywords: Gait analysis, Vitamin E, mdx mice.</p>
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Title	Environmental enrichment effects on zebrafish: Implications on behavior, seizure response, molecular markers, and growth
Authors	Isabela Corrêa Areias ¹ Ana Tada Fonseca Brasil Antiorio ² Viviane Cristina Fais ¹ Claudia Vianna Maurer-Morelli ¹
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Session	3 – Ciência de Animais de Laboratório

Abstract and Keywords	<p>Zebrafish have been used as an experimental model to study various brain diseases, including epilepsy and seizures. These animals require a program of environmental enrichment (EE) to provide proper maintenance and animal well-being. According to the Brazilian legislation, the use of EE is a requisite for laboratory animals; otherwise, the rationale for the objection should be explained on a scientific basis. However, there is no standard program for zebrafish EE, and its impact on experimental results is not fully known. Therefore, it is necessary to evaluate the most appropriate methodology in different research areas. This study aimed to investigate whether an enriched environment could alter behavior in the Novel Tank and Light-Dark Tank Tests, the pentylenetetrazole-induction epileptic response, the transcript profile of brain molecular markers (<i>c-fos</i>, <i>bdnf</i>, and <i>nr3c1</i>), and animal growth. Sixty zebrafish larvae at 5 days post-fertilization were divided into: control group (CG=30), kept without environmental enrichment, and experimental group (EG=30), kept in an environment with various items such as red gravel, artificial plants, colorful resin corals, hiding caves, and conch shells that were alternated weekly for eight weeks. There were no differences between groups in the Novel Tank Test and the latency to reach a complete seizure (tonic-clonic behavior with loss of posture). In the Light-Dark Tank Test, the CG increased transitions between the two halves of the tank. Among the molecular markers, the expression of the glucocorticoid receptor <i>nr3c1</i> was significantly higher in the EG. Additionally, the EG presented a shorter craniocaudal length than the control group. In conclusion, the results show that the use of EE is suitable for studies of seizures/epilepsy in zebrafish. Nonetheless, it interfered with the behavior, which could be minimized by a period of acclimatization. For future studies, the impact of misgrowth should be further investigated. CEUA/UNICAMP approval number 4539-1.</p> <p>Keywords: <i>Danio rerio</i>; animal welfare; environment; pentylenetetrazole; neurosciences.</p> <p>Support: PIBIC-CNPq e FAPESP CEPID-BRAINN 13/07759-3.</p>
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Title	Effects of dyslipidemia due to overexpression of human APOCIII on body mass, triglyceride levels, blood glucose and cholesterol in elderly transgenic mice
Authors	Julia Regina de Andrade Nilton Rodrigues Teixeira Junior Diogo Rodrigues Jimenes Jairo Augusto Berti Carmem Patrícia Barbosa
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Session	3 Ciência de Animais de Laboratório

Abstract, Ethics Committee Number*, and Keywords	<p>Hypertriglyceridemia results from an increase in triglycerides (TG) in the blood, which are composed of apolipoproteins such as apoCIII and transported by chylomicrons. The increase in apoCIII can cause hypertriglyceridemia, and the transport and metabolism of TG can be influenced by aging.</p> <p>This research (CEUA No. 6201190623) used transgenic mice (apoCIII-tgn) that overexpressed apoCIII and non-transgenic mice (NTG) divided into 6 groups: 1. apoCIII-tgn-3m; 2. NTG-3m; 3. apoCIII-tgn-8m; 4. NTG-8m; 5. apoCIII-tgn-20m; 6. NTG-20m. Data analysis used the GraphPad Prism 8® program. For comparison and interaction of the effects of age and hypertriglyceridemia, two-way ANOVA and Tukey were applied for comparison.</p> <p>There was a gradual increase in body mass in relation to age ($p < 0.0001$), regardless of hypertriglyceridemia. TG1 levels showed a significant difference, especially in relation to hypertriglyceridemia, with CIII groups showing significantly higher TG levels compared to NTG groups ($p < 0.0001$). The effect of age was also relevant, reducing in the CIII groups at 3 and 20 months ($p = 0.0076$). TG2 levels showed a significant difference in hypertriglyceridemia, with an increase in CIII compared to NTG ($p < 0.0001$) and a gradual increase in relation to age in CIII ($p = 0.0413$). Plasma cholesterol was significantly higher in CIII compared to NTG ($p < 0.0001$). Plasma glucose showed a significant difference in relation to age, increasing at 20 months, regardless of hypertriglyceridemia.</p> <p>The effects of apoCIII overexpression on body mass are gradual in relation to age, regardless of dyslipidemia. TG1 increased in the CIII, with age influencing and reducing values at 3 and 20 months. TG2 increased with age in the hypertriglyceridemic groups. Cholesterol was higher in transgenic mice and blood glucose increased with age. It is hoped that these analyses will broaden our understanding of the influence of human apoCIII overexpression and aging on these data in transgenic mice.</p> <p>Keywords: Triglyceridemia; cholesterolemia; aging.</p>
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Title	Assessment of the length-weight relationship (condition factor K) from the Brazilian tortoise <i>Chelonoidis carbonarius</i>
Authors	Letícia Ribeiro de Oliveira Bruna Caroline da Silva Lima Claudia Regina Bonini Domingos
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Session	Laboratory Animal Science

Abstract, Ethics Committee Number*, and Keywords	<p>The tortoise species <i>Chelonoidis carbonarius</i> is one of the major species found in Brazil. In general, recent literature offers few specific data relating to this group, for behavioral factors and indices based on morphological parameters. This study aimed to evaluate the condition factor (K) of the <i>Chelonoidis carbonarius</i> species at the São José do Rio Preto City Zoo.</p> <p>Body weight and curvature carapace length (CCL) data were collected from 12 tortoises (3 females and 9 males). For the data obtained, the highest value was 0.978 (male; 3.42 kg and 35 cm long), and the lowest 0.861 (male; 8.52 kg and 46.9 cm long), with the mean of the total values being 0.923 and standard deviation 0.043. The data shows that, even with variations in the weight and length values collected, the condition factor of the animals studied is significantly similar to the group's overall average, which may suggest similar responses from the individuals concerning the habitat conditions in which they are found.</p> <p>Keywords: Condition factor, Brazilian tortoise, <i>Chelonoidis carbonarius</i>.</p> <p>Ethics Committee Number: 257/2024</p>
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Title	Analysis of the effects of the psychostimulant methylphenidate hydrochloride on the flow, biochemical composition and salivary redox state of young adult male rats
Authors	José Vitor Furuya de Lima ¹ Lauani Murakami Lopes ¹ Renan José Barzotti ^{1,2} Larissa Victorino Sampaio ^{1,2} Arieli Raymundo Vazão ^{1,3} Pedro Penati Pimpinato ¹ Guilherme Eduardo Rocha Silva ^{1,3} Rayara Nogueira de Freitas ^{1,3} Rafaela Yumi Gregório Fuzishima ¹ Ana Clara Emilio Padovezi ¹ Maria Clara Pacce Bispo ¹ Isabel Dourado de Oliveira ¹ Ana Laura Favaro Nalin ¹ Ana Cláudia de Melo Stevanato Nakamune ¹ Antonio Hernandez Chaves Neto ^{1,2,3}
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Session	Panel presentation

Abstract, Ethics Committee Number*, and Keywords	Methylphenidate hydrochloride (MTF) is the first-choice psychostimulant for the treatment of children and adults with Attention Deficit Hyperactivity Disorder (ADHD). However, abusive use, known as “intellectual doping”, has been growing among young adolescents and is becoming a serious public health problem among Brazilian university students. The objective of this study was to analyze the effects of MTF on flow, biochemical composition and salivary redox status. To this end, young adult male Wistar rats (4 weeks) were randomly divided into 2 groups (n=10): Control (saline solution) and MFT (3 mg/kg/day), which were administered for 28 days via intragastric gavage. The chosen dose is similar to the therapeutic window for treating ADHD in humans, in addition to being effective in improving spatial learning, memory, attention and locomotor activity in rats. After treatment, pilocarpine-induced saliva was collected for analysis of salivary parameters (CEUA FOA/UNESP n° 255/2023). The normality of the results was analyzed using the Shapiro-Wilk test and comparisons were
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made using the unpaired Student's t test. MTF did not affect salivary pH, but reduced buffering capacity ($p > 0.01$) and salivary flow rate (mL/min/g of salivary gland) ($p < 0.01$). In turn, MTF increased total protein ($p < 0.01$) and amylase activity ($p < 0.01$). Furthermore, MTF promoted an increase in calcium ($p > 0.01$) and phosphate ($p > 0.05$) concentrations, while sodium, potassium and chloride concentrations did not differ between the groups. Total antioxidant capacity ($p > 0.05$) and protein oxidative damage ($p > 0.05$) were lower in the MTF group, while lipid oxidative damage concentrations were similar between groups. The present study suggests that treatment with MTF causes a reduction in flow, damage to salivary composition and an imbalance in the redox state, which can be considered a risk factor for oral health.

(CEUA FOA/UNESP n° 255/2023)

Fomento: FAPESP (2023/12031-0 e 2023/12875-3)

Keywords: Methylphenidate; Saliva; Salivary alpha-Amylase; Oxidative stress.



Title	FairDose, an open-source automated method of oral drug administration for rodents
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Session	Session 1, area 3

Abstract, Ethics Committee Number*, and Keywords	<p>Orogastric gavage, a technique commonly used in pharmacological research can be time-consuming to perform and represents a source of significant risk and distress to animals, ultimately compromising experimental results. Alternative methods to chronic orogastric gavage have been suggested, such as creating micelles for drug delivery in <i>ad libitum</i> drinking water or adding the drug to <i>ad libitum</i> food formulation for animal ingestion. Another option is to use palatable solutions for oral gavage, either by adding them to the drug formulation, applying them directly to the tip of the gavage needle, or administering them with a pipette. In general, these methods are less invasive and cost-effective when used sparingly. This often leads to sufficient drug absorption and less stress compared to traditional methods. However, repeating these procedures for long periods can potentially increase the risk of esophageal trauma followed by death, aspiration pneumonia, and stress with significant weight loss, in addition to negatively impacting animal welfare. All the mentioned issues, along with imprecise dosing, impact the data analysis and the number of animals used in experiments. Thus, to optimize work and animal welfare, we developed FairDose, an open-source automated method of oral drug administration. This</p>
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low-cost device is based on integrating the best of two open-source systems: FARESHARE and PiDose. They can identify each group-housed animal using an RFID tag, weigh them, and administer a drug solution or regular water drops by licking at a spout. This system represents a refinement in the long-term chronic oral treatment of rats and mice, in line with the Principles of Humane Experimental Technique by minimizing the suffering and distress that research animals might experience in this experimental setting.

Keywords: Orogastic gavage; animal welfare; drug administration; long-term treatment; experimental technique.



Title	Prenatal and early life stress effects on rodent microglia, astrocyte, and oligodendrocyte density and morphology: a systematic review and meta- analysis
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Session	Laboratory Animal Science

Abstract, Ethics Committee Number*, and Keywords	<p>Exposure to stress during early developmental stages can have long-lasting adverse consequences, given the vulnerability of the fetus during pregnancy and the sensitivity of newborns to environmental influences post-birth. Disruption in both periods has been associated with an increased risk for the development of psychopathologies later in life, potentially due to alterations in brain maturation. Glial cells, including microglia, astrocytes, and oligodendrocytes, are crucial for brain function and are vulnerable to stress-induced changes, so they could be a potential mechanism associated with adverse outcomes. While literature points to a disrupted glial function induced by stress during early development, results from rodent studies have been conflicting. To address this we performed a systematic review and meta-analysis of rodent studies that investigated the effects of prenatal stress (PNS) and early life stress (ELS) on microglia, astrocyte, and oligodendrocyte density and morphology within the offspring. A total of 95 studies were included, and meta-analysis revealed that animals exposed to PNS or ELS exhibited a significant increase in microglia density, as well as decreased oligodendrocyte density. Furthermore, animals exposed to ELS protocols showed increased microglia soma size. However, there were no significant differences in astrocyte density compared to controls. Meta-regression indicated that stress protocol, sex, age and type of tissue analyzed were significant covariates influencing outcomes. In conclusion, this study provides evidence that stress exposure during early development may disrupt glial cells, especially those involved with immune function and myelination processes, which may have implications for long-term brain function. These findings highlight the importance of preclinical studies in understanding the mechanisms underlying susceptibility to psychiatric disorders.</p> <p>Keywords: Prenatal stress, Early life stress, Microglia, Astrocyte, Oligodendrocyte, Glia.</p>
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Title	Bibliometric analysis of compassion fatigue and occupational stress in laboratory animal science professionals
Authors	Jivago, Jose Luiz Paula Rolo Krewer, Carina da Costa
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Session	Ciência de Animais de Laboratório

Abstract, Ethics Committee Number*, and Keywords	<p>Growing interest in the intersection between compassion fatigue, occupational stress, and animal welfare in laboratory contexts has driven planned investigations into how these factors impact professionals working with laboratory animals. Understanding these dynamics is crucial for developing more ethical laboratory practices that promote compassionate satisfaction. We carried out an extensive bibliometric analysis using the Web of Science, Scopus, PubMed, and EBSCO databases, with the help of the VOSviewer and Bibliometrix tools. We used a detailed search strategy, focusing on terms such as "Compassion Fatigue", "Laboratory Animals", and "Workplace Stress", to capture relevant publications from 2000 to the present. We identified a total of 235 studies, distributed as follows: 23 in Web of Science, 190 in Scopus, 13 in PubMed, and 9 in EBSCO. A significant increase in the production of articles related to the topic was presented from 2019 onwards. A co-authorship analysis revealed three main research clusters, highlighting a strong collaboration network focused on ethical management practices, animal welfare, and occupational support. The areas of occupational stress and compassion fatigue have emerged as important research topics. The results highlight the importance of an integrated approach that considers human well-being, animal well-being, and scientific validity. The identified collaboration networks suggest a solid foundation for future interdisciplinary research. It is recommended that research institutions adopt policies that promote ethical work practices and effectively support the mental health of professionals involved in animal care.</p> <p>Keywords: Compassion Fatigue, Occupational Stress, Laboratory Animal Welfare, Bibliometric Analysis, Ethical Practices</p>
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Title	Boric acid for topical treatment of Psoroptic mange in rabbits used for hematophagy of <i>Rhodnius prolixus</i> – case report
Authors	Desenir Adriano Pedro Mateus Odorico Linhares do Nascimento Gustavo Dornelles Machado Caroline dos Santos da Fonseca Priscila Angelica Vicente de Oliveira Natalia Lopes de Carvalho Marcel Frajblat
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Session	03 – Laboratory Animal Science

Abstract, Ethics Committee Number*, and Keywords	<p>Rabbits commonly suffer from mange caused by the ectoparasite <i>Psoroptes cuniculi</i>. The mite causes lesions in the ear canal, forming gray or brown crusts with a dry, thick and flaky appearance. Affected animals experience itching, agitation and may develop otitis and vestibular syndrome, impacting their health and well-being. Conventional treatment involves antiparasitic drugs associated or not with anti-inflammatories and/or antibiotics. Although effective, these drugs can cause gastrointestinal damage to rabbits, in addition to making their use in feeding hematophagous insects unfeasible for a prolonged period, when intended for this purpose. This work aimed to report a case of treatment of sarcoptic mange with the use of boric acid in rabbits used for hematophagy of <i>R. prolixus</i>. Five rabbits with characteristic sings of early-stage sarcoptic mange were treated with boric acid, preceded by the application of mineral oil in both ears of each animal, followed by the administration of ½ teaspoon of boric acid powder and subsequent ear massage, to fix the powder to the oil. Four days post-treatment, the animals showed no signs of mange and appeared macroscopically free of irritation or other changes. Groups of adults and 5th stage nymphs of <i>R. prolixus</i> were fed with rabbit blood after 15 and 4 days of treatment. None of the fed insects died or showed anybody or behavioral variation. Boric acid proved to be effective for the treatment of early stage sarcoptic mange in rabbits and was safe feeding adults and 5th stage nymphs of <i>R. prolixus</i> with the treated animals' blood, with a minimum interval of 4 days post-treatment. More studies are needed to investigate the safety of the treatment at microscopic and biochemical levels for rabbits and insects fed with the blood of these animals.</p> <p>Keywords: Animal Welfare. <i>Oryctolagus cuniculus</i>. Rabbit ear mite.</p>
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Title	The use of an OMV vaccine for the immune response to Theiler's Murine Encephalomyelitis Virus (TMEV) - GDVII
Authors	Daniela Cagnoto Noronha ¹ Daniel Gianfrancisco ² Daniele Masselli Rodrigues Demolin ² Marcelo Lancellotti ³ Nathalia Rocha de Oliveira ¹ Mayara Cristina Moreira Silva ¹ Marcus Alexandre Finzi Corat ¹
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Session	03 - Ciência de Animais de Laboratório

Abstract, Ethics Committee Number*, and Keywords	<p>The Theiler's Murine Encephalomyelitis Virus (TMEV) - GDVII is a natural enteric pathogen of mice that is of great interest as it is part of unwanted contaminants in an SPF colony. This virus and its pathological development have significant relevance for producing model studies and treating demyelinating diseases of the central nervous system due to its high similarity to neurodegenerative diseases such as poliomyelitis in humans and distemper in dogs. Therefore, in this stage of our work, we aim to produce vaccines using Outer Membrane Vesicles (OMV) from bacteria to stimulate the immune system of mice to produce antibodies against this pathogen. The use of <i>Neisseria meningitidis</i> OMV as a methodology for vaccine production is safe because it involves harmless, biocompatible vesicles that are easy to produce. For OMV vaccine manufacturing, after 48 hours of infection of BHK-21 (Baby Hamster Kidney) cell line cells in disposable 75cm² plastic bottles (Costar, USA) with TMEV-GDVII virus, approximately 1 x 10⁹ OMV were added and shaken at room temperature for 24 h at 150 rpm. The supernatant containing the virus-OMV complex was inactivated at 56°C. We administered 100 µL of the vaccine subcutaneously to 6 c57/bL6/J lineage animals to be analyzed after 15 days in the serum of the challenged animals by Indirect Immunofluorescence (IFI) for specific antibody production. For this, after blocking with non-specific human serum, the 1:20 sera of vaccinated mice were placed in contact with slides containing the GDVII antigen. Negative and positive control tests were conducted with negative and positive sera, respectively, prepared previously in the laboratory. We performed readings of the slides under a Fluorescence Microscope, and positivity was considered when fluorescence was evident, and the characteristic cytopathic effect was observed.</p> <p>Ethics Committee Number (CEUA): 6316-1/2023 Keywords: vaccine, OMV, indirect immunofluorescence</p>
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Title	Effects of resveratrol on body weight and white adipose tissue in adult rats subjected to the maternal obesogenic diet
Authors	¹ Ramos, A. H. L. S.; ² Mendonça, C.R.; ² Bandeira, V. C. A.; ¹ Amadeu, G.V.S ¹ Silva, P. E.; ² Silva, M. L. P. ; ² Galindo, L. C. M. ; ^{2,3} Vasconcelos, D. A. A
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Session	Poster

Abstract, Ethics Committee Number*, and Keywords	<p>Maternal obesogenic diet (MOD) can lead to long-term obesity and insulin resistance in offspring. Resveratrol is a polyphenol has been studied for its effects related to modulating metabolic processes and reducing the accumulation of white adipose tissue (WAT). This study aimed to analyze the effects of resveratrol administration during pregnancy and lactation on body weight and adipose tissue in adult rats submitted to a maternal obesogenic diet. The pregnancy rats received experimental diets and/or resveratrol during pregnancy and lactation, were divided into three groups: control diet AING93 (DCMat); obesogenic diet enriched with fat and sugar (DOMat); and obesogenic diet associated with resveratrol (DOMat+Res). The offspring rats were monitored from birth, receiving a standard diet throughout the post-weaning period until adulthood, when the animals were euthanized for tissue collection and subsequent analysis. Ethics Committee on Animal Use of the Federal University of Pernambuco nº 0020/2021. In terms of weekly body weight, the DOMat group increased weight compared to the DCMat group from the 63rd week (DCMat=273.84g±7.92 vs *DOMat=305.02g±7.14) until the 90th week (DCMat=338.14g±6.65 vs *DOMat= 387.99g±13.26). In total weekly weight gain, the DOMat group demonstrated an increase compared to the control group (DCMat=263.1g±3.71 vs *DOMat=305.5g±12.86). The group DOMat+Res once again attenuated weight gain in the group that received only DOMat. In the adipocyte area of the retroperitoneal adipose tissue, animals with DOMat showed a greater area of adipocytes compared to DCMat (DCMat=568um²±18.27 vs *DOMat=603.1um²±18.48). The increase in adipocyte area in animals that received DOMat+Res was reversed (DOMat=603.1um²±18.48 vs *DOMat+Res=441.1um²±9.12). The results of the study suggest that the administration of resveratrol was promising for attenuating body weight gain and the hypertrophy of adipocytes in adult rats subjected to a maternal obesogenic diet.</p> <p>Keywords: Maternal obesity; Resveratrol; White adipose tissue; Metabolism.</p>
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Title	Characterization of the copper decavanadate effects on blood vessel development using the chicken embryo chorioallantoic membrane (CAM)
Authors	¹ Almeida, N.A., ¹ Oliveira, E.T., ¹ Mariano-Lara, L.M.M., ¹ Kempis, R., ¹ Barbosa, V., ¹ Gonçalves, G.V., ¹ Zela, J.C., ³ Rodrigues, D.S., ¹ Taborda-Rocha, L., ² Sá, E.L., ² Nunes, G.G., ¹ Pereira, L.F.*
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Session	3- Ciência de Animais de Laboratório

Abstract, Ethics Committee Number*, and Keywords	The process of wound healing is a complex series of events that involves various stages such as cell division, synthesis of new extracellular matrix, neovascularization, chemotaxis, and scar tissue formation and remodeling. However, some wounds do not heal as expected, leading to chronic wounds. The Vanadium compounds have been extensively studied due to their interaction with proteins and their potential application in medicinal chemistry. Among them, decavanadate has demonstrated a wide medical application. Also, copper is essential in biochemistry, catalyzing different enzymes. Thus, copper inserted in a decavanadate structure should improve his biological activity. The chicken embryo chorioallantoic membrane (CAM) is a tissue that forms a network of blood vessels that helps study angiogenesis. Our methodology was approved by the institutional ethical committee (Nº 850). The fertilized eggs were incubated for 7 days, a window was opened in the shell, and CAM was injected with different concentrations (None, 0.1,0.5,1.0,5.0,15 $\mu\text{g}.\text{mL}^{-1}$) of the CuV_{10} and sealed with adhesive tape. CAM was photographed with a stereoscopic microscope seven
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days later to count the blood vessels. The pictures were analyzed using Image-Pro Plus® program. Statistical analyses performed with mean±SEM, ANOVA, and Tukey's test. Afterward, CAM was removed and fixed for histological analysis. Assays with CAM revealed that decavanadate (V₁₀) was lethal in all doses. Solutions of CuV₁₀ triggered an increase in CAM's blood vessel density in a dose-dependent manner in addition to changing the vessels' caliber, as compared with the control. Finally, the copper sulfate test decreased the number of vessels in the membrane. Studies have shown that cooper decavanadate can increase the number of vessels in the CAM system, indicating a potential positive impact on wound healing. However, further assays are necessary to verify its effect on CAM healing.

Keywords: Blood vessels; CAM; Vanadate; CuV₁₀.



Title	Use of the PDCA method in the management of the rabbit facility of the Health Sciences Center (CCS) at UFRJ
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Session	03 – Laboratory Animals Science

Abstract, Ethics Committee Number*, and Keywords	<p>The PDCA method (Plan - Do – Check – Act) is a management tool used to solve problems and optimize tasks. This technique assists in improving the performance of activities, decision-making and, consequently, the results of a process. It can be used in any type of organization, whether it's a project, a private company or a public service. The Federal University of Rio de Janeiro has a facility housing a maintenance colony of New Zealand rabbit, used for hematophagy of <i>Rhodnius prolixus</i>. In order to refine the work carried out in the facility as well as the installation itself, this study aimed to use the PDCA method as a management tool. The first step of the project was to identify the problems and possibilities for improvements of the rabbit facility and carry out planning of how they would be carried out enhancement changes (Plan). Next, the planned measures were implemented (Do). After, the changes were analyzed and whether they were brought about improvements (Check). Finally, the efficient changes became the standard, while the inefficient ones were revisited within the cycle. This work took place between May 2023 and January 2024 and, after this period of applying the method, we obtained 94 improvements in the facility as a result. There are 44 of them acquisitions of furniture and materials, 21 refinements in the routine animal care activities, 10 innovations and reforms, and 19 refinements in administrative activities. Therefore, it was found that the PDCA method brought positive results as a management tool applied in the maintenance facility for New Zealand rabbits at UFRJ.</p> <p>Keywords – Management. New Zealand rabbit. PDCA cycle.</p>
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Title	Effect of copaiba oil on oxidative stress in hypothalamic obese rats
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Session	Ciência de Animais de Laboratório

Abstract, Ethics Committee Number*, and Keywords	<p>Obesity can lead to irreversible alterations in cellular metabolism, impacting mitochondrial respiratory function and biogenesis. Copaiba Oil (OC) is known for its antioxidant properties, and here we investigated this effect on the liver of rats with hypothalamic obesity. For this, Wistar rats were induced hypothalamic obesity through subcutaneous injections of Monosodium Glutamate (MSG; 4g/kg of body weight) during the first postnatal week (n=12). Control (CON) groups (n=12) received equimolar saline injections. At 21 days of age, both CON and MSG groups were divided and OC supplementation was administered, via gavage (0.5 mL/kg) three times per week for eight weeks, while non-supplemented animals (NS) received saline (0.9%) following the same schedule. Four experimental groups were established: CONNS, CONOC, MSGNS, and MSGOC(n=6/group). At 90 days, the animals were euthanized, and plasma and liver oxidative stress levels were assessed using CAT, SOD, and TBARS assays. All protocol was approved by the Animal Use Ethics Committee (CEUA) of Unioeste (number 13-20). Interestingly, elevated oxidative stress was observed in the liver of MSGNSanimals, while a notable reduction occurred with OC supplementation in MSGOCanimals. OC supplementation reinstated CAT and SOD levels to normal and decreased TBARS, indicating inhibition of lipid peroxidation. Enzymatic antioxidants in the liver and plasma (SOD and CAT) play pivotal roles in scavenging reactive oxygen species (ROS) such as O₂•⁻ and H₂O₂. Considering OC's antioxidant properties interaction with these free radicals during the study is plausible. These findings suggest that Co-oil supplementation may be an effective intervention for alleviating oxidative stress linked to obesity.</p> <p><i>Copaifera Officinalis; antioxidant; bioactive compound; liver.</i></p>
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Title	A new experimental animal facility: The challenges from implementation to operation
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Affiliations	Biotex – Experimental Bioterium at Faculty of Pharmaceutical Sciences; LAFTex – Phytochemistry, Pharmacology and Experimental Toxicology Laboratory; FCF – Faculty of Pharmaceutical Sciences; UNICAMP – University of Campinas; Campinas – Brazil
Session	03 - Laboratory Animal Science

Abstract, Ethics Committee Number*, and Keywords	<p>The use of animal experimental models is essential for the development of biomedical research. Therefore, to ensure the attainment of reproducible results, these animals require a facility that meet requirements for breeding, handling, and animal welfare. Therefore, with this in mind, the Faculty of Pharmaceutical Sciences - FCF, of UNICAMP (State University of Campinas), established in 2018 the process of implementation of the Experimental Animal Facility, Biotex, within the premises of standardizing the environmental and animal management according to the good practices in experimentation defined by CONCEA (National Council for Animal Experimentation Control). Therefore application for funds to adapt the animal facilities according to CONCEA with further registration at CIUCA (Registry of Institutions for Scientific Use of Animals), establishing internal biosafety norms and internal regulations were undertaken. The structure of the animal facility aligned with 22 out of the 24 mandatory criteria of NR-57. All experimentation protocols are submitted for CEUA approval by prior to experimental assays. as of January 2024 the animal facility began operation. Many challenges such as high cost of implementation, infrastructure maintenance, training of the entire team, as well as the commitment to ensuring animal welfare were overcome. However, planning and responsible resource management, as well as ethical behavior are essential to ensure quality management for reliable and reproducible data.</p> <p>Keywords: Laboratory Animal Science, animal welfare, animal facility.</p>
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Title	Efeito antioxidante do extrato fracionado da soja no músculo diafragma de camundongos distróficos mdx
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Affiliations	¹ Departamento de Anatomia - DANat, Instituto de Ciências Biomédicas, Universidade Federal de Alfenas (UNIFAL-MG), Alfenas, MG, Brasil. ² Departamento de Alimentos e Medicamentos, Faculdade de Ciências Farmacêuticas, Universidade Federal de Alfenas (UNIFAL-MG), Alfenas, MG, Brasil.
Session	Laboratory Animal Science

Abstract, Ethics Committee Number*, and Keywords	<p>A Distrofia Muscular de Duchenne (DMD) é uma doença genética que causa mutação no gene da distrofina, proteína importante para o funcionamento normal dos músculos. Sua falta resulta em fraqueza, degeneração muscular, alteração da homeostase intracelular de íons cálcio, ativação de proteases endógenas e necrose da fibra muscular. O acúmulo intracelular de cálcio resulta na captação anormal pela mitocôndria, aumentando a produção de espécies reativas de oxigênio (EROs). Estudos comprovaram que a ação antioxidante das isoflavonas com a utilização do extrato fracionado da soja (EFS) contendo 2 mg/kg de genisteína em camundongos mdx se dá pelos possíveis efeitos benéficos de redução do estresse oxidativo no músculo distrófico pela atividade das isoflavonas na modulação dos ERs e regulação da atividade de enzimas antioxidantes. O camundongo mdx é um modelo animal frequentemente usado para estudar DMD, pois possui mutação homóloga à encontrada em humanos. Os animais foram tratados com EFS a partir do 14º dia de vida por 46 dias, e após eutanásia, coleta do músculo diafragma, mais afetado entre os músculos, para avaliação do estresse oxidativo crônico a partir dos grânulos de lipofuscina, peroxidação lipídica pela quantificação dos níveis de 4-HNE e sistema antioxidante enzimático através da quantificação dos níveis de SOD2 e CAT. O extrato seco de soja foi realizado por extração exaustiva e percolação etanólica. Análises HPLC-ESI-IT-MSn e FIA-ESI-IT-MSn foram realizadas para determinar a quantidade de genisteína total no EFS. Todo protocolo experimental seguiu os princípios éticos da COBEA e aprovado pelo CEUA da UNIFAL-MG (0019/ 2021). Camundongos da linhagem C57BL/ 10 e camundongos mdx foram divididos em grupo controle (Ctrl), mdx tratados com salina (mdxSAL), mdx tratados com prednisona (mdxP) e mdx tratados com EFS (mdxS). A quantificação cromatográfica identificou uma média de 0,042 mg/ml de genisteína no EFS. Não foram observadas diferenças significativas nas quantificações por Western</p>
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Blotting (CAT, SOD2 e 4HNE). Os grânulos de lipofuscina apresentaram dados significativos do grupo mdxS. (%: Ctrl=0,0008±0,0007; mdxSAL=0,0456±0,0052; mdxP=0,0287±0,0046; mdxS=0,0241±0,0077*). Baseado no exposto, é possível concluir que o EFS tem propriedades antioxidantes benéficas para o músculo diafragma distrófico do modelo mdx.

Estresse oxidativo, fitoestrógeno, isoflavonas de soja, distrofia muscular de Duchenne, camundongos *mdx*.



Title	The effects of silver vanadate (AgVO₃) on a blood vessel network using the chicken embryo chorioallantoic membrane (CAM)
Authors	¹ Almeida, N.A., ¹ Oliveira, E.T., ¹ Mariano-Lara, L.M.M., ¹ Kempis, R., ¹ Barbosa, V., ¹ Gonçalves, G.V., ¹ Zela, J.C., ³ Rodrigues, D.S., ¹ Taborda-Rocha, L., ² Sá, E.L., ² Nunes, G.G., ¹ Pereira, L.F.*
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Session	3- Ciência de Animais de Laboratório

Abstract, Ethics Committee Number*, and Keywords	Vanadium is a metal found in nature and living organisms such as fungi and macroalgae. Its biological activities, including antibiotics, have attracted attention in the medicinal field. Recently, it has demonstrated its action on various tumors, such as breast, prostate, and lung. In various biological fields, the chicken embryo chorioallantoic membrane (CAM) has been used as an alternative to traditional research methods on blood vessels. This study aimed to examine the impact of silver vanadate on a network of CAM vessels. All animal procedures underwent approval by the institutional ethical committee (number: 01657.90). Fertilized eggs were kept in an incubator (70% RH, 37-38°C). After an incubation period of 7 days, AgVO ₃ (none, 0.1, 1, 5, 15 µg.mL ⁻¹), the vanadate ion, and AgNO ₃ were implanted directly onto the CAM by opening the eggshell. The eggs were then closed and returned to incubation for seven days. After that, the eggs were reopened and photographed with a stereoscopic microscope to visualize their macroscopic appearance. The samples were analyzed using the
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IMAGE PRO PLUS® 4.5 program, and histological study samples were taken. The results were described as Mean \pm SEM and analyzed using the ANOVA and Tukey test. The vanadate ion was found to be lethal at all concentrations. Meanwhile, AgNO₃ decreased the number of vessels by 15-50%, and the highest dose was lethal. The data from AgVO₃ were also significant, showing a respectively decreased vessel density of 55.28% (at 0.1 $\mu\text{g.mL}^{-1}$), 34.89% (at 1 $\mu\text{g.mL}^{-1}$), and 28.28% (at 5 $\mu\text{g.mL}^{-1}$). However, the dose of 15 $\mu\text{g.mL}^{-1}$ did not show significant results. In conclusion, silver vanadate is an efficient angiogenesis inhibitor in the CAM vessel network. It can reduce blood support in regions with or without tumors. Further studies are necessary to explore the effects of Silver Vanadate on blood vessels.

Keywords: Blood vessels; CAM; AgNO₃; Vanadate; AgVO₃.



Title	Adipocyte-specific Dicer deletion alters clock genes and adrenergic signaling in the heart of mice
Authors	Henver S. Brunetta ¹ , Stepheny C. de Campos Zani¹ , Gabriel P. Ruiz ¹ , Anna Jung ³ , Alexander Bartelt ³ , Julio C. Ferreira ⁴ , Marcelo A. Morj ^{1,2}
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Session	Biologia e Doenças Cardiovasculares

Abstract and Keywords	<p>The adipose tissue communicates with other organs via a myriad of pathways, including via microRNAs (miRNA). Adipocyte-specific Dicer deletion (AdicerKO) alters the profile of circulating miRNAs, resulting in metabolic dysfunction and premature aging. However, the effect of AdicerKO in the heart has not been investigated. We tested the hypothesis that Dicer ablation in adipocytes affects heart function via miRNAs. For that, 10-12 weeks old male C57Bl/6J mice (Dicer^{fl/fl}-AdipoCre⁻ (WT) or Dicer^{fl/fl}-AdipoCre⁺ (AdicerKO)) were fed a low-fat diet (10% kcal fat) for 20 weeks (animal care committee protocol 5632-1/2020) and, after anesthesia, the left ventricle (LV) was collected and snap frozen for further analysis. <i>In vitro</i>, Dicer was silenced in brown adipocytes (WT1) using siRNA and they were co-cultured with cardiomyocytes (H9C2) for 48 hrs. Bulk mRNAseq showed a dysregulation in the circadian cycle gene expression in AdicerKO mice. To further investigate this, we euthanized another cohort of mice every 4 hours throughout the day. We observed that circadian rhythm related genes <i>Nr1d1</i> and <i>Nr1d2</i> had a different expression pattern in AdicerKO compared to WT over a 24-hr cycle. Likewise, Dicer silencing in brown adipocytes <i>in vitro</i> downregulated miRNA abundance while increasing <i>Nr1d1</i> and <i>Nr1d2</i> expression in H9C2 cells. KEGG analysis of the downregulated mRNA in LV of AdicerKO revealed an enrichment of genes associated with adrenergic signaling, we then tested the acute effects of norepinephrine (NE) on the adrenergic pathway in the LV of AdicerKO mice. After NE injection, p-phospholamban and p-CaMK abundance was decreased in LV samples from AdicerKO mice at 4 PM, but not at 4 AM, indicating a circadian-dependent impairment in the adrenergic signaling. In conclusion, Dicer ablation in adipocytes affects clock genes in cardiomyocytes, resulting in circadian-dependent adrenergic resistance, probably due to changes in the adipose tissue-derived miRNA abundance.</p> <p>Key words: Dicer, organ crosstalk, adipose tissue, circadian rhythm, miRNA.</p>
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Title	CETP-mediated oxidative stress impairs PVAT's anticontractile function in males
Authors	Lazaro, Carolina Martins; Netto, Israelle Freitas; Oliveira, Helena C. F.; Davel, Ana Paula.
Affiliations	Department of Structural and Functional Biology, Institute of Biology, State University of Campinas - UNICAMP, Campinas, SP, Brazil.
Session	Biologia e Doenças Cardiovasculares

Abstract, Ethics Committee Number*, and Keywords	<p>The plasma cholesteryl ester transfer protein (CETP) mediates cholesteryl ester transfer from HDL to VLDL/LDL, reducing HDL-cholesterol, a key atheroprotective lipoprotein. CETP also impairs endothelial function in males due to local oxidative stress. Perivascular adipose tissue (PVAT) modulates vascular tone and redox status. Therefore, we investigated if CETP impacts PVAT anticontractile and antioxidant functions. Transgenic male mice (4-6 month) expressing human CETP gene and non-transgenic controls were compared (n=5-8/group; CEUA 5353-1/2019). Thoracic aortas were dissected with (w) or without (wo) PVAT for phenylephrine (PE) concentration-response curves. In aortic PVAT we evaluated nitric oxide (NO) levels (DAF-2DA fluorescence), eNOS and peNOS^{Ser1177} protein levels (Western-blot), ROS production (DHE fluorescence), gene expression (qRT-PCR) and lipid content (histology). Data were analyzed by ANOVA or Student's t-test (*P<0.05) and expressed as mean±SEM or % of control. Mice expressing CETP lost PVAT anticontractile function (Rmax: wo PVAT= 3.0±0.9 vs. w PVAT= 2.9±0.8, NS), accompanied by decreased NO (-25%), eNOS (-50%) and peNOS (-42%) expression. Increased PVAT ROS production (+34%), altered gene expression related to oxidative stress (+58% <i>Nox1</i>, +35% <i>Nox2</i>, -20% <i>Sod2</i>, -17% <i>Sod3</i>) and inflammation (-16% <i>Cd163</i>, -39% <i>IL10</i>, -30% <i>Arg1</i>, +92% <i>Tnfa</i>) were found in PVAT expressing CETP. Incubation of aortic PVAT rings with NOX inhibitors (DPI and GSK2795039) and with SOD mimetic (Tempol) restored PVAT anticontractile function in PE-curves from CETP mice. PVAT from CETP mice exhibited higher lipid content (+19%) and leptin expression (+86%) while brown adipose tissue markers were reduced (-33% <i>Prdm16</i>, -39% <i>Cidea</i>) indicating whitening of aortic PVAT. In conclusion, these results highlight the adverse impact of CETP expression on PVAT function and phenotype in males, due to decreased NO bioavailability and oxidative stress.</p> <p>Keywords: CETP; PVAT; Oxidative Stress.</p> <p>Financial support: FAPESP (São Paulo Research Foundation).</p>
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Title	Periodontal disease exacerbates coronary endothelial dysfunction in elderly rats: prevention through prebiotic treatment (β-glucan)
Authors	Grazielle Caroline da Silva ¹ Eduardo Damasceno Costa ² Virgínia Soares Lemos ³ Luciano José Pereira ⁴
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Session	Biologia e Doenças Cardiovasculares

Abstract and Keywords	<p>The relationship between periodontal disease (PD) and cardiovascular diseases has been the subject of study due to growing evidence of their interconnection. The study evaluated coronary vascular function in rats with PD during aging and the effect of prebiotic intake (β-glucan - <i>Saccharomyces Cerevisiae</i>). Young and aged rats were subjected to PD induction by ligation (14 days). Aged rats received β-glucan (50 mg/kg) or saline orally (gavage - 4 weeks). A myograph system assessed vascular reactivity. The expression of the protein was quantified by western blotting and the level of serum cytokines was assessed by ELISA. PD in aged rats was associated with reduced acetylcholine (ACh)-induced relaxations of coronary artery rings (aged rats $67 \pm 3.39\%$ versus PD-aged rats $54 \pm 4.5\%$). No change in ACh relaxation was observed in young rats ($99.6\% \pm 4.0$), even those with PD ($97.85\% \pm 4.5$). PD-aged rats demonstrated increased contractile sensitivity to 5HT (aged rats $6.49 \text{ mN/mm} \pm 0.2$ versus PD-aged rats $8.08 \text{ mN/mm} \pm 0.3$). No change in contractile sensitivity in the coronary artery of young rats with DP ($5.06 \text{ mN/mm} \pm 0.4$ versus $4.97 \text{ mN/mm} \pm 0.3$). The endothelial dysfunction was related to eNOS downregulation, pronounced impairment of the EDH-mediated relaxation, increased IL-1β and TNF-α proinflammatory cytokines, and also upregulation of NADPH oxidase and COXs. Treatment with β-glucan effectively reduced bone loss in PD and delayed endothelial dysfunction in the coronary artery (sham aged treated $96.6\% \pm 4.0$ and PD aged treated $79.2\% \pm 4.5$) and restored contractile sensitivity of coronary artery rings ($5.25 \text{ mN/mm} \pm 0.6$ versus $5.03 \text{ mN/mm} \pm 0.3$). Our data show that yeast β-glucan ingestion prevented oxidative stress and synthesis of proinflammatory markers and prevented eNOS reduction induced by PD in aged rats. These results suggest that β-glucan has a beneficial effect on the coronary vascular bed.</p> <p>Keywords: Coronary disease, endothelial dysfunction, aging, periodontal disease</p>
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Title	Functional Evaluation between the interaction of PTK2 (FAK) kinase and p68 (helicase) during genotoxic stress in cardiac myocytes
Authors	Ana Paula Samogim ¹ Isabela Aparecida Moretto ¹ André Alexandre de Thomaz ² Craig Nigel Robson ³ Aline Mara dos Santos ¹
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Session	Biologia e Doenças Cardiovasculares

Abstract and Keywords	<p>Protein tyrosine kinase 2 (PTK2/FAK) is a non-receptor tyrosine kinase crucial for many cellular functions, including survival, migration, and cell growth. Studies elucidated that PTK2 can interact and regulate transcription factors related to cell survival and DNA damage response, such as p53, GATA and MEF2c, however, its nuclear functions remain poorly understood. Co-immunoprecipitation experiments were performed revealing that PTK2 interacts with the p68 (DDX5), a RNA helicase known to regulate pre-mRNA processing and transcriptional events, such as p53 and β-catenin co-activation/activation. Our hypothesis is that PTK2 regulates the function and subnuclear location of p68 during genotoxic stress, with an impact on the survival of cardiac myocytes. These study aimed to: validate the PTK2-p68 interaction; verify whether PTK2 activation modulates the p68 subcellular distribution; investigate if PTK2-p68 interaction can influence the activation of p53 and β-catenin in cardiac myocytes under genotoxic stress. In this study we used cell culture with H9c2 (mouse cardiomyoblast) and AC16 cells (human cardiac cell). Total extracts of cardiomyocytes were analysed by western-blotting assays and co-immunoprecipitation. Cells were treated with PTK2 inhibitor (PF-573228) and doxorubicin to induce PTK2 inhibition and genotoxic stress, respectively. Super-resolution microscopy techniques (SR-SIM) were used to analyse protein distributions in the nucleus. Lentivirus infection assays were performed to create overexpressed-p68 AC16 human cardiomyocytes. Our data confirmed the PTK2-p68 interaction in cardiomyocytes under basal and genotoxic stress conditions. Dox-treatment</p>
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reduced the expression of PTK2 and p68 and increased the phosphorylation levels of both proteins. The dox-treatment also induces the formation of PTK2-p68 nuclear clusters, which coincide with activated spliceosomes and alternative splicing subnuclear regions. Also, PTK2-p68 interaction sites coincide with β -catenin condensates during genotoxic stress in H9c2. Analysis of overexpressed-p68 and control human cardiomyocytes treated with dox and PF-573228 showed a decrease in PTK2 and p68 expression. Our findings show a notable interaction between PTK2 and p68 in H9c2 and AC16 cells under genotoxic stress, potentially influencing splicing sites and β -catenin co-activation. Additionally, doxorubicin treatment led to increased phosphorylation of PTK2 and p68, suggesting a regulatory role for PTK2 in nuclear dynamics and for p68 function during genotoxic stress.



Title	Sympathetic nerve activity, endothelial dysfunction, aortic stiffening and exercise capacity in long COVID patients: A Long-Term Cardiovascular Sequelae Study
Authors	Bruna E Ono ^{1,2} , Artur Sales ^{1,2} , Thais S Rodrigues ³ , João E Izaias ^{1,2} , Fernanda M C Colombo ³ , Maria Claudia C Irigoyen ³ , Natália G. Rocha ⁴ , Helena N.M. Rocha ⁴ , Gabriel F. Teixeira ⁴ , Renata R T Castro ⁵ , Renata Moll-Bernardes ¹ e Allan R K Sales ^{1,2,3}
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Session	BIOLOGIA E DOENÇAS CARDIOVASCULARES

Abstract, Ethics Committee Number*, and Keywords	<p>Our group showed that long COVID patients have mid-term cardiovascular sequelae, such as neurovascular dysfunction and attenuated exercise capacity. However, whether these cardiovascular changes are present in the long-term still unknown. Thus, we hypothesized that long COVID patients have sympathetic neural overactivity, endothelial dysfunction, aortic stiffening, and reduced exercise capacity. 18 long COVID patients (Age: 49±8years and BMI: 30.80±3.58 Kg/m²) and 19 well-matched controls (Age: 44±9years and BMI: 29.36±4.55Kg/m²) were enrolled for the study (CAAE: 31468020.1.0000.524. Muscle sympathetic nerve activity (MSNA, Microneurography), brachial artery flow-mediated dilation (BAFMD, Ultrasound-Doppler), carotid-femoral pulse wave velocity (CFPWV, Applanation tonometry), systolic and diastolic blood pressure (SBP and DBP, semi-automatic), heart rate (HR, Electrocardiogram), left ventricular ejection fraction, global longitudinal strain (LVEF and GLS, echocardiography) and peak oxygen consumption (VO₂peak, ergospirometry) were measured. Furthermore, circulating markers of oxidative stress (carbonyls, NADPH, SOD and FRAP), endothelial cell-derived extracellular vesicles (ECVEs) and angiotensin II (Ang II) were measured in plasma samples. Long COVID patients were studied 26±2 months after SARS-CoV-2 infection. They had higher MSNA and GLS ($p<0.001$), and lower BAFMD ($p<0.03$), LVEF ($p<0.002$) and VO₂peak ($p<0.015$) than controls. Interestingly, VECEs ($p=0.02$) and carbonyls ($p=0.0003$) were greater in long COVID than controls and SOD and FRAP were lower ($p<0.05$ for both). There was no difference between groups for SBP, DBP and Ang II. VO₂peak was inversely associated with MSNA ($p<0.002$) and GLS ($p<0.0008$) and directly associated</p>
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with BAFMD ($p < 0.03$). Our findings revealed that long COVID patients exhibit sympathetic overactivity, vascular dysfunction, reduced VO_{2peak} , and increased circulating levels of VECs and oxidative stress, even 2 years after COVID diagnosis.

Keywords: SARS-CoV-2, sympathetic activity, endothelium.



XXXVIII REUNIÃO ANUAL DA FESBE
XXII REUNIÃO ANUAL DA BRAVO
XVIII CONGRESSO DA SBCAL
III CONGRESSO DOHAD BRASIL
II CONGRESSO DA SBBA
2 A 5 DE JULHO 2024, CAMPINAS/SP

FRONTEIRAS DAS CIÊNCIAS BIOMÉDICAS E SEUS DESAFIOS

Title	Is the AC16 cell lineage a good experimental model to study cardiomyocyte hypertrophy?
Authors	Eloisa Olher Gomes Gabriela Cavazza Cerri Wenddy Wyllie Damasceno Sougey Maria Luiza de Moraes Barreto-Chaves
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Session	4 - Biologia e Ciências Cardiovasculares

Abstract, Ethics Committee Number*, and Keywords	<p>Heart failure is commonly preceded by cardiac hypertrophy (CH), characterised by the increase of cardiac mass as a compensatory response. However, depending on the stimulus and its intensity, CH can become non-adaptive, leading to cardiomyocyte death, fibrosis deposition and contractile dysfunction. Therefore, the importance of studies that seek to elucidate the mechanisms involved in the progression of CH becomes evident. Nevertheless, the conduct of studies involving human subjects includes many obstacles. Thus, several experimental models have been developed with the intention of getting closer to human biological functionality. One of them is the AC16 cell lineage, originated from a primary cell culture of adult ventricular tissue that were fused with a human fibroblasts cell lineage. The objective of this project is to characterise this cell lineage to subsequently study the possible links between CH (induced by T3 or isoproterenol) and inflammasomes activity. In order to elaborate a cell growth graphic, we cultivated AC16 cells in proliferation and in differentiation mediums at different timing (24h to 96h). As results we observed that AC16 cells present an elongated phenotype and an oval nucleus, with no ability to contract. We also noticed that up to 72h of cultivation in the proliferation medium the cellular proliferation is in log phase, and at 96h there is a decrease in the number of cells present. Conversely, in the differentiation medium there is proliferation up to 48h after the incubation, but after that the number of cells stabilises, indicating the lag phase. Also, the AC16 cells were treated with T3 or Isoproterenol at different concentrations for 24h and our preliminary results indicate that they are able to hypertrophy under these stimuli. Our next steps include an immunocytochemistry and RT-PCR essays of cardiomyocytes and fibroblasts typical proteins and genes.</p> <p>keywords: AC16 lineage; cardiac hypertrophy; T3; Isoproterenol.</p>
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Title	Association between Ankle-Brachial Index and cardiovascular risk factors
Authors	Lara Baldim Bessi ¹ Nathália Ferragut ¹ Camila Linhares Taxini Passos ^{1,2}
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Session	Biologia e Doenças Cardiovasculares

Abstract, Ethics Committee Number*, and Keywords	<p>Atherosclerosis has an important role in the genesis of cardiovascular diseases, because it precludes the circumferential expansion of the arteries, accelerating the process of luminal stenosis, reducing blood flow. The Ankle-Brachial Index (ABI) is an effective non-invasive method for indirect measurement of peripheral blood flow. The main objective is to investigate the occurrence of cardiovascular diseases through ABI in patients with cardiovascular risk factors. This is a prospective descriptive study, approved by The Committee for Ethics in Research of the University of Araraquara (CAAE: 64600022.0.00005383), executed through the application of a form and the measurement of systolic pressures of the upper and lower limbs. There were considered patients of Araraquara City, São Paulo, Brazil, over the age of 18 years old, without active ulcer on lower limbs and agreeing with the Informed Consent Letter, were excluded patients with lower limb amputations and pregnant women. The forms were used to survey patients' comorbidities. The data of the maximum systolic pressures in the brachial and dorsalis pedis or tibial arteries were used to analyse the frequency of ABI, values under 0,9 and over 1,3 are considered altered. From 60 volunteers, 36 are females and 24 males. Twenty five of the interviewed reported current or previous smoking of which 32% have altered ABI; forty presented high Body Mass Index, of which 30% have altered ABI; twenty six are diabetics, of which 38,5% presented altered ABI. The change in ABI values may be due to atherosclerosis when decreased, or calcinosis when increased, therefore ABI can be an important tool to determine early stages the peripheral circulation modifications, helping as a guide to direct cardiovascular treatments and improving patients quality of life.</p> <p>Keywords: Atherosclerosis, smoking, body mass index, cardiovascular risk, ankle-brachial index.</p>
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Title	Pazopanib causes generalized vascular dysfunction and increased systemic blood pressure in patients with clear renal cell carcinoma
Authors	Bruna E Ono ^{1,2} , Artur Sales ^{1,2} , Thais S Rodrigues ³ , João E Izaias ^{1,2} , Fernanda M C Colombo ³ , Maria Claudia C Irigoyen ³ , José Mauricio S C Mota ² , Renata Moll-Bernardes ¹ & Allan R K Sales ^{1,2,3}
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Session	Biologia e Doenças Cardiovasculares

Abstract, Ethics Committee Number*, and Keywords	<p>Pazopanib is a tyrosine kinase inhibitor (ITKS) used as a first-line treatment for clear renal cell carcinoma (CRCC). Although Pazopanib is associated with an increase in survival in these patients, it can cause systemic arterial hypertension (SAH). However, the pathophysiological mechanisms associated with this clinical manifestation are poorly understood. We hypothesize that oral use of Pazopanib causes endothelial dysfunction, increases aortic stiffening, increases peripheral vascular resistance, leading to a significant increase in systemic blood pressure. 7 CRCC participants (64±6yrs), undergoing first-line treatment with continuous Pazopanib 800 mg/day and 2 CRCC participants undergoing active screening (57±2yrs), without use of pazopanib, were enrolled for the study (CAAE: 52685821.9.0000.5249). They were followed for 4 weeks of treatment and were evaluated at baseline and at two and four weeks of treatment). The brachial artery flow-mediated dilation (BAFMD, Ultrasound-Doppler), brachial artery vascular resistance (ABRV, Ultrasound-Doppler), microvascular function (reactive hyperemia index, IHR, EndoPat), carotid-femoral pulse wave velocity (CFPWV, Tonometry), heart rate (HR, Electrocardiogram) and casual and 24-hour systolic and diastolic blood pressure (SBP and DBP - Ambulatory Blood Pressure Monitoring). Pazopanib decreased the BAFMD after 2 and 4 weeks of follow-up. (34% and 20% respectively). Furthermore, Pazopanib decreased the IHR at 20% , increased RVAB at 28%, CFPWV at 19%, SBP at 11% and DBP at 18% within week 2, and these changes were maintained in the fourth week of treatment. No changes were observed in the control group. Our findings revealed that Pazopanib causes a rapid and important generalized vascular dysfunction at 4 weeks of treatment, resulting in a significant increase in BP. Notably these findings strongly indicate the need for therapeutic strategies that can prevent the adverse effects of Pazopanib use on the cardiovascular system of CRCC patients.</p> <p>Keywords: cancer, angiogenesis inhibitor and blood pressure</p>
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Title	Influence of physical exercise and dermatan sulfate on thrombosis and vascular remodeling after arterial injury in female mice
Authors	Elisa Gomes Santos de Oliveira ¹ Mayra Gabriella Simplício Bomfim ² (Co-advisor): Cristina Pontes Vicente ² (Advisor): Claudio Chrysostomo Werneck ¹
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Session	04 - Biologia e Doenças Cardiovasculares

Abstract, Ethics Committee Number*, and Keywords	<p>Physical exercise is vital for cardiovascular health, combating the leading causes of global death: cardiovascular diseases (CVDs). Diseases caused by blood clots are commonly treated with anticoagulant drugs; however, these are associated with hemorrhagic risks. In contrast, the glycosaminoglycan Dermatan Sulfate has shown great antithrombotic potential associated with a lower hemorrhagic risk, potentially being an ally in the treatment of thrombosis cases.</p> <p>Studies have addressed differences between men and women regarding CVDs. In this study, we sought to relate the effects of DS drug administration and physical exercise practice on thrombus formation and blood vessel re-endothelialization after ferric chloride injury in female mice. The thrombus formation time was evaluated immediately after the injury, and the percentage of vessel obstruction by neointima was obtained 15 days later. Sedentary animals had the thrombus formed in 4.59 min and 91.49% of the vessel obstructed by neointima, while exercised animals had thrombus in 5.76 min and 76.92% obstruction. The groups that received drug administration had a time of 6.63 min and 68.43% obstruction in sedentary animals and 8.65 min and 62.65% in exercised animals, with a statistically significant difference in comparative time between sedentary and exercised with DS ($p < 0.05$).</p> <p>The association between DS and physical exercise demonstrated the potentiation of their beneficial effects in the prevention and treatment of thrombosis in female animals. When compared to data from studies conducted on male animals, it was possible to observe that females obtained less pronounced beneficial results. This difference may be due to the greater susceptibility of disease worsening in females when they are affected at a young age, as suggested by some studies, and by other factors not analyzed in this research.</p> <p>Ethics Committee Number 6056-1/2022</p> <p>Keywords: Thrombosis, physical exercise, anticoagulant, dermatan sulfate, female gender.</p>
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Title	The role of myosin Va in intracellular transport of focal adhesion kinase (PTK2) in H9C2 myocytes under genotoxic stress
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Session	

Abstract, Ethics Committee Number*, and Keywords	<p>Introduction: Protein tyrosine kinase 2 (PTK2) is a non-receptor tyrosine kinase, essential for mechanosignaling, cell migration and proliferation. PTK2 acts in the nucleus by interacting with and regulating transcription factors for ubiquitination and degradation; in the cytoplasm, it plays a canonical role in focal adhesions and cell migration. However, the mechanisms of PTK2 transport between the cytoplasmic and nuclear compartments have yet to be elucidated. Myosin Va is a mechanoenzyme that converts chemical energy into mechanical force by hydrolyzing ATP along the actin filaments. It acts in the transportation and anchoring of cellular structures and molecules. Previous results from our group show that PTK2 interacts with myosin Va, suggesting that MYOVa is crucial in the distribution and subcellular localization of PTK2. Our aim is to demonstrate that myosin Va acts in the transport and localization of PTK2 from the subcellular and nuclear compartments in H9c2 cardiomyocytes under genotoxic stress.</p> <p>Methodology: We evaluated these conditions by myosin Va <i>knockdown</i> (siRNA MYO5A; 48h-24h recovery) and myosin Va inhibition (pentabromopseudoline; 12h); in H9c2 cardiomyocytes control and dox (doxorubicin; 12h) by functional experiments and super resolution microscopy (SR-SIM). Analyses were performed using Image J software (Fiji). Results: Our data show that silencing of MYOVa by RNA interference (siRNA) decreases FAK localization in focal adhesions and increases the concentration of this kinase in the nucleus. Inhibition of myosin Va corroborates this finding in regard to focal adhesions; as a decrease in the PTK2 localization was observed in focal adhesions after myosin Va inhibition. Analysis of PTK2 concentration in the nucleus in the inhibited group is still ongoing. Conclusion: These findings indicate that myosin Va may regulate the intracellular transport of PTK2, more specifically being essential for the localization of PTK2 in focal adhesions and also at the nucleus.</p> <p>Keywords: PTK2, Myosin Va, genotoxic stress</p>
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Title	Genetic deletion of MrgD receptor modifies obesity-induced cardiac remodeling in mice
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Session	Pôster

Abstract, Ethics Committee Number*, and Keywords	<p>The Renin-Angiotensin System (RAS) is one of the main mechanisms involved in the development of cardiovascular diseases. MrgD receptor was recently discovered as one of the receptors of the counterregulatory axis of RAS, but its role in cardiac pathophysiology remains to be elucidated, especially in obesity related cardiac outcomes. Adult male mice were divided in: SC (wild type + control diet), HF (wild type + high fat diet), KO (MrgD knockout + control diet) and KOHF (MrgD knockout + high fat diet). Ethics approval n. 2132023. Body mass (g), cholesterol level (mg/dL), systolic blood pressure (SBP, mmHg), left ventricle (LV) mass (mg) and wall thickness (mm) were assessed. Statistical analyses were performed by one way ANOVA and Holm-Sidak pos hoc. All groups (HF: +31,5%, p<0.0001; KO: +14,1%, p<0.001; KOHF: +28,6%, p<0.0001) had higher body mass than SC group. HF and KOHF groups showed increased body mass (HF: +15,2% p<0.001; KOHF: +12,8% p<0.001) also in relation to KO group. Cholesterol level (HF: +21,5%, p<0.001 vs SC; +27,5%, p<0.0001 vs KO; KOHF: +10,3%, p<0.05 vs SC; +15,8%, p<0,01 vs KO) and SBP (HF: +27,0%, p<0.01 vs SC; +27,1%, p<0.01 vs KO; KOHF: +18,4%, p<0.05 vs SC; +18,5%, p<0.05 vs KO) were higher in HF and KOHF groups in relation to SC and KO groups. LV mass (+17,8%, p<0.05) and wall thickness (+17,6%, p<0.05) was increased in HF group in relation to SC group. KO group had higher LV mass (+21,0%, p<0.01) than SC group, but thinner LV wall (-11,8%, p<0.05</p>
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vs SC; -25,0%, $p < 0.0001$ vs HF; -11,8%, $p < 0.05$ vs KOHF) than all groups. In KOHF group, LV mass (+34,2%, $p < 0.0001$ vs SC; +14,0, $p < 0.05$ vs HF; +10,9%, $p < 0.05$ vs KO) was increased in relation to all groups, but LV wall (-15,0%, $p < 0.01$) was thinner than HF group. While MrgD appears not to be involved in the regulation of SBP, its deletion might be involved in obesity-induced cardiac remodeling.

Keywords: Renin-Angiotensin System; MrgD Receptor; Cardiac remodeling.



Title	TUDCA attenuates left ventricular hypertrophy in protein malnourished mice
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Session	Biology and Cardiovascular Diseases

Abstract, Ethics Committee Number*, and Keywords	<p>In the heart, protein malnutrition increases susceptibility to cardiovascular diseases, which are the main causes of global mortality. Studies have shown that TUDCA can balance calcium metabolism in the heart, prevent apoptosis and extracellular matrix deposition in the myocardium of animals subjected to a high-calorie diet. However, the effects of TUDCA on the hearts of rodents subjected to protein restriction are not fully understood. In this sense, the study seeks to characterize the cellular mechanisms involved in cardiac dysfunction induced by protein malnutrition and the potential therapeutic role of the bile acid TUDCA. For this, animals of the C57/BI/6 lineage were divided into four experimental groups (CEUA nº 5564-1/2020). These animals were subjected to normal or hipo-protein (restricted group) diets for 16 weeks and in the last 15 days they were treated with TUDCA (300 mg/kg) or PBS. After the experimental protocol, the animals were euthanized and the hearts were weighed and the left ventricles (LV) dissected to prepare histological slides for staining with Hematoxylin-Eosin or Masson's Trichrome to evaluate hypertrophy and the percentage of fibrosis, respectively. The results showed differences in heart weight/body weight when comparing the restricted and restricted treated with TUDCA groups, demonstrating that TUDCA was able to reduce hypertrophy. Morphometric and histological analysis showed larger cardiomyocytes in restricted animals compared to the normal or restricted diet group. Furthermore, a larger area of interstitial and perivascular collagen was observed in the restricted group when compared to the control or with TUDCA-restricted animals. Thus, the results demonstrate that TUDCA has therapeutic potential for alleviating the cardiovascular effects generated by protein restriction. However, more research must be carried out.</p> <p>Keywords: Cardiomyocytes; Heart; Collagen</p>
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Title	Cardiovascular risk factors in postmenopausal women with Type 2 diabetes
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Session	04-Biologia e Doenças Cardiovasculares (pôster)

Abstract, Ethics Committee Number*, and Keywords	<p>Type 2 diabetes (T2DM) and obesity are public health problems. After menopause, metabolic complications intensify, increasing the risk of developing cardiovascular diseases. The objective of this study was to evaluate the relationship between metabolic and cardiorespiratory parameters in postmenopausal women with T2DM assisted by the public health system in Lavras (MG). The project was approved by CEP/UFLA (n. 2442296). Data regarding body composition (BMI, WC and BF), HbA1C and lipemia were collected. An exercise stress test (ST) was performed to measure cardiorespiratory fitness (CF), through which DP, VO₂max, speed, HR, SBP were obtained. Data were analyzed using the mean ± standard deviation or Spearman's correlation test (p<0.05). The 16 volunteers were aged 47 - 65 years old, were taking metformin and were classified as sedentary, overweight or obese. No significant changes were observed in the parameters evaluated in the ST. The majority of women achieved more than 85% of their predicted maximum HR. The average speed was 3.36 ± 0.71 Km/h and the estimated VO₂max was 39.80 ± 7.24 mL/Kg/min. CF was classified as poor in 37.5% and regular in 62.5% of participants. SBP increased by 53.95 ± 22.31% but at the end of the recovery period it was 4.56 ± 6.28% lower than at rest. A DP greater than 30,000 suggests a good prognosis and was observed in 32.5% of participants. Considering HbA1C values, a positive correlation was observed with diabetes duration, suggesting that glycemic control gets worse over time. Considering VO₂max values, a negative correlation was observed with BMI, WC and BF, while for BF values, a negative correlation was observed with speed, indicating that body composition (BC) significantly interferes with CF. No significant correlation was observed with lipemia values. The data suggest that BC is a determinant of CF and improvements in glycemic control and body composition can result in a better quality of life for these women.</p>
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Title	Renovascular hypertensive rats (2K1C) have a reduced intracranial compliance induced by raised ventricular volume compared to normotensive rats
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Session	04- Biologia e Doenças Cardiovasculares

Abstract, Ethics Committee Number*, and Keywords	<p>Background and Objective: Intracerebroventricular (ICV) infusions of increased volumes of saline can be used to study intracranial compliance. Since the two-kidneys,1-clip (2K1C) rat model of renovascular hypertension has an enhanced baseline level of ICP, the present study aimed to verify the effect of increasing intracerebroventricular (ICV) infusions of 0.15 M NaCl on ICP and intracranial compliance (ICC) in 2K1C rats.</p> <p>Methods: Male Holtzman rats (180-210 g) were randomly separated to be NT (sham surgery, n = 5) or with 2K1C (n = 5), done by a partial left renal artery occlusion. Six weeks after rats were anaesthetized with urethane (1.2 g/kg body weight, i.v.), tracheostomized, placed under artificial ventilation and had an ICP sensor and a needle inserted into the lateral ventricle for, respectively, ICP recording and ICV 0.15 M infusions (30, 60 and 90 μL, at 100 μL/min). There was a 20-minute interval between each infusion. ICC was calculated by the ratio of volume change to ICP change [ICC = ΔV (μL)/Δ ICP (mmHg)].</p> <p>Results: Baseline MAP, ICP and P2/P1 of 2K1C (166 \pm 6 mmHg; 15.7 \pm 1.7 mmHg; 1.6 \pm 0.1, respectively) were higher than the ones in NT rats (94 \pm 5 mmHg; 10.1 \pm 1.4 mmHg; 0.7 \pm 0.1 respectively; p<0.05 vs 2K1C). ICV infusions induced greater increase in ICP in 2K1C (19 \pm 0.5; 42 \pm 1.2 and 55 \pm 5.5 mmHg, vs NT: 8 \pm 0.6; 25 \pm 3.2 and 36 \pm 4.9 mmHg, respectively at 30, 60 e 90 μL; p<0.05). In addition, 2K1C presented a reduced ICC at the 30 μl infusion (5.6 \pm 1.4 vs NT: 11.4 \pm 0.9; p<0.05).</p> <p>Conclusions: These findings suggest that renovascular hypertension impairs ICC in addition to the well-documented increase in ICP.</p> <p>Keywords: intracranial pressure; hypertension; brain compliance; wave morphology; challenge of increased intracranial pressure.</p> <p>CEUA: 16/2023</p>
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Title	Impact of sucralose consumption on body composition and cardiac health in nephrectomized rats
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Session	Posters

Abstract, Ethics Committee Number*, and Keywords	<p>Artificial sweeteners, such as sucralose, are widely consumed and are associated with some chronic diseases. Consumption of artificial sweeteners has been observed to cause effects such as excess body fat and an imbalance in cardiac tissue oxidative levels. Study aimed to understand the impact of sucralose consumption on body composition and cardiometabolic parameters in nephrectomized rats. Male <i>Wistar</i> rats (90 days), divided in Sham (surgical stress, n=7) and Nephrectomy (Nx, removal of 5/6 of the kidneys, n=27). After 4 weeks, divided in Sham, Nx (n=7), Nx + Sucrose (NxSuc, 10%, n=7), Nx + Sucralose at the maximum limit recommended by <i>Federal Drug Administration</i> (FDA) (NxSF, 5mg/kg/day, n=7), Nx + Sucralose at the maximum limit recommended by <i>European Food Safety Authority</i> (EFSA) (NxSE, 15mg/kg/day, n=6). Sweeteners were offered in a drinking fountain. After 8 weeks, body composition, systolic blood pressure (SBP), electrocardiogram (ECG), cardiac parameters, biochemical profile, and levels of thiobarbituric acid reactive substances (TBARS) were assessed (N°9803060520). NxSF had a lower Lee index compared to Nx (0.1644±0.0048 vs 0.1703±0.0030, g/cm, p=0.0490) and NxSE (0.1644±0.0048 vs 0.1713±0.0048, g/cm, p=0.0194). NxSF reduced SBP (153.3±13.02 vs 177.3±9.60, mmHg, p=0.0017), compared to Nx. NxSE showed lower values of % of adipose tissue (9.08±0.87 vs 13.26±2.91, %, p=0.0233) and fat mass (35.00±4.84 vs 54.14±11.10, g, p=0.0194) than Nx. Sucralose intervention did</p>
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not influence ECG and cardiac parameters. HDL-c levels were higher in NxSE compared to Nx (93.68 ± 8.00 vs 36.79 ± 16.57 , mg/dL, $p=0.0003$) and NxSF (93.68 ± 8.00 vs 56.97 ± 36.78 , mg/dL, $p=0.0251$). NxSF decreased TBARS plasma levels when compared to Nx (2.04 ± 0.16 vs 6.71 ± 3.32 , nm/mL, $p=0.0035$). Sucralose FDA recommendation showed a reduction in SBP and lipid peroxidation, and Sucralose EFSA recommendation improved body composition and HDL-c levels in nephrectomized rats.

Keywords: Sucralose, cardiovascular parameters, body composition



Title	Role of perivascular adipose tissue in thoracic aortic reactivity of isoproterenol-treated female mice
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Session	4. Biology and Cardiovascular Diseases

Abstract, Ethics Committee Number*, and Keywords	<p>Cardiovascular diseases (CVD) are the leading cause of death for 35% of women in 2019. A key pathophysiological mechanism involved in the genesis and maintenance of CVD is the hyperactivation of adrenergic receptors. In males, hyperactivation of beta-adrenergic receptors (β-AR) with isoproterenol (ISO) mimics sympathetic hyperactivation-induced cardiovascular damage such as cardiac and vascular oxidative stress and inflammation. More recently, we demonstrated impaired perivascular adipose tissue (PVAT) anticontractile function in response to ISO in aorta of male mice. However, less is known about the effect of β-AR overactivation in female sex. Therefore, our study aimed to examine the impact of β-AR hyperactivation on thoracic aorta reactivity and PVAT anticontractile function in female mice. Female C57BL6/JUnib mice (12 weeks old, CEUA # 6387-1/2024) were treated with ISO (15 μg/g/day, s.c.) or vehicle for 7 days. At the end of the protocol, blood pressure and body weight were measured. After euthanasia, the weights of the heart, uterus, and ovaries were registered. Thoracic aorta was isolated and concentration-response curves to phenylephrine in aortic rings in the presence or absence of PVAT. ISO treatment did not change body, uterus and ovaries weight or blood pressure in the females. Heart weight/body weight ratio was increased in ISO compared to the control group confirming the β-AR hyperactivation protocol. The presence of PVAT reduced aortic contraction to phenylephrine in both control and ISO groups, in a similar way. These data suggest that females may be protected from β-AR hyperactivation-induced PVAT anticontractile dysfunction.</p> <p>Key words: aorta; female mice; isoproterenol; beta-adrenergic receptors. Financial Support: CAPES</p>
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Title	Analysis of the effects of TUDCA on nuclear protein quality control of PTK2 kinase in H9C2 cardiomyocytes subjected to oxidative stress by Doxorubicin
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Session	4. Biology and Cardiovascular Diseases

Abstract, Ethics Committee Number*, and Keywords	<p>Doxorubicin (DOX) is a chemotherapy drug known for its high cardiotoxic potential, mainly due to the generation of reactive oxygen species. Current preventive strategies for DOX-induced cardiotoxicity are limited, prompting the search for alternatives. Focal adhesion kinase (PTK2) enhances cell resistance during DOX treatment. However, studies conducted at the Biological Physics and Cell Signaling Laboratory at the State University of Campinas showed that the stress caused by DOX results in structural destabilization of PTK2, a process that leads to its aggregation or post-translational modification by protein quality control (PQC) system on the nucleus. Therefore, to enhance the pro-survival effects of PTK2 in DOX-treated cardiomyocytes, we propose the use of tauroursodeoxycholic acid (TUDCA), which acts as a chemical chaperone. H9c2 cells were divided into the following groups: control; DOX (1µM); TUDCA (30µM); DOX+TUDCA (concomitant). All groups underwent treatment for 12h. Structured illumination super-resolution microscopy (SR-SIM) data demonstrated a significant reduction of PTK2 clusterization in the nucleus of cells co-treated with DOX and TUDCA compared with cells treated with DOX alone. SR-SIM analyses also showed a significant increase in the presence of the HSP70 chaperone in the areas close to the PTK2 clusters in the DOX+TUDCA group compared with DOX. Western blotting assays demonstrated that treatment with TUDCA significantly increased the levels of PTK2, and VCP chaperones on DOX-treated cardiomyocytes. Regarding the cell viability rate, as determined by the MTT assay, no significant difference was observed between the groups. However, when cells were recovered for 24h, a significant increase in cell viability was noticed in the co-treated group, indicating a beneficial effect of TUDCA on cardiomyocyte survival. Thus, this study indicates that TUDCA induces a possible improvement in the stabilization of PTK2 by acting with intranuclear PQC components, consequently favoring survival and may reduce the toxicity of DOX in cardiomyocytes.</p> <p>Keywords: Aggregation, Post-Translational Modification; Chaperone; Cardiotoxicity.</p>
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Title	Utilization of grape pomace flour in functional food development: nutritional potential and health implications
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Session	7 – Nutrição e Metabolismo

Abstract, Ethics Committee Number*, and Keywords	<p>Several studies have highlighted the benefits of phenolic compounds, flavonoids, antioxidants, and dietary fibers in mitigating chronic diseases. However, many by-products from food and beverage processing, rich in these compounds, are often wasted, causing significant environmental impact. This study aimed to explore alternatives to integrate grape pomace, derived from juice and wine processing, into the human diet. To this end, grape pomace was processed, milled, and sifted through 60 mm sieves to obtain a flour used in the preparation of functional foods. Whole wheat bread, jam, and ketchup were developed from the flour. The pomace flour underwent bromatological analysis for centesimal and phytochemical determination. The resulting foods were subjected to acceptance evaluation and bioactive compound analysis. Total phenolic compounds in the pomace and foods were determined by the Folin-Ciocalteu method, flavonoids by the aluminum chloride method, and antioxidant activity by DPPH assay. The moisture content was 7 ± 0.09, ash 5.4 ± 0.12, proteins 11 ± 0.12, lipids 9.5 ± 0.08, and carbohydrates 66.7 ± 0.15 g/100g. Phytochemical analysis showed the presence of phenolic compounds, tannins, and flavonoids in both the flour and developed foods, as well as significant antioxidant activity. Public acceptance was approximately 85% for bread, 90% for jam, and 65% for ketchup. The results demonstrated that grape pomace flour has promising potential in the development of functional foods, indicating its utility in preventing non-communicable chronic diseases associated with inflammation, thanks to the bioactive compounds retained in the developed products.</p> <p>Keywords: functional foods; bioactivated compound; antioxidant; inflammation.</p> <p>Acknowledgements: FAPESC n° 54/2022, TO n° 2023TR000885, CNPq Doctoral Scholarship n° 69/2022.</p>
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Title	Cardioprotective and nephroprotective effects displayed by acCGP nanoparticles loaded with essential oil from citrus sinensis in spontaneously hypertensive rats
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Session	04 - Biologia e Doenças Cardiovasculares

Abstract, Ethics Committee Number*, and Keywords	<p>This study aimed to evaluate the renal and cardiac protective effects of acetylated cashew gum polysaccharide nanoparticles loaded with EO (acCGP@EO) in spontaneously hypertensive rats. The nanoparticles produced were characterized by TEM, XDR, DSC and TG and used to treat spontaneously hypertensive rats. Wistar (normotensive control) and SH rats were treated daily with EO or acCGP@EO for 30 days, at a dose of 75 mg.Kg⁻¹ (CEUA-UFG n°049/22). The data were analyzed by two-way ANOVA followed by Tukey's post hoc test. The acCGP@EO nanoparticles presented a spherical morphology with a size of around 161.1 nm and a Zeta potential of -19.1mV and the XDR, DSC and TG results revealed a good compatibility between acCGP and EO during the formation of the nanoparticles. EO and acCGP@EO showed an antihypertensive effect (p<0.05, vs SHR group), occasioning weight reduction (17.4% compared to SHR, p<0.05), without interfering with food and water intake, urinary volume, and fecal content (p>0.05). Treatment also caused a decrease in the urinary sodium content (23.41% for OE; 20.92% for acCGP@OE, p<0.05) while increased the creatinine (2-fold increase, p<0.05 vs. SHR group) and urea clearance (59.5%, p<0.01 vs. SHR group), indicating a possible improvement in the natriuretic behavior of SH rats. Additionally, SH animals treated with EO and acCGP@EO experienced a reduction in TBARS levels (26.06% - cardiac tissue and 13.5% - kidney cells, vs SHR group, p<0.05) and an increase in the activity of the antioxidant enzymes SOD (21.0%, p<0.05 vs SHR group, in the kidney tissue; 58.82%, p<0.05 vs SHR group, in cardiac tissue), and CAT (39.89%, p<0.05 vs SHR group, in the kidney tissue). Altogether, these results show that acCGP@OE is a promising treatment against hypertension-associated nephropathy and evokes potent antihypertensive and cardioprotective effects.</p> <p>Keywords: cashew gum polysaccharide; antihypertensive; nanotechnology.</p>
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Title	Peripubertal high fat diet exposure induces obesity and hypertension in male Wistar rats
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Session	04 - Biologia e Doenças Cardiovasculares

Abstract, Ethics Committee Number*, and Keywords	<p>The consumption of a high fat diet (HF) by Wistar rats induces biometric and cardiometabolic dysfunctions in adult life. However, little is known about the cardiovascular function of rats exposed to peripubertal HF diet. Therefore the objective of this study is to evaluate the biometric and cardiometabolic parameters of Wistar rats fed a high fat diet during peripuberty. The protocol was approved by the ethics committee on Use of Animals from the Maringa State University (CEUA/UEM: 2910011021). Between post natal day (PN) 30 and PN 75, male Wistar rats (n=16) were divided into two groups: the High Fat diet (HF) (35% fat), and the control (4,5% fat diet) groups. Blood pressure (recorded by plethysmography) and biometric parameters were evaluated at PN 30, PN 40, PN 50, PN 60 and PN 70. Student's-t test was used to evaluate the statistical differences using the GraphPad Prism software. $P < 0,05$ was considered a significant difference. No significant differences between the groups were found in the area under the curve from the body weight evolution during peripuberty. Retroperitoneal ($P < 0,0001$) and mesenteric ($P < 0,0001$) fat depots were increased in the HF group at all evaluated ages. HF group showed higher systolic blood pressure at PN 40 (+20 mmH, $P < 0,0001$), PN 50 (+24 mmH, $P < 0,0001$), PN 60 (+12 mmH, $P = 0,0004$) and PN 70 (+20 mmH, $< 0,0001$), when compared to control group. The peripubertal consumption of a high fat diet induces obesity and hypertension during the dietary challenge.</p> <p>Keywords: High Fat diet, hypertension, peripuberty.</p>
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Title	Peripubertal low protein diet exposure induces cardiometabolic dysfunction in male Wistar rats
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Session	04 - Biologia e Doenças Cardiovasculares

Abstract, Ethics Committee Number*, and Keywords	<p>Exposure to a low-protein diet in perinatal life induces cardiometabolic dysfunction related hypertension in adulthood. However, little is known about the cardiovascular and metabolic profile during the peripubertal insult. This research aims to evaluate the effects of peripubertal exposure to a low-protein diet on cardiovascular and metabolic profile. The procedures were approved by the Ethics Committee on Use of Animals from the Maringa State University (protocol nº 2910011021). Male <i>Wistar</i> rats were exposed to Low-Protein diet (LP; 4% protein) (n=8-15) or Normal-Protein diet (NP; 20,5%) (n=8-15) during the peripuberty, from post natal day (PN) 30 until PN 70. The biometric, metabolic and cardiovascular parameters were evaluated at PN 30, PN 40, PN 50, PN 60, and PN 70. Systolic blood pressure (SBP) was recorded by plethysmography. Blood samples were collected for biochemical metabolic analysis. Statistical analysis were performed with the Student's-t test, using the GraphPad prisma software. The LP group showed lower body weight (P=0,0001), food and water intake (P<0,021, P<0,0001) through the dietary challenge, and smaller nasoanal length at PN 60PN (P<0,0001). Moreover, the LP rats showed a higher systolic blood pressure from the PN 40 until the end of the special diet exposure (30PN, P=0,2299; 40PN, P<0,0001; 50PN, P<0,0001; 60PN, P<0,0001; 75PN, P<0,0001). Metabolic dosages showed that the LP group has higher levels of glucose (P<0,01), and changes in the lipid profile, with lower levels of triglycerides (P<0,01), total cholesterol (P<0,05), and HDL-cholesterol (P<0,0001). The LP diet exposure during peripuberty induces cardiovascular and metabolic dysfunctions during the dietary challenge exposure.</p> <p>Keywords: Low Protein Diet. Hypertention. Peripuberty</p>
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Title	The coexistence of undernutrition and obesity throughout life impairs pancreatic vascular function
Authors	Leticia Barssotti ¹ Daniele M. Guizoni ¹ Camila B. R. Vitoria ¹ Everardo M. Carneiro ² Ana Paula C. Davel ¹
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Session	04 - Biologia e Doenças Cardiovasculares

Abstract, Ethics Committee Number*, and Keywords	<p>The coexistence of undernutrition and obesity throughout life characterizes the double burden of malnutrition (DBM). Both undernutrition and obesity are risk factors for cardiovascular and metabolic diseases. Pancreatic islet microvasculature is crucial for beta cell secretory activity and glycemic homeostasis. However, the impact of DBM on vascular function remains understudied. We hypothesize that postnatal undernutrition programs vascular dysfunction in the endocrine pancreas, and causes more damage when associated with obesity in adulthood. To test this, post-weaned male mice (CEUA 6055-1/2022) were fed a control diet (C) or protein-restricted diet (R) for 6 weeks, then divided to receive C or high-fat/high-sucrose diet (H) for 10 weeks (CC, RC, CH, and RH groups). Following this, vessels were isolated, and vascular responses in conductance (aorta) or resistance arteries (mesenteric and pancreatic arteries) were assessed using a wire myograph. CH and RH groups exhibited increased weight gain and perigonadal fat, greater in RH. Blood pressure remained unchanged. In the aorta, CH and RH animals showed hypocontractility and decreased endothelium-dependent relaxation to acetylcholine, suggesting that the H diet induces aortic dysfunction per se. However, in the lieno-pancreatic artery, there was an increased relaxation to acetylcholine, as well as an increased contraction to phenylephrine and high KCl observed only in the RH group. Interestingly, the small mesenteric artery, a resistance artery with similar diameter, was unaffected. Our data suggest that protein restriction in early life, followed by a hypercaloric diet in adulthood affects resistance artery function in the pancreas but not in mesentery. So, targeting vascular dysfunction in the endocrine pancreas should be relevant for cardiometabolic diseases.</p> <p>Keywords: obesity, protein restriction, pancreatic islets, vascular function, endothelium, resistance artery</p>
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Title	Cardiotoxic effects of chemotherapy with Doxorubicin and Cyclophosphamide and the possible benefits of leucine supplementation in pregnant tumour-bearing rats
Authors	Beatrice Candido Alves ,Igor Fernando dos Santos, Carla de Moraes Salgado, Rogério Willians dos Santos, Lais Rosa Viana, Maria Cristina Cintra Gomes Marcondes
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Session	04 – Biology and cardiovascular diseases

Abstract, Ethics Committee Number*, and Keywords	<p>The most common chemotherapy treatment for breast cancer during pregnancy involves the administration of doxorubicin and cyclophosphamide. However, chemotherapy can cause severe damage to the heart tissue which limits treatment and contributes to long-term morbidity and mortality. Therefore, we aim to carry out a pre-clinical study to identify the effects of chemotherapy treatment on the morphology, morphometry and cardiovascular parameters of pregnant Walker 256 tumour-bearing rats, assessing whether a leucine-rich diet modulates the alterations in the cardiac tissue. Female Wistar rats were distributed into 4 pregnant groups: 1- healthy control; 2- Walker 256 tumour-bearing; 3- healthy fed a leucine-rich diet; 4- Walker 256 tumour-bearing + leucine-rich diet. The tumour was implanted in the subcutaneous in groups 2 and 4. All the animals were evaluated daily during 21 days of the pregnancy and then euthanised to collect samples. For morphometric analysis, we assessed the weight, size of the heart, and the thickness of the right and left ventricular walls. As for the morphological and histological analyses, heart tissue samples stained with haematoxylin and eosin were used to assess the characteristics of the cardiomyocytes, as well as measure the area/size. In both groups (2 and 4), exponential tumour growth was observed with a reduction in food intake and total mother body weight, but maintained the heart weight. On the other hand, only the tumour-bearing group 2 showed a reduction in heart area with histologic alterations as greater cardiac cell area. Leucine nutritional supplementation minimised the heart area changes, despite having a larger cardiac cell area. Although no significant changes were evident in the previous data, we are currently engaged in developing new experiments to increase the number of samples per group, as well as the effect of leucine supplementation over the deleterious effects of tumour evolution. Ethics committee number: 6404-1/2024.</p> <p>Keywords: Cardio toxic, Walker 256 tumour, Doxorubicin, Cyclophosphamide, Leucine.</p>
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title	Consumption of Minas Frescal cheese enriched with probiotic (<i>WEIZMANNIA COAGULANS</i> GBI-30) decreased total cholesterol in an experimental model of Type II diabetes mellitus
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Session	Posters

Abstract, Ethics Committee Number*, and Keywords	Type 2 diabetes mellitus (T2DM) is closely related to changes in the gut microbiota and the development of cardiovascular diseases. Probiotics have been identified as a strategy to mitigate this damage. The study aims to analyze the effects of probiotic-enriched Minas cheese on cardiovascular risk in rats with streptozotocin-induced DM2. Twenty-one male <i>Wistar</i> rats were fed a high-fat diet for 4 weeks and received an intraperitoneal injection of streptozotocin (35 mg/kg) in the third week. The animals were divided into 3 groups (n=7): Type 2 Diabetes Mellitus (DM), Type 2 Diabetes Mellitus Minas Frescal Cheese (DMC), and Type 2 Diabetes Mellitus Probiotic Minas Frescal Cheese (DMPC) (CEUA/UFF No.6165130722). For 2 weeks, each animal in cheese groups received 20 g/day of cheese. <i>Wezmannia coagulans</i> GBI-30 concentration was 10 ⁸ to 10 ⁹ colony-forming units. After 6 weeks, fasting glucose was measured. The relative heart mass, serum biochemical markers, atherogenic coefficient and
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thiobarbituric acid reactive substances (TBARS) in plasma were assessed. Relative heart mass was similar between groups. Probiotic Minas cheese did not affect the fasting blood glucose, postprandial blood glucose, triglycerides, HDL-c or VLDL-c levels. Probiotic Minas cheese decreased the total cholesterol level (DMPC: 112.9 ± 19.13 vs DMC: 151.0 ± 38.32 (mg/dL), $p = 0.0348$) in comparison to conventional cheese. Although, no significant differences were observed in the atherogenic index of plasma and atherogenic coefficient among the groups. Also was not observed significant difference in plasma TBARS levels among groups. The consumption of a probiotic Minas cheese with *Weizmannia coagulans* GBI-30 for 2 weeks in an experimental model of T2DM decreased the total cholesterol level compared to Minas frescal cheese. Further investigations are needed to complement this study's results.

Keywords: Diabetes Mellitus; cardiovascular risk; probiotics; minas frescal cheese



Title	Depicting the role of BCLAF1-PTK2 interacting complex in the regulation of cardiomyocyte survival during Doxorubicin-induced oxidative stress
Authors	Isabela Aparecida Moretto (1) Beatriz Rocha Lídio Rodrigues (1) Pedro Victor de Carvalho Costa (1) Ana Paula Samogim (1) Maria das Graças de Souza Carvalho (1) Mariana Conceição da Silva (1) Ingridi Rafaela de Brito (1) Fernando Bladimir Valdivieso Rivera (3) Fernanda Luisa Basei (2) Murilo Vieira Geraldo (1) Carlos Henrique Grossi Sponton (3) Jörg Kobarg (2) Giovanna Lopes de Araújo (4) Fabio Cesar Gozzo (4) Hernandes Faustino de Carvalho (5) André Alexandre Thomaz (5, 6) Aline Mara dos Santos (1, 5)
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Session	04 - Biologia e Doenças Cardiovasculares

Abstract, Ethics Committee Number*, and Keywords	Protein tyrosine kinase 2 (PTK2) is a non-receptor tyrosine kinase essential for regulating distinct cellular functions, including migration, growth, and maintenance of survival under stress conditions. Co-immunoprecipitation coupled to mass spectrometry revealed Bclaf1, a stabilizing protein, as an interacting partner for PTK2. This study aimed to characterize the molecular function of the PTK2-Bclaf1 interaction in H9c2 cardiomyocytes under stress induced by doxorubicin (dox). Proximity ligation assays confirmed the direct interaction between PTK2 and Bclaf1, while super-resolution microscopy (SR-SIM) revealed the formation of nuclear biomolecular condensates of Bclaf1 containing PTK2 in cardiomyocytes under stress. Cell ROX analysis demonstrated that oxidative stress (OS) increases over the dox-treatment time, as well as the organization of Bclaf1-PTK2 condensates. AlphaFold and PSpredictor analyses confirmed the unstructured nature of Bclaf1 and its high propensity for liquid-liquid phase separation (LLPS). FRAP experiments further confirmed the high fluidity of the nuclear biomolecular condensates. In
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dox-treated cardiomyocytes, PTK2 localized into the Bclaf1 condensate cavities was shown to be protected from ubiquitination induced by dox-generated OS. The treatment with the proteasome inhibitor MG-132 culminated in nuclear PTK2 aggregation and ubiquitination, confirming the action of the ubiquitin-proteasome system on PTK2 proteostasis. Moreover, the ubiquitination site on PTK2, K926, was identified using mass spectrometry. Otherwise, Bclaf1 knockdown disrupted the nuclear agglomeration of PTK2, increasing PTK2 ubiquitination, which resulted in cell death. These findings indicate that Bclaf1 biomolecular condensates are generated by LLPS and function sequestering and stabilizing PTK2, allowing cardiomyocyte to resist genotoxicity caused by doxorubicin-induced oxidative stress.

Keywords: cell survival, biomolecular condensates, cardiomyocytes



Title	Oxidized LDL is associated with carotid intima layer in hypertensive patients
Authors	Saturne Barthold, Eduarda O.Z. Minin, Luís F.R.S. Carvalho-Romano, Edmilson R. Marques, Layde R. Paim, Elisangela C.P. Lopes, Camila F.L. Vegian; José A. Pio-Magalhães, José Roberto Matos-Souza, Otavio R. Coelho-Filho, Andrei C. Sposito, Wilson Nadruz Jr, Roberto Schreiber
Affiliations	Department of Internal Medicine, School of Medical Sciences, State University of Campinas, São Paulo, Brazil
Session	Biologia e Doenças Cardiovasculares

Abstract, Ethics Committee Number*, and Keywords	<p>Carotid intima-media thickness (cIMT) is considered a measure of subclinical atherosclerosis. However, as it comprises the carotid intima (cIT) and media (cMT) layers, it may not be an accurate marker of atherosclerosis. We previously demonstrated that cIT has a stronger association with carotid plaques and coronary calcification than cMT and cIMT, indicating that cIT may be a better marker of atherosclerotic burden. Previous studies have shown that oxidized LDL (oxLDL) levels are related to carotid atherosclerosis, but to date there are no studies associating oxLDL levels with carotid wall layers. This cross-sectional study measured serum oxLDL levels and evaluated their association with carotid wall layers in 117 hypertensive patients followed at a university outpatient clinic. The study was approved by the Ethics Committee (CAAE:56841616.5.0000.5404). cIT, cMT, and cIMT were calculated from high-resolution images of the left and right common carotid arteries obtained using a Vivid q device equipped with a linear array transducer set at 10 MHz. Plasma oxLDL levels were analyzed using the ELISA method. The association between carotid wall layers and oxLDL was evaluated by multivariable regression analysis adjusted for age, gender, systolic blood pressure, diabetes, body mass index, smoking, statins and antihypertensive medications use. The sample had age=59.7±11.4 years, 44% males and values of oxLDL, cIMT, cMT and cIT of 73.7±40.8 U/L, 0.74±0.13 mm, 0.48±0.10 mm and 0.26±0.06 mm, respectively. Multivariable regression analysis showed that oxLDL levels associated with cIT (beta=21.7±8.0; p=0.008) and cIMT (beta=9.3±4.0; p=0.023), but not with cMT (beta=6.3±5.2; p=0.23). In summary, our data demonstrate that cIT, a more specific marker of atherosclerosis is associated with plasma oxLDL levels. This finding may expand the knowledge about the role of oxLDL in the development of atherosclerosis in hypertensive patients.</p> <p>Keywords: oxidized LDL, carotid intima-media thickness</p>
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Title	Influence of physical activity and gala apple (<i>Malus domestica</i> Bork) in knockout mice for the LDL receptor gene (LDL R-/-) fed a hyperlipidic diet
Authors	Lara Beatriz Lombardi Laleska Eduarda Moreira Gabriel Zotti Plat José Antonio Dias Garcia
Affiliations	Universidade Professor Edson Antônio Velano-UNIFENAS, Alfenas, Brasil
Session	Biology and Cardiovascular Diseases (04)

Abstract, Ethics Committee Number*, and Keywords	<p>The population is increasingly concerned about quality of life, therefore, the search for functional foods and physical activity is on the rise. With the aim of evaluating the effect of swimming and apples on left ventricular hypertrophy (LVH) and dyslipidemia (DI), this work was carried out. The animals weighed $23 \pm 3g$, divided into five groups (N=10): group S, received standard food (4% total fat); group HL, high-fat diet; HLN group, high fat diet and was subjected to swimming; HLM group, high-fat diet and apple; HLMN group, high-fat diet, swimming and apple. After 75 days, blood samples and serum isolates were collected to evaluate total cholesterol, blood glucose, insulin, C-reactive protein and HOMA-IR. The heart was isolated, and the left ventricle separated and weighed (mg) in relation to the ventricular weight/weight of the animal (g) calculated, histological sections processed to quantify the collagen area and the diameter of the stained cardiomyocytes. Approved CEUA protocol 04A/2011. In serum analysis, mice in the HL group showed high levels of TC, LDL, VLDL, TG, insulin, HOMAIR and CRP and reduced HDL in relation to mice in groups S, HLN, HLM and HLMN. Apple treatment and physical activity in the HLMN group prevented the increase in HDL, VLDL, TG, glucose, insulin, HOMAIR and CRP. Insulin, CRP and HOMAIR levels in groups treated with a high-fat diet increased when compared to animals that received a standard diet, indicating the presence of insulin resistance. Glucose showed no difference between the groups studied. Results demonstrated an increase in the proportion of left ventricular weight in relation to the weight of the animal in the HL group. The apple and swimming partially prevented LVH, the increase in the diameter of cardiomyocytes and the deposit of collagen in the HLMN group. Therefore, physical activity with apples in animals that received a high-fat diet helped with ID.</p> <p>Keywords: Cardiovascular Diseases; Diet; Physical activity</p>
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Title	Influence of coconut oil on dyslipidemia and left ventricular hypertrophy of knockout mice for the LDL receptor gene (LDL R-/-) fed a hyperlipidic diet
Authors	Laleska Eduarda Moreira Lara Beatriz Lombardi Gabriel Zotti Plat José Antonio Dias Garcia
Affiliations	Universidade Professor Edson Antônio Velano-UNIFENAS, Alfenas, Brasil
Session	Biology and Cardiovascular Diseases (04)

Abstract, Ethics Committee Number*, and Keywords	<p>Currently, there is a popular interest in alternative therapies for dyslipidemia, which is the association between an increase in triglycerides, low-density lipoprotein (LDL) and cholesterol or a reduction in high-density lipoprotein (HDL), which favor the appearance of cardiovascular diseases and increased serum glucose levels and insulin resistance. The objective of this work was to analyze the influence of coconut oil on dyslipidemia. Each animal weighed 22 ± 2g, divided into five groups (N=10): group S, received standard food (4% total fat); SCO group, standard food and coconut oil at a dose of 2g/kg; group HL, high-fat diet; HLCO group, high-fat diet and oil; HLSI group, high-fat diet and simvastatin 20mg/kg. After 15 days of experiment, the animals were anesthetized, blood was collected and serum was isolated to determine total cholesterol and its fractions, blood glucose, insulin, C-reactive protein and calculated HOMA-IR. The heart was isolated, the left ventricle separated, weighed (mg) and the ventricular weight/animal weight ratio (g) calculated, histological sections processed to quantify the collagen area and the diameter of cardiomyocytes stained with HE. The project was approved by the ethics committee by opinion no. 17A/2011. Coconut oil did not prevent hypercholesterolemia and the decrease in HDL in mice on a high-fat diet (HLCO group), but it prevented hypertriglyceridemia in both the HLCO and SCO. Simvastatin stopped 50% of hypercholesterolemia, 100% of hypertriglyceridemia and the decrease in HDL compared to the HL group. Both treatments partially prevented hyperinsulinemia and completely prevented the HOMA-IR index. Therefore, it is concluded that the oil completely prevented cardiomyocyte hypertrophy and partially prevented the percentage of collagen in the left ventricular myocardium and slightly LVH, bringing benefits to cardiac health.</p> <p>Keywords: Cardiovascular Diseases; Alternative Treatment; Cholesterol; Inflammation</p>
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Title	Evaluation of a microrna profile in obese and lean patients with heart failure
Authors	Roberto Schreiber ¹ , Eduarda O. Z. Minin ¹ , Layde Rosane Paim ¹ , Luis Miguel da Silva ¹ , Lígia M. Antunes-Correa ¹ , Vinicius Citelli Ribeiro ¹ , Adriana Aparecida Bau ¹ , Camila Nicolela Geraldo Martins ¹ , Andrei Sposito ¹ , Jose Roberto Matos-Souza ¹ , Licio A. Velloso ¹ , Wilson Nadruz ¹ , Michael Jerosch-Herold ² , Otávio R. Coelho Filho ¹ .
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Session	Biologia e Doenças Cardiovasculares

Abstract, Ethics Committee Number*, and Keywords	<p>Heart failure (HF) results in increased morbidity and mortality, and body mass index (BMI) is a risk factor for its development. However, obesity is associated with reduced mortality among patients with HF, a phenomenon known as the “Obesity Paradox.” MicroRNAs (miRNAs) play a role in regulating gene expression in numerous diseases. To date, our understanding of the impact of these miRNAs on obesity in conjunction with FH remains limited. We established a profile of differentially expressed miRNAs in obese patients with HF compared to lean patients with HF. We also evaluated this association between patients with reduced ejection fraction (HFrEF) and with preserved ejection fraction (HFpEF). The study was approved by the Ethics Committee (CAA:63097016520115404) Sixty-five patients with HF, 29 lean (BMI=25.8±2.5kg/m²) and 36 obese (BMI=34.5±4.1kg/m²), were prospectively evaluated. Serum miRNA expression was assessed by the TaqMan OpenArray system, a platform capable of evaluating 754 microRNAs. Eighteen miRNAs were differentially expressed in serum from obese patients with HF compared to lean patients with HF. Ten miRNAs in obese patients with HFrEF and five in HFpEF were differentially expressed when compared to lean. miR-132 was upregulated in obese patients with HFrEF and HFpEF, while miR-1291 was downregulated in HFpEF compared to lean. We observed a positive correlation between BMI and miR-132 (r=0.50;p=0.001), miR-148b (r=0.51;p<0.001), miR-103a-3p (r=0.37;p=0.011) and miR-374b-5p (r=0.32;p=0.034) in HF patients. Gene set enrichment analysis identified EIF4E3 and TRA2B genes common to all four miRNAs. In summary, we present a profile of 18 miRNAs differentially expressed in obese patients with HF compared to lean ones. BMI was correlated with 4 of these miRNAs and two potential target genes were identified. Further studies are needed to confirm our results and explore the mechanisms linking miRNAs to the obesity paradox.</p> <p>Keywords: heart failure, microRNAs, obesity paradox</p>
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Title	Possible mechanism of arterial pressure maintenance in obese ovariectomized female rats
Authors	Sara de Moraes Leite Helena de Oliveira Deróbio Gabriela Maria Lucera José Vanderlei Menani Eduardo Colombari Debora Simões de Almeida Colombari
Affiliations	School of Dentistry, Araraquara, São Paulo State University
Session	Biology and Cardiovascular Diseases

Abstract, Ethics Committee Number, and Keywords	<p>Obesity is a major health problem and affects men and woman. We have demonstrated before that female obese rats (ovary intact) did not have a difference in baseline arterial pressure compared to euthrophic female. However, we still do not know if obesity will cause change in baseline arterial pressure in ovariectomized obese rats and if the estrogen replacement will do any change. Holtzman female rats (280 - 300 g) were fed with standard diet (SD, 11% calories from fat) or high fat diet (HFD, 45% calories from fat) for 6 weeks. In the same week of the commencing of the diets regiment, the rats were bilaterally ovariectomized (OVX) and at the end of the 6th week, 17-β-estradiol (40 µg of body weight, sc) was injected for 7 days. The groups were then: SD-OVX (n = 4), SD-OVX/E2 (n = 3), HFD-OVX (n = 5), HFD-OVX/E2 (n = 3). In the 7th day, the femoral artery and vein were canulated for mean arterial pressure (MAP) recording and drugs injection. In the next day, in freely moving rats, we observed that baseline MAP was not different between all groups (SD-OVX; SD-OVX+E2; HFD-OVX; HFD-OVX+E2; respectively: 119 ± 5; 125 ± 10; 123 ± 2 and 118 ± 0,2 mmHg). After 30 min, losartan administration (10 mg/kg, iv) produced very sutle changes in MAP in all group (Δ MAP, -6 ± 2; -7 ± 9; - 7 ± 2 and -7 ± 2, respcevely). After 30 min of losartan injection, hexametonium (20 mg/kg, iv) produced a substantial decrease in MAP, which was also similar between groups (Δ MAP, -61 ± 5, -68± 8 , -64 ± 4, -69 ± 4 mmHg, respectively). From the preliminary data, it seems that at euhydrated conditions, there is no difference in baseline and in the mechanisms controlling MAP in euthrophic and obese OVX female rats. We will next challenge these females to a water deprivation that increases the renin-angiotensin system (RAS). Since RAS is overactiveated in obese rats, a difference in controlling MAP under this circumstance might appear.</p> <p>Keywords: obesity, female, menopause, angiotensin II, sympathetic nervous system.</p> <p>CEUA: 10/2023</p>
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Title	The use of kefir as an intervention in gestational dysbiosis
Authors	Jéssica Alves Ribeiro ¹ , Taise Fonseca Pedroso ² , Gabriel Da Silva Cordeiro ² , Kênia Moreno De Oliveira ² , Lailson Oliveira De Sousa ³ , Mileane De Souza Busch ⁴ , Karoline Da Silva Farias ¹ , Marco Aurélio Ramirez Vinolo ² , Everardo Magalhães Carneiro ² , Karina Cogo Müller ¹ , Débora Parra Baptista ³ , Georgia Correa Atella ⁴ , Liana Verinaud ² , Catarina Raposo Dias Carneiro ¹ , Valquiria Aparecida Matheus ¹ .
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Session	Biology and Gastrointestinal Diseases

Abstract and Keywords	<p>Offspring born to individuals with an imbalance in the intestinal microbiota (dysbiosis) during the gestational period are predisposed to metabolic, immunological, and neurological disorders. This project hypothesizes that this predisposition is linked to the low availability of short-chain fatty acids (metabolites of the intestinal microbiota) and suggests using probiotics to restore these levels. Kefir was chosen for its ability to modulate the microbiota and immunity. Pregnant C57BL6J females were divided into 3 groups, treated during pregnancy: control group (CT), without intervention; dysbiosis group (ABX), received intervention with an antibiotic mixture (1 mg/mL of ampicillin and 0.5 mg/mL of neomycin) in drinking water; kefir group (K), received 200 µL of kefir daily, by gavage (CEUA 6231/2023). The bacteria <i>Lactobacillus kefiranofaciens</i> and the fungus <i>Saccharomyces cerevisiae</i> are the predominant microorganisms (sequencing). Kefir contained 3.05% protein, providing significant nutritional value. Compared to the other groups, ABX offspring had lower exploratory activity (Open Field test), greater intestinal permeability (FITC-Dextran test), greater expression of the claudin and occludin genes (proteins that modulate intestinal permeability, assessed by PCR), and significantly larger cecum (indicative of dysbiosis). Despite showing exploratory activity similar to the CT group, K offspring exhibited higher intestinal permeability than controls, though not to the level of ABX offspring. Since the size of the cecum in the K group resembled the CT group, it's possible to infer that the K group did not have dysbiosis. The increased permeability may be related to kefir's antimicrobial profile. Next steps include measuring the concentration of short-chain fatty acids in maternal and offspring feces, evaluating the antimicrobial effect of kefir, and testing it in pregnant females with established dysbiosis.</p> <p>Keywords: Probiotic, Offspring, Microbiota</p>
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Title	Effects of protein restriction post-weaning on small intestine morphofunction
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Affiliations	Laboratório de pâncreas endócrino e metabolismo - LaPEM, OCRC, Universidade Estadual de Campinas
Session	Biologia e Doenças Cardiovasculares

Abstract, Ethics Committee Number, and Keywords	<p>Introduction: Protein undernutrition may cause deleterious effects on the functioning of the gastrointestinal tract (GIT), which may lead to obesity and type 2 diabetes. However, there is a lack of information regarding this relationship. Aim: To analyze the morphometry of the small intestine (SI) and ileum morphology in mice under protein restriction post-weaning. Methods: From 30 to 120 days-old <i>C57Bl/6</i> male mice were divided into control (C), fed a 14% protein diet, and Restricted (R), fed a 6% protein diet. Data was analyzed by Shapiro-Wilk followed by Student's t-test or Mann-Whitney U ($P < 0,05$; CEUA UNICAMP nº 5564-1/2020). Results: As expected, R mice had lower final body weight (BW) ($22,2 \pm 0,7$ g), feed efficiency ($0,03 \pm 0,008$), and mesenteric fat pads ($4,99 \pm 1,34$ mg/g BW) despite the hyperphagia ($1994 \pm 286,3$ g.days⁻¹) when compared to C mice ($26,5 \pm 0,5$ g, $0,04 \pm 0,01$, $6,68 \pm 1,04$ mg/g BW and $1247 \pm 44,2$ g.days⁻¹, respectively). Protein restriction did not alter the length ($3,6 \pm 0,2$ cm/nose-anus length, NAL) or weight of the SI ($8,9 \pm 9,6$ mg/g BW) in R group compared to C group ($3,4 \pm 0,3$ cm/NAL and $48,2 \pm 7,3$mg/g BW). In the ileum, although protein deprivation did not modify the submucosal ($145,4 \pm 42$ μm) and muscular ($366,4 \pm 11,9$ μm) layers' thickness, crypts had a 23,9% reduction in depth ($66,2 \pm 15$ μm) and 10,5% in diameter ($380,2 \pm 60,1$ μm), associated with a 29,7% increase in the distance between crypts ($232,8 \pm 143,7$ μm) in comparison to C group ($13,9 \pm 4,9$ μm, $38,8 \pm 19,887 \pm 26,6$ μm, $43,6 \pm 6,7$ μm and $163,7 \pm 145,1$ μm, respectively). Finally, inadequate protein intake diminished thickness ($57,2 \pm 13,5$ μm) and augmented the height ($149,2 \pm 35,7$ μm) of the villi, without altering the number of goblet cells ($5,5 \pm 3,6$) compared to C group ($67,1 \pm 27,6$ μm, $132,1 \pm 38,1$ μm and $5,4 \pm 3$, respectively). Conclusion: Post-weaning protein restriction induced crypts hypotrophy and villi elongation in the ileum without modifying the weight or length of the SI.</p> <p>Keywords: Ileum, protein undernutrition, after weaning</p>
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Title	The administration of triiodothyronine (T3) reduced the expression of inflammatory cytokines and improved intestinal permeability in mice
Authors	Rodrigo Pereira Prates ¹ Paulo Henrique Evangelista-Silva ¹ Paula Bargi-Souza ² Rafael Simone Saia ¹ Francemilson Goulart da Silva ¹
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Session	Poster

Abstract, Ethics Committee Number*, and Keywords	<p>The high-fat diet (HFD) can cause an increase of inflammatory cytokines secretion that may damage the intestinal barrier and favoring the passage of bacterial products from the intestine to the blood. It is known that supraphysiological T3 administration reduce inflammatory cytokines expression in the muscle, fats, and blood of obese rats, but it is not still known whether T3 exerts effect on the intestinal obese animals by regulating cytokines expression and its permeability. To understand T3 effects on the gut of obese and non-obese animals, male C57BL/6 mice were divided into two groups: standard diet (LFD) or HFD for 12 weeks. At the end of these weeks, both groups were treated for 30 days with saline (LF+S, HF+S) or T3 (0.25 µg g⁻¹) (LF+T3, HF+T3). After euthanasia, the following experiments were performed: 1) RT-qPCR for the TSH-β gene in the pituitary gland and IL-1β, IL-6, TNF-α, IFN-γ, IL-10, and COX-2 in the jejunum; 2) intestinal permeability assay to FITC-Dextran (FD-4) and -3) measurement of villus width (CEUA protocol no. 7283120421). T3 efficiency treatment was confirmed by decreased TSH-β gene expression, loss of fat mass and high food intake. In non-obese mice, T3 administration reduced intestinal permeability, villus length and IL-1β and IFN-γ expression, while in obese animals, T3 decreased both IFN-γ expression and villus length. Our data suggest that T3 exerts an anti-inflammatory action upon the jejunum of both non-obese and obese mice, and its action was efficient only in non-obese to decrease jejunum permeability.</p> <p>Keywords: Obesity; Inflammation; Permeability; High Fat Diet; Mice.</p>
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Title	Evaluation of the synergistic effect of 4-methylesculetin and piperine in DSS model of intestinal inflammation in mice.
Authors	Andrade, J.S.Z.*¹ ; Ribeiro, G.C. ¹ ; Vidal, B.A. ¹ ; Souza, B.B. ¹ ; Cruz, V.M. ¹ ; Quaglio, A.E.V. ² ; Curimbaba, T.F.S. ¹ ; Di Stasi, L.C. ¹ .
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Session	Biology and gastrointestinal diseases.

Abstract, Ethics Committee Number*, and Keywords	<p>Inflammatory bowel disease (IBD) is a chronic inflammation of the gut associated with oxidative stress, which induce the degradation and cell membranes, perpetuating the inflammatory process. 4-methylesculetin (4-ME) is a coumarin described as intestinal anti-inflammatory and antioxidant product, modulating inflammation by decreasing myeloperoxidase (MPO) and avoiding glutathione (GSH) depletion. Piperine (PIP) is an alkaloid that acts as an anti-inflammatory compound through the modulation of NF-κB transcription factors, and being widely used as a product that facilitates the absorption and availability of other chemical compounds. The intestinal anti-inflammatory effects of 4-ME and PIP previously described indicate that these compounds act through different mechanisms, indicating the possibility that, when administered in combination, their effects can be enhanced via synergistic action, generating a product with potential applications to controlling the progression of IBD. So, the aim of this study was to evaluate the synergistic effects of 4-ME and PIP in the DSS-model of intestinal inflammation. For that we used male C57BL mice that received DSS 3% in drinking water for 7 days and 5mg/kg 4-ME, 25mg/kg PIP or 4-ME+PIP for the same doses. Disease activity index (DAI) was record daily and after the experiment, colonic samples were collected for MPO and GSH analysis. Data were compared considering $p < 0,05$ and presented as <i>mean ± SEM</i>. Biochemical analysis demonstrated that both the isolated groups and the combined group caused a significant decrease in MPO activity (Healthy $74,1 \pm 3,9$; DSS $120,4 \pm 10,09$; 4-ME $88 \pm 5,73$; PIP $74,2 \pm 6,8$; 4-ME+PIP $89,5 \pm 6,6$), treated animals also showed a smaller weight reduction associated with a reduced DAI score. The data obtained indicates that 4-ME and PP reduce intestinal inflammation, but this association did not promote synergistic effects.</p> <p>Ethics Committee CEUA N°992</p> <p>Keywords: Inflammatory bowel disease, 4-ME, Coumarin, Alkaloid, PIP.</p>
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Title	Evaluation of the synergistic effects of daphnetin and piperine in DSS- induced intestinal inflammation model in mice
Authors	Ribeiro, G.C¹ ; Vidal, B.A. ¹ ; Andrade, J.A.S. ¹ ; Souza, B.B. ¹ ; Cruz, V.M. ¹ ; Quaglio, A.E.V. ² ; Curimbaba, T.F.S. ¹ ; Di Stasi, L.C. ¹
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Session	Biology and Gastrointestinal Diseases

Abstract, Ethics Committee Number*, and Keywords	<p>Inflammatory bowel disease (IBD) is a multifactorial and chronic disease characterized by inflammation of the gastrointestinal tract, resulting in Crohn's disease (CD) and ulcerative colitis (UC), both without definitive pharmacological treatment. IBD is related to dysregulation of the immune system, dysfunctions in the intestinal mucosa and microbiota, and oxidative stress. Daphnetin (DAPH), a coumarin derivative, has several pharmacological properties, including anti-inflammatory and antioxidant. Piperine (PIP), an alkaloid, displays anti-inflammatory activity and was able to improve bioavailability of curcumin. Based on this, we evaluated the intestinal anti-inflammatory activity of DAPH and PIP as well as the potential synergistic effects of the association between them.</p> <p>For this, 9-week-old C57BL/6 mice were divided into 3 experimental groups ($n=8$) following the dextran sulfate sodium (DSS) model (3% in drinking water): daphnetin (DAPH, 5mg/kg), piperine (PIP, 25mg/kg) and daphnetin associated with piperine (5mg/kg; 25mg/kg), receiving treatment for 7 days by oral administration. For comparison, healthy and DSS-control groups were included. The macroscopic characteristics of colon and spleen, the Disease Activity Index, and biochemical analyses of anti-inflammatory and antioxidant markers (myeloperoxidase and glutathione) were determined. The compounds studied decreased MPO activity showing a significant anti-inflammatory activity (DAPH 82.58 ± 6.46; PIP 74.24 ± 6.83; DAPH+PIP 88.06 ± 6.46; vs DSS-control 117.0 ± 11.40; mean \pm SEM). The association between DAPH and PIP did not prove to improve pharmacological effects of said isolated compounds. None of the other parameters were altered after treatments.</p> <p>Ethics Committee CEUA n° 992</p> <p>keywords: inflammatory bowel disease; colitis; coumarin; alkaloid.</p>
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Title	Evaluation of the synergistic effects of daphnetin and piperlongumine in a DSS intestinal inflammation model in mice
Authors	VIDAL, B.A.¹ ; RIBEIRO, G.C. ¹ ; ANDRADE, J.S.Z. ¹ ; SOUZA, B.B. ¹ ; CRUZ, V.M. ¹ ; QUAGLIO, A.E.V. ² ; CURIMABABA, T.F.S. ¹ ; DI STASI, L.C. ¹ .
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Session	Biology and Gastrointestinal Diseases

Abstract, Ethics Committee Number*, and Keywords	<p>Inflammatory Bowel Disease (IBD) does not have definitive pharmacological treatment due to its complex and multifactorial etiology, mainly related to oxidative stress and intestinal dysbiosis. Daphnetin (DAPH), a coumarin derivative, has high pharmacological value and antioxidant action, inhibiting the production of ROS and increasing the production of endogenous antioxidant mediators, whereas piperlongumine (PPL), a piperine alkaloid, can reduce the production of pro-inflammatory cytokines. Based on this, the present study aimed to evaluate the intestinal anti-inflammatory activity of DAPH and PPL as well as the potential synergistic effects of the association between them. For this, C57/BL6 mice were randomly divided into experimental groups (n=8/group) and received DSS (3%) in drinking water for 7 days to induce intestinal inflammation, along with following treatments: DAPH (5 mg/kg), PPL (10 mg/kg) and combination of DAPH (5 mg/kg) with PPL (10 mg/kg). For comparison, healthy (n=9) and DSS-control (n=9) groups were included. Clinical and biochemical parameters were evaluated. Data were compared considering $p \leq 0.05$ and presented as mean\pmSEM. The treatments presented a lower weight decrease as well as a lower indication of rectal bleeding and diarrhea in the Disease Activity Index, when compared with DSS group. PPL performed better anti-inflammatory and antioxidant potential among the substances. All treatments reduced myeloperoxidase levels (HEALTHY 74.13\pm3.90; DSS 120.4\pm10.09; DAPH 82.58\pm6.46; PPL 89.05\pm5.95; DAPHPL 81.15\pm5.36) and only PPL counteracted glutathione depletion (HEALTHY 1112\pm88.04; DSS 434.96\pm54.85; PPL 695.03\pm68.11). Therefore, the substances have anti-inflammatory activity, but the association did not prove to be more or less effective than the isolated ones.</p> <p><i>Keywords: Inflammatory bowel disease, coumarin, alkaloid, DSS.</i></p> <p><i>Ethics Committee Number: CEUA 992</i></p>
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Title	Anti-obesity effect of citral through metabolic and intestinal modulation in high-fat diet-induced obese mice
Authors	Maycon Tavares Emílio-Silva ¹ Vinicius Peixoto Rodrigues ¹ Antonio Jesús Ruiz-Malagon ² Mariana Moraes Fioravanti ¹ Rie Ohara ¹ Gabriela Bueno ¹ Felipe Lima Dario ¹ Alba Rodríguez-Nogales ² Julio Gálvez ² Lúcia Regina Machado da Rocha ¹ Clélia Akiko Hiruma Lima ¹
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Session	Biology and Gastrointestinal disease - Oral presentation

Abstract, Ethics Committee Number*, and Keywords	<p>Obesity is directly associated with a disbalance of the gut environment and fat accumulation in adipose tissue. However, the mechanisms involved in changes in the intestinal epithelium to reverse obesity are poorly understood. We aimed to investigate the impact of citral (CT) against damages associated with lipopolysaccharide (LPS) and high-fat diet (HFD) <i>in vitro</i> and <i>in vivo</i> models that mimic obesity. Adult Male C57BL/6J mice were fed a standard diet (SD) and HFD for 17 weeks (CEUA No. 6702310820), together with daily oral treatment with CT (25, 100, or 300 mg/kg) and its vehicle (1% Tween 80 at 10 mL/kg). Body mass gain (g) and caloric ingestion (g/day) were assessed twice weekly. Finally, a glucose tolerance test, lipid profile, adipose index, and gene expression of the colon were performed. The human normal colon epithelial cell line (NCM-356) stimulated with LPS (10 ng/mL) for 48h was used to assess cell viability and scratch wound-healing assay. Statistical analysis was performed using one- and two-way ANOVA followed by Tukey's post-test ($p < 0.05$). Daily treatment with CT (300 mg/kg) protected against the increase in body mass gain when compared to the HFD mice. Also, a significant reduction in adiposity index was associated with a decrease in MDA levels in adipose tissue and serum levels of total and LDL cholesterol caused by CT treatment. The anti-hyperglycemic effect of treatment with CT also was demonstrated in HFD-induced obese mice. CT shows an important anti-inflammatory effect in the colon preventing the increase in NLRP3 inflammasome gene expression caused by HFD. CT also showed a significant proliferative effect <i>in vitro</i> at concentrations until 50 $\mu\text{g/mL}$ when</p>
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compared with the control group, also CT reversed this LPS-caused epithelial intestinal disorder in the stimulated wound closure. In conclusion, CT has anti-obesogenic effects associated with inflammatory and metabolic alterations modulated by intestinal changes against obesity. Funding: FAPESP.

Keywords: hypercaloric diet; adipose tissue; low-grade chronic inflammation; monoterpene.



Title	Study of enteric neurons and enteric glial cells in chronic experimental ulcerative colitis in mice deficient for the P2X7 receptor (P2X7R -/-)
Authors	Souza, Roberta, F Caetano, Marcos, A, F Castelucci, Patricia
Affiliations	Department of Anatomy, University of São Paulo, Brazil.
Session	5 – Biology and Gastrointestinal Diseases

Abstract and Keywords	<p>Introduction: Inflammatory Bowel Disease (IBD) is a term used to describe prolonged inflammation of the gastrointestinal tract, including Chron’s disease (CD) and Ulcerative colitis (UC). Ulcerative colitis affects enteric neurons and causes continuous mucosal inflammation, extending from the rectum to the proximal colon. The P2X7 receptor (P2X7R) is activated by increased levels of extracellular ATP in intestinal inflammation and participates in the regulation of the inflammatory response. Objective: This project aims to analyze enteric neurons and enteric glial cells in chronic experimental ulcerative colitis in Knockout (KO) mice deficient for the P2X7R gene (P2X7R -/-) and C57BL/6 Wild Type (WT).</p> <p>Methods: Male mice were used. Colitis was induced by 3 cycles of 2% (2g/100ml) of Dextran Sodium Sulfate (DSS) dissolved in drinking water for 5 days (cycles 1 and 2 2% (2g/100ml) and 1.5% (1.5g/100ml) in cycle 3), followed by drinking water for the next 14 days (KO/DSS and WT/DSS groups). The status of the animals was monitored by general examination and body weight evolution. The KO/SHAM and WT/SHAM groups received water through the same period. The animals were euthanized after 57 days and the distal colon was removed. This study was approved by the Ethics Committee on Animal Use of the University of São Paulo, Brazil, protocol number CEUA 5491270323. Tissues were prepared by immunohistochemical methods with double labeling of the nitric oxide synthase neuronal (NOSn), acetylcholine transferase (ChAT), P2X7 Receptor (P2X7R), and glial fibrillary acidic protein (GFAP). The number of NOSn-immunoreactive (-ir) neurons (neuron/ganglion), ChAT-ir, P2X7R-ir, and glial cells positive for GFAP-ir were counted. Data were compared using ANOVA and Tukey’s test, $p < 0.05$ was statistically significant. (WT/SHAM vs WT/DSS, KO/SHAM vs KO/DSS). Results: The NOSn-ir neurons/ganglion in the WT/DSS group (2.8 ± 0.1) decreased by 78.8% compared to that in the WT/SHAM group (3.5 ± 0.1) respectively ($p < 0.05$), no differences between KO/DSS (3.4 ± 0.1) and KO/SHAM (3.9 ± 0.1) groups; ChAT-ir neurons/ganglion of WT/DSS group (5.7 ± 0.1) decreased by 69.3% compared to that in the WT/SHAM group (8.2 ± 0.1), and in the KO/DSS group (5.0 ± 0.1) decreased by 65.6% to that in the KO/SHAM group (8.3 ± 0.1) respectively ($p < 0.05$). The P2X7R-ir/ganglion of WT/DSS group (9.1 ± 2.2) increased by 97.3% compared to that in the WT/SHAM group (8.8 ± 2.4) respectively ($p < 0.05$). The GFAP-ir/ganglion in the WT/DSS group (9.1 ± 2.2) decreased by 74.5% compared to that in the WT/SHAM group (8.8 ± 2.4), and in the KO/DSS group (5.8 ± 0.1) decreased by 70.8% to that in the KO/SHAM group (8.3 ± 0.1) respectively ($p < 0.05$). Histological studies</p>
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revealed that the mucosa, lamina propria, and submucosal ganglia in the WT/SHAM and KO/SHAM groups were normal appearance. The submucosal of the WT/DSS group displayed increased thickness and KO/DSS group approaches of normal appearance.

Conclusions: Our data conclude that myenteric neurons and glial cells of the distal colon were affected by ulcerative colitis and, that P2X7R Knockout mice were efficient in neuroprotection. Thus, these results demonstrate that the P2X7 receptor may be an important target in the therapeutic strategy.

Keywords: Purinergic receptor, Enteric Nervous System, Inflammatory Bowel Disease, P2X7R Knockout mice.



Title	Butyrate produced by gut microbiota protects myenteric neurons loss following experimental ulcerative colitis by reducing TNF-α
Authors	Marcos Antônio Ferreira Caetano Roberta Figueiroa de Souza Jheniffer Rayane de Lima Duarte Patricia Castelucci
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Session	05 - Biologia e Doenças Gastrointestinais

Abstract, Ethics Committee Number*, and Keywords	<p>The enteric nervous system (ENS) is affected by inflammatory bowel diseases (IBDs). Butyrate is a short-chain fatty acid, produced by gut microbiota from the fermentation of dietary fibers, which binds to GPR43 receptor, improving intestinal health, and reducing the production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α). This work aimed to explore the GPR43 receptor and Butyrate's effects on myenteric neurons in experimental ulcerative colitis (EUC). C57BL/6 (8-week-old) male mice received an intrarectal injection of 100μL of 2,4,6-Trinitrobenzenesulfonic-acid (TNBS group). SHAM group received vehicle (ethanol 35%). After UC induction, some animals were treated for 7 days with Sodium Butyrate (100mg/kg, Butyrate group) via gavage. SHAM and TNBS groups received saline (CEUA-ICB/USP-5482071122). Large intestines were collected 7 days after TNBS/ethanol injection for immunofluorescence double labeling for GPR43 with Calretinin (Calr) and with neuronal nitric oxide synthase (nNOS). Double labeling of TNF-α with Calr and nNOS was also performed. The number of neurons/ganglia immunoreactive(-ir) for GPR43, Calr, and nNOS, and fluorescence intensity (CTCF) of TNF-α were analyzed. Colocalization of GPR43 with Calr-ir and nNOS-ir neurons, and of TNF-α with Calr-ir, nNOS-ir was observed. TNBS reduced GPR43-ir (20.3%), Calr-ir (38.5%), and nNOS-ir (41.1%) neurons compared to SHAM group. Butyrate treatment restored these neurons by 20.9%, 35.4%, and 38.3%, respectively. TNF-α CTCF increased by 40.6% in TNBS group and decreased by 27.7% in Butyrate group compared to TNBS group. There was a CTCF reduction of GPR43 (28.4%) in TNBS group, and an increase by 35.7% in Butyrate group compared to TNBS group. Calr and nNOS CTCF had no difference between groups. Butyrate protects neuronal loss and reduces TNF-α CTCF. Also, colocalization with GPR43 was observed, suggesting that ENS can respond to Butyrate binding, being a possible therapy tool for IBDs.</p> <p>Keywords: Inflammatory Bowel Disease; Enteric Nervous System; Gut Microbiota; Free fatty-acid receptor; Tumor necrosis factor alpha</p>
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Title	FeTPPS (a peroxynitrite scavenger) protects the intestinal epithelial barrier during polymicrobial sepsis
Authors	Lucas de Souza Ortolan Rafael Simone Saia
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Session	05 - Biologia e Doenças Gastrointestinais

Abstract, Ethics Committee Number*, and Keywords	<p>Sepsis is associated to activation of the inflammatory response and the production of numerous reactive species, including peroxynitrite, which is a potent inducer of cell death. FeTPPS is a selective peroxynitrite scavenger, with therapeutic potential in diseases related to the overproduction of this oxidant. Our aim was to investigate the role of peroxynitrite on intestinal epithelial barrier dysfunction induced by polymicrobial sepsis and the inflammatory response. Mice were treated with FeTPPS (1 or 5mg/kg, <i>i.p</i>) or saline immediately after cecal ligation and puncture (CLP) or sham surgery. After 24h, the parameters were investigated such as ileal permeability was assessed by injection of dextran (FD4; 5mg/mL); microbiological quantification in blood, peritoneal lavage and mesenteric lymph node; and determination of cytokines in serum. Septic animals ($3,39 \pm 0,52$) showed increased plasma FD4 concentration when compared to control ($1,23 \pm 0,18$), which was attenuated in mice treated with FeTPPS 1 ($1,43 \pm 0,27$) and 5mg/kg ($1,31 \pm 0,26$). Septic mice showed increased bacterial count in blood ($9,0 \times 10^4 \pm 1,8 \times 10^4$ CFU/mL), peritoneal lavage ($3,1 \times 10^5 \pm 1,7 \times 10^5$ CFU/mL) and mesenteric lymph nodes ($2,5 \times 10^5 \pm 1,1 \times 10^5$ CFU/g tissue) were higher when compared to control groups. However, FeTPPS reduced the bacterial count in blood ($8,8 \times 10^3 \pm 7,2 \times 10^3$), peritoneal lavage ($9,3 \times 10^3 \pm 7,2 \times 10^3$) and mesenteric lymph nodes ($2,2 \times 10^4 \pm 7,8 \times 10^3$). Serum concentrations of IL-6 and TNF-α were significantly increased (pg/mL) in septic animals when compared to control groups, which were attenuated by with the treatment. IL-10 concentrations were lower in septic animals ($209,1 \pm 79,1$), and treatment with FeTPPS at the higher dose stimulated the synthesis of this anti-inflammatory cytokine ($758,9 \pm 223,1$). Our findings demonstrated that peroxynitrite promotes the ileal epithelial barrier dysfunction, associated with bacterial translocation and increased synthesis of pro-inflammatory mediators in polymicrobial sepsis.</p> <p>Committee Number: 1149/2022R1.</p> <p>Keywords: scavenger; peroxynitrite; intestinal permeability; bacterial translocation; inflammation; oxidative stress.</p>
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Title	Carboxymethylcellulose and polysorbate 80: Effects of food additives on NAFLD-associated hepatocarcinogenesis
Authors	Gabriel P. Bacil ¹ Heloiza Alves Ferreira ¹ Julia Oliveira Noveti ¹ Guilherme R. Romualdo ² Luís Fernando Barbisan ¹
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Session	Poster session I.

Abstract, Ethics Committee Number*, and Keywords	<p>Epidemiological findings indicate a prominent role of hepatocellular carcinoma (HCC), featuring as the 3rd deadliest kind of cancer worldwide and displaying a major interplay with non-alcoholic fatty liver disease (NAFLD), the most common cause of chronic liver disease. In this context, adherence to a Westernized diet (WD) and the high intake of ultra-processed food and alimentary additives, especially emulsifier compounds, have been targeted as potential drivers of HCC. We sought to assess the effects of carboxymethylcellulose (CMC) or polysorbate 80 (P80), at populational-relevant doses, on NAFLD-associated hepatocarcinogenesis. C57BL/6J mice were allocated in 11 experimental group and received intraperitoneal doses of diethylnitrosamine (25 mg/Kg of body weight, bw, 1x/week, G2 and G5-G11) or vehicle (G1, G3, and G4), for 4 weeks, and were kept in a WD (30% fat and 0.2% cholesterol-added chow, and 44/55% glucose/fructose-water diluted solution, G5-G11) or basal diet (G1-G4), for 24 weeks. Additionally, mice received intragastrically isolated (G6-G9) or combined (G10 and G11) doses of CMC (370/740 mg/Kg of bw) and P80 (100/200 mg/kg of bw) or vehicle, for 24 weeks (5x/week). Liver tumor, non-tumor liver and adipose tissue samples were collected for further analyses, and a glucose tolerance test was done (CEUA 1410/2022). Tumor multiplicity and volume were not modulated by food additives. WD-receiving groups featured a glucose intolerance profile ($p < 0.0001$) and increased final body weight ($p < 0.0001$) and hepatic relative weight ($p < 0.0001$) while only P80 and CMC+P80 at lower dose increased the adiposity index ($p < 0.0001$). The WD protocol led to an extensive macro/microvesicular steatosis profile ($p < 0.0001$) and pronounced NAFLD activity score ($p = 0.0006$). Our findings suggest that lower doses of CMC and P80 might exert promoter effects on NAFLD-associated hepatocarcinogenesis, by enhancing the metabolic disorder-related and modulating the tumoral microenvironment.</p> <p>Keywords: NAFLD; hepatocarcinogenesis; carboxymethylcellulose, polysorbate 80; food additives; Western diet.</p>
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Title	Activity of free and nanoemulsified myrcene in modulating ulcerative colitis induced by dextran sodium sulfate in adult C57BL/6J male mice: the involvement of antioxidant mechanism
Authors	Isabela Galende Guidolin ¹ Vinícius Peixoto Rodrigues ¹ Maycon Tavares Emílio-Silva ¹ Felipe Lima Dario ¹ Gabriela Bueno ¹ Jonatas Lobato Duarte ² Laura Vitória Fortunato de Oliveira ¹ Mariana Moraes Fioravanti ¹ Felipe Leonardo Fagundes ¹ Marlus Chorilli ² Clelia Akiko Hiruma-Lima ¹
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Session	Biology and Gastrointestinal Diseases – Oral presentation

Abstract, Ethics Committee Number*, and Keywords	<p>Ulcerative colitis (UC) is an inflammatory bowel disease that affects the colon and rectum. The severity and development of UC is influenced by different factors. Myrcene (MC) is a monoterpene with antioxidant and anti-inflammatory properties and due to its lipophilic characteristic, an alternative to increase the MC bioavailability is through nanoemulsions. Thus, we aimed to evaluate the activity of free and nanoemulsified MC in dextran sodium sulfate (DSS)-induced UC in mice. The male C57BL/6J mice (n=10) were given drinking water with 3% (w/v) DSS for five days, simultaneously also orally given 1% or 0.5% Tween 80 (vehicle, 10 mL/kg), three different doses of free and nanoemulsified MC (3.75; 7.5 and 11.25 mg/kg). A non-colitic (blank) group was included to ensure tissue quality. Finally, the data of severity of the disease, the length of the colon (cm), and tissue oxidative stress were assessed. The results were subjected to the one-way ANOVA and Tukey's post hoc test (p<0.05) (CEUA 946616112). Exposure to DSS over the days increased progressively the severity of the disease in the mice. Treatment with all doses of free MC significantly reduced disease severity when compared to the vehicle group (p<0.05 and p<0.01). While the nanoemulsion MC only significantly reduced signals compared to the vehicle group at a dose of 7.5 mg/kg (p<0.05). Furthermore, free MC (3.75 mg/kg) had an attenuating effect on colon shortening when compared to the vehicle group (p<0.01 and p<0.0001). This effect was not observed in the nanoemulsion. The</p>
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group treated at a dose of 3.75 mg/kg with MC nanoemulsion showed an increase in superoxide dismutase activity compared to the vehicle and blank groups ($p < 0.01$). Free and nanoemulsified MC reduced the degree of severity of the DSS-induced UC, such as morphological changes and an increase in antioxidant capacity during the analyzed period.

Keywords: Inflammatory bowel disease, monoterpenes, nanoemulsion.



Title	Effects of Yacon (<i>Smallanthus sonchifolius</i>) root syrup on non-alcoholic fatty liver disease in obese mice
Authors	Isadora Penedo de Souza ¹ Guilherme Ribeiro Romualdo ² Daniel Rodrigues Cardoso ³ Ana Paula Dionísio ⁴ Luís Fernando Barbisan ¹
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Session	Poster Session 1

Abstract, Ethics Committee Number*, and Keywords	<p>Non-alcoholic fatty liver disease (NAFLD) affects ~30% of the population and is linked to a Western-style diet (WD). Dietary re-education (DR) and functional foods may help balance the hepatic-adipose axis altered by WD. This study evaluated the protective effects of Yacon (<i>Smallanthus sonchifolius</i>) root syrup (YRS) in NAFLD during the DR period. Female C57BL/6 mice were submitted to a standard diet (SD) (G1) or WD-induced NAFLD (30% lard diet and high sugar solution) (G2-G7). After 3 months, groups stayed on SD (G1) and WD (G2), or received a reduced lard diet (20%) (partial DR G3-G5) or SD (total DR G6-7), and were treated with YRS intragastrically (7×/week, at 8.5 [G4] or 17 [G5, G7] g/kg b.w.) or corn syrup (G3, G6), and received water for 3 weeks (CEUA n°8274270522). Glucose tolerance test (GTT) was performed, and animals were euthanized. Liver, adipose tissue (AT), and serum samples were collected. WD increased body weight (p=0.0003), relative AT total weight (p=0.0004), adipocyte hypertrophy (p=0,0051), and glucose intolerance (p<0.0001), but none of the treatments reduced these parameters. Relative retroperitoneal AT weight was reduced by all interventions (p<0.0001) (G3-G7). The groups did not differ in the relative liver weight (p=0.0206), however, a reduction in NAS scores, increased by WD, were observed in partial DR+YRS 8.5g/kg (G4) and total DR (G6) for all score parameters (macro [p=0.0003] and microvesicular [p<0.0001] steatoses and hypertrophy [p<0.0001]), and the total score (p= 0.0001). Hepatic inflammatory foci differed only in G6 (total DR) when compared to G2 (WD) (p=0.0331). All WD groups showed an increase in serum cholesterol levels, reduced only by partial DR+YRS 17g/kg (G7) (p=0.0003). DR resulted in benefits for obese female mice, and YRS showed potential benefits, reducing some hepatic and AT parameters.</p> <p>Keywords: Non-alcoholic fatty liver disease; Western diet; Yacon (<i>Smallanthus sonchifolius</i>)</p>
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FRONTEIRAS DAS CIÊNCIAS BIOMÉDICAS E SEUS DESAFIOS

Title	Effects of wasabi (<i>Wasabia japonica</i>) rhizome and its main compound allyl isothiocyanate against irinotecan chloride-induced adverse effects: <i>in vivo</i> and <i>in vitro</i> bioassays
Authors	Ana Clara Gemignani ¹ Giullia Cavicchioli Barbosa ² Leticia Cardoso Valente ² Guilherme Ribeiro Romualdo ² Luís Fernando Barbisan ¹
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Session	Poster Session 1

Abstract, Ethics Committee Number*, and Keywords	<p>Irinotecan chloride (CPT-11) is used to treat various neoplasms, but with several side effects. Wasabi rhizome (Wab) is used in oriental culinary, containing mainly allyl isothiocyanate (AITC). Thus, this study evaluated whether Wab or AITC reduces the CPT-11-induced adverse effects. Female C57BL6/J mice received single doses of CPT-11 [75 mg/kg, i.p.] for 6 days or CPT-11 plus single doses of Wab (0.5 or 1 g/kg, gavage) or AITC (2 mg/kg, gavage) for 6 days. Then, peripheral blood samples (comet assay) and liver, kidney, and intestines (histology) were collected. In addition, human colon epithelial cell line HCT-116 was treated with CPT-11 and AITC to calculate the half maximal effective concentration (EC50) by viability test (MTT). Next, we evaluated whether AITC (1/10 and 1/20 EC50, 94 and 47 μM), would modify the cytotoxic response of CPT-11 (EC50 and 1/2 EC50, 57.5 and 28.75 μM). Data were analyzed using ANOVA or Kruskal-Wallis tests, $p < 0.05$, CEUA N° 6666300323). In animal assay, no difference in body weight, liver and kidney weights, or lengths of the intestines were observed among groups, but CPT-11 caused intestinal mucositis. In comet assay analyses, groups receiving IRT and treated with 0.5 g Wab and 2 mg AITC showed lower levels (% DNA damage) in leukocytes when compared to the IRT group ($p < 0.0003$). In HCT-116 cells, AITC improved the cell viability (MTT) at 24 and 48 h ($p < 0.0001$) and reduced the genotoxicity at 24h ($p = 0.0003$) after CPT-11 exposure. AITC did not alter the effects of CPT-11 on the restitution of the intestinal barrier. AITC + CPT-11 showed antagonism compared to CPT-11 alone regarding cell viability.</p>
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Additionally, AITC (1/10 and 1/20 EC50) did not modify the antitumor effects of CPT-11 (1/2EC50 or EC50) in HCT-116/CCD-18co tumor spheroids. The novel findings indicate that wasabi and AITC may attenuate the side effects caused by CPT-11 while AITC did not impair its chemotherapy effect.

Keywords: Wasabi, AITC, CPT-11 side effects.



Title	Investigating the role of the flavonoid cyanidin in intestinal inflammatory damage
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Session	Biology and Gastrointestinal disease - Poster presentation

Abstract, Ethics Committee Number*, and Keywords	<p>Polypharmacy is associated with disbalances in intestinal homeostasis, mainly in the formation of ulcers with a pro-inflammatory response with gut barrier disruption. Cyanidin is a flavonoid present in flowers, fruits, and seeds with an important anti-inflammatory and gastroprotective activity. Thus, this work aimed to investigate whether the administration of cyanidin can decrease the deleterious effects caused by polypharmacy (PPI + NSAID) in the small intestine. Male Swiss mice were treated to induce intestinal ulcers using lansoprazole 20 mg/kg, acetylsalicylic acid 10 mg/kg, and celecoxib 20 mg/kg (protocol number 001.12.2017). Cyanidin was administered at a dose of 5mg/kg. Quantify interleukins (IL-10, IL-6, IL-1β) and cytokine (TNF) was performed, and measured gene expression of TNF, IL-10, IL-6, TLR-4, HMOX-1, MMP 2 and 9, COX-1, MUC-3, ZO-1, CL-1. Monolayers of colonic epithelial cell lines (Caco-2) were mounted in Ussing chambers to assess barrier function and to determine transepithelial resistance (TER). To analyze the permeability response to injury, we utilized TNF and IFN (25 ng/mL) with cyanidin (10 or 100 μM) for 48 h in transwell plates, with measurement of total intestinal permeability using 4 kD FITC-dextran. Analysis of mouse intestine indicated that cyanidin significantly reduced expression of IL-6 and TNF, TLR4, and HMOX-1 ($p < 0.05$), and increased gene expression of MUC-3, CL-1, occludin, COX-1, and IL-10 ($p < 0.05$). Cyanidin (100 μM) maintained barrier function as shown by transepithelial electrical resistance (TER) and accelerated tight junction reassembly in Caco-2 cells in a calcium switch assay, and significantly reversed the detrimental effects of the inflammatory cytokine on FITC-dextran flux in Caco-2 cells ($p < 0.05$). These</p>
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results suggest that cyanidin decreases intestinal inflammatory damage while maintaining the integrity of the intestinal epithelium. Other trials are underway to elucidate these mechanisms. Funding: NSERC and FAPESP.

Keywords: Peptic ulcers; Polypharmacy; Anthocyanidins; Caco-2 cells; Swiss mice.



Title	The role of myrcene in obesity associated with colitis induced by dextran sodium sulfate (DSS) in C57BL/J6 male mice
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Affiliations	São Paulo State University (UNESP), Institute of Biosciences, Botucatu, SP, Brazil.
Session	Oral-Biology and Gastrointestinal Diseases

Abstract, Ethics Committee Number*, and Keywords	<p>Obesity is a disease that aggravates inflammatory bowel diseases (IBD). Colitis (CO) is an IBD characterized by chronic inflammation in the colon that compromises the integrity of the intestinal barrier, triggering an inflammatory response leading to oxidative stress altering markers such as myeloperoxidase (MPO), Metalloproteinase (MMP)-9 and superoxide dismutase (SOD). Myrcene (Myr) is a monoterpene with antioxidant and anti-inflammatory action on the gastric mucosa in rodents. The aim of the study was to evaluate the action of Myr in experimental CO aggravated by obesity induced by high-fat diet (HFD) in C57BL/J6 male mice (n=15). Obesity was induced for 18 weeks by HFD while the lean group received standard diet (SD). CO was induced by 3% DSS in drinking water for seven days. After induction, mice were orally treated for seven days: 1% Tween 80 (vehicle 10mL/kg; TW), Myr (7.5 mg/kg) and the blank group. During treatment the disease activity index (DAI) was measured. The colon was collected, measured and weighed for morphological evaluations and to assess MPO, MMP-9 and SOD (Ethics Committee N°. 5437170821). The Kruskal-Wallis test, one-way ANOVA followed by the Tukey test or the two-way ANOVA followed by the Bonferroni test were used to determine statistical significance (p<0.05). HFD increased body weight, adiposity index and glycemic alterations in the obese animals compared to the SD animals. Induction of CO was more severe in obese mice than in lean animals, with a higher mortality rate among obese mice (58.4%). Treatment with Myr reversed the colon shortening in the animals and decreased the DAI compared with TW group. Myr reduced MPO and increased SOD in the colon of all animals compared to TW. Myr contributes to the</p>
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improvement of CO by reversing the shortening of the colon, providing reconstruction of the barrier by reducing MMP-9 activity, promoting antioxidant and anti-inflammatory action in the colon by increasing SOD and reducing MPO, respectively.

Keywords: Obesity; Colitis; Myrcene; High-fat-diet.



Title	Evaluation of the immune profile in experimental animals treated with kefir
Authors	Danaê Fernanda Avanze Cação ¹ , Gabriela Teixeira Aschenbrenner ¹ , Jéssika Takaki ² , Malu Benedetti Nascimento ¹ , Jéssica Alves Ribeiro ¹ , Monara Kaélle Sérvulo Cruz Angelim ³ , Lailson Oliveira De Sousa ⁴ , Pedro Manoel Mendes De Moraes Vieira ² , Débora Parra Baptista ⁴ , Mirna Lúcia Gigante ⁴ , Liana Verinaud ² , Catarina Rapôso Dias Carneiro ¹ , Valquiria Aparecida Matheus ¹ .
Affiliations	1 - Faculty Of Pharmaceutical Sciences – UNICAMP, Campinas, Brazil 2 – Universidade Paulista, São Paulo, Brazil 3 - Institute Of Biology - UNICAMP, Campinas, Brazil 4 - Faculty Of Food Engineering Unicamp – UNICAMP, Campinas, BRASIL
Session	Biologia e Doenças Gastrointestinais

Abstract, Ethics Committee Number*, and Keywords	<p>The intestinal microbiota plays a role in modulating the host's immune response. Its imbalance, or dysbiosis, is associated with metabolic and immunological disorders. During the gestational period, maternal dysbiosis can predispose offspring to the development of these disorders. Kefir, recognized for its ability to restore the intestinal microbiota, emerges as a promising tool to modulate immunity at different stages of life. This study aims to evaluate how kefir influences the composition of circulatory immune cells in non-pregnant adult females, pregnant females, and their respective offspring. Non-pregnant and pregnant females of the C57BL6J lineage were distributed into 3 groups and received the following treatments for 21 days: 1) control group (CT), without intervention; 2) dysbiosis group (ABX), received intervention with an antibiotic mix (1 mg/mL of ampicillin and 0.5 mg/mL of neomycin) in drinking water; 3) kefir group (K), received 200 µL of kefir daily via gavage (CEUA 6232/2023). The offspring received no intervention. Hemogram analysis showed that none of the groups presented significant alterations in indices related to erythrocytes, whether non-pregnant, pregnant, or offspring. There was an increase in the total amount of leukocytes in the offspring and their respective mothers of the K group compared to the other groups. In non-pregnant K females, this increase was discreet. ABX offspring presented a decrease in the total number of leukocytes, suggesting an immunological deficit in offspring of mothers with gestational dysbiosis. K offspring had an increase in the number of monocytes and a greater presence of reactive lymphocytes compared to the offspring of the other groups. The results indicate that maternal dysbiosis reduces leukocytes in offspring, while offspring of pregnant who consumed kefir had an increase in leukocytes, monocytes, and reactive lymphocytes. Kefir treatment has the potential to balance the offspring's immune response. Next steps include investigating the effect of kefir on immunological parameters in non-pregnant and pregnant adult females with established dysbiosis, as well as in their offspring.</p> <p>Keywords: Microbiota, Offspring, Dysbiosis</p>
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Title	Butyrate protects mice myenteric plexus pan-neuronal loss following experimental ulcerative colitis
Authors	DUARTE, Jheniffer Rayane de Lima, CONCEIÇÃO, Laura Barbosa, CAETANO, Marcos Antônio Ferreira, CASTELUCCI, Patricia.
Affiliations	Department of Anatomy, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil
Session	05 - Biology and Gastrointestinal Diseases

Abstract, Ethics Committee Number*, and Keywords	<p>Inflammatory bowel diseases (IBD) entail gastrointestinal tract inflammation. Ulcerative colitis is a serious health problem, potentially fatal. Butyrate, a short-chain fatty acid (SCFA), produced by gut microbiota through dietary fiber fermentation, SCFA binding to GPR41 can activate intracellular pathways, enhancing intestinal barrier and health functions. The study aimed to analyze GPR41 receptor in PGP9.5 (pan-neuronal marker) immunoreactive neurons (-ir) and investigate sodium butyrate's impact on myenteric plexus neurons in mice with experimental ulcerative colitis. 8-week-old male C57BL/6 mice in the TNBS group received 100µL of 2,4,6-Trinitrobenzenesulfonic acid via intrarectal injection. The Sham group received ethanol. The BUT group was administered Sodium Butyrate via gavage after TNBS injection. Animals were euthanized after 7 days for disease activity index (DAI) analysis. A double labeling reaction of GPR41 and PGP9.5 receptors was conducted to assess colocalization, neuron count per ganglion, and neuron area. (Approved by CEUA-ICB/USP nº 6507140420). The DAI showed weight loss, increased fecal bleeding, and soft stools in the TNBS group, which improved in the BUT group. The Sham group remained unchanged. GPR41-ir receptors were present in PGP9.5-ir enteric neurons. PGP9.5-ir neuron count decreased by 9% in the TNBS group (13 ± 0.2) compared to the Sham group (14 ± 0.2; $p < 0.001$). The BUT group (15 ± 0.2; $p < 0.001$) exhibited a 14% recovery compared to TNBS. The cell body neuron area ($235 \pm 4 \mu\text{m}^2$) and cytoplasmic area ($127 \pm 3 \mu\text{m}^2$) of PGP9.5-ir neurons reduced by 13% in the TNBS group compared to Sham but did not differ from the BUT group. The TNBS-induced experimental ulcerative colitis replicated clinical, macroscopic, and microscopic manifestations. Sodium butyrate treatment mitigated clinical effects and PGP9.5-ir neuron reduction in the BUT group.</p> <p>Keywords: Inflammatory Bowel Disease; Enteric Nervous System; Sodium Butyrate; Short-chain fatty acids; GPR41.</p>
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Title	Effects of short-term treatment with anti-lipogenic drugs fenofibrate and ezetimibe on steatotic liver disease in male C57BL6 mice fed a high-fat diet
Authors	Julia Noveti ¹ Gabriel Prata Bracil ¹ Rodrigo Pereira Prates ² Francemilson Goulart-Silva ² Luis Fernando Barbisan ¹
Affiliations	¹ Department of Structural and Functional Biology, Institute of Biosciences of Botucatu, Sao Paulo State University (UNESP), Botucatu, SP, Brazil. ² Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo (USP), São Paulo, SP, Brazil.
Session	Poster session 1

Abstract, Ethics Committee Number*, and Keywords	<p>Recent studies highlight steatotic liver disease (SLD) as the fast-growing cause of chronic liver disease worldwide, also affecting almost 33% of the global population. Although there are several preclinical studies and efforts, still scarce potential therapeutic strategies approved by governmental agencies. In this context, prospecting for potential effects of anti-dyslipidemia and anti-lipogenic drugs, like fenofibrate (FF) and ezetimibe (EZ), might be an effective strategy against SLD. Thus, we sought to assess the therapeutic effects of FF and EZ in a murine model of SLD. Male C57BL/6J mice were kept on a high-fat diet (HFD)-induced protocol (30% of fat or 60% of Kcal), for 11 weeks. In the last 2 weeks of protocol, mice also received intragastric doses of FF [400 mg/Kg of body weight (b.w.)] and EZ (5 mg/Kg of b.w.) or their combination (FF+EZ). At the euthanasia, hepatic samples were collected for analysis of nonalcoholic fatty liver disease activity score (NAS) and the hepatic fibrotic background, according to the Ethics Committee of Animal Care (ICB/USP N° 8861200821). Both FF and FF+EZ treatments reduced the final body weight ($p=0.0336$), compared to the HFD group, but no differences were observed regarding the hepatic relative weight. The HFD induced an extensive microvesicular steatosis profile with pronounced hepatocellular hypertrophy. Only FF and FF+EZ groups alleviated the microvesicular steatosis background ($p=0.0026$), and all treatments reduced the hepatocellular hypertrophy occurrence ($p=0.0026$). No effects regarding the lobular inflammation were observed. Of note, fibrosis occurrence was not pronounced in the HFD group. Surprisingly, only the combination of FF+EZ reduced the final NAS ($p=0.0066$). Considering our findings, the association of</p>
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two anti-lipogenic drugs might be an effective strategy to alleviate the SLD, by modulating the hepatic steatotic background.

Keywords: fenofibrate; ezetimibe; steatotic liver disease; high-fat diet; C57BL/6J



Title	Carboxymethylcellulose and polysorbate 80 emulsifiers did not modify aberrant crypt foci development using a chemically induced colon carcinogenesis model in female C57BL/6J mice.
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Session	Poster session 1

Abstract, Ethics Committee Number*, and Keywords	<p>Recent estimates target colorectal cancer (CRC) as the 3rd/2nd most incident/deadliest type of cancer worldwide, with pronounced occurrence in industrialized countries. The fast-growing incidence of CRC is often attributable to westernized diet and a high intake of ultra-processed food (UPF) – commonly enriched with food additive emulsifiers - which might be linked to the chronic injury of the gut tract. Thus, we sought to assess the modifying effects of carboxymethylcellulose (CMC) and polysorbate 80 (P80) emulsifiers on early stages of colon carcinogenesis, administered at populational-relevant doses. Female C57BL/6J mice (6 weeks old) received intraperitoneal doses of 1,2-dimethylhydrazine [DMH, 30 mg/Kg of body weight (b.w.), 1x/week] (G1-G4, n=12/group) or vehicle (G5-G8, n=8/group) for 6 weeks. Then, G1-G4 groups received dietary deoxycholic acid (DA) (0.02% w/w), while G5-G8 groups were fed a basal chow for 22 weeks. Additionally, G2 and G6, G3 and G7 and G4 and G8 groups received dietary P80 (2% w/w), CMC (2% w/w), or their combination (CMC+P80), respectively, for 22 weeks (CEUA N° 5719220324). At necropsy, the large intestine (LI), liver, and spleen samples were collected for histopathological analysis. The LI was fixed flat and stained with 0.2% methylene blue for 3–5 min for aberrant crypt (AC) foci (ACF) analysis. Dietary P80, CMC or their combination did not alter final body weight, chow/water intake, or colon length. G1-G4 groups showed an increase in hepatic relative weight (p<0.0001) compared to G5-G8 groups, while G3 and G4 groups presented an increase in splenic relative weight (p<0.0001), compared to G1 and G5-G8 groups. ACF density and AC/ACF ratio were not significantly modified by dietary emulsifiers (G2-G4 groups) compared to the G1 group. No ACF was detected in the G5-G8 groups. These findings indicate that these emulsifiers administered at populational-relevant doses did not alter the early steps of colon carcinogenesis.</p> <p>Keywords: colorectal cancer; polysorbate 80; carboxymethylcellulose; emulsifiers; ultraprocessed</p>
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Title	Investigation of the protective potential of citronellol against gastric ulcers in rats
Authors	Luciano Rogério Destro Giacóia Antonio Miguel Leutwiler Garcia Isadora Passos Frigero Juan Carlos Ramos de Oliveira Ariane Leite Rozza
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Session	Poster

Abstract, Ethics Committee Number*, and Keywords	<p>Gastric Ulcers (GU) are lesions that develop in the stomach, affecting approximately 10% of the adult population. These lesions can compromise layers from the mucosa to the muscularis, resulting in reduced blood flow, inflammation, and oxidative stress. The primary treatment for GU involves the use of proton pump inhibitors; however, these medications can cause adverse effects, including gastric cancer. Citronellol, a monoterpene found in the leaves of the <i>Cymbopogon</i> genus, has various proven biological activities, including anti-inflammatory and antioxidant properties. This study aimed to evaluate the effect of citronellol in protecting against ethanol-induced GU in rats. Thirty-five male Wistar rats were randomly distributed into five groups (n=7): vehicle (2% tween 80), omeprazole (20 mg/kg), and citronellol (25, 50, and 100 mg/kg). The rats underwent oral treatment (gavage) for 7 days, always at the same time. On the seventh day, after fasting, one hour post-treatment, gastric ulcers were induced using absolute ethanol (5 ml/kg, by gavage). After one hour, the animals were euthanized, and the stomach, kidneys, liver, and spleen were collected. The ulcerated area was analyzed using ImageJ software. Data were analyzed via ANOVA, followed by Tukey's test with $p < 0.05$, with the aid of Prism software. The protocol was approved by the Animal Use Ethics Committee (CEUA-IBB 5458020623). The results showed that citronellol provided a gastroprotective effect at all three doses (25, 50, and 100 mg/kg) compared to the vehicle ($p < 0.001$, $p < 0.05$, and $p < 0.001$, respectively); however, there was no difference between the citronellol doses. The body weight of the rats and the relative weight of the kidneys, spleen, and liver did not differ between groups, suggesting an absence of toxicity. We conclude that citronellol has a gastroprotective effect, and further analyses are being conducted to elucidate the mechanisms of action.</p> <p>Keywords: gastric ulcer; citronellol; natural products</p>
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Title	Chronic intracerebroventricular treatment with irisin reduces insulin synthesis and secretion in diet-induced obesity mice
Authors	Tassiana Cristina Talpo Kelly Cristina Pereira Bem Guilherme Augusto da Silva Nogueira Joel Alves da Silva Junior Helena Cristina de Lima Barbosa
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Session	6 - Sistema Endócrino

Abstract and Keywords	<p>Obesity is marked by hypersecretion of insulin by pancreatic beta cells, leading to endoplasmic reticulum (ER) stress. The hypothalamus exerts control over insulin secretion via autonomic nervous system (ANS). Irisin, an adipomyokine released in circulation after exercise, crosses blood-brain barrier, is expressed in hypothalamus, and could mediate effects of exercise on insulin secretion via ANS. CEUA-UNICAMP:5995-1/2022. C57BL/6J male mice, 4w, were divided in 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 300ng/day. After 13w in diets, right lateral ventricle cannulation surgery was performed and animals were submitted to ICV treatment for 7d; followed by glucose tolerance test and blood collection. Tissues were collected after euthanasia. Sample size: 4-9 animals. Results were analyzed by One-way ANOVA ($P < 0,05$), followed by Tukey post-test. HFD showed higher fasting blood glucose ($118.8 \pm 6 \text{mg/dL}$) and insulinemia ($3.6 \pm 0.7 \text{ng/mL}$), glucose intolerance ($27348.8 \pm 1774 \text{AUC}$), hyperinsulinemia ($290.3 \pm 43 \text{AUC}$), insulin resistance ($30.3 \pm 6 \text{HOMA-IR}$), higher GSIS (glu 8.3mM: 0.9 ± 0.1 and glu 16.7mM: $2.8 \pm 0.4 \text{ng/mL.islet.h}$), higher insulin content ($2620.2 \pm 137 \text{ng/mL.islet}$) and increased ER stress in islets (Xbp1: 2.0 ± 0.2, Atf4: 1.5 ± 0.1, Chop: 1.8 ± 0.09, Bip: 2.2 ± 0.1 and Bcl-2: 2.0 ± 0.2, fold change of CTL), compared to CTL ($90.4 \pm 3 \text{mg/dL}$, $0.7 \pm 0.08 \text{ng/mL}$, $17901.1 \pm 799 \text{AUC}$, $73.4 \pm 7 \text{AUC}$, $4.5 \pm 0.5 \text{HOMA-IR}$, 0.5 ± 0.06 and $1.6 \pm 1 \text{ng/mL.islet.h}$, $1200 \pm 125 \text{ng/mL.islet}$). HFD-Iri showed lower fasting insulin ($1.9 \pm 0.3 \text{ng/mL}$), trend towards decreased hyperinsulinemia ($182.1 \pm 31 \text{AUC}$, $P = 0.08$), improved insulin sensitivity ($16.2 \pm 2 \text{HOMA-IR}$), lower GSIS (glu 8.3mM: 0.5 ± 0.07 and glu 16.7mM: $1.5 \pm 0.1 \text{ng/mL.islet.h}$), lower insulin content ($1330.7 \pm 170 \text{ng/mL.islet}$) and decreased ER stress in islets (Xbp1: 1.2 ± 0.1, Atf4: 1.1 ± 0.07, Chop: 1.4 ± 0.1, Bcl-2: 1.3 ± 0.2, fold change of CTL), compared to HFD. Chronic ICV treatment with irisin protected mice against obesity challenges, improving insulin sensitivity, reducing insulin synthesis and secretion, and reducing ER stress in pancreatic islets.</p> <p>Keywords: Irisin. Obesity. Pancreatic Islet. Endoplasmic Reticulum Stress.</p>
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Title	Expression of miR-146a in the subcutaneous adipose tissue of young and postmenopausal sedentary women
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Session	6 - Sistema endócrino

Abstract, Ethics Committee Number*, and Keywords	<p>In women, menopause marks the transition from reproductive to non-reproductive life and is characterized by various physiological and metabolic changes that can lead to a chronic inflammatory state, making them more prone to age-related diseases. Subcutaneous adipose tissue (SAT) can be an important source for studying these changes over time, as secretory and endocrine functions. In previous <i>in vitro</i> and animal studies, miR-146a found in SAT is known to be associated with senescence and inflammation regulation, but there are still no studies evaluating the expression of this miRNA in SAT of women at different stages of life. Thus, this study aimed to evaluate the expression of miR-146a in sedentary women of reproductive age and after menopause. Nineteen eutrophic women participated in this study, divided into young group (YG/n=13; age=28.7±5.4 years; BMI=21.53±2.82) and postmenopausal group (PMG/n=6; age=60±4.2 years; BMI=25.93±2.36), who underwent SAT biopsy. To classify the level of physical activity, we utilized the International Physical Activity Questionnaire (IPAQ). The study was approved by the Research Ethics Committee of the University of Campinas (appraisal number: 1.967.450/2017). The expression of miR-146a was evaluated by RT-qPCR analysis. YG had significantly lower values compared to PMG:0.131±0.104 vs YG:0.757±0.668 (relative expression), p=0.003, independent t-test. As expected, PMG had increased miR-146a compared to YG at baseline, showing that this microRNA is related to important metabolic changes associated with senescence and inflammation of SAT, becoming a source for future investigations regarding menopause and its relationship with hormonal loss occurring in this phase, female aging, and other factors that may influence its regulation.</p> <p>Keywords: microRNAs, Adipose tissue, Postmenopausal women.</p>
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Title	Moderate chronic sleep perturbation reduces weight and visceral adiposity in rats
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Session	Poster

Abstract and Keywords	<p>The studies associating sleep deprivation with metabolic outcomes come from clinical observation, where it is difficult to isolate the causality factor, or from preclinic studies, where the daily duration of sleep deprivation is too long. Thus, we proposed to evaluate the impact of moderate chronic sleep perturbation (SP) on biometric and metabolic parameters in rats, trying to mimic the human behavior of going to bed later and waking up earlier during the week. Adult animals of both sexes (n=10) were submitted to a SP protocol (multiple platform water), between 06:00-08:00 h and 16:00-18:00 h, for 4 consecutive weeks, with interruption of the protocol on the weekends. Body weight and food intake were analyzed throughout the protocol. Biochemical, histological and organ masses analyzes were also performed during the protocol progression. The symmetry and homogeneity of the data were analyzed and unpaired 'Student' t-Test or Mann-Whitney were applied and considered a significant effect when reached the value of $p \leq 0.05$. Data is expressed as mean \pm SD or median and interquartile intervals. CEUA-UFSC n° 9662240920. SP resulted in lower body weight gain in both sexes and changed the pattern of food intake, with oscillations between a tendency to reduce food intake during sleep perturbation days with a compensatory increase in rest periods. Rats from both sexes submitted to SP exhibited lower adipose mass without any change in plasma or hepatic triacylglycerol, leptin or non-esterified fatty acids values and a reduction on total cholesterol values. Histological analyses confirmed lower adipocytes area and perimeter in rats exposed to SP. Moderate chronic SP in rats reduces weight and visceral adiposity in association with altered food intake regardless of sex. This study reinforces the need for attention to the quantity and quality of sleep and its impact on metabolic health.</p> <p>Key words: circadian cycle, sleep disturbance, obesity, diabetes</p>
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Title	Effects of neonatal monosodium glutamate-obese rats and its treatment with exenatide on muscarinic acetylcholine receptor subtypes in rat hippocampus
Authors	Amanda de Lima Santos Musto ¹ , Pedro Henrique Garcia Figueiredo ¹ , Jeferson Rubens Mamona da Silva ¹ and Fernando Maurício Francis Abdalla ¹
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Session	Poster

Abstract and Keywords	<p>Introduction: Studies have revealed the existence of five distinct muscarinic acetylcholine receptor (mAChR) subtypes (M₁-M₅). The modulation of excitatory transmission by mAChRs seems particularly relevant to learning and memory processing in the hippocampus. The memory can be modified by various experimental conditions such as calorie restriction. However, little information about a possible relationship between mAChRs and obesity has been shown. Objective: Here we investigate the effects of obesity induced by glutamate (MSG) and its treatment with exenatide (MSG+E), an antiobesogenic drug derived from the venom of the Gila monster <i>Heloderma suspectum</i>, on the affinity, density and subtypes of mAChRs in rat hippocampus. Methods: Healthy rats were used as controls, and MSG-obese rats were selected via the Lee index > 0.300 (the experimental procedures were approved by the Research Ethical Committee from Instituto Butantan; #2452031022). The effects of MSG-induced obesity on affinity and density of mAChRs were evaluated by binding assay and their subtypes by immunoprecipitation assays. Results: Specific binding yielded a K_D of 0.48±0.11, n=3; 0.78±0.44, n=3 and 0.20±0.10 nM, n=3 for CT, MSG and MSG+E, respectively, which did not differ among them (p>0.05). The B_{max} obtained for MSG (115.80±22.80, n=3) and MSG+E (133.85±32.45, n=3) was lower than for CT (987.23±99.48 fmol/mg protein, n=3) (p<0.05). Immunoprecipitation assays induced a decrease in the expression of M₁ subtype of MSG (1.72±0.61, n=4) when compared to CT (4.84±0.65, n=4). MSG+E recovered the expression of M₁ (4.43±0.19 fmol/mg protein, n=4), similar to CT (P<0.05) (M₂ to M₅ subtypes did not differ among CT, MSG and MSG+E; p>0.05). Conclusion: Our results indicate that obesity induced by glutamate strongly influences the expression of M₁ subtype. The exenatide reversed this effect, suggesting an important role on hippocampal muscarinic cholinergic system. This action of obesity might be a key step mediating cellular events important for learning and memory.</p> <p>Keywords: obesity; monosodium glutamate; hippocampus; muscarinic receptor subtypes; rats.</p>
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Title	Better cardiometabolic/inflammatory profile is associated with differences in the brown adipose tissue activity of overweight sedentary middle-aged women with type 2 diabetes
Authors	Ivan Luiz Padilha Bonfante ^{1, 2, 9} , Higor da Silva Segantim ^{3, 10} , Kauê Neto Soares Mendonça ^{3, 10} , Murilo Augusto Belmiro de Oliveira ^{3, 10} , Milena Monfort-Pires ^{4,5, 6} , Renata Garbellini Duft ^{1,7} , Keryma Chaves da Silva Mateus ¹ , Mara Patricia Traina Chacon-Mikahil ¹ , Celso Darío Ramos ⁸ , Licio Augusto Velloso ^{4,5} , Cláudia Regina Cavaglieri ¹
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Session	6 - Sistema endócrino

Abstract, Ethics Committee Number*, and Keywords	<p>Brown adipose tissue (BAT) activity has been associated with a better cardiometabolic profile and reduced risk of developing non-communicable chronic diseases (NCD), in addition to being associated with a healthier phenotype in obesity. However, it is unknown whether greater BAT activity could be associated with a healthier metabolic profile in overweight sedentary middle-aged women with type 2 diabetes already diagnosed with type 2 diabetes (T2DM). Thus, the present work to evaluate if BAT activity is associated with metabolic and molecular markers in women with T2DM. Based on a cluster study, women with T2DM (age= 5.57 ± 5. 19 years; BMI= 29.72 ± 3.43) were divided into groups according to higher (n= 8) or lower-BAT (n= 6) activity levels. Functional,</p>
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biochemical, inflammatory, and molecular markers (thermogenic and autophagic gene expression in white subcutaneous fat and cold-induced 18-Fluoroxyglucose Positron Emission Computed Tomography (18FDG-PET/CT) were measured and compared between groups. The study was approved by the Research Ethics Committee of the University of Campinas (appraisal number 1.597.626 and 2.030.070). Women with higher BAT activity present lower count of leukocytes, platelets, along with lower TYG-index, z score of metabolic syndrome values, triglycerides, VLDL, LDL, and TNF α levels. In addition, higher expression of thermogenic genes (CD-137, TMEM-26, UCP-1 and ZIC-1) and (autophagic/insulin sensitivity gene (BECN-1) in white subcutaneous fat of women with higher BAT activity. Differences in the metabolic activity of BAT in women with T2DM are associated with a better cardiometabolic/inflammatory profile and expression of thermogenic genes.

Key words: Brown adipose tissue; metabolism; type 2 diabetes; obesity



Title	Insulin signaling in hypothyroid female rats exposed to bisphenol A (BPA)
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Session	Endocrine System

Abstract, Ethics Committee Number*, and Keywords	<p>Endocrine disruptor chemicals (EDC) are compounds capable to affect the homeostasis of the endocrine system. A ubiquitous EDC from plastic is BPA, which has been described to disrupt insulin signaling pathway, predisposing to insulin resistance. Hypothyroidism is associated with insulin resistance and impaired glucose metabolism. The prevalence of hypothyroidism in diabetic patients is higher than in non-diabetics, especially in women. However, the association between BPA and hypothyroidism on glycemic homeostasis is unknown. Here we evaluated the glycemic homeostasis, insulin signaling and thyroid function of hypothyroid female rats, exposed or not to BPA. Ethics committee (CEUA): 048/21. Adult female Wistar rats were divided into: control (vehicle), BPA (0.39 mg/kg/day in drinking water for 43 days), MMI (methimazole 0.03% in drinking water for 21 days), and BPA+MMI; n=10/group. Glucose and insulin tolerance tests were performed. After euthanasia, thyroid, white and brown adipose tissues (WAT and BAT) were weighed.</p> <p>Evaluations: Serum thyroid hormones, thyroid peroxidase (TPO) activity, thyroid hydrogen peroxide generation and AKT in WAT.</p> <p>Data analysis: One-Way-ANOVA and Dunnett's post-test.</p> <p>Results consider $p < 0.05$. MMI and BPA+MMI groups exhibited decreased BAT weight (-33% and -33%), increased thyroid weight (+70% and +110%) and reduced food intake (-14% and -13%), besides decreased T3 (-74% and -66%) and T4 (-90% and -91%). MMI group showed increased TPO activity (+11.500%), while BPA+MMI group showed increased thyroid hydrogen peroxide (+84%). MMI group, the area under the curve in the ITT was increased (+18%). The phosphorylated AKT:total AKT ratio did not differ among groups. Hypothyroidism appears to be associated with insulin resistance. However, this effect does not seem to involve AKT phosphorylation abnormality in the WAT. Increased thyroid hydrogen peroxide generation in BPA+MMI group suggests that the thyroid gland is subjected to oxidative stress.</p> <p>Keywords: BPA, EDC, Insulin and Hypothyroid</p>
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Title	Perinatal exposure to organophosphorus pesticides and glycemic outcomes in adult offspring
Authors	Beatriz Souza da Silva ¹ , Mariana de Souza Pomacena ¹ ; Yasmim Petronilho de Souza ¹ ; Mayara da Silva Almeida ¹ ; Nathália Medeiros Nehme ¹ ; Iala Milene Bertasso ⁺ ; Luana Lopes de Souza ⁺ ; Egberto Gaspar de Moura ⁺ ; Rosiane Aparecida Miranda [†] ; Patrícia Cristina Lisboa [†] .
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Session	06-Endocrine System

Abstract, Ethics Committee Number*, and Keywords	<p>Brazil is one of the largest consumer worldwide of the organophosphorus pesticides (OPPs), such as glyphosate and acephate. Its use impacts the population health, inducing glycemic dysregulation that can lead to diabetes. Our hypothesis is that maternal exposure to these pesticides can induce glycemic dyshomeostasis in the offspring from both sexes later in life. On the 7th day of gestation until the end of lactation, pregnant rats were exposed by gavage to vehicle (filtered water; Control group); or glyphosate (5mg/kg of body weight, bw-GLY5; or 0.5mg/kg of bw-GLY0.5); or acephate (4.5mg/kg of bw-ACE4.5; or 0.45mg/kg of bw-ACE0.45), n=8/group (protocol: CEUA/004/2020). We assessed bw gain, pancreas weight, glycemia, insulinemia, oral glucose tolerance test (OGTT), insulin resistance index (IRI), and glucose-stimulated insulin secretion (GSIS) in isolated pancreatic islets with different glucose concentrations (5.6, 11.1, 16.7 and 22mM) of the offspring from both sexes at adulthood. For statistical analysis, we used One Way Anova followed by Dunnett's post-test, considering p<0.05 as significant. Males GLY5 showed decrease in pancreas weight (-35%). Females GLY5 and ACE4.5 exhibited glucose intolerance after 30min of glucose overload (+25%; +19%; respectively), ACE4.5 at 60min (+13%) and males ACE 4.5 at 15 and 60min in OGTT (+18%; +25%; respectively). Both sexes from ACE4.5 group presented increased in IRI (+2fold). In females, the GSIS of GLY5 decreased at 5.6mM and increased at 16.7mM (-65%; 1.8x; respectively), while in ACE4.5 decreased at 5.6, 11.1 and 22mM (-68%; -50%; -49%; respectively). Males GLY5 presented a GSIS decrease at 16.7 and 22mM (-64%, -80%, respectively) while GLY0.5 a reduction only at 22mM (-67%). Males ACE4.5 increased GSIS at 5.6mM and decreased at 11.1mM (+1.8x; -75%; respectively). Our data suggest that OPPs exposure early in life compromises the glycemic homeostasis of the offspring later in life, regardless of dose and sex.</p> <p>Pesticides, Metabolic Programming, Glycemic.</p>
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Title	Effects of the combination of T3 and insulin (3U) on hemodynamic parameters and protein expression in alloxan-induced diabetic rats' heart
Authors	Henrique Izaias Marcelo Johnatas Maldonado Campos Armando Ribeiro Florido Neto Gisele Kruger Couto Luciana Venturini Rossoni Maria Tereza Nunes
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Session	Endocrine System

Abstract, Ethics Committee Number*, and Keywords	<p>Diabetes mellitus (DM) has been associated with cardiovascular risks and the development of hypothyroidism in rats. Studies in our laboratory revealed that treatment of rats induced to DM with T3 in combination with half of the physiological insulin replacement dose (3U, s.c.) restored glycemia and serum TSH to values similar to those of the Control (C) group. In this study we investigate the repercussion of this treatment on the heart of DM rats. Fifty male <i>Wistar</i> rats were divided into control (C); diabetic (DM), (induced by alloxan 150 mg/kg); DM treated with insulin 3U, s.c. (DMI3U) or T3 (1,5µg/100g, i.p.- DMT3) and T3 plus insulin (DMT3I) groups, in the same doses reported above. After 4 weeks, blood glucose and glucose decay rate were evaluated. Rats were then anesthetized (urethane, 1,6mg/kg, i.p.) and subjected to catheterism for hemodynamic studies. The rats were euthanized and T3 target proteins' expression was analysed by <i>Western Blot</i>. DM rats presented hyperglycemia and insulin resistance vs. C. DMT3I improved these parameters to the level of C. The Right Ventricle (RV) weight remained unchanged, while the LV weight was decreased in the DM groups, except for the DMT3I group, which presented a value similar to that of the C. There was no alteration in systolic blood pressure, while diastolic blood pressure was increased in the DM group vs. C, and the same in the DMT3I and C group. Heart rate values did not change. GLUT4 expression was reduced by DM, and all treatments reversed this result. T3 plus insulin treatment did not alter the α/β MHC ratio, β_1- and β_2-AR, and normalized the expression of β_3-AR, which was increased in DM. The BAX/Bcl-2 ratio was increased in the DM rats and reduced to control levels in DMT3I group. This treatment reduced caspase-9 compared to DM. This treatment was able to restore glycemia preserving the ventricles, hemodynamics, the expression of functional proteins and the cellular viability of the DM rats' heart. ECN: 103/2016.</p> <p>Keywords: Diabetes; hemodynamic; triiodothyronine; heart; protein expression.</p>
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Title	Triiodothyronine treatment (associated or not with insulin) reduced serum brain-derived neurotrophic factor and preserved cognitive function of alloxan-induced diabetic adult Wistar rats
Authors	Johnatas Maldonado-Campos Henrique Izaias Marcelo Armando Ribeiro Florido-Neto Maria Tereza Nunes
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Session	Sistema Endócrino

Abstract, Ethics Committee Number*, and Keywords	<p>Diabetes <i>mellitus</i> (DM) is a chronic disease resulted from relative/absolute insulin deficiency and disturbed tissue sensitivity to it. Thyroid hormones (THs) are essential during neurodevelopment but little is known about their roles in adult brain. DM is associated with hypothyroidism and cognitive impairments. BDNF is normally upregulated by THs and was shown to have anxiolytic effects and to regulate memory and cognition. In this study, 49 two-month-old male Wistar rats (CEUA: 103/2016) were induced to DM by intraperitoneal (i.p.) alloxan injection (150mg/kg of BW), and randomly divided into 4 groups: DM (n=9); T3 (DM + i.p. T3 injection; 1,5ug/100g BW; n=10); 3U (DM + subcutaneous injection of 3 units of insulin ; n=10); and T33U (DM plus a combination of T3 and insulin 3U; n=10), in which glycaemia and TSH levels returned to the control group values (C; non diabetic rats, n=10). After 56 days, the animals were submitted to Open Field (OFT) and Novel Object Recognition (NORT) tests and 4 days later, anesthetised (i.p. thiopental; 80mg/kg BW) and euthanised by decapitation. Plasma BDNF levels were assessed through ELISA and were reduced in DM (1125pg/mL ± 211.9; p=0.0044); T3 (1003pg/mL ± 211.9; p=0.0009) and T33U (1226pg/mL ± 211.9; p=0.0158) groups vs C group (1936pg/mL). Groups showed no difference in anxiety-like behaviours, evaluated through OFT, and in short and long-term memory integrity, which were measured by recognition and discrimination indexes assessed through NORT. In summary, data support evidence our group has been finding that T3 may act differently in health and disease conditions and that, despite the decrease in BDNF levels, DM rats' cognitive parameters investigated were not impaired in groups treated with T3 associated or not with insulin.</p> <p>Keywords: diabetes <i>mellitus</i>; thyroid hormone; BDNF; cognition.</p>
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Title	Insulin storage form using cryo-electron tomography
Authors	Francisco Breno S. Teófilo ¹ ; Gabriel G. Araujo ² ; Joel Alves da S. Junior ¹ ; Leonardo Talachia Rosa ³ ; Hernandes F. Carvalho ¹ .
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Session	Session 6: Sistema Endócrino

Abstract, Ethics Committee Number*, and Keywords	<p>Insulin plays an indispensable role in the metabolism of glucose, lipids, and proteins. This is evident in the central role that insulin plays in understanding the physiological processes of various diseases, and consequently, it has received significant attention over the years. The structure of the monomeric unit of insulin is well known, as is the fact that these monomers associate into dimers and then into hexamers through a zinc-dependent aggregation process. The latter have been regarded as the storage form of insulin in insulin secretory granules (ISGs) present in beta-pancreatic cells. However, the higher-order structures formed by the hexamers in the ISGs remains poorly explored. Thus, our main objective is to study how insulin high-order aggregates are arranged in situ inside ISGs, using high-resolution cryo-electron tomography (<i>cryo-ET</i>). For this purpose, we isolated rat pancreatic islets, disintegrated them into single cells, and subsequently isolated the ISGs using differential centrifugation and density gradients. The resulting sample was maintained in a sucrose-containing buffer and vitrified by plunge-freezing in liquid ethane. Cryo-ET acquisitions were performed at the LNNano/CNPEM using a Titan Krios G3i cryo-electron microscope operated at an accelerating voltage of 300 kV. Grids for negative stain TEM were also prepared. Crystalline structures were found in the analyses, confirming that the granules were preserved during the procedures. Our proposal here is to present the main results of the data collected, in dialogue with previous publications, and to present perspectives for understanding how insulin is stored from a structural biology standpoint.</p> <p>Keywords: insulin; structural biology; cryo-et.</p>
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Title	Association of T3 with insulin as a potential therapeutic strategy for glycemic, inflammatory cytokines and muscle mass control in experimental Diabetes Mellitus (DM)
Authors	Armando Ribeiro Florido Neto Maria Tereza Nunes
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Session	6- Endocrine System

Abstract, Ethics Committee Number*, and Keywords	<p>Glucose homeostasis is set by a dynamic control between glucose release and uptake/utilization by cells. Its regulation depends mainly on the interaction between insulin and peripheral organs (liver, skeletal muscle– SM- and adipose tissue), since its impairment observed in Diabetes Mellitus (DM) and inflammatory diseases leads to glucose intolerance. The SM plays a key role in glucose metabolism and energy consumption. DM and hypothyroidism lead to insulin resistance (IR), increased inflammatory cytokines (IC), reduced glucose uptake and loss of muscle mass. Studies from our laboratory showed that alloxan diabetic rats present hypothyroidism, IR and increased IC expression in SM, which was reduced by triiodothyronine (T3) treatment. This study aimed to investigate in this experimental model the repercussions of T3 and insulin association treatment on the glycemic control, TSH levels and IR. Inflammatory status (TNF-α, IL-1β, IL-6, NF-Kbp65 e IL-10), key proteins of the glucose uptake/utilization (GLUT4, hexokinase-HK, glucokinase-GK) and activity (SERCA, MHCII) of SM were evaluated by western blotting (CEUA ICB n^o 103/2016). Male Wistar rats were induced to DM by alloxan, assorted into groups treated with the replacement dose of Insulin (6U) and half of it (3U) associated or not with T3 (1.5 μg/100 g of BW) for 28 days, and euthanized by decapitation. Diabetic rats presented increased fasting glucose and TSH levels, IR and IC expression, and decreased GLUT4, HK, GK, SERCA and MHCII expression. T3 and/or insulin treatment improved all the parameters assessed. Besides, when T3 was associated with 3U insulin all parameters evaluated returned to the control group (non diabetic) values, as occurred with the diabetic group treated with insulin (6U). These data point out that the association of T3 with insulin 3U treatment could delay the IR that occurs with the chronic insulin treatment of DM1, reduce costs, and be a potential strategy for treatment of DM to be further explored.</p> <p>Key words: Diabetes, T3, insulin, skeletal muscle, cytokines, therapeutic potentiality</p>
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Title	Functional parameters related to fertility and reproduction of knockout females for the <i>a7nAChR</i> receptor
Authors	Caroline Alves dos Santos ¹ Priscilla Karla Lopes ² Ana Paula Varela Sanches ¹ Letícia Ignácio-Souza ¹
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Session	Endocrine System

Abstract, Ethics Committee Number*, and Keywords	<p>Obesity is an inflammatory disease and the study of anti-inflammatory response, in specific to the <i>a7nAChR</i> nicotinic subunit emerging as a potential pathway to reduce pro-inflammatory cytokine levels and thus, control the co-morbidities. To this end, genetically modified animals are indispensable tools and it was demonstrated in the literature that <i>a7nAChR</i> KO animals have smaller litters or reduced fertility. Thus, this study intends to investigate the functional parameters related to fertility and reproduction in females KO for the <i>a7nAChR</i> receptor.</p> <p>For this, data from the Genetically Modified Colony Maintenance Record were retrieved to evaluate some functional parameters. After, the estrous cycle of representative females was assessed. Data are presented in absolute and relative frequency and the rates and indices calculated in arithmetic mean and standard deviation and the estrous cycle in qualitative graph (CEUA/UNICAMP protocol no. 5274/2019).</p> <p>Comparing Knockout and Heterozygous couple, the last one in fact had more successful matings but, interestingly, had a delay for pregnancy confirmation and more neonatal deaths, generating a lower prolificacy index; demonstrating that this mating does not appear to be viable. Furthermore, when evaluating the estrous cycle of KO females, it was possible to observe that their cycle appears to be shorter, as around 35% of females went through estrus twice in a period compared to 25% of <i>WildType</i> females. The analyzes also indicate that the cycle in KO females is precoc; it appears that they already show characteristics of the estrus period on at least one of the days evaluated at 6 weeks old, while Wild females still did not present characteristics of sexual maturity.</p> <p>Our results show important functional modifications in the <i>a7nAChR</i> KO female estrous cycle suggest that the lack of <i>alpha 7</i> receptor may be anticipating the reproductive life of females and leading to shorter cycling in adulthood.</p> <p>Keywords: Fertility, Inflammation, <i>a7nAChR</i> Receptor.</p>
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Title	Bisphenol S disturbs glucose metabolism and pancreatic morphology in mice with or without a high-fat diet
Authors	Barreto-Reis, E. ^{1,2} Alves, A.P.P. ^{1,3} Luca, B. G. ^{1,2,4} Souza, K. M. G. ⁴ Stockler-Pinto, M. ² Miranda-Alves, L. ³ Frantz, E.D.C. ^{1,5,6} Santos, C. M. ⁴ Magliano, D.C. ^{1,2,3}
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Session	Pôster

Abstract, Ethics Committee Number*, and Keywords	Obesity is an inflammatory disease related to insulin resistance and type 2 diabetes mellitus. Endocrine disruptors (ED) are known to contribute to obesity development through its obesogenic activity. Bisphenol S (BPS) is a widely distributed ED that may present risks to pancreas and glucose metabolism. The aim of the study is to assess the effects of BPS exposure and/or high-fat (HF) diet on pancreatic morphology and glucose metabolism of male mice. C57BL/6 mice fed a standard-chow or HF diet, exposed or not to BPS (25µg/kg/day) for 12 weeks, resulting the groups: C, CB, HF and HB. Body, pancreas and pancreatic islet masses were measured as well as pancreatic α and β cells masses. Plasma insulin and glucose levels were assessed, and HOMA-IR was determined. Pancreas underwent histological and immunohistochemical staining. Mean \pm standard deviation, one-way ANOVA with Holm-Sidak post-test ($p < 0,05$). All groups showed higher body (CB: +12%, $p < 0,0001$; HF: +51%, $p < 0,0001$; HB: +47%, $p < 0,0001$), pancreatic islets (CB: +136%, $p < 0,01$; HF: +273%, $p < 0,0001$; HB: 144%, $p < 0,0001$), pancreatic α -cells (CB: +445%, $p < 0,05$; HF: $p < 0,0001$; HB: $p < 0,0001$) and β -cells (CB: +133%, $p < 0,05$; HF: +302%, $p < 0,0001$; HB: +263%, $p = 0,0001$) masses,
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	<p>together with elevated insulin (CB: +104, $p < 0,05$; HF: +100, $p < 0,05$; HB: +189%, $p < 0,0001$) and glucose (CB: +12%, $p < 0,05$; HF: +11%, $p < 0,05$; HB: +22%, $p < 0,0001$) plasma levels and HOMA-IR (CB: +112%, $p < 0,01$; HF: +136%, $p < 0,01$; HB: +266%, $p < 0,0001$) compared to C group. HB group had a worsening in glycemic profile compared to other groups. Only HF and HB groups increased pancreas mass (HF: +84%, $p < 0,0001$; HB: +74%, $p < 0,01$) and islet diameter (HF: +59%, $p < 0,01$; HB: +57%, $p < 0,01$) compared to C group. The immunohistochemical staining showed an altered cell distribution, marked by the presence of α-cells in the center of islet and a stronger staining of β-cells in all experimental groups. BPS enhances insulin resistance and alters pancreatic morphology, suggesting a potential obesogenic and diabetogenic activity.</p> <p>ETHICS COMMITTEE: CEUA 1929240521</p> <p>KEYWORDS: Obesity. Insulin resistance. Type II diabetes mellitus. Bisphenol S. Pancreas. Morphology.</p>
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Title	Phytoestrogen therapy reduced carcinogenic development of the mammary gland after endocrine disruption in aged females
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Session	06 - Sistema Endócrino

Abstract, Ethics Committee Number*, and Keywords	<p>Endocrine therapies are often used for women during menopause, such as phytotherapeutic molecules that mimic endogenous hormones. We aimed to evaluate the effects on the mammary gland (MG) of the phytoestrogen genistein (GIN) after exposure to a carcinogenic dose of BPA (endocrine disruptor) during pregnancy and lactation in aged female gerbils. After BPA, females (mothers) were exposed from 12 to 18 months of age to doses of GIN with attested estrogenic action. After, the MG was collected and analyzed by histopathological (IHC) and molecular (WB) routines. Females exposed only to BPA developed ductal carcinomas (26.1%±6.4). To verify the development of severe tumor features, a group was induced with N-ethy-N-nitrosurea (ENU). In this group, there was an increase in estrogen receptor alpha (ERα) (IHC: 83.4%±12.3; WB: 2.1±0.6), as well as an increase in MG microinvasive carcinomas (21.8%±7.9). Regarding other receptors, there was a modulation related to the progesterone receptor (PR) (IHC: 33.7%±12.4; WB: 1.9±0.4) and AR (IHC: 39.8%±4.9; WB: 1.6±0.7). These proliferative lesions were confirmed by phospho-histone H3 (proliferation >70%) in contrast with the results obtained by caspase 3 (<15%), which identifies cell death. In comparison to these groups, GIN-treated females restored homeostatic features, only with an increase in ductal regions and hyperplasias (55.8%±7.6). Also, ERα and PR rates were comparable to the control group, not exposed to any disruptor. Regarding the proliferation and apoptotic rates, there was a dynamic related to caspase 3, in which its active form was highly expressed (IHC: 15.6%±5.1; WB: 2.6±0.4)</p>
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compared to other groups. This phenomenon can be explained by the modulation of the Bcl-2 (IHC:1.2%±0.7; WB:0.9±0.3), relevant to apoptosis regulation. Thus, our results demonstrated the protective effect of GIN to impair the development of pre- and malignant features on MG, even following exposure to an endocrine disruptor, such as BPA.

Keywords: Estrogen; cancer; receptors; apoptosis.



Title	Reduction in H₂S production is involved in impaired insulin secretion and pancreatic islet vascularization following protein restriction
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Affiliations	1 – Vascular Biology Laboratory (LaBiVasc), UNICAMP, Campinas, Brazil; 2- Obesity and Comorbidities Research Center (OCRC), UNICAMP, Campinas, Brazil; 3 – Laboratory of Pharmacology of Free Radicals, Inflammation and Pain, USP, Sao Paulo, Brazil.
Session	06 – Sistema Endócrino

Abstract, Ethics Committee Number*, and Keywords	<p>Pancreatic islets are richly vascularized with a positive correlation between blood flow to the pancreas and its endocrine function. Hydrogen sulfide (H₂S) promotes vasodilation and protects the function of endocrine cells exposed to hyperglycemia. We hypothesized that reduced H₂S levels due to protein restriction may lead to vascular and endocrine pancreas issues. Therefore, our aim was to investigate whether protein restriction affects vascularization and insulin secretion in pancreatic islets, focusing on H₂S. Post-weaned male mice were divided into two groups: control (C) fed normal protein diet and protein restriction (R) fed low-protein diet, for 90 days (CEUA #6207-1). Isolated pancreatic islets from R group exhibited lower insulin secretion compared to C group. In C group, H₂S donor (Lawesson, 1μM) reduced insulin secretion in normoglycemic (at 11.1 mM glucose) but not in hyperglycemic state (22.2 mM glucose). On contrary, H₂S inhibition (PAG 1mM) did not affect insulin secretin in normoglycemic state but reduced it at high glucose exposure. Neither the H₂S donor nor the inhibitor affected insulin secretion in the R group. The expression of the H₂S synthesizing enzyme cystathionine γ-lyase (CSE) was reduced in R pancreatic islets. Both C and R mice underwent islet transplant surgery to the anterior chamber of the eye to receive islets grafts (i) either from C or R mice, resulting in the following groups: C+Ci, C+Ri, R+Ci and R+Ri. Higher glucose tolerance (GTT) in R+Ri was normalized in R+Ci mice, indicating functional C islets grafts transplanted to R mice. Images of transplanted islets were captured weekly for 4 months. Capillarity increased in the islet grafts of C+Ci over time indicating neovascularization, but this was less observed in the R+Ri or R+Ci. Our data suggest a reduced H₂S effect in the impaired insulin secretion of protein restricted mice. In addition, altered pancreatic islet capillarization may contribute to pancreatic islet dysfunction.</p> <p>Keywords: Protein restriction, hydrogen sulfide, pancreatic islet vascularization.</p>
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XXXVIII REUNIÃO ANUAL DA FESBE
XXII REUNIÃO ANUAL DA BRAVO
XVIII CONGRESSO DA SBCAL
III CONGRESSO DOHAD BRASIL
II CONGRESSO DA SBBA
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FRONTEIRAS DAS CIÊNCIAS BIOMÉDICAS E SEUS DESAFIOS

Title	Hypothyroidism increases mitochondrial dynamics machinery in the testis of adult rats
Authors	Lorena Cristina de Souza e Souza, Juliana Santos Romão, Karen de Jesus Oliveira
Affiliations	Laboratório de Fisiologia Endócrina e Metabologia, Departamento de Fisiologia e Farmacologia, Instituto Biomédico, Universidade Federal Fluminense, Niterói/RJ
Session	Session 6

Abstract, Ethics Committee Number*, and Keywords	<p>Thyroid hormones (TH) play a crucial role in testicular development and function, spermatogenesis and hormonal function, and in hypothyroidism reproductive dysfunction is observed. Mitochondria are vital components in male reproduction, essential for testosterone production, cell differentiation, and spermatogenesis in the testis. Mitochondria continuously undergo changes in their number and morphology through fusion and fission processes, a homeostatic mechanism known as mitochondrial dynamics. An imbalance in mitochondrial dynamics is linked to pathological conditions, however, whether defects in mitochondrial dynamics are related to the reproduction dysfunction seen in hypothyroidism remains unknown. In addition, the regulation of mitochondrial dynamics in the testis by TH has not been described. Adult male Wistar rats (n=18) were divided into two groups: euthyroid and hypothyroid. Hypothyroidism was induced with methimazole (0.03%) diluted in the drinking water, provided for 3 weeks. The testis were weighed and frozen for analysis. mRNA expression of the mitochondrial dynamics machinery was analysed by qPCR. All protocols were approved by CEUA/UFF n° 2488110221. Hypothyroidism was confirmed by undetectable levels of total and free T4. Hypothyroid rats showed reduced testis weight (p=0.0082). All genes analysed involved in the mitochondrial fission process increased in the hypothyroid group (<i>Dnm1l</i>, p=0.0079; <i>Mff</i>, p=0.0164; <i>Fis1</i>, p=0.0201;). Regarding mitochondrial fusion, an increase in <i>Opa1</i> (p=0.0311) and <i>Mfn1</i> (p=0.0021) expression was also found, while MFN2 showed no statistical difference. Therefore, the increased expression of these genes suggests that both fusion and fission processes are stimulated, indicating that in hypothyroidism, there might be changes in mitochondrial dynamics that may contribute to the reproductive dysfunctions observed in this condition.</p> <p>Keywords: Thyroid hormone, fusion, fission, reproduction.</p>
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Title	Clinical and anthropometric evolution of children of women who had COVID-19 during pregnancy
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Session	Nutrition and Metabolism

Abstract and Keywords	<p>The impact of maternal SARS-CoV-2 infection on newborns is not yet well established. Prospective cohort study with women who had COVID-19 during pregnancy and their children at 3 months of age in the pre-vaccination period. 233 women who were tested were included. At 90 days postpartum, all families were called for reevaluation, 85 pairs (mothers and children) attended at that time. A standardized questionnaire was applied to families, consultation of the child's records and measurement of anthropometric indicators and pediatric clinical assessment. At 90 days of life, maternal and infant blood and breast milk were collected for the determination of IgA and IgG anti-RBD antibodies to SARS-CoV-2 by ELISA and total antibodies anti-RBD of SARS-CoV-2 by chemiluminescence, considering as positive values above ≥ 1.1 anti-SARS-CoV-2 IgA and IgG and ≥ 1.0 for total antibodies. This study was approved by the research ethics committee (CAAE: 34587520.3.0000.0082, opinion 4.184.253). At 3 months postpartum, 61 (71.8%) of the infants were breastfeeding. It was observed that the anthropometric indicators of length and weight for both sexes and head circumference for females were lower compared to the reference for the same age. At 90 days postpartum, an increase in positivity in serum levels for anti-SARS-CoV-2 IgG ($p < 0.001$) and IgA ($p = 0.022$) was observed in women. There was a significant drop in the percentage of positive anti-SARS-CoV-2 IgA values in breast milk ($p < 0.001$). As for infants, a drop in total anti-SARS-CoV-2</p>
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titers could be seen between the two moments ($p=0.008$). Serum total antibodies, IgA and IgG anti-SARS-CoV-2 in the mother, baby and breast milk were identified up to 90 days postpartum. The infants did not present serious clinical complications or changes in neuropsychomotor development. Infants had worse anthropometric indicators when compared to the reference.

Keywords: COVID-19, breastfeeding, infants, antibodies.



Title	Effects of <i>Lactoseibacillus rhamnosus</i> LB1.5 on adipose and liver tissues in adult male mice fed a high fat diet
Authors	Schmidt N.P. ^{1*} Molz P. ¹ Fraga B. ¹ Silveira P.D. ¹ Ferri M.H. ¹ Santos D.R. ² Montanez J.A.C. ² Breyer G.M. ³ Motta A.S. ³ Porawski M. ^{1,4} Barshack A.G. ⁴ Guedes R.P. ^{1,4} Giovenardi M. ^{1,4}
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Session	7

Abstract and Keywords	<p>Non-pharmacological strategies have been investigated with the aim of minimizing the negative outcomes observed in individuals with overweight and obesity. We evaluated the effect of probiotic on the histomorphology and redox state of adipose and liver tissue from adult male fed high-fat diet (HFD). Male (N=40) isogenic (C57BL/6) mice, aged 21 days, started their feeding with a standard diet (CONT), a standard plus probiotic diet (CONT+PROB), a (HFD), or a high-fat diet plus probiotics (HFD+PROB) for 13 weeks. <i>Lact. rhamnosus</i> LB1.5 (1.3×10^8 CFU/mL) was administered by gavage 3x/week (CEUA/UFCSPA nº 722/21). At 110 days of age, the animals were euthanized, adipose and hepatic tissues were collected. Results were analyzed by two-way ANOVA followed by the Bonferroni test ($p < 0.05$). In adipose tissue, we observed that HFD had greater tissue weight ($p = 0.0001$) and adipocyte size and a lower number of adipocytes/area ($p = 0.0001$) when compared to the CONT, however, there was no effect of probiotic supplementation. Regarding the redox state, there were no differences in TBARS (diet effect: $p = 0.118$, supplementation effect: $p = 0.378$) and sulfhydryls (diet effect: $p = 0.166$, supplementation effect: $p = 0.775$) between the groups. In liver tissue, the HFD showed hepatocellular ballooning and severe steatosis ($p = 0.0007$) when compared to CONT, but no effect of probiotic supplementation. As for the redox state, HFD showed increased lipid damage (diet effect: $p = 0.013$, supplementation effect: $p = 0.701$) when compared to CONT, and the HFD+PROB showed an increase in antioxidant defenses compared to the HFD ($p = 0.0279$). HFD induced fat accumulation in adipose tissue, liver, and greater oxidative damage in the liver. Supplementation with <i>Lact. rhamnosus</i> LB1.5, at the dose and frequency administered, did not</p>
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attenuate the accumulation of fat in tissues, but was able to increase the antioxidant response in the liver of the HFD.

Probiotic, Oxidative Stress, Steatosis, Antioxidant, TBARS



Title	Daily supplementation with guaraná Powder (<i>Paullinia cupana</i>) prevents protein oxidative damage in a paracetamol-induced hepatotoxicity model
Authors	² Camila Maciel dos Santos ^{1,2} Clécia Dias Teixeira ² Paloma Leticia Gonçalves ¹ Gustado Silveira Breguez ³ Wanderson Geraldo de Lima ¹ Melina Oliveira de Souza
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Session	Nutrition and Metabolism

Abstract and Keywords	<p>Toxic doses of paracetamol (APAP) can trigger hepatotoxicity due primarily to the accumulation of the metabolite NAPQI and, consequently, an imbalance in the hepatic oxidative state. Preventative treatments using polyphenols present in foods have been studied widely. Guaraná (<i>Paullinia cupana</i>) is an Amazonian fruit rich in these compounds. The study aimed to evaluate the effect of preventive treatment with guarana powder on oxidative damage in hepatotoxicity induced by a toxic dose of APAP. 32 male Wistar rats were divided into 4 groups: control (C), guaraná (G), APAP (P), and guaraná+APAP (GP). G and GP groups received a 300 mg/kg dose of guarana powder diluted in filtered water for 7 days and groups P and GP received a single dose of 3g/kg of APAP on the 8th day. Both were administered via orogastric gavage. After 24 hours of intoxication, the animals were euthanized. The project was approved by the Ethics Committee/UFOP (3488300122). The statistical analysis was performed by ANOVA TWO-WAY and Bonferroni post-test. The serum activity of ALT and AST enzymes showed the effect of intoxication ($p < 0.001$), preventive treatment ($p < 0.001$; $p = 0.002$), and interaction ($p < 0.001$), with an increase in both enzymes in group P. Preventive treatment with guarana prevented this increase. In photomicrographs of the liver tissue, the C and G groups showed preserved morphology, the P group showed significant damage and guarana (GP group) was able to prevent the morphological damage caused by APAP. The hepatic concentration of malondialdehyde (MDA) showed an effect of intoxication ($p < 0.001$), with an increase in concentration in the P and GP groups. The hepatic concentration of protein carbonyl showed an effect of intoxication ($p < 0.001$), preventive treatment ($p < 0.001$), and interaction ($p < 0.001$), with an increase in the P group and prevention of damage in the GP group. It is suggested that guarana can prevent liver failure caused by toxic dose of APAP due to its antioxidant action.</p> <p>Keywords: Paracetamol, Guarana, <i>Paullinia cupana</i>, Polyphenols, Hepatotoxicity, Antioxidant</p>
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Title	Stress resistance effects in <i>Macrobrachium rosenbergii</i> post-larvae supplemented with <i>Curcuma longa</i> extract
Authors	Pedro Trabulsi Junqueira Franco Milena Cia Retcheski Daniel Massato Vital Hide Ana Paula Pelinson da Fonseca Luciano Tormen Sílvia Romão Luisa Helena Cazarolli Caroline Cristina Ribeiro Simões de Souza
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Session	Nutrição e metabolismo

Abstract and Keywords	<p>This work studied the effects of <i>Curcuma longa</i> extract as a dietary supplement for <i>Macrobrachium rosenbergii</i>. Shrimp post-larvae received diets with different concentrations of turmeric extract (0.05, 0.2, 1%) for 60 days, and at the end of the supplementation period a transport simulation (TS) test was carried out and, in challenged animals, survival and antioxidant status were studied. Phenolic compound content and <i>in vitro</i> antioxidant potential of the turmeric extract were evaluated through a DPPH radical scavenging assay, total antioxidant capacity, and the Fe(III) to Fe(II) reducing activity assay. The <i>in vitro</i> antioxidant activity of the turmeric extract was like BHT, a common antioxidant, and the total phenolic content was similar (0.98 ± 0.02 mmolAG/g) to that described in the literature. In animals from the TS test, turmeric extract promoted a dose-dependent increase in animal survival. Furthermore, turmeric supplementation increased the activity of the enzymes catalase, glutathione reductase, glutathione peroxidase, glutathione S-transferase, and it reduced glutathione (GSH) content. A reduction in the levels of lipid peroxidation (TBARS) and protein carbonyl was also observed in the hepatopancreas of all experimental groups. However, superoxide dismutase activity was not influenced by turmeric supplementation. The addition of turmeric extract to the diet of <i>M. rosenbergii</i> post-larvae presents great potential for application in commercial shrimp farming, since it improved the physiological conditions of the animals, improving the use of nutrients and consequently the animals' responses to stressful conditions, such as transport.</p> <p>KEYWORDS: turmeric, resistance, freshwater shrimp, antioxidant status.</p>
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Title	Identification of new markers of adipocyte size conserved in rodents and humans
Authors	de Moraes, D Grillo, L G P Cal, T C M F Figueiredo, L S Brunneta, H S Mori, M A
Affiliations	Laboratory of Aging Biology, Department of Biochemistry and Tissue Biology, Institute of Biology, University of Campinas (UNICAMP), Campinas, Brazil.
Session	Nutrition and Metabolism

Abstract and Keywords	<p>Obesity is a global epidemic associated with severe metabolic disorders, including type 2 diabetes and cardiovascular diseases - a group of conditions that characterize the metabolic syndrome. Instead of relying solely on anthropometric parameters, recent research has shed light on the relationship between metabolic imbalance and adipocyte hypertrophy, indicating that the expansion of adipocytes in volume, but not in quantity associates better with poor metabolic health. In this study, we investigated genes that are closely related to adipocyte size across mammalian species. Using bioinformatic analyses, we assessed differential gene expression correlated with adipocyte size from 918 slides of human visceral and subcutaneous adipose tissue samples available at the GTex database and we found 27 genes statistically correlated with adipocyte size. These genes were then validated using an independent set of human, mice, and rat tissues - in mice, there was also a comparison between normal and high-fat diet. Adipocytes were isolated and separated in different sizes by serial mesh filtering. Nine genes appeared consistent with the bioinformatic prediction in at least two of the three types of tissues. Validations confirmed that the genes encoding Matrix Metalloproteinase 28 (Mmp28), Dehydrodolichyl Diphosphate Synthase (Dhdds), Natriuretic Peptide Receptor 3 (Npr3), and Alpha-1-antitrypsin Heavy Chain Inter-5 (Itih5) had a strong positive correlation with adipocyte size across species. This study unveils markers associated with adipocyte size in rodents and humans, pointing to putative regulators of adipocyte hypertrophy and metabolic function.</p> <p>Keywords: adipose tissue, adipocyte hypertrophy, bioinformatics, adipocytes, metabolic syndrome</p>
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Title	Replacement of a high-fat diet to a normocaloric diet does not influence total food intake but reduces daytime food intake and normalizes fat accumulation and metabolic parameters in obese mice
Authors	Vanessa Cristina de Souza Melo ¹ Jean Franciesco Vettorazzi ² Julia Nicoloy Bohn Couto ³ Julia Martins Cabreira ³ Sandra Lucinei Balbo ³
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Session	Nutrition and Metabolism

Abstract, Ethics Committee Number*, and Keywords	<p>The consumption of ultra-processed foods, rich in fat, has contributed to the global increase in obesity and other metabolic disorders resulting from excess adipose tissue and hepatic steatosis. Physical exercise and dietary reeducation constitute the first-line treatment for weight loss; however, to accelerate weight loss, the use of restrictive diets is common but are unsustainable in the long term and result in weight regain. On that account our aim was evaluate the effects of replacing a high-fat diet to a normocaloric diet (<i>ad libitum</i>) on food intake, fat accumulation, glycemic homeostasis and hepatic steatosis . For this, the animals were divided into two groups: one feed with high-fat diet and other with standard diet for 8 weeks. Then, half of the animals on the high-fat diet had their food replaced for a standard diet. Body weight was measured weekly. After 7 weeks, we assessed food intake and tested glucose and insulin tolerance. The animals were euthanized 8 weeks after the dietary intervention, and the liver tissue was collected for histological analysis. We observed that the high-fat diet induced obesity and metabolic changes such as hyperglycemia, insulin resistance, dyslipidemia, and hepatic steatosis. The replacement for a standard diet reduced food intake during the day, even without influencing total caloric intake, and was able to restore body weight, normalize blood glucose and lipid profile, beyond improve the hepatic steatosis. We concluded that dietary intervention with a standard diet reduce daytime food intake and acting, at least in part, to reverses obesity and comorbidities, suggesting that this replacement may modulate the expression of genes that regulate circadian rhythm. Further studies are needed to confirm whether this substitution modulate the expression of genes that regulate circadian rhythm and affect the body weight gain.</p> <p>Keywords: obesity, high-fat diet, hepatic steatosis, food intake. Committee Number: 22-20 (CEUA-UNIOESTE).</p>
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Title	Metabolic implications of high-protein diet and probiotics administration in Wistar rats
Authors	Cunha, L.F., Almeida, I.P.M., Giovenardi, M., Barschak, A.G.*
Affiliations	Departamento de Ciências Básicas da Saúde, Universidade Federal de Ciências da Saúde de Porto Alegre, RS, Brasil
Session	7

Abstract and Keywords	<p>Protein-rich diets have become popular among physically active individuals with the aim to improve quality of life and aesthetics. Also, probiotics prescriptions have also been increasing in an attempt to improve gut health, but the implication of its association with high protein diets are still unclear. The aim of this study was to investigate the metabolic implications of high-protein diets and the consumption of probiotics (<i>Lactobacillus acidophilus</i> and <i>Bifidobacterium lactis</i>) in Wistar rats. The study involved 32 eight-weeks-old male rats randomized into four groups: Standard diet (CT), standard diet with probiotics (CTP), high-protein diet (DT), and high-protein diet with probiotics (DTP) for ten weeks. Weekly weight gain was measured. Behavioral analyses (open field and elevated plus maze) were conducted in the tenth week before euthanasia. Blood samples were analyzed biochemically. The high-protein diets resulted in less weight gain, despite no significant difference in caloric consumption between groups. The CTP group had a lower Lee Index and visceral fat when compared to the CT group, and the DT group had a lower amount of mesenteric and visceral fat than the CT group, but the probiotics did not have a significant impact. LPS displayed significant differences between CT and CTP, and CT and DT. No notable differences were found in plasmatic short-chain fatty acids. Variations in amino acids were noted between CTP and DTP groups for aspartate, alanine, and leucine, and between CT and DT, and CTP and DTP for glutamate and methionine. Behavioral analyses revealed no significant differences among groups. The high-protein diet improved weight, fat gain and fat amount compared to the control diets. Probiotics were not able to improve any of the analyzed parameters. Further studies are warranted for validation.</p> <p>Keywords: high-protein diet, probiotics, metabolism Financial Support: UFCSPA, CNPq, CAPES Ethical Committee CEUA UFCSPA approval protocol: 355/2023</p>
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Title	Preoperative supplementation with beta-hydroxy-beta-methylbutyrate (HMB) suppresses ATF4-Parkin signaling and alters the hepatic regenerative process in mice
Authors	Ana Laura Vieira da Silva ¹ Franciely Alves da Silva ² Marcos Vinícius Esteca ¹ Isabela Aparecida Divino ¹ Fernanda Carneiro ³ Adriana Souza Torsoni ² Eduardo Rochete Ropelle ³ Igor Luchini Baptista ¹
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Session	Session 2

Abstract, Ethics Committee Number*, and Keywords	<p>The liver has exceptional capacity for regeneration allowing it to restore its original mass and size after significant resections. However, the regenerative process relies on sufficient energy supply to assure cell proliferation and maintain homeostatic functions. β-Hydroxy-β-Methylbutyrate (HMB) impacts metabolic pathways by promoting protein synthesis, mitochondrial biogenesis and reducing protein breakdown. This study explored the effects of HMB supplementation on mitochondrial quality and its influence on liver regeneration. Male C57BL/6J mice were supplemented via gavage with 600 mg/kg of HMB for 10 days, and then were subject to the partial hepatectomy (PHx). The following 7 days corresponded to the liver regeneration period (CEUA no. 5712-1/2021). The group supplemented with HMB showed a decrease in liver weight (PHx 1.6 vs PHx+HMB 1.22 a.u.) and in the liver weight/body weight ratio (PHx 0.07 vs PHx+HMB 0.05 a.u.). There was a reduction in the activity of the liver enzymes ALT (PHx 4.87 vs PHx+HMB 0.29 a.u.) and AST (PHx 4.69 vs PHx+HMB 0.87 a.u.). The levels of proteins related to cellular stress control, including Parkin (PHx 2.52 vs PHx+HMB 0.92 a.u.) and ATF4 (CTRL 1 vs PHx+HMB 0.42 a.u.), were also reduced. PGC-1α, a protein downstream of Parkin-ATF4, had its content increased (CTRL 1 vs PHx+HMB 1,68 a.u.). The content of proteins involved in mitochondrial dynamics, such as DRP1 (CTRL 1 vs PHx+HMB 0.67 a.u.) and Mfn2 (PHx 1.33 vs PHx+HMB 0.89 a.u.) decreased, while mitochondrial markers such as VDAC2 (PHx 1.12 vs PHx+HMB 2.31 a.u.) and Tom20 (PHx 1.09 vs PHx+HMB 1.37 a.u.) increased. Cell cycle proteins like Ki-67 and Cyclin D1 were respectively reduced (PHx 0.097 vs PHx+HMB 0.031 a.u.) and increased (PHx 2.64 vs PHx+HMB 3.9 a.u.). These results show that HMB supplementation prior to PHx modulates mitochondrial quality and cell cycle pathways and, consequently, the liver regeneration.</p> <p>Keywords: Liver regeneration; mitochondrial quality; cell cycle; Parkin.</p>
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Title	Effects of intermittent fasting on the histomorphometry and redox state of the submandibular glands of Wistar male rats
Authors	Renan José Barzotti Larissa Victorino Sampaio Rayara Nogueira de Freitas Guilherme Eduardo Rocha Silva José Vitor Furuya de Lima Ana Claudia de Melo Stevanato Nakamune Antonio Hernandes Chaves Neto
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Session	Nutrition and Metabolism

Abstract, Ethics Committee Number*, and Keywords	<p>Intermittent Fasting (IF) is a dietary strategy that has shown promising results in regulating glucose, weight loss, and cellular stress resistance. Additionally, salivary glands exhibit sensitivity to dietary and nutritional patterns. Therefore, the aim of this study was to analyze the effect of IF on the histomorphometry and redox state of the submandibular glands. For this purpose, 20 Wistar male rats were divided into 2 groups (n = 10 rats/group): the <i>Ad Libitum</i> (AL) group had continuous access to water and food, while the IF group was deprived of food for 24 hours on alternate days for a period of 12 weeks. Body weights, water and food consumption were recorded throughout the treatment. At the end of the experiment, the animals were anesthetized, euthanized, and their submandibular glands were excised, with the right gland destined for biochemical analysis and the left gland for histological processing (CEUA FOA/UNESP n° 257-2023). The results were compared using unpaired Student's t-test ($p < 0.05$). IF resulted in lower body weight gain ($p < 0.0001$), final weight ($p < 0.0001$), food consumption ($p = 0.00008$), feed efficiency ($p < 0.0001$), and absolute glandular weight ($p = 0.0233$), while the relative weight was similar at the AL group. Histomorphometry analyses didn't detect differences significant in the acinar, ducts, and stroma areas between the groups. Total oxidant capacity, concentration of lipid peroxidation, and carbonylated proteins also showed similar values between groups. However, IF reduced uric acid concentration and total antioxidant capacity ($p < 0.0001$), without influencing reduced glutathione concentration. Furthermore, IF decreased superoxide dismutase ($p = 0.0233$) and glutathione peroxidase activities ($p = 0.0032$), while increasing catalase activity ($p < 0.0001$). Although IF did not cause histomorphometric changes or oxidative damage in the submandibular glands, the results of antioxidant defense suggest disturbances in the redox state.</p> <p>Keywords: Intermittent Fasting; Food Restriction; Time-Restricted Feeding; Submandibular Gland; Oxidative Stress.</p>
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Title	Effects of tauroursodeoxycholic acid (TUDCA) treatment on hypothalamic inflammation in a malnourished mice model
Authors	Gabriela Moreira Soares Bruna Lourençoni Alves Kênia Moreno de Oliveira Joel Alves da Silva Junior Licio Augusto Velloso Everardo Magalhães Carneiro
Affiliations	Obesity and Comorbidities Research Center, University of Campinas, SP, Brazil
Session	7 - Nutrição e Metabolismo

Abstract, Ethics Committee Number*, and Keywords	<p>Early childhood malnutrition triggers hypothalamic inflammation disrupting anorexigenic/orexigenic signals. This contributes to increased food consumption, predisposing undernourished individuals to develop obesity and metabolic diseases. Here, we analyzed hypothalamic inflammation in a protein-restricted mice model and the effects of the anti-inflammatory compound TUDCA. Male C57BL/6 mice were fed a control (14% protein - C) or an isocaloric low-protein (6% protein - R) diet for 14 weeks. In the last two weeks, half of the mice received PBS (C/R) or 300 mg/kg TUDCA (CT/RT). Analyses: body weight (BW), serum (albumin, total protein, and leptin), diet consumption, gene expression (hypothalamic neuropeptides and inflammation markers). Data are mean \pm SEM, $P \leq 0.05$, One-Way ANOVA/Tukey's test. All experiments were approved by the Animal Care Committee at UNICAMP (Protocol#5907-1/2021). R mice showed reduced BW, albumin, and total protein levels (C: 28.0 ± 1.0 x R: 21.1 ± 0.5g, $p < 0.0001$; C: 2.3 ± 0.1 x R: 1.9 ± 0.1g/dL, $p = 0.02$; C: 5.3 ± 0.2 x R: 4.6 ± 0.2g/dL, $p = 0.03$). Food consumption, leptin levels (C: 10.4 ± 0.1 x R: 11.3 ± 0.1kcal, $p = 0.01$; C: 252.6 ± 40.8 x R: 570.4 ± 49.3 pg/mL, $p = 0.01$), and expression of the orexigenic neuropeptide Agrp (R: 3.6 ± 0.5FC, $p = 0.0004$) were higher in R mice. Hypothalamic expression of pro inflammatory cytokines (Tnf α, R: 1.5 ± 0.1, $p = 0.02$; Il1β, R: 1.8 ± 0.2FC, $p = 0.02$) and astrocyte and microglial markers (Gfap, R: 1.9 ± 0.2FC, $p = 0.01$; Iba-1, R: 1.6 ± 0.2FC, $p = 0.01$) were increased in R mice. Treatment with TUDCA reduced food consumption, leptin levels, and Agrp expression (R: 11.3 ± 0.1 x RT: 10.5 ± 0.3kcal, $p = 0.04$; R: 570.4 ± 49.3 x RT: 126.8 ± 22.2 pg/mL, $p < 0.0001$; RT: 2.0 ± 0.4FC, $p = 0.04$). Expression of the inflammatory markers Tnf-α, Il-1β, Gfap, and Iba-1 was lower in RT mice compared to R mice (RT: 1.0 ± 0.1, $p = 0.01$; RT: 1.0 ± 0.2, $p = 0.02$; RT: 0.8 ± 0.1, $p = 0.0005$; RT: 0.8 ± 0.1FC, $p = 0.0016$). TUDCA treatment reduces hypothalamic inflammation which may lead to reduced Agrp expression and leptin levels, thus altering feeding behavior in mice.</p> <p>Keywords: tauroursodeoxycholic acid; hypothalamic inflammation; Agrp; food consumption; undernutrition</p>
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Title	Abrupt withdrawal of prednisone treatment as a promising protocol to alleviate nutritional and biochemical biomarkers in Wistar rats
Authors	Mirella Stoianov Rocha Andressa Pereira Silva Gabrielly Felix de Freitas Adryan Jheferson da Silva Neres João Vitor Alves Damacena Andre Cantarelli Vilela Kleber Eduardo de Campos
Affiliations	Laboratório de Fisiologia de Sistemas e Toxicologias Reprodutiva. Instituto de Ciências Biológicas e da Saúde. Universidade Federal de Mato Grosso, Câmpus do Araguaia, Brasil.
Session	7 - Nutrição e Metabolismo

Abstract, Ethics Committee Number*, and Keywords	<p>Prednisone is a glucocorticoid used as immunosuppressant. However, there is no reduction protocols, in this case is essential studies to apprise the adverse effects. The objective of the study was to evaluate the nutrition and biochemical biomarkers in rats subjected in total reduction of prednisone (PRED), and it was approved by Ethics Committee Number (CEUA-Araguaia 23108.044685/2023- 06). All Wistar rats were daily treatment with PRED or vehicle (SyrSpend®) for 28 days. The VE group (n=10) was treated only vehicle; PRED (n=7) with PRED 5mg/Kg/day; the last groups (n=7 each) got PRED 5mg/Kg/day for 14 days and in de 15° day was replaced for vehicle (WITHD); or reduce for 2.5mg/Kg/day and in the 21st day was replaced for vehicle, a weaning treatment (WEAN). Nutritional biomarkers were weekly measured. On the 27th day the oral glucose tolerance test (OGTT) was evaluated followed by biochemical determinations in day 28. The results were statistically analyzed, with 5% significance. The Specific Rate of Weight Gain and Coefficient of Weight Gain per Caloric Consumption of WITHD rats were higher respectively in the 3rd (57,9% and 50%) and 4th weeks (58,0% and 50%) vs 1st week, as also the Food Efficiency Coefficient increased in all weeks (2nd, 38,9%; 3rd 52,2% and 4th 50%) vs first week. Respectively, glycemic increase in the OGTT of VE, WITHD and WEAN at 30' and 60 (131.6±21.1; 136.1±6.6; 139.9±13.3mg/dL) vs fasting (96.6±7; 91.6±21.1; 93.8±6.7mg/dL). PRED and WEAN rats had increased triglycerides (110.4±19.6; 117.4±42.2 vs VE 70.7±11.6mg/dL, with HDL reduction in VE and WITHD (34.2±7.3; 33.2±5.8 vs VE 46.8±4.4mg/dL). Likewise, PRED increased ALT activity (111.2±32.4 vs VE 55.2±18.7U/L); and WEAN reduced (62.2±6.6) vs VE; and WITHD rats reduced AST activity (130.5±33.9 vs VE 197.5±16.2U/L). The adverse effects of PRED were more attenuated in the WITHD indicating the best method for future studies.</p> <p>Keywords: Prednisone. Withdrawal. Wean. Adverse effects. Lipids.</p>
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Title	Metabolic phenotype of Bdnf knockdown in a novel hypothalamic neuronal population
Authors	Dayana Silva Ariane Zanesco Natália Mendes Lício Velloso
Affiliations	Laboratory of Cell Signaling - Obesity and Comorbidities Research Center, University of Campinas, Brazil.
Session	7 Nutrição e Metabolismo

Abstract, Ethics Committee Number*, and Keywords	<p>The regulation of energy balance is crucial for health maintenance, with the hypothalamus playing a central role. The hypothalamus receives peripheral signals that regulate signals involved in vital responses such as satiety, food intake, and thermogenesis. Obesity results from an imbalance in this control, leading to chronic anabolism due to inflammatory damage to hypothalamic neurons, often triggered by excessive dietary fat consumption. Bdnf, a neurotrophic factor essential for neurogenesis and neuronal survival, also contributes to energy expenditure and suppresses inflammation induced by a high-fat diet.</p> <p>We have previously identified neurons expressing Fezf1, a transcription factor crucial for the migration of neurons of the olfactory bulb during development, also expressing a high amount of Bdnf. The precise role of Fezf1 neurons in regulating energy metabolism and the underlying mechanisms of Bdnf production remain elusive.</p> <p>This study aimed to elucidate the role of Bdnf expression in Fezf1 neurons. Male and female Fezf1-cre/Bdnf-floxed and Bdnf-floxed mice were subjected to an 8-week 45% high-fat diet (HFD) and exposed to 4°C for 6 hours on the last day. Results indicated that female mice lacking Bdnf expression in Fezf1 neurons were protected against weight gain (Control HFD 30g, KO HFD 20g $p < 0.005$), improved glucose tolerance (Control HFD 60000 AUC, KO HFD 40000 AUC $p < 0.05$) and have a decrease in white adipose tissue (Control HFD 0.05g, KO HFD 0.02g $p < 0.005$) compared with control groups. One-way ANOVA was used for multiple comparisons.</p> <p>These findings underscore the importance of Bdnf in Fezf1 neurons in modulating metabolic responses, particularly under high-fat diet conditions. Understanding the intricate mechanisms governing energy balance regulation holds promise for developing strategies to combat obesity.</p> <p>Ethics committee number: 5191-1/2020 – CIBIO 04/2020</p> <p>Keywords: Bdnf, hypothalamus, energy balance</p>
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Title	Effects of TUDCA treatment on aging-related sarcopenia
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Session	7- Nutrição e Metabolismo (Poster)

Abstract, Ethics Committee Number*, and Keywords	<p>Aging is associated with the development of metabolic disorders and body changes, such as skeletal muscle mass loss, called sarcopenia. For the treatment of these complications, bile acids have stood out. Recent studies have demonstrated that the bile acid TUDCA can act in the regulation of energy and glycemic metabolism, as well as in the attenuation of muscle mass loss in several experimental models. In this study, 3-month-old (Ctl) and 18-month-old (Old) C57BL/6 mice were used (CEUA: 5612-1/2020). To evaluate the effects of TUDCA on sarcopenia, Old mice were treated for 20 days with an intraperitoneal injection of TUDCA at 300 mg/kg (Old+TUDCA) or its vehicle (PBS). Results were analyzed by One-way ANOVA ($P < 0,05$), followed by Tukey post-test, $n=8-10$. TUDCA-treated mice presented a body weight loss (BW) (g) (29.20 ± 0.678 Ctl x 33.08 ± 1.025 Old x 29.54 ± 0.841 Old+TUDCA), despite presenting an increase in skeletal muscle weight (%BW) (gastrocnemius: 0.544 ± 0.015 Ctl x 0.427 ± 0.014 Old x 0.498 ± 0.023 Old+TUDCA; soleus: 0.027 ± 0.0006 Ctl x 0.020 ± 0.001 Old x 0.024 ± 0.001 Old+TUDCA; TA: 0.179 ± 0.009 Ctl x 0.12 ± 0.004 Old x 0.161 ± 0.006 Old+TUDCA) and improved muscle function, judging by a higher MVCC (%BW) (151.0 ± 7.83 Ctl x 77.4 ± 6.55 Old x 106.1 ± 7.54 Old+TUDCA) and time score on Kondziela's test (121.9 ± 7.08 Ctl x 7.3 ± 1.43 Old x 39.84 ± 7.34 Old+TUDCA). The increase in muscle weight in these mice was accompanied by increased protein content of phosphorylated P70S6K (0.323 ± 0.092 Old x 0.756 ± 0.108 Old+TUDCA, fold change of Ctl), and AKT (0.5 ± 0.1021 Old x 1.238 ± 0.1496 Old+TUDCA) when the mice were stimulated with insulin. TUDCA treatment also reduces the expression of pro-inflammatory cytokines (TNFα: 1.6 ± 0.2446 Old x 0.2338 ± 0.097 Old+TUDCA; IL-1b: 5.129 ± 1.135 Old x 0.4231 ± 0.093 Old+TUDCA). Our results indicate that TUDCA may be an alternative for mitigating the harmful effects resulting from aging, related to the loss of muscle mass and strength.</p> <p>Keywords: Tauroursodeoxycholic acid, sarcopenia, aging, skeletal muscle.</p>
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Title	Evaluation of the effect of berberine in hyperglycemic rats
Authors	Maretti JG ¹ ; Bressan, LC ² ; Pereira, CS ² ; Andrade, ER ¹ ; Garcia, MP ¹ ; Araújo, CO ¹ ; Amaral, MEC ³
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Session	07 - Nutrição e Metabolismo

Abstract, Ethics Committee Number*, and Keywords	<p>Introduction: Type 1 diabetes mellitus is an autoimmune disease, is a chronic disease characterized by insulin deficiency due to pancreatic β-cell loss and leads to hyperglycemia. Berberine is a natural alkaloid found in some plants that has shown potential in studies to help control blood sugar levels. Objective: This study aims to evaluate the effects of berberine on the glycemic and lipid metabolism of hyperglycemic rats. Methods: The project was approved by the Ethics Committee of the Herminio Ometto Foundation University Center (n^o026/2022). Wistar rats were divided into three groups: control and diabetic group (alloxan induced), which received vehicle (sodium carboxymethylcellulose 0,5%) and berberine diabetic group treated with berberine (210mg/kg/day) dissolved in saline 0,9% for a period of 15 days. Results: After the experimental period, the animals were subjected to ITT and euthanized. Cardiac puncture was performed to obtain serum and liver tissue and hypothalamic tissue was obtained for western blotting and histological analyses. Berberine treatment did not modify glycemic and lipid homeostasis in diabetic animals. Berberine increased hepatic glycogen stores and the glucose decay rate (KITT) was not different between control and berberine diabetic group, suggest an increase in insulin sensitivity attributed to berberine. In the hypothalamus tissue have not significant changes either in the histological or in the western blotting quantification of catalase levels (anti-oxidative marker) and CD8 levels (anti-inflammatory marker). Conclusion: Results suggest that berberine improved insulin sensitivity, not present a risk of hypoglycemia and no hypothalamic change were verified.</p> <p>Keywords: insulin, diabetes, berberine, CD8, catalase</p> <p>Financial Support: Bolsa: PIC/Herminio Ometto Foundation University Center.</p>
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Title	Oral green tea supplementation changes bone and biochemical parameters in healthy and obese rats
Authors	Bianca Freitas de Barros ¹ Cyntia Gomes Teles ¹ Karen Pereira Coutinho ² Bruna Almeida Nascimento ² Milla Christie Rodrigues de Lira ³ Paulo Roberto Nuyens Leme ² Anderson Junger Teodoro ⁴ Mariana Sarto Figueiredo ⁴
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Session	7- Nutrição e Metabolismo

Abstract, Ethics Committee Number*, and Keywords	<p>Abstract: Green tea is a source of bioactive compounds, rich in antioxidant and anti-inflammatory effects, which are important to prevent obesity. We aimed to evaluate the effects of aqueous extract of green tea (<i>Camellia sinensis</i>) supplementation in healthy and obese adult rats on body composition, bone and biochemical parameters. Forty male Wistar rats divided into two experimental groups: 1) Control group (C,n=20) and 2) and High fat (HF) group (HF,n=20) until 90 days old (PN90). At PN90, C group was subdivided: 1) Control Saline – commercial diet/ gavage saline (CS,n=10), 2) Control Green Tea – commercial diet/ gavage green tea (CGT,n=10); and HF group was subdivided: 3) HF saline – HF diet/gavage saline (HFS,n=10) and 4) HF green tea – HF diet/ gavage green tea (HFGT,n=10), for 30 days (dose 100mg/kg). In PN120, were analyzed body composition, bone and biochemical parameters. At PN120, no change were observed in body mass among group. HFS and HFGT presented lower food intake (-37%, p< 0.05 and -49%, p<0.05, respectively vs CS). CGT and HFGT showed lower body mineral density (-5% and -6%, p<0.05, respectively vs HFS). In femur, no changes were observed in bone composition, anatomic parameters and radiodensity. HFS and HFGT presented higher femur rupture force (+24% and +25%, p<0.05, respectively vs CS). HFS showed higher breaking strenght (+39%, p<0.05 vs CS), without changes in maximum strength and Module Young. In serum, HLCV presented lower calcium (-9% vs CS and -13% vs HFS, p<0.05), magnesium (-16% vs CS, p<0.05), alkaline phosphatase (-34% vs CS and -33% vs CGT, p<0.05) and vitamin D (-36% vs HFS, p<0.05). Thus, HF diet increase adaptative bone resistance. On the other hand, green tea supplementation impact negativelly in bone health and biochemical parameters.</p> <p>Committee number: 3444210721</p> <p>Keywords: <i>Camellia sinensis</i>, Bone healthy, Biochemical parameters, Obesity.</p>
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Title	Comparison of the effects of <i>Camellia sinensis</i> and <i>Hibiscus sabdariffa</i> supplementation on biochemical and serum redox balance in healthy and obese rats
Authors	Cyntia Gomes Teles ¹ Bianca Freitas de Barros ¹ Giovanna de Menezes Abreu ² Karen Pereira Coutinho ³ Bruna Almeida Nascimento ³ Carolina de Oliveira Ramos Petra de Almeida ⁴ Alana Louzada Millions Monteiro ² Anderson Junger Teodoro ⁵ Mariana Sarto Figueiredo ⁵
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Session	7- Nutrição e Metabolismo

Abstract, Ethics Committee Number*, and Keywords	Abstract: <i>Camellia sinensis</i> and <i>Hibiscus Sabdariffa</i> are sources of antioxidants which can prevent and treat non-communicable diseases. We aimed to evaluate effects of <i>Camellia sinensis</i> and <i>Hibiscus sabdariffa</i> supplementation on biochemical parameters and serum redox balance in healthy and obese rats. Sixty male rats at 30 days old were divided in: 1) Control group: commercial diet (C,n=30); and 2) High fat group: high fat diet (HFD) (HF,n=30). At PN90, C group were subdivided: 1) Control Saline group - commercial diet/gavage saline (CS,n=10); 2) Control Green Tea group - commercial diet/gavage green tea (CGT,n=10); 3) Control Hibiscus group - commercial diet/gavage green tea (CHT,n=10) and HF group were subdivided in: 4) HF Saline group – HFD/gavage saline (HFS,n=10), 5) HF Green Tea group - HFD/gavage green tea (HFGT,n=10) and 6) HF Hibiscus group - HFD/gavage green tea (HFHT,n=10) during 30 days (dose 100mg/kg). At PN120, were analyzed biochemical parameters and redox balance. In PN120, HFGT and HFHT presented lower body mass (-10% and -10%, p<0.05 vs HFS). CGT showed higher HDL (+21%, p<0.05 vs CS) and lower FRAP (-30%, p<0.05). CHT showed lower triglycerides (-33%, p<0.05), cholesterol (-20%, p<0.05), HDL (-17%, p<0.05), VLDL (-33%, p<0.05), total protein (-6%, p<0.05), creatinine (-12%, p<0.05); and higher DPPH (+53%, p<0.05), FRAP (+48%, p<0.05) and catalase (1.5x fold increase, p<0.05). HFGT
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presented lower albumin (-27%, $p < 0.05$), total protein (-17%, $p < 0.05$) pyruvic glutamic transaminase (-30%, $p < 0.05$), alkaline phosphatase (-30%, $p < 0.05$) and bilirubin direct (-33%, $p < 0.05$). HFHT showed lower triglycerides (-41%, $p < 0.05$), cholesterol (-29%, $p < 0.05$), HDL (-24%, $p < 0.05$), VLDL (-45%, $p < 0.05$), LDL (-33%, $p < 0.05$); and higher FRAP (+38%, $p < 0.05$) and catalase (1.4x fold increase, $p < 0.05$). Thus, hibiscus extract supplementation improve lipid profile and increase antioxidant activity in comparison to green tea extract.

Committee number: 3444210721

Keywords: *Camellia sinensis*, *Hibiscus sabdariffa* L., Biochemical parameters, Redox balance.



Title	Effects of aqueous extract of <i>Hibiscus Sabdariffa</i> L. supplementation in healthy and obese rats on body composition, bone and biochemical parameters
Authors	Alana Louzada Millions Monteiro ¹ João Fernando Bernado da Costa ² Cyntia Gomes Teles ² Bianca de Freitas Barros ² Giovanna de Menezes Abreu ¹ Karen Pereira Coutinho ³ Milla Christie Rodrigues de Lira ¹ Pâmela Braga Louback ³ Mariana Sarto Figueiredo ⁴
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Session	7-Nutrição e Metabolismo

Abstract, Ethics Committee Number*, and Keywords	Abstract: <i>Hibiscus sabdariffa</i> L. presents functional characteristics according to its antioxidant profile and its consumption has been stimulated by the presence of bioactive compounds, which can play an important role in non-communicable diseases prevention. The aimed was to evaluate the effects of supplementation with <i>Hibiscus sabdariffa</i> extract on body composition, biochemical and bone parameters in healthy and obese rats. Forty <i>Rattus norvegicus</i> Wistar male at 30 days old were divided into two experimental groups: 1) Control group: commercial diet (C,n=20); and 2) High fat group: high fat diet (HF,n=20) until 90 days old (PN90). At PN90, C group were subdivided in: 1) Control Saline group - commercial diet/gavage saline (CS,n=10); 2) Control Hibiscus group - commercial diet/gavage hibiscus extract (CH,n=10); and high fat group were subdivided in: 3) High Fat Saline group – high fat diet/gavage saline (HFS,n=10) and 4) High Fat Hibiscus group - high fat diet/gavage hibiscus extract (HFH,n=10) during 30 days. At PN120, were analyzed body composition, bone and biochemical parameters. At PN120, HFH presented lower body mass (-10,67%, p < 0,05 vs HF). However, HFH presented higher body fat mass (+47%, p<0.05 vs CH) and lower lean mass (-12%, p<0.05 vs CS). HFH showed lower triglycerides (-22%, p<0.05), LDL (-41%, p<0.05) and glutamic-pyruvic transaminase (26%, p<0.05), without changes in others parameters. At PN120, HFH presented higher bone mineral content (+11%, p<0.05), bone area (+8%, p<0.05), femur length (+3%, p<0.05) and lower femoral width (-6%, p0.05)
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when compared to CS. Oral supplementation with aqueous hibiscus extract for 30 days promote significant changes in body composition, improve lipid profile and bone compartments. Hibiscus tea can be an important strategy to prevent or treat obesity and comorbidities associated.

Committee number: 3444210721.

Keywords: *Hibiscus Sabdariffa* L., Body composition, Biochemical parameters, Bone parameters



Title	Effects of <i>Euterpe edulis</i> supplementation in Wistar rats on body composition, biochemical parameters and serum redox balance
Authors	Milla Christie Rodrigues de Lira ¹ Carolina de Oliveira Ramos Petra de Almeida ² Giovanna de Menezes Abreu ¹ Pamela Navarro Brito ³ Julia Teixeira de Oliveira Meirelles ³ Leticia de Almeida Sant'Anna Trindade ¹ Clarice Maia Vinagre de Oliveira ³ Yasmin Cunha Silva ⁴ Valdir Florêncio Veiga Junior ⁵ Anderson Junger Teodoro ⁶ Mariana Sarto Figueiredo ⁶
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Session	7-Nutrição e Metabolismo

Abstract, Ethics Committee Number*, and Keywords	Abstract: <i>Euterpe edulis</i> Martius is an açai species found in Brazil is rich in bioactive compounds such as antioxidants and polyphenols, which can promote health benefits and can protects against non-communicable diseases. We aimed to evaluate the effects of açai juçara pulp supplementation in adult rats on body composition, biochemical parameters and serum redox balance. Forty male Wistar rats aged 90 days old (PN90) were divided: 1) Control group: commercial diet/ gavage saline (C, n=10); 2) Açai juçara 50 group: commercial diet/ gavage with 50mg/kg/day of açai juçara pulp (50AJU, n=10); 3) Açai juçara 100 group: commercial diet/ gavage with 100mg/kg/day of açai juçara pulp (100AJU, n=10); and 4) Açai juçara 200 group: commercial diet/ gavage with 200mg/kg/day of açai juçara pulp (200AJU, n=10) during 30 days. At PN120, were analyzed body composition, biochemical parameters and serum redox balance. Açai supplementation did not change body composition, tissues relative weight and bone parameters. 50AJU and 100AJU showed lower aspartate aminotransferase (-29% and 31%, p<0.05). 100 AJU presented lower uric acid (-25%, p<0.05) and lower FOX (-2%, p<0.05), followed by higher DPPH (+20%, p<0.05), FRAC (+24%, p<0.05), ORAC (+45%, p<0.05), catalase (+28%, p<0.05), SOD (+18%, p<0.05), Thiol (1.56 fold increase, p<0.05). 200AJU presented lower fasting glycemia (-11%, p<0.05), urea (-10%, p<0.05), creatinine (-15%, p<0.05), uric acid (-31%, p<0.05),
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alanine aminotransferase (-20%, $p < 0.05$), aspartate aminotransferase (-47%, $p < 0.05$), alkaline phosphatase (-19%, $p < 0.05$), FOX (-3%, $p < 0.05$) protein carbonyl (-32%, $p < 0.05$). 200AJU presented higher serum ORAC (+53%, $p < 0.05$) and Thiol (+95%, $p < 0.05$). Thus, açai juçara supplementation improves serum redox balance and can be an important nutritional strategy to increase antioxidants in daily diet.

Committee number: 1645110921

Keywords: *Euterpe edulis*; Açai juçara, Body composition, Biochemical parameters, Redox balance



Title	Single nuclei RNA-sequencing reveals novel cellular diversity and enriched communication in human cervical brown adipose tissue
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Session	07 - Nutrição e Metabolismo

Abstract, Ethics Committee Number*, and Keywords	<p>Brown adipose tissue (BAT) is composed by thermogenic adipocytes that efficiently utilize glucose and triglycerides and act as an endocrine organ by secreting batokines. Hence, increased BAT activity is associated with improved metabolic health in humans. In adults, thermogenic adipose tissue is localized in deep cervical planes, exhibiting a gradient from white adipose tissue (WAT) to BAT from superficial to deep layers. To understand the cellular compositions of human adipose tissue and compare the differences between human BAT vs. WAT, we collected 30 paired samples of superficial, intermediate and deep human cervical adipose tissue from 15 participants who underwent anterior cervical discectomy fusion or thyroid surgery and subjected the frozen tissues to single-nucleus RNA sequencing (snRNA-seq) (#NCT04352244). White adipocytes (WAd) were the dominant cell type, comprising 38% of the 37,596 good-quality nuclei captured. Further, the WAd from deep cervical samples expressed higher levels of brown adipocyte-associated genes, such as EBF2 and COBL, and enrichment of oxidative phosphorylation pathway score, indicating increased thermogenic potential. Bona fide brown adipocytes (BAd), marked by the expression of PPARGC1A, were predominantly located in the deep cervical</p>
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samples (>80%) but comprised less than 2% of the dataset. Among the non-adipocyte cells, a novel neuronal-like population marked by CASR was exclusively detected in the deep cervical samples. In addition to distinct cellular composition, cells from BAT had stronger and more complex cell-cell communication. Many key signaling pathways including the BMP and Chemerin were identified as BAT-specific. Moreover, brown adipocytes and adipocyte progenitors were the main source of communication signals. The current study comprehensively characterized a major human BAT at the single cell level. This knowledge could be pivotal to target the adipose tissue for therapies against obesity and related disorders.

Keywords: Brown adipose tissue; Adipocyte; Single cell RNAseq; Metabolism; Obesity; Thermogenesis; Bioinformatics



Title	The influence of sodium butyrate on the autophagic pathway in <i>Saccharomyces cerevisiae</i> cells
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Session	7 – Nutrição e metabolismo

Abstract and Keywords	<p>Autophagy is one of the main cellular recycling pathways in a process of degradation of dysfunctional cytoplasmic components. Problems in this pathway are related to a series of diseases, such as neurodegeneration and cancer. Butyrate is a metabolite originating from the enteric fermentation of fibers that has been associated with multiple regulatory effects on cellular homeostasis. The objective of this work was to analyze the influence of sodium butyrate on the autophagy process using <i>Saccharomyces cerevisiae</i> (BY4741) and isogenic mutants of autophagic pathway genes ($\Delta atg8$, $\Delta gcn4$ and $\Delta pep4$). When evaluated on strain BY4741, concentrations of 50, 100, 150 and 200μM of sodium butyrate did not impact growth kinetics in 24h, indicating absence of toxicity. This result was confirmed with the evaluation of cell viability in BY4741 after 2h, 6h and 24h of treatment, where no significant difference was observed from the control. The 100μM concentration was chosen to evaluate cell vitality in the mutant strains through the metabolic activity marker resazurin. When compared to the control strain (BY4741), the deletion of genes related to autophagy reduced vitality to 68.93\pm4.46% in $\Delta atg8$, to 72.46\pm4.90% in $\Delta gcn4$ and to 60.43\pm 5.15% in $\Delta pep4$. Butyrate treatment did not impact vitality in any of the strains evaluated. Autophagy was induced by nitrogen deprivation for 24h and evaluated for cell viability. Strains $\Delta atg8$, $\Delta gcn4$ and $\Delta pep4$ without butyrate treatment showed, respectively, increases of 240.9%, 165.9% and 148.2% when compared to BY4741; however, when treated with butyrate (100μM) there was a reduction in viability of the $\Delta atg8$ and $\Delta gcn4$ strains to 161.4% and 126.3% of BY4741, respectively; and an increase to 166.5% in $\Delta pep4$. These results show that butyrate is not toxic at the concentrations tested and that 100μM is capable of influencing the viability of strains deleted of autophagic system proteins.</p> <p>Keywords: Sodium butyrate, autophagy, <i>Saccharomyces cerevisiae</i>.</p>
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Title	Noradrenergic system regulates hepatic autophagy to activate gluconeogenesis and ketogenesis during acute cold stress
Authors	Henrique J. N. Morgan; Aline Z. Schavinski; Ana P. Assis; Luciane Carla Alberici; Isis C. Kettelhut; Luiz C. C. Navegantes
Affiliations	Ribeirão Preto Medical School, University of São Paulo, Brazil.
Session	Nutrition and Metabolism

Abstract, Ethics Committee Number*, and Keywords	<p>Autophagy, one of the main cellular degradation processes, is essential to maintain gluconeogenesis and ketogenesis in the liver. Its dysregulation is involved in hepatic pathologies such as steatosis. Even though the well described action of pancreatic hormones in regulating autophagy, the role of sympathetic nervous system remains unclear. We aimed to investigate the role of noradrenergic system in autophagy as a regulator of gluconeogenesis and ketogenesis during acute cold stress. For this, neonate male mice were sympathectomized (6OHDA; 100mg.kg⁻¹.dia⁻¹) and 10 weeks later were exposed to cold (4°C) for 3-6h, a model of hepatic noradrenergic activation. Cold increased the autophagy flux (2fold) performed by leupeptin treatment (40mg.Kg⁻¹), blood glucose (214.2±6.9 vs 135.8±7.2mg/dL RT), ketone body (1.7±0.1 vs 1.0±0.1mmol RT) and hepatic noradrenaline (70%), effects that were abolished or attenuated in 6OHDA mice. Plasm levels of glucagon, corticosterone and fatty acids were increased while insulinemia was reduce in saline and 6OHDA mice exposed to cold. In innervated mice, cold also increased protein and genes, by western blot and Rt-qPCR, of gluconeogenesis (G6Pase; PEPCK), ketogenesis (CPT1a; ACAA2) and autophagic (LC3; ULK1), these effects were attenuated or abolish in 6OHDA mice. Hepatic beta-oxidation, performed in Oroboros in the presence of palmitoyl-carnitine, was unchanged during cold but reduced in 6OHDA. Liver immunofluorescence of LC3 and bodidy revealed an increase of co-localization during cold, indicating lipophagy, that was attenuated by 6OHDA. The leupeptin-induced blockage of autophagy in cold-exposed mice inhibited the hyperglycaemia and attenuated the increase in ketone body plasma levels. Data suggest cold-inducible sympathoexcitation leads to the activation of gluconeogenesis and ketogenesis, which, at least in part, are dependent on hepatic autophagy.</p> <p>Fapesp: 2021/05848-4 CEUA: 065-2021 Keywords: liver; metabolism; noradrenaline.</p>
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Title	Effects of chronic green tea intake in healthy and obese adult rats on body composition and biochemical parameters
Authors	Karen Pereira Coutinho ¹ Giovanna de Menezes Abreu ² Carolina de Oliveira Ramos Petra de Almeida ³ Cynthia Gomes Teles ⁴ Bianca Freitas de Barros ⁴ Karla de Araújo Coutinho ¹ Julia Teixeira de Oliveira Meirelles ¹ Clarice Maia Vinagre de Oliveira ¹ Anderson Junger Teodoro ⁵ Mariana Sarto Figueiredo ⁵
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Session	7-Nutrição e Metabolismo

Abstract, Ethics Committee Number*, and Keywords	Abstract: Bioactive compounds present in green tea (<i>Camellia sinensis</i>) play a role in non-communicable disease. We aims to evaluate effects of oral aqueous green tea extract supplementation in healthy and obese adult rats on body composition and biochemical parameters. Forty <i>Rattus norvegicus</i> Wistar male at 30 days old were divided into two experimental groups: 1) Control group: commercial diet (C,n=20); and 2) High fat group: high fat diet (HF,n=20) until PN90. At PN90, C group were subdivided in: 1) Control Saline group - commercial diet/gavage saline (CS,n=10); 2) Control Green Tea group - commercial diet/gavage aqueous green tea extract (CGT,n=10); and high fat group were subdivided in: 3) High Fat Saline group – high fat diet/gavage saline (HFS,n=10) and 4) High Fat Green Tea group - high fat diet/gavage aqueous green tea extract (HFGT,n=10) during 30 days. At PN120, were analyzed body composition and biochemical parameters. At PN120, CGT and HFGT presented lower body mass gain (-63% and -87%, p<0.05, vs CS), lower body fat mass (-26% vs CS and -26% vs HFS, p<0.05). HFGT showed reduction in peri-mesenteric adipose tissue weight (-32%, p<0.05, vs HFS) and higher brown adipose tissue (+42%, p<0.05, vs CGT). CGT reduced brown adipose tissue (-38% vs CS, p<0.05). HFGT presented lower triglycerides (-42% vs HFS, p<0.05), total cholesterol (-31% vs CS and -27% vs HFS, p<0.05), HDL-c (-19% vs CS and -26% vs CGT, p<0.05), VLDL-c (-42%, p<0.05 vs HFS). CGT showed lower fasting glucose (-
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9.4% vs CS, $p < 0.05$) and HFGT presented lower fasting glucose (+12% vs CGT, $p < 0.05$). Oral green tea supplementation in healthy and obese animals reduce body fat mass and white adipose tissue compartments and improve lipid profile, and may be an important nutritional strategy for the treatment of NCDs that affect population worldwide.

Ethics Committee: 3444210721

Keywords: Green tea; Bioactive Compounds; Body composition; Lipid profile.



Title	The impact of genetic and environmental condition in the response to β3-adrenergic stimulus in three mice strains
Authors	Ana Luisa Gallo-Ferraz ^{12*} , Marcela Reymond Simoes ¹ , Bruna Bombassaro ¹ , Guilherme Augusto da Silva Nogueira ¹ , Licio Augusto Velloso ¹ *Corresponding Author: analuisa.gferraz@gmail.com
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Session	07 - Nutrição e Metabolismo

Abstract, Ethics Committee Number*, and Keywords	<p>The identification of active brown adipose tissue (BAT) in adult humans in 2009 raised its potential as a therapeutic target for obesity and other metabolic diseases. Upon activation, the BAT consumes metabolic substrates such as fatty acids and glucose to produces heat and with this increases energy expenditure. Studies have identified the β3-adrenergic receptor (Adrb3) as the main activator of thermogenic program in BAT, and studies using synthetic agonists of this receptor have provided important advance in the field. However, it is currently unknown how genetic factors can affect the BAT responsivity to Adrb3 agonists. Here, we evaluated BAT responsivity to a β3-adrenergic agonist (CL-316,243) acute treatment in three genetically distinct strains of mice; Balb/c, Swiss and C57BL/6. After three hours of fasting, mice were treated with CL-316,243 (1mg/kg) and temperatures and glucose were measured during the following 2 hours. At room temperature (RT) we observed that the three mice strains have difference in the expression of beta 3 adrenergic receptor (Adrb3) as well in BAT temperature. However, at thermoneutrality (TN) mice increased body and BAT temperature if compared with phenotype observed at RT. Taken together the results show that genetic and thermic conditions influence CL-316,243 responses and these two factors must be considered when evaluating drug response in obesity context.</p> <p>Keywords: Thermogenesis, Energy Expenditure, Pharmacotherapy Ethics Committee: CEUA 5922-1</p>
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Title	Effects of taperebá extract (<i>Spondias mombim</i>) supplementation on bone parameters in healthy Wistar rats
Authors	Giovanna de Menezes Abreu ¹ Carolina de Oliveira Ramos Petra de Almeida ² Clarice Maia Vinagre de Oliveira ³ Cynthia Gomes Teles ⁴ Bianca Freitas de Barros ⁴ Milla Christie Rodrigues de Lira ¹ Karla de Araújo Coutinho ³ Alana Louzada Millions Monteiro ¹ Paulo Roberto Nuyens Leme ³ Mariana Sarto Figueiredo ⁵
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Session	07 - Nutrition and Metabolism

Abstract, Ethics Committee Number*, and Keywords	<p>Abstract: Development of diseases has been associated with poor eating habits, such as low fruit consumption. Taperebá (<i>Spondias mombim</i>) (TAP) is a Brazil native fruit, rich in nutrients and bioactive compounds (BC). BC present anti-inflammatory and antioxidant effects, which can contribute to maintain health and prevent diseases such as bone damage. The aim of the study was to evaluate the effects of freeze-dried taperebá pulp extract supplementation in different doses on bone parameters in adult male rats. Forty male Wistar rats, 90 days old (PN90), were divided into: 1) Control group – gavage saline (C,n=10), 2) 50TAP group - gavage TAP dose 50mg/kg/day (50Tap,n=10), 3) 100TAP group – gavage TAP dose 100mg/kg/day (100Tap, n=10), and 4) 200TAP group – gavage TAP dose 200mg/kg/day (200Tap, n=10), during 30 days. At PN120, were analyzed serum parameters (calcium, phosphorus and magnesium) and bone parameters in femur. 100Tap group showed an increase in serum calcium compared to C (10.60mg/dL±0.31; 9.91mg/dL±0.53, p=0.02). 100Tap group showed an increase in serum phosphorus compared to C (7.64mg/dL±1.55; 5.98mg/dL±1.10, p=0.02). No changes were observed in serum magnesium and in composition and biomechanics of femur among groups. On the other hand, it was observed that 100Tap group showed an increase in femur radiodensity compared to group C (472.70HU±69.08; 390.70HU±40.19, p=0.03). Results suggest that the dose 100mg/kg/day was able to improve bone health and consumption of taperebá may have a potential preventive role in strength the composition and damage in bone structure. Thus, taperebá as part of daily habitual diet can contribute to bone health through its potential effects on biochemical parameters and femur radiodensity.</p> <p>Committee number: 9501060121.</p> <p>Keywords: Native Brazilian Fruits; Bone Health; Bioactive compounds.</p>
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Title	Effects of <i>Mauritia flexuosa</i> L. f. oil (buriti oil) supplementation on body composition and lipid profile in male rats
Authors	Karla de Araújo Coutinho ¹ Letícia de Almeida Sant' Anna Trindade ² Pamela Navarro Brito ¹ Cyntia Gomes Teles ³ Gabriel de Alcantara Noblat ² Clarice Maia Vinagre de Oliveira ¹ Julia Teixeira de Oliveira Meirelles ¹ Paulo Roberto Nuyens Leme ¹ Milla Christie Rodrigues de Lira ² Anderson Junger Teodoro ⁴ Mariana Sarto Figueiredo ⁴
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Session	7 – Nutrição e Metabolismo

Abstract, Ethics Committee Number*, and Keywords	<p>Abstract: <i>Mauritia flexuosa</i> L. f. (buriti) oil is composed of oleic acid, palmitic acid and present lower amounts of linolenic, linoleic, stearic and myristic fatty acids, which confer properties important in body composition regulation, biochemical parameters and intermediary metabolism. It is known that a balanced and diversified diet in vegetables and fruits composed of nutrients and bioactive compounds are essential for disease prevention and health maintenance. We aimed to evaluate the effects of oral buriti oil supplementation on body composition and lipid profile in adult male rats. Thirty male Wistar rats, 90 days old (PN90), were divided in: 1) Control saline group – commercial diet/ gavage saline (C, n=10), 2) Soybean oil group – commercial diet/ gavage soybean oil (SO, n=10) and 3) Buriti oil group – commercial diet/ gavage buriti oil (BURI, n=10) during 30 days consecutively. At PN120, were analyzed food intake, body composition and biochemical parameters. At PN120, OS and BURI groups presented lower food intake (-25% and 26% vs C, p<0.05, respectively), without changes in body mass gain. BURI showed lower percentage of body fat mass (-24% vs C, p<0.05), without changes in lean mass, bone area, body mineral density and body mineral content. OS group presented lower triglycerides (-32%, p<0.05) and VLDL-c (-37%, p<0.05). BURI group showed lower triglycerides (-27%, p<0.05), HDL-c (-10%, p<0.05) and VLDL-c (-33%, p<0.05). No changes were observed in total cholesterol, LDL-c and fasting glycemia among groups. Thus, our study indicates that buriti oil supplementation can reduce body mass and improve lipid profile, which can be a nutrition strategy to treat or prevent obesity and dislipidemic.</p> <p>Committee number: 9204110520.</p> <p>Keywords: <i>Mauritia flexuosa</i>, Buriti oil, Body composition, Biochemical parameters.</p>
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Title	Brown adipose tissue and IL10: impact on the thermogenesis of neonates and the role of breastfeeding
Authors	Bruna Bombassaro ¹ , Marcela Reymond Simões ² , Ana Luisa Gallo Ferraz ² , Guilherme Augusto da Silva Nogueira ² , Sergio Marba ³ , Jamil Pedro de Siqueira Caldas ⁴ , Licio Augusto Velloso ² , Eliana Pereira de Araújo ¹ .
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Session	7 – Nutrição e Metabolismo

Abstract, Ethics Committee Number*, and Keywords	<p>The identification of a functional brown adipose tissue (BAT) identification in human adults has stimulated the search for new strategies to treat metabolic conditions as obesity and the importance of this tissue, once known only in early life. In low birth weight and pre-term infants specially, hypothermia is a prevalent and important condition that can lead to life-threatening outcomes, such as infections and arrhythmia. Maintaining body temperature stability is a great challenge neonates face after birth, as they are no longer protected by the mother's environment, and they are not able to promote shivering thermogenesis relying on brown adipose tissue to effectively maintain body temperature. Interleukin-10 (IL-10) is known for its role as an anti-inflammatory cytokine; however, a prior study from our group has shown it is also involved in the correct structure and function of BAT mitochondria. The lack of IL-10 in adult mice impairs thermogenesis and appropriate BAT structure. In this study, the absence of functional IL-10 in newborn KO mice was evaluated in the context of cold exposure, showing that these mice are cold intolerant in the first days of life, but this phenotype can be reverted upon fostering in wildtype females. The oral supplementation of IL-10 also improved the IL-10 KO mice response to cold as well as in wildtype neonate mice and was also capable of inducing increase in gut secretin transcript, a recently described BAT thermogenesis inducer. The intraperitoneal IL-10 administration did not prevent the temperature loss and didn't increase secretin transcript, showing a novel oral IL10 role on thermogenesis. In humans, IL-10 is present in both neonate serum at birth and in mother's milk and they show correlation. Thus, IL-10 rises as a potential milk factor promoting thermoregulation in neonate mice.</p> <p>Keywords: brown adipose tissue, cold intolerance, IL-10, milk</p> <p>CEUA: 5409-1/2019 and 5950-1/2022</p> <p>CAAE 30471220.2.0000.5404</p>
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Title	Hyperlipid diet and low dose of streptozotocin induces glucose intolerance and insulin resistance in Swiss mice
Authors	Thainara Agnis Dourado Wéilton Barreto da Silva Edgar Willibaldo Allebrandt Neto Daniela de Souza Vial Dahmer Elaine Cristina de Lara Spada Leilane Natsumi Alves Kanbara Suélem Aparecida de França Lemes
Affiliations	Laboratory of Biochemistry Professora Nair Honda Kawashita, Department of Chemistry, Federal University of Mato Grosso, Cuiabá, Brazil
Session	Poster

Abstract, Ethics Committee Number*, and Keywords	<p>Chronic non-communicable diseases have generated an excessive number of premature deaths and loss of quality of life. These impacts can be reduced with the use of functional foods in the diet that contribute to metabolic control, immunity of the individual and maintenance of body composition. Different studies highlight the antidiabetic, anti-inflammatory, antioxidant and diuretic activity of <i>Acrocomia aculeata</i> pulp oil (Jacq.) Lodd. ex Mart, an abundant species in Mato Grosso and Mato Grosso do Sul where it is popularly known as macaúba or bocaiuva. Therefore, our proposal was to validate a model of type 2 diabetes in mice and verify whether the consumption of <i>A. aculeata</i> helps in lower food intake and thus lead to an improvement in glycemic control, thus preventing the appearance of comorbidities associated with type 2 diabetes mellitus. Swiss male mice with approximately 7 weeks of age (\cong 40g) were conducted according to CEUA/UFMT (n^o 23108.031902/2022-17), and divided into two groups: Non-Diabetic (ND) (N=11, received commercial Labina[®] food) and Diabetic (D) (N=10, high-fat diet: 66.5% commercial Labina[®] diet, 13.5% lard and 20% sugar). On the 13th day of diet, the animals received a single dose of streptozotocin (\cong 50 mg/kg) intraperitoneally. Both groups received <i>A. aculeata</i> in the outside of water-solubilized flour. The results revealed a number of discrepancies in relation to the classic model of type 2 diabetes, such as an increase in body mass (D 12% > DN), reduction in water intake (D < 30% ND) and decreased serum urea levels (D 47% < ND), while they showed elevated levels of carbonylated proteins (D 143% > ND), which suggests a direct relationship between induced hyperglycemia and oxidative stress. However, the lack of exact reproduction of the classical model of type 2 diabetes highlights the need to consider the present model as an experimental representation of glucose intolerance, hypercholesterolemia, oxidative stress, and possible insulin resistance.</p> <p>Keywords: <i>Acrocomia aculeata</i>; antidiabetic activity; Diabetes Mellitus</p>
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Title	Behavioural evaluation of experimental model of cancer cachexia associated with ageing under the effects of a leucine-rich diet and spontaneous physical activity
Authors	Bruno Sérgio Maia Madeira Leisa Lopes-Aguiar Carla de Moraes Salgado Guilherme Augusto Silva Nogueira Rogério Williams dos Santos Maria Cristina Cintra Gomes-Marcondes
Affiliations	Campinas, Brazil
Session	07 - Nutrition and Metabolism

Abstract, Ethics Committee Number*, and Keywords	<p>Keywords: cachexia, senility, leucine, spontaneous physical activity, and behavioural activity.</p> <p>Introduction: Cancer cachexia is a condition characterised by body weight loss, especially skeletal muscle and adipose tissue, affecting particularly ageing cancer patients, which causes functional limitations, low physical performance and high mortality. Studies suggest benefits of a leucine-rich diet and physical activity as a coadjuvant treatment, but their impact on ageing has not been defined yet. This study investigates the effect of these mediations in senile rats with Walker 256 tumour. Materials and methods: Ageing animals were subjected to spontaneous physical activity at activity wheels and then distributed into 8 exercised (E) and sedentary (S) groups: control diet (CE; CS); leucine-rich diet (LE; LS); Walker tumour-bearing and control diet (WE; WS); Walker tumour-bearing and leucine-rich diet (WLE; WLS). The behavioural assessment of the rats was carried out using images from night vision cameras. After 21 days or the pre-agonic period, the animals were euthanised. Videos were analysed using EthoVisionXT12 software, and data was processed by Two-way ANOVA. Results: The distance covered ($F=4.0$, $P=0.01$) and time spent moving ($F=4.4$, $P=0.01$) were influenced by tumour factors and leucine. The time without movement was influenced by tumour and leucine diet ($F=3.8$, $P=0.01$), by physical activity factor ($F=4.4$, $P=0.04$) and by the interaction of tumour, diet and exercise ($F=4.0$, $P=0.01$). The distance travelled was significantly reduced in the WLS group compared to the CS group (9190.1 ± 6910.7 vs 44767.0 ± 49155.7, $P=0.03$). The time without movement was also shorter in the CE and WS groups when compared to the CS group (38097.9 ± 3553.8 vs 85397.1 ± 60981.7, $P=0.01$; 30855.7 ± 11582.1 vs 85397.1 ± 60981.7, $P=0.01$). No changes were observed in speed, acceleration, and mobility in the different groups. Conclusion: Preliminary data indicate that</p>
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	<p>age-related cachexia, under a leucine-rich diet and spontaneous physical activity, impacted the behaviour of the animals.</p> <p>Ethics committee: n°: 5843-1/2021 and 6110-1/2022.</p>
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Title	Different effects of estradiol on food intake comparing hypertensive and normotensive rats
Authors	S. Lemos, E. D. Pereira Jr., J. V. Menani, L. A. DE Luca Jr., C. A. F. Andrade.
Affiliations	Dept. of Physiol. and Pathology, São Paulo State University (UNESP), School of Dentistry, Araraquara – SP, Brazil.
Session	Nutrição e Metabolismo

Abstract, Ethics Committee Number*, and Keywords	<p>In addition to their benefic influence on arterial pressure, estrogens participate in mechanisms of ingestive behavior and hydromineral balance. We have shown that β-estradiol (E2) reduces the intense sodium appetite of female spontaneously hypertensive rats (SHRs). E2 also inhibits food intake in normotensive rats, but its effect on food intake of female SHRs is unknown. We evaluated the effect of E2 treatment on the feeding of adult ovariectomized (OVX) SHRs and normotensive (Holtzman) rats, individually housed in metabolic cages (CEUA/FOAr no:08/2022). Rats were treated with E2 (10 μg/0.1 ml/rat) or vehicle (VEH, sunflower oil, 0.1 ml) subcutaneously (sc) for 8 days. The efficiency of E2 was confirmed by uterine index in normotensives [VEH (n = 8): 66 ± 8, vs. E2 (n=10): 192 ± 14 mg/100 g b.w.] and SHRs [VEH (n=5): 106 ± 8, vs. E2 (n=5): 274 ± 32 mg/100 g b.w.]. In normotensives, E2 decreased daily food intake (20.8 ± 2.1, vs. VEH: 27.0 ± 0.9 g/24 h) and 24 h food deprivation-induced food intake (1.8 ± 0.1, vs. VEH: 2.4 ± 0.1 g/2 h/100 g b.w.). In SHRs, however, E2 changed neither daily food intake (15.2 ± 0.8, vs. VEH: 17.4 ± 1.3 g/24 h) nor 24 h food deprivation-induced food intake (2.5 ± 0.1, vs. VEH: 2.7 ± 0.1 g/2 h/100 g b.w.). In both normotensives, E2 had no effect on daily water intake (25.9 ± 3.8 vs. VEH: 39.0 ± 7.0 ml/24 h) or SHRs (20.8 ± 5.7 vs. VEH: 31.2 ± 11.7 ml/24h), as well as food intake-induced water intake (E2: 2.5 ± 0.4 vs. VEH: 2.8 ± 0.4 ml/2 h/100g b.w. and E2: 3.5 ± 0.2 vs. VEH: 3.5 ± 0.3 ml/2 h/100g b.w., respectively, for normotensive and SHRs). The results suggest that female SHRs are refractory to the anorexic effect of estrogens.</p> <p>Keywords: hypertension, estrogens, ingestive behavior.</p>
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Title	Renin-angiotensin system modulation in brown adipose tissue by aerobic training and enalapril in an obesity-induced model
Authors	Luiza da França Losito ¹ ; Gabriela Rodrigues Medeiros ^{1,2} ; Lyss Meireles ¹ ; Gabriel Ximenes ¹ ; Guilherme dos Santos Reis ¹ ; Beatriz Alexandre-Santos ^{1,2} ; D'Angelo Carlo Magliano ² ; Nathalia Silva Carlos Oliveira ³ ; Antonio Claudio Lucas da Nóbrega ¹ ; Eliete Dalla Corte Frantz ^{1,2} .
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Session	Pôster

Abstract, Ethics Committee Number*, and Keywords	<p>Obesity promotes renin-angiotensin system (RAS) classical axis overactivation, impairing brown adipose tissue (BAT) morphology and function. To compare the effects of RAS modulation by aerobic training and/or enalapril in BAT, C57BL/6 mice were fed a control (SC) or high fat (HF) 16-weeks-diet. After 8 weeks, HF animals were subdivided into 4 groups (n=8/group): HF, HF+Enalapril (HF-E), HF+Training (HF-T) and HF+Enalapril+Training (HF-ET). BAT mass, lipid content, nuclear distancing and thermogenesis were evaluated, such as RAS components. Data was analyzed by one-way ANOVA with Holm-Sidak post-test. P value was considered significant when $P \leq 0.05$. HF group showed nuclear rarefaction in BAT compared to SC (HF: -62%, $P=0,016$), as the HF-ET group repaired BAT histoarchitecture (HF: +214,94%, $P=0,001$; HF-T: +81,37%, $P=0,047$). The HF group had lower UCP-1 than SC (HF: -47,82%; $P=0,043$), while the interventions increased UCP-1 (HF-E: +144,04%, $P=0,015$; HF-T: +198,69%, $P=0,0004$; HF-ET: +164,13%, $P=0,004$). As for PGC-1α only trained groups showed an increase compared to HF (HF-T: +599,06%, $P= 0,001$; HF-ET: +418,41%, $P=0,025$) and HF-T showed an additional increase when compared to enalapril-only group (HF-E: +148,29%, $P=0,033$). Concerning the RAS, classical axis expressions were increased in the HF group compared to SC (HF: +99,7%, $P=0,013$), and the interventions were able to decrease those parameters (HF-E: -45,27%, $P=0,013$; HF-T: -53,13%, $P=0,0091$; HF-ET: -54,38%, $P=0,0117$). RAS counterregulatory</p>
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axis markers expressions were increased only with the interventions when compared to HF (HF-E: +96,23%, $P=0,043$; HF-T: +161,16%, $P=0,0002$; HF-ET: +127,41%, $P=0,0037$). We preliminarily conclude that HF diet-induced obesity caused extensive BAT tissue damage, and the interventions partially attenuated the deleterious morphological and physiological effects of the disease.

CEUA/UFF: 2504060718.

Keywords: obesity, renin-angiotensin system, brown adipose tissue, aerobic training.



Title	Berberine mechanism immunomodulation in hyperglycemic rats
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Session	07 Nutrição e Metabolismo

Abstract, Ethics Committee Number*, and Keywords	<p>Introduction: Type 1 diabetes results from the autoimmune destruction of insulin-producing beta cells in the pancreas. Type 1 diabetes is associated with human leucocyte antigen, implicating T cells in disease pathogenesis. Berberine may be used for metabolic disorders treatment and the alkaloid has been having positive results against diabetes mellitus. The aim of this study was to evaluate the berberine effect on liver tissue, considering the interleukins, TGF, VEGF and T lymphocytes in hyperglycemic rats.</p> <p>Methods: The Committee Ethic from FHO have approved this project (n°26/2022). Wistar rats were divided into three groups: Control and diabetic groups (alloxan induced) received vehicle (sodium carboxymethylcellulose 0,5%) for 15 days. Berberine Diabetic group received dissolved berberine (210mg/Kg/day) in saline for 15 days.</p> <p>Results: Our findings in hepatic tissue with Western Blotting technique, revealed IL-6 (an inflammatory marker) and VEGF (an angiogenesis marker) levels were similar for the groups. However, significant increase was observed Berberine Diabetic in IL-10 levels (an anti-inflammatory marker) compare to Diabetic group (p=0,03). Additionally, a decrease in TGF-β1 (a transforming growth factor marker) levels was noted in Berberine Diabetic group (p=0.02) compared to Control group. An elevation in CD4 levels was observed in Berberine Diabetic groups compared to Control group (p=0,006), along with an increase in CD8 levels in Berberine Diabetic group versus Diabetic and Control group (p=0,002). These markers signify activity within the immunologic system. In humans, CD8 T cells predominantly infiltrate the islets, yet their activation and propagation probably require CD4 T cell help.</p> <p>Conclusion: Taken together berberine studies to date suggest that IL-10, TGF, T cells could be included for the pathogenic process rescue in T1D and berberine is a potent oral immunomodulatory agent.</p> <p>Keywords: diabetic mellitus, berberine, pancreas, liver, VEGF, IL-10 Financial support: PIBIC/CNPq – Centro Universitário Fundação Hermínio Ometto, FHO.</p>
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XXXVIII REUNIÃO ANUAL DA FESBE
XXII REUNIÃO ANUAL DA BRAVO
XVIII CONGRESSO DA SBCAL
III CONGRESSO DOHAD BRASIL
II CONGRESSO DA SBBA
2 A 5 DE JULHO 2024, CAMPINAS/SP
FRONTEIRAS DAS CIÊNCIAS BIOMÉDICAS E SEUS DESAFIOS

Title	Plasticity of adipose tissue in obese animals: effects of genetic deletion of the MrgD receptor and angiotensin (1-7)
Authors	Ana Beatriz Proença Souza ¹ Nicia Pedreira Soares ² Ana Livia Ladislau de Souza Vieira ¹ Yasmin Kolz Brozeghini ² Gabriela de Castro Magalhães ² Pedro Henrique Mayrink Soares ² Gabriel Ximenes de Oliveira ¹ Guilherme dos Santos Reis ¹ D'Angelo Carlo Magliano ¹ Robson Augusto Souza Santos ² Maria Jose Campagnole Santos ² Antonio Claudio Lucas da Nobrega ¹ Eliete Dalla Corte Frantz ²
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Session	Poster

Abstract, Ethics Committee Number*, and Keywords	<p>Obesity overactivates the classical renin-angiotensin system (RAS) axis and damages adipose tissue (AT). Conversely, the counterregulatory RAS axis, Angiotensin (Ang)-(1-7)/MAS receptor and alamandine/MrgD receptor, can influence thermogenesis and exhibit anti-obesity effects on the plasticity of white AT (WAT) and brown (BAT). This study investigates the role of MrgD and Ang-(1-7) in AT plasticity in obese animals. Male C57BL6 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) was administered by gavage (30 µg/kg/day), in the last 14 days of the experiment. Data were analyzed by two-way ANOVA and Holm-Sidak post-test. HF animals showed increased body mass, subcutaneous WAT mass, and adiposity index compared to controls, WT (+31.57%, p=0.0001; +177.35%, p=0.083; +159.91%, p=0.0002, respectively) and MrgD-ko (+12.68%, p=0.0002; +102.45%, p=0.074; +78.72%, p=0.0038, respectively). Ang-(1-7) treatment improved these parameters only in WT animals compared to HF (-14.44%, p=0.0004; -50.47%, p=0.0438; -40.25%, p=0.156, respectively). HF animals exhibited glucose intolerance compared to controls (WT: +36.84%, p=0.0001 and MrgD-ko: +26.52%, p=0.0027), while Ang-(1-7) treatment improved glucose intolerance in both groups compared to HF (WT: -15.79%, p=0.0152 and MrgD-ko: -15.63%, p=0.031). HF animals showed reduced BAT surface temperature compared to controls (WT: -7.05%, p=0.0002 and MrgD-ko: -7.94%, p=0.0001), while Ang-(1-7) increased temperature only in WT animals compared to HF (+6.34%, p=0.0019). Our findings suggest that Ang-(1-7) alleviates, in part, obesity effects, particularly in WT animals with MrgD receptors, emphasizing its therapeutic potential in addressing AT plasticity and metabolic dysfunction.</p> <p>*CEUA/UFMG 2132023.</p> <p>KEYWORDS: Obesity, renin-angiotensin system, adipose tissue plasticity</p>
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Title	Interesterified fat intake disrupt glucose homeostasis and alters ceramide profile in mice
Authors	Josiane Érica Miyamoto ¹ , Beatriz Piatezzi Siqueira ² , Raísa Magno dos Santos ² , João Vítor da Silva Domingues ² , Penelope Lacrisio Reis Menta ² , Jeanne Guitton ³ , Adriana Souza Torsoni ² , Letícia Martins Ignacio-Souza ² , Marcio Alberto Torsoni ² , Christophe Magnan ⁴ , Hervé Le Stunff ⁴ , Marcelo Alves da Silva Mori ¹ , Marciane Milanski ²
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Session	07 - Nutrição e Metabolismo

Abstract, Ethics Committee Number*, and Keywords	<p>Chemical interesterification of oils and fats alters the positional distribution by rearranging the fatty acid chains in a completely random manner, thereby generating new triacylglycerol (TAG) structures. This process has been widely used by food industry as replacement for partially hydrogenated vegetable oils (source of industrial trans fat). Previous data evidenced that normocaloric interesterified lipid diet impairs glucose tolerance, results in abnormal peripheral insulin signaling and expression of cellular stress markers in several tissues in mice. In the present study, we aimed to investigate the metabolic parameters and the lipidome of mice fed an interesterified fat enriched diet. Adult male Swiss mice were randomly divided into four diet groups: normolipidic and normocaloric diet with 10% of total energy from palm oil (PO) or interesterified palm oil (IPO), high-fat with 45% of total energy from palm oil (POHF) or interesterified palm oil (IPOHF). The mice received the diet for 8 weeks. The chemical interesterification process generates an altered TAG profile, increasing the saturated fatty acid content in the sn-2 position. Disrupted glucose tolerance in IPO was found in the hyperinsulinemic-euglycemic Clamp. DEXA analysis shows increased adiposity and reduced lean mass in IPO and HF groups. Sphingolipidome profile revealed accumulation of ceramides in hypothalamus, cerebral cortex and adipose tissue in IPO and HF groups. Interestingly, these alterations were found in mice fed a normolipidic diet with interesterified fat, indicating that the consumption of this fat in a normocaloric diet is enough to disrupt the metabolism in several parameters evaluated. Understanding how modified lipids can affect the lipidome could define critical differences between native TAG and the modified ones on their metabolic fate. These findings, along with other studies, raise concerns about the safety of using interesterified fat in processed foods.</p>
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The projects were approved by Comitê de Ética em Pesquisa Animal da Universidade Estadual de Campinas (CEUA: n° 4864- 1/2018) and Buffon-Université Paris Cité" Ethics Committee (#2016040414129137, French Ministry of Research).

Key words: Interesterified fat, palm oil, adipose tissue, lipidomic, ceramides, diacylglycerol



Title	The increased of oxidative damage in the cardiac muscle of rats treated with a low-protein; high-carbohydrate diet for 15 days
Authors	Edgar Willibaldo Allebrandt Neto Elaine Cristina de Lara Spada Daniela de Souza Vial Dahmer Wéilton Barreto da Silva Thainara Agnis Dourado Livia Machado Bittar Pontes Suelem Aparecida de França Lemes
Affiliations	Laboratory of Biochemistry Professora Nair Honda Kawashita- Cuiabá- MT- Brazil
Session	Poster

Abstract, Ethics Committee Number*, and Keywords	<p>Oxidative stress (OS) occurs when there is an imbalance between reactive species (RS) and antioxidant defense systems (ADS). One of the tissues that suffer from this imbalance is the heart muscle, the amount of macronutrient intake may be related to the increase in these RS. Newly weaned male Wistar rats, approximately 30 days old ($\cong 100g$ N=6) (CEUA/UFMT n^o 23108.006061/2021-11), were randomly divided into two groups: Control group (C- received a diet with 17% protein and 64% carbohydrates) and the low-protein; high-carbohydrate (LPHC) group (received a diet with 6% protein and 74% carbohydrate). The animals were kept in metabolic cages in a 12-hour light-dark cycle, temperature of $25 \pm 1^{\circ}C$ for a period of 15 days, with diet and water <i>ad libitum</i>. At the end of the experimental period, the animals were euthanized and cardiac muscle was collected and weighed. The results are expressed as mean \pm SEM (Student's t-test, $p < 0.05$). To evaluate the biomarkers of OS, the malonaldehyde (MDA) and the carbonylated protein contents were performed. In enzymatic ASD, catalase (CAT) and superoxide dismutase (SOD) activities were performed. No statistical difference was observed in the relative weight of the heart (C: $0.50 \pm 0.01g$; LPHC: $0.53 \pm 0.02g$). We observed an increase in the MDA (59%) and carbonylated protein (100%) contents in LPHC animals ($1.99 \pm 0.12mM$.gram of tissue; $0.17 \pm 0.01nmol$.mg of protein, respectively) when compared with the control group ($1.48 \pm 0.06mM$. gram of tissue; $0.09 \pm 0.01nmol$.mg of protein, respectively). In CAT activity, an increase of 32% was observed in LPHC animals ($28.21 \pm 0.77U$.min.mg of protein), when compared to control animals ($21.43 \pm 0.21U$.min.mg of protein). There was no statistical difference in SOD activity (C: $2.15 \pm 0.28U$.min.mg of protein; LPHC: $2.04 \pm 0.13U$.min.mg of protein). The LPHC diet induced an increase in the oxidation of lipids and proteins in the cardiac muscle. Of the enzymes evaluated, CAT was the enzyme that obtained the greatest response to stress.</p> <p>Key words: Hypoprotein-hyperglycidic diet, oxidative stress, cardiac muscle, <i>Wistar</i> rats.</p>
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Title	Effects of sulforaphane supplementation on cellular senescence markers in patients with chronic kidney disease
Authors	Marcia Ribeiro ¹ Danielle Nascimento ¹ Ligia S. Lima ¹ Isadora Britto ¹ Ludmila Cardozo ² Marcelo Ribeiro-Alves ³ Denise Mafra ^{1,2}
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Session	Nutrition and metabolism

Abstract, Ethics Committee Number, and Keywords	<p>Current evidence indicates that chronic kidney disease (CKD) is a clinical model of premature aging. Kidney failure causes several important macro and microstructural body changes, increasing the number of senescent cells (SCs). These cells resist apoptosis, leading to upregulating kinases such as p16INK4a (p16) and p53. In addition, SCs induce the production of pro-inflammatory cytokines. This study aimed to evaluate the effects of sulforaphane (SFN) supplementation on mRNA expression of p16 and p53 in non-dialysis CKD patients. This was a longitudinal, double-blind, placebo-controlled study of 25 patients with CKD (stages 3-5). The patients were randomly allocated to the intervention group (2 capsules containing 200 µg of SFN each per day) or the placebo group (2 capsules containing 200 µg of cornstarch per day) for one month. mRNA expression of selected senescence genes (p16 and p53) in isolated peripheral blood mononuclear cells was evaluated by real-time quantitative polymerase chain reaction. Ten patients in the intervention group [59.5 ± 11.9 years, six women, BMI of 28.4 ± 7.3 kg/m² and glomerular filtration rate (GFR) of 42.9 ± 10.6 mL/ min/ 1.73 m²] and 15 patients in the placebo group [60.4 ± 11.9 years, seven women, BMI of 30.0 ± 7.2 kg/m² and GFR of 35.7 ± 12 mL/ min/ 1.73 m²] completed the study. After intervention with SFN, no significant change was observed in the expression of genes p16 (p= 0.6) and p53 (p= 0.7). In conclusion, our findings suggest that the supplementation with two capsules of SFN per day (400 ug/day) for one month did not significantly modulate cellular senescence gene expression markers. However, this does not negate the potential of SFN as a non-pharmacological strategy for CKD, and further research is warranted.</p> <p>Keywords: Sulforaphane, Chronic kidney disease, cellular senescence.</p> <p>The research project was approved by the Ethics Committee of the Faculty of Medicine/UFF (number 39904520.8.0000.5243).</p>
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Title	Plasma levels of trimethylamine-n-oxide (TMAO) in patients with chronic kidney disease under different treatments
Authors	Isadora Britto ¹ Marcia Ribeiro ¹ Danielle Nascimento ¹ Ligia S. Lima ¹ Ludmila Cardozo ² Marcelo Ribeiro-Alves ³ Denise Mafra ^{1,2}
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Session	Nutrition and metabolism

Abstract, Ethics Committee Number, and Keywords	<p>In chronic kidney disease (CKD), elevated uremic toxins produced by the gut microbiota, such as trimethylamine n-oxide (TMAO), influence inflammation exacerbation and contribute to oxidative stress. These complications are putatively linked to the development of cardiovascular diseases, an important cause of death in CKD patients. This cross-sectional study assessed TMAO plasma levels in non-dialysis CKD patients (ND), patients undergoing hemodialysis (HD), and peritoneal dialysis (PD). TMAO plasma levels were assessed by reversed-phase high-performance liquid chromatography (RP-HPLC). Fifteen ND patients [64 (IQR=12.5) years, BMI 25.2 kg/m², eight women]; fourteen PD patients [57.5 (IQR=8.5) years, BMI of 27.87 (IQR=8.3) kg/m², nine women]; and, thirty-four HD patients [43.5 (IQR=45.5) years, BMI of 24.44 (IQR=6.16) kg/m², nineteen women] were analyzed. Patients undergoing HD had higher levels of TMAO [68.6 (IQR=37.17)] when compared to the ND [11.4 (IQR=7.06)] and PD [53.55 (IQR=29.28)] (p<0.01). In conclusion, HD patients had higher TMAO levels than ND and PD patients. Monitoring TMAO plasma levels in hemodialysis patients is important, as elevated levels have been associated with cardiovascular disease risk and mortality in this population. Dietary modifications, such as including bioactive compounds in the daily diet, can modulate the gut microbiota, reduce TMAO production, and decrease the risk of CVD. The research project was approved by the Ethics Committee of the Faculty of Medicine/UFF (number 39904520.8.0000.5243).</p> <p>Keywords: Chronic kidney disease; Uremic toxins; Trimethylamine N-oxide (TMAO).</p>
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Title	Impact of digital monitoring on diabetic patients: a longitudinal analysis of glyceimic factors
Authors	Victor Hugo Leal Magalhães ¹ Mariana Pirani Rocha Machado ¹ Fernanda Regina Casagrande Giachini Vitorino ¹
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Session	Poster

Abstract, Ethics Committee Number*, and Keywords	<p>The use of digital media to monitor diabetic patients offers a revolutionary approach to patient care, providing a range of significant benefits for glyceimic control. This strategy enables the creation of platforms for continuous patient education, empowering them to better understand their condition and adopt healthy lifestyle habits, resulting in improved diabetes management and consequently, a significant enhancement in quality of life. The study obtained approval from the Research Ethics Committee (CEP) involving human subjects at the Federal University of Mato Grosso, under Opinion Number: 4.624.066. All actions in this study complied with the Ethics Criteria in Research with Human Subjects, aiming to minimize risks and discomfort for participants. This study aimed to conduct digital monitoring of diabetic patients, evaluating the evolution of glyceimic related factors during the study. Glyceimic and biochemical data from diabetic patients (n=10), both sexes, were assessed at the beginning (day 0) and end (day 180) of a cycle of interactions using a digital communication application. Information related to diet, exercise, the correct use of medication, among others, was sent three times a week to the participants. Glycemia (159±16 vs. 130±10 mg/dL; p=0.18), glycosylated hemoglobin (7.3±0.5 vs. 7.7±0.6%; p=0.58), triglycerides (175±24 vs. 169±25 mg/dL, p=0.88); microalbuminuria (16.4±4.9 vs. 8.5±3.6 mg/g; p=0.15) did not change between the beginning and end of the study, respectively. Our results show that the intervention was beneficial for reducing glycemia in some patients, while others showed worsening. Therefore, this type of intervention. Although remote patient monitoring offers many benefits, it may not be the best option for all patients. Some individuals may prefer or need in-person interactions with their doctors because of comfort, communication, or medical complexity.</p> <p>Keywords: Digital media; Diabetic patients; Glyceimic control; Patient education.</p>
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Title	Phenylhydrazine-induced anemia worsens glucose tolerance and leads to insulin resistance regardless of the presence of vagus nerve and spleen
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Session	07 – Nutrição e Metabolismo

Abstract, Ethics Committee Number*, and Keywords	<p>Disturbances in iron homeostasis may be related to insulin resistance (IR) and inflammation. Vagus nerve (VN) and spleen participate in the anti-inflammatory reflex. Here we investigated the impact of VN and spleen absence on glucose tolerance and IR in anemic rats. Ethics committee approval nº 13-22. On postnatal day (PND) 60, male Wistar rats underwent subdiaphragmatic vagotomy (SV); splenectomy (SPL); SV+SPL or sham surgery. On PND90, the animals were phenylhydrazine-induced anemia (PHZ; 3 doses; 40 mg/kg). Non-anemic group received saline (0.9%). Oral glucose tolerance test (2 g/kg) was performed on PND116 and the area under curve (AUC) was evaluated. After euthanasia (PND120) biochemical parameters (glucose (Glc), triglycerides (TG), total cholesterol (TC), and iron) were measured. Triglyceride-Glucose index (TyG) was used as a marker of IR. VN and spleen ablation improved oral Glc tolerance, resulting in a reduction in Glc AUC in SV (31%), SPL (42%), and SV+SPL (37%) groups compared to SHAM rats. In contrast, PHZ caused oral Glc intolerance. Thus, PHZ groups displayed a 30% increase in Glc AUC compared to non-anemic animals. At PND120, fasting Glc were higher in SV (14%) and SV+SPL (15%) groups, compared to SHAM. SV (68%), SPL (44%), and SV+SPL (73%) groups also showed higher TC levels compared to SHAM. PHZ did not affect Glc and TC levels. TG levels were higher in SV (59%), SPL (59%), and SV+SPL (60%) groups, compared to SHAM, as well as, in PHZ groups (23%), in relation to non-anemic animals. Serum iron was approximately 40% lower in SV+SPL rats compared to SHAM and SV groups. Moreover, SHAM_{PHZ} (46%), and SV_{PHZ} (26%) groups also presented lower serum iron than the respective non-anemic groups. VN and spleen ablation, as well as, PHZ caused IR, resulting in higher TyG values in SV (7.1%); SPL (6.8%); SV+SPL (6.8%), and PHZ groups (2.3%). In conclusion, PHZ-induced anemia worsens Glc tolerance and IR, regardless of the presence of VN and spleen.</p> <p>Keywords: Glucose tolerance; Anemia; Iron; Vagus nerve; Spleen.</p>
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Title	Mild mitochondrial stress protects abnormal regulation of proopiomelanocortin neurons against palmitate-induced damage by increasing CPT-1 function
Authors	Lara Regina-Ferreira ¹ Fernando Valdivieso-Rivera ^{1,2} Monara K. S. C. Angelim ³ Larissa Menezes dos Reis ³ Vanessa O. Furino ¹ Joseane Morari ¹ Lizandra Maia de Sousa ⁴ Sílvia R. Consonni ⁴ Carlos H. Sponton ^{1,2} Pedro M. Moraes-Vieira ^{1,3} Lício A. Velloso ^{1,5}
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Session	Oral



Abstract, Ethics Committee Number*, and Keywords	<p>Proopiomelanocortin (POMC) neurons located in the mediobasal hypothalamus, act as sensors of systemic signals that indicate the energy reserves stored in the body. The consumption of saturated fatty acids induces damage to pro-opiomelanocortin neurons, generating a positive energy balance, predisposing the development of obesity. The induction of mild mitochondrial stress can protect these neurons against damage caused by fatty acids, mitigating the development of obesity. However, the cellular mechanisms behind the protective effect of mild mitochondrial stress have not been elucidated. In this study, we induced moderate mitochondrial stress in a proopiomelanocortin neuron cell line through the inhibition of Crif1, using a small interfering RNA. Proopiomelanocortin neuron cell line exposed to high level of saturated fatty acid demonstrated anomalous regulation of enzymes that process POMC and severe mitochondrial stress; this was accompanied by a decrease in ATP production and impairment in the fusion/fission cycle. On the other hand, in the partial inhibition of Crif1, all damage caused by exposure to a high concentration of saturated fatty acid was completely reversed; demonstrating an increase in ATP production and improvement in the fusion/fission cycle. Furthermore, partial inhibition of Crif1 promoted a reduction in the dependence on glycolysis for respiration, making the proopiomelanocortin neuron cell line more likely to use saturated fatty acid as a source of energy substrate. These changes depend on the enzymatic activity of CPT-1. Therefore, we identify for the first time, the shift of energy substrate preference toward greater fatty acid oxidation as the mechanism behind the beneficial effects of mild mitochondrial stress on hypothalamic proopiomelanocortin neurons.</p> <p>CIBio/IB No. 2018/Tipo 2-01.</p> <p>Keywords: Hypothalamus, energy balance; energy substrate, obesity, mitochondrial stress, respiration.</p>
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Title	Comparative lipidomic analysis of dry blood spots and plasma samples collected during a dietary challenge
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Session	07 - Nutrition and Metabolism

Abstract, Ethics Committee Number*, and Keywords	<p>Dry blood spots (DBS) consist of a minimally invasive strategy for blood sampling that also has the advantage of low cost and better stability of the samples, while preserving several groups of metabolites. A dietary challenge is an intervention that allows the investigation of the postprandial metabolism and usually consists of the intake of a defined meal and blood sampling in the following hours. Studies that validate the applicability of DBS to collect blood during a dietary challenge for posterior lipidomics analysis are lacking. This study aimed to compare the lipidome of DBS and plasma samples collected simultaneously during a dietary challenge in healthy women. The study was approved by the Research Ethics Committee of the School of Pharmaceutical Sciences of the University of São Paulo (CAAE: 15438019.7.0000.0067), and was conducted with 10 volunteers subjected to a mixed-meal dietary challenge. Capillary blood and DBS samples were collected after a 10-hour fasting ($t = 0$), and at 60, 90, 120, and 150 min after meal ingestion. Ultra-high performance liquid chromatography coupled with mass spectrometry (UHPLC-MS) was used to evaluate the lipidome of DBS and plasma samples. A total of 338 lipids from 13 classes were found in plasma and 307 lipids from 15 classes were identified in the DBS. Despite differences in the matrices, similar results were obtained. Most metabolites were found in both plasma and DBS, with differences in intensity. Free fatty acids and triglycerides were the lipid classes that displayed the highest responses to the meal intake. From these classes of lipids, the metabolites most responsive to the intervention or with the highest intensity have comparable kinetics patterns and relative</p>
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participation on total lipids in both plasma and DBS samples. The results validate the use of DBS for lipidome analysis during the postprandial period, providing a patient-friendly, practical, and cost-effective approach for studying lipid metabolism.

Keywords: DBS; Postprandial; Lipidomics; Metabolism.



Title	Evaluation of novel mechanisms mediating innate immunity of <i>C. elegans</i> in response to metformin
Authors	Letícia de Moraes Souza Deisi L. Braga Marcelo A. Mori
Affiliations	Institute of Biology/UNICAMP, Campinas, Brazil
Session	Poster presentation

Abstract, Ethics Committee Number*, and Keywords	<p>The processes of aging and decline in immune system function are related, and calorie restriction (CR) delays these processes. Metformin is a drug that acts analogous to CR having immunomodulatory and longevity-increasing properties in animal models, including <i>C. elegans</i>. In worms, to extend lifespan, metformin needs to act on live bacteria that the worm feeds on. Recognition of bacteria in the intestine of <i>C. elegans</i> may require double-stranded RNA transporters, such as SID-2. <i>C. elegans</i> treated with metformin show upregulation of genes related to the immune system, including <i>sodh-1</i>. Therefore, this study aimed to understand if metformin confers protection against infection by pathogenic bacteria and whether its longevity-increasing effect is dependent on <i>sodh-1</i> and <i>sid-2</i>. For this, we performed three replicates of experiments to evaluate the lifespan of wild-type worms (N2), <i>sid-2</i> and <i>sodh-1</i> mutants, fed with <i>Escherichia coli</i> (non-pathogenic bacteria) and <i>Pseudomonas aeruginosa</i> (pathogenic bacteria), under treatment with vehicle or metformin. In <i>E. coli</i>, metformin increased lifespan of N2 and <i>sid-2</i> mutants, but not of <i>sodh-1</i> mutants. In <i>P. aeruginosa</i>, metformin also increased survival of N2, while presenting an inconclusive result for the <i>sodh-1</i> and <i>sid-2</i> mutants due to high variability. In <i>E. coli</i>, the deletion of <i>sodh-1</i> extended lifespan relative to N2, but <i>sid-2</i> did not alter survival. In <i>P. aeruginosa</i>, the absence of these genes did not influence lifespan. Therefore, our conclusion is that metformin extends lifespan in <i>E. coli</i> and <i>P. aeruginosa</i>, while this depends on <i>sodh-1</i> in the former. Deletion of <i>sodh-1</i>, but not <i>sid-2</i>, also increases lifespan in <i>E. coli</i>, whereas in <i>P. aeruginosa</i> these genes do not modulate lifespan. Further experiments are needed to evaluate if <i>sid-2</i> and <i>sodh-1</i> are required for the protection against infection conferred by metformin.</p> <p>Keywords: aging, metformin, <i>C. elegans</i>, innate immunity</p>
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Title	Unveiling the transcriptional regulation of GPR3 gene in brown adipocytes
Authors	Vanessa de Oliveira Furino ^{1,2} Fernando Bladimir Valdivieso Rivera ^{1, 2} Carlos Eduardo Pinheiro Leher ^{1, 2} Flavia Caroline Gan ^{1, 2} Lício Augusto Velloso ^{1, 2, 3} Carlos Henrique Grossi Sponton ^{1, 2, 4}
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Session	Poster

Abstract, Ethics Committee Number*, and Keywords	<p>Thermogenic activation of brown adipose tissue (BAT) occurs upon exposure to cold, and counteracts obesity-induced metabolic dysfunction. Cold exposure enhances BAT thermogenesis mainly through the β-adrenergic receptor (βAR) signaling pathway. In a clinical setting, pharmacological activation of β3AR results in adverse cardiovascular effects, limiting its therapeutic application. In contrast, GPR3 has recently been demonstrated to be a ligand-independent G protein-coupled receptor that is activated under cold stress in BAT. GPR3 activation enhances energy expenditure and improves energy homeostasis in mice. Interestingly, cold-induced lipolysis leads to increased GPR3 expression, but its transcriptional regulation remains unknown. Therefore, the goal of this study is to elucidate the transcriptional regulation of GPR3. Our in silico analysis identified several transcription factors that controls the expression of GPR3 in BAT. In this study, we focused on the nuclear receptors Nr1c1 and Nr1c2 based on the presence of a highly homologous ligand-binding domain between Nr1c1/Nr1c2 and Pparg. Single-nucleus RNA sequencing analysis demonstrated that Nr2c1/Nr2c2 has a homogeneous distribution in different subpopulations in humans and mice. Moreover, in silico ablation of Nr2c1/Nr2c2 promoted the downregulation of metabolic pathways, such as oxidative metabolism, lipid oxidation, and mitochondrial function. Our preliminary data suggests that the nuclear receptors Nr1c1 and Nr1c2 as a cold-induced determinants of GPR3 expression in BAT.</p> <p>Keywords: Energy Metabolism; Transcriptional Regulation; Brown adipose tissue.</p>
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Title	E4BP4 prevents obesity-induced mitochondrial fragmentation via transcriptional repression of ceramide synthase 6 in brown adipose tissue
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Abstract, Ethics Committee Number*, and Keywords	<p>The accumulation of ceramides in adipocytes is a hallmark of obesity-induced metabolic dysfunction. In particular, C16 ceramide, which is synthesized by ceramide synthase isoform 6 (Cers6), is associated with mitochondrial dysfunction. C16 binds to the mitochondrial fission factor, leading to increased mitochondrial fission and, consequently, decreased mitochondrial efficiency. In contrast, the activation of thermogenic adipocytes (brown and beige adipocytes) by cold exposure or pharmacological stimuli leads to decreased Cers6 expression and de novo synthesis of C16 ceramide. Despite these findings, transcriptional regulation of ceramide synthesis remains unknown. Our data revealed that the DNA-binding transcription factor E4bp4, also known as nuclear factor interleukin 3 (Nfil3), is an important repressor of ceramide synthesis genes in thermogenic adipocytes. E4bp4 expression is induced by cold or β-adrenergic receptor agonist. Gain-of-function assays (<i>in vivo</i> – CEUA n° 6102-1/2022) have demonstrated that E4bp4 represses the expression of Cers6 in thermogenic adipocytes. E4bp4 binds to a 65 kb upstream distal enhancer to repress Cers6 expression and consequently decreases C16 levels. Genetic deletion of the distal enhancer using CRISPR/Cas9 was sufficient to mitigate Cers6 expression and C16 levels in the brown adipocytes. Moreover, ablation of the Cers6 distal enhancer mitigated mitochondrial fission, leading to enhanced adipocyte thermogenesis. Our preliminary data indicate that E4bp4 leads to the improvement of mitochondrial function by repressing C16 ceramide-induced mitochondrial fission in thermogenic adipocytes.</p> <p>Keywords: ceramides, adipocytes, mitochondrial-fission, transcriptional-regulation</p>
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Title	Preconception treatment with açai juçara (<i>Euterpe edulis</i> Martius) did not interfere with metabolic parameters of advance-aged female Wistar rats
Authors	Mayra Dias Rodrigues ¹ Brenda Francisconi Diaz ² Fernando Henrique Borges ¹ Rhauany Pelisson Guergolette ¹ Larissa Rugila dos Santos Stopa ¹ Ernane Torres Uchoa ¹ Karla Bigetti Guergolette ² Graziela Scaliante Ceravolo ¹
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Session	Nutrição e metabolismo

Abstract, Ethics Committee Number*, and Keywords	<p>Preconception care is defined as a series of interventions aimed at identifying and modifying biological, behavioural and social hazards to a woman's health or pregnancy outcomes through prevention and management. Preconception care includes good nutrition and the consumption of açai juçara (<i>Euterpe edulis</i> Martius) can be a good alternative for the inclusion of antioxidants polyphenols in the diet. However, it is not known if the preconception treatment with açai juçara would affect metabolic parameters in healthy advance-aged females. So, this study aimed to evaluate the effects of the juçara pulp, before pregnancy, on the maternal metabolic and litter parameters of pregnant rats at an advanced reproductive age. All animal procedures were approved by the Ethics Committee on the Use of Animals, protocol n° 33/2022. At postnatal day 168, healthy female Wistar rats were divided into two groups receiving juçara pulp (JU group) or tap water (control/CTR group) by gavage for six weeks and then put for mating with males. When pregnant, maternal weight gain was recorded weekly. On gestational day (GD) 19, oral glucose tolerance test was performed, and obtained a glucose uptake curve and the area under the curve. In the next day (GD 20), the rats were euthanized, blood serum was collected, and the litter of each mother was weighed and counted. The serum was stored at -80°C until dosages of total cholesterol, high-density lipoprotein and triglycerides were made. For statistical analysis, the T-test was used, differences when $p < 0.05$. The results are expressed as mean±SEM, n=number of rats/group. Preconception treatment with juçara pulp reduced</p>
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basal glucose levels (CTR: $89.63 \pm 2,80$, $n=8$ vs JU: $81.00 \pm 2,05$, $n=7$), but didn't interfere in the other metabolic parameters evaluated, in the maternal weight gain and in the litter parameters either. These results suggest that the consumption of juçara pulp can be secure for women in preparation for pregnancy at an advanced age.

Keywords: Advanced age. Juçara. Preconception care.



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Title	Bisphenol S triggers insulin resistance, induces hepatic mitochondrial biogenesis dysfunction and endoplasmic reticulum stress
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Session	Pôster

Abstract, Ethics Committee Number*, and Keywords	<p>Obesity, characterized as a risk factor for insulin resistance, is linked with metabolic-associated fatty liver disease (MAFLD). In response to obesity and MAFLD, mitochondrial biogenesis (MBG) dysfunction and endoplasmic reticulum stress (ERS) occur. Bisphenol S (BPS) is potentially obesogenic and very little studied. The aim of the study is to analyze the effects of BPS exposure, with or without an association of a high-fat diet, on biochemical and hepatic parameters. C57BL/6 adult male mice were divided into: standard diet (SC), standard diet exposed to BPS (SCB, 25 µg/kg/day), high-fat diet (HF), high-fat diet exposed to BPS (HFB, 25 µg/kg/day) for 12 weeks. Oral glucose tolerance test (OGTT), plasma and molecular analyses were evaluated. Meanstandard deviation, one-way ANOVA followed by Holm-Sidak post-test ($p < 0,05$). All experimental groups showed a greater fasting glucose of OGTT (SCB: +17%,$p < 0,05$; HF: +34%,$p < 0,001$; HFB: +35%,$p < 0,0001$) and insulin (SC: +104%,$p < 0,05$; HF: +100%,$p < 0,05$; HFB: +189%,$p < 0,0001$), HOMA-IR (SCB: +112%,$p < 0,01$; HF: +136%,$p < 0,01$; HFB: +266%,$p < 0,0001$), expression of ATF4 (SCB: +85%,$p < 0,05$; HF: +209%,$p < 0,05$; HFB: +297%,$p < 0,01$), CHOP (SCB: +50%,$p < 0,05$; HF: +76%,$p < 0,01$; HFB: +109%,$p < 0,001$), MFF (SCB: +85%,$p < 0,05$; HF: +130%,$p < 0,01$; HFB: +268%,$p < 0,0001$), DRP1 (SCB: +20%,$p < 0,05$; HF: +24%,$p < 0,05$; HFB: +56%,$p < 0,0001$) and MFN2 (SCB: +72%,$p < 0,05$; HF: +150%,$p < 0,01$; HFB: +204%,$p < 0,0001$) compared to the SC group. HFB group presented a higher fasting glucose of OGTT (+16%,$p < 0,05$) and insulin (+42%,$p < 0,05$), HOMA-IR (+73%,$p < 0,001$), expression of ATF4 (+115%,$p < 0,05$), CHOP (+39%,$p < 0,05$), MFF (+99%,$p < 0,0001$), DRP1 (+30%,$p < 0,01$) and MFN2 (+45%,$p < 0,01$), besides a lower expression of OPA1(-38%,$p < 0,01$) compared to the SCB group. HFB group showed increased fasting insulin (+44%,$p < 0,05$), HOMA-IR (+55%,$p < 0,01$), expression of MFF (+60%,$p < 0,001$) and DRP1 (+25%,$p < 0,01$), in addition to a decreased OPA1 (-24%,$p < 0,01$) expression compared to the HF group. BPS triggered insulin resistance and induced hepatic MBG impairment and ERS.</p> <p>KEYWORDS: Obesity. MAFLD. Bisphenol S. Insulin resistance. Mitochondrial biogenesis. Endoplasmic reticulum stress. COMITÉ ÉTICO: CEUA 1929240521</p>
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Title	Investigation of the influence of the FGF21 on the modulation of hypothalamic neuropeptides and its correlation with offspring metabolism
Authors	Alves, L.V.G. ¹ ; Baqueiro, M. B. ² ; Nascimento, L. C.P ² ; Silva, M. J ² , Torsoni, M.A ² ; Torsoni, A. S ² .
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Session	07

Abstract, Ethics Committee Number*, and Keywords	<p>Dysregulation of central hypothalamic physiological mechanisms responsible for appetite control is a significant contributor to the development of obesity. Among the factors influencing hypothalamic appetite control is fibroblast growth factor 21 (FGF21), which has garnered increasing attention for its role in various metabolic processes, including appetite regulation, weight control, brown adipose tissue thermogenesis, and modulation of hunger and satiety neuropeptides. Animal experiments were conducted with approval from the Ethics Committee on the Use of Animals at the University of Campinas (CEUA/UNICAMP), protocol number 5639-1/20. Female C57 mice were fed a control diet (CT; 10% fat; 70% carbohydrates, n=10) or a high-fat diet (HF; 45% fat; 35% carbohydrates, n=10) during pre-gestation and lactation. Milk intake and offspring weight gain were evaluated during lactation. After weaning, gene expression (qPCR) of Fgf21, TNFα, IL1B in the liver (n=6-7/group) and genes involved in appetite control in the arcuate nucleus (n=5-7/group) were performed. Offspring from HF dams exhibited increased body weight compared to offspring from CT dams, starting from the 12th day of life. Milk intake was higher in female offspring from HF dams during the first week of lactation (HF 0.16\pm0.09 vs CT 0.05\pm0.02 p=0.02). Additionally, a trend towards increased cumulative milk intake was observed in HF females (HF 0.11\pm0.10 vs CT 0.03\pm0.03 p=0.06).</p> <p>Although no significant differences were observed in hypothalamic neuropeptides and hepatic Fgf21 expression, maternal consumption of a lead to increased milk intake, elevated body weight and higher levels of pro-inflammatory cytokines in the offspring. It is hypothesised that Fgf21 levels may be altered in the circulation of offspring from HF dams.</p> <p>Ethics Committee Number: CEUA 56391/20 Keywords: Hyperphagia, Fgf21, Metabolic Programming</p>
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Title	The anti-inflammatory effect of bergamot attenuates diet-induced cardiac remodeling by regulating the protein expression of the collagen/metalloproteinase axis
Authors	Taynara Aparecida Vieira ¹ Juliana Silva Siqueira ¹ Erika Tiemi Nakandakare-Maia ¹ Núbia Alves Grandini ¹ Silméia Garcia Zanati Bazan ¹ Fabiane Valentine Francisqueti-Ferron ¹ Artur Júnio Togneri Ferron ² Camila Renata Corrêa ¹
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Session	Poster

Abstract, Ethics Committee Number*, and Keywords	<p>Inflammation resulting from obesity acts on the pathogenesis of cardiac remodeling through different mechanisms, among them, the modulation of collagen deposition by the activity of metalloproteinases. Bergamot is a fruit rich in flavonoids and exhibits anti-inflammatory action. However, little is explored regarding this action in its leaves. Thus, this study aimed to investigate the anti-inflammatory effect of bergamot leaves extract (BLE) in attenuating cardiac remodeling in obese rats through the regulation of the protein expression of the collagen/metalloproteinase axis. Wistar rats (n=18) were distributed into two groups: control diet (C, n=6) and high sugar-fat diet (HSF, n=12) for 20 weeks. After the cardiac remodeling was detected by echocardiography, the animals were again distributed to receive BLE (50mg/kg/day) or placebo (water) via gavage for 10 weeks: C (n=6), HSF (n=6) and HSF+BLE (n=6). This study was approved by the Animal Ethics Committee (1393/2021). After the 30th week, the following were evaluated: adiposity index, echocardiographic profile, cardiac inflammatory markers, MMP-2 activity in its active form and protein expression of myocardial type III collagen; (p<0.05). The HSF group exhibited a high adiposity index (HSF 8.77±2.64 vs C 3.09±1.02, p=0.007), cardiac remodeling and dysfunction, inflammation, decreased active MMP-2 activity (HSF 0.43±0.09 vs C 0.71±0.07, p=0.009), as well as increased expression of type III collagen (HSF 1.32±0.27 vs C 1.00±0.18, p=0.038). Conversely, animals receiving the extract did not experience a reduction in adiposity index, but showed improvement in cardiac remodeling and function, as well as inflammation, active MMP-2 activity (HSF+BLE 0.84±0.22 vs HSF 0.43±0.09, p<0.001) and collagen expression (HSF+BLE 0.68±0.11 vs HSF 1.32±0.27, p<0.001). Therefore, the anti-inflammatory effect of BLE improved diet-induced cardiac remodeling by regulating the protein expression of the collagen/metalloproteinase axis.</p> <p>Keywords: Obesity; Inflammation; Cardiac remodeling; Collagen; Bergamot</p>
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Title	Could obesity induced by a high fat diet during the last programming window, adolescence, be avoided? A case for PPAR-alpha
Authors	Ana Letícia Manso Assakawa Rafael Pereira Lopes Letícia Ferreira Barbosa Ana Carolina Carvalho de Sá Beatriz Marques Ferreira Maria Natália Chimirri Peres Scarlett Rodrigues Raposo Claudio Guilherme de Assis Oliveira Luís Fernando Fernandes Miranda Paulo Cezar de Freitas Mathias Lucas Paulo Jacinto Saavedra
Affiliations	Department of Genetics, Cell Biology and Biotechnology – UEM
Session	Nutrição e Metabolismo

Abstract, Ethics Committee Number*, and Keywords	<p>Stimuli during critical phases of development can program the organism for health or disease at adulthood. Recent studies have shown that adolescence can be the last susceptible phase. Thus, we hypothesize that a high-fat diet (HFD) in this phase causes malprogramming and the only way to hamper this is through interventions in this period. The activation of PPARα promotes lipid oxidation and could be a potential therapeutic target. Experiment 1: to consolidate adolescence as a programming window, HFD (5.8 Kcal/g) was given to male Wistar rats from postnatal day (PN) 30 to 60 forming the experimental groups HFD (n=6-16) and control (NFD n=4-14). Experiment 2: To test our hypothesis that the activation of lipid oxidation during adolescence could protect against obesity and metabolic dysfunction induced by HFD, we administered an PPARα agonist (Fenofibrate-F, i.p.50mg/kg) HFD-F (n=7-18) or vehicle HFD-V (n=6-16) during the same period. Animals from both experiments were evaluated at adulthood (PN120) CEUA: 4945240423. HFD leads to increased weight gain (NFD 20176\pm1085 vs HFD 21322\pm899A.U p<0.01), perigonadal fat (NFD 4.7\pm0.8 vs HFD 5.7\pm1.1g p<0.05), retroperitoneal fat (NFD 6.0\pm1.1 vs HFD 7.5\pm1.6g p<0.05), plasma triglycerides (NFD 66.2\pm16.1 vs HFD 85.1\pm23.5mg/dL p<0.05) and brown adipose tissue (BAT) lipid droplet area (NFD 63.1\pm2.3 vs HFD 67.1\pm1.8% p<0.05), also decreased BAT sympathetic nerve activity (SNA)(NFD 27.7\pm8.4 vs HFD 17\pm2.5spikes/5s p<0.05). PPARα activation decreased weight gain (HFD-V 21322\pm899 vs HFD-F 1002\pm1002A.U p<0.0001), perigonadal fat (HFD-V 5.7\pm1.1 vs HFD-F 4.6\pm1.4g p<0.05), retroperitoneal fat (HFD-V 7.5\pm1.6 vs HFD-F 6.2\pm1.4g p<0.05) and plasma triglycerides (HFD-V</p>
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	<p>85.1±23.5 vs HFD-F 66.3±17.3mg/dL p<0.05), moreover increased BAT SNA (HFD-V 17±2.5 vs HFD-F 23.1±5.7spikes/5s p<0.05). In conclusion, HFD during adolescence programs to obesity and BAT dysfunction at adulthood, which is hampered by PPARα activation in the same phase. DOHaD, Obesity</p>
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Title	Metformin during adolescence can improve the effects of neonatal overnutrition
Authors	Scarlett Rodrigues Raposo Rafael Pereira Lopes Leticia Ferreira Barbos Ana Carolina Carvalho de Sá Maria Natália Chimirri Peres Lucas Paulo Jacinto Saavedra Luiz Gustavo Antunes Marcos Vinicius Martins Ana Leticia Assakawa Ananda Malta Paulo Cesar de Freitas Mathias
Affiliations	Departament of Cell Biology and Genetics, State University of Maringá, Maringá, Brasil.
Session	Nutrition and Metabolism

Abstract, Ethics Committee Number*, and Keywords	<p>Childhood obesity is a major public health problem as it leads to metabolic dysfunction (MD) in adulthood. This is well elucidated by the DOHaD concept (Developmental Origins of Health and Disease), as interventions during critical developmental stages (gestation/lactation/adolescence) can program for health or disease. Metformin is a first-choice drug to treat MD, found in obesity and diabetes type 2, among others. Considering the plasticity present during adolescence, we hypothesize that metformin treatment can attenuate the MD acquired during lactation by the rat neonatal overfeeding model. Our project was approved by the ethics committee under protocol number 4718150623. To induce obesity, we reduced the litter size to 3 pups per dam (SL), while the control remained with 9 pups (NL). We treated with metformin from 30 to 60 days-old, with a dose of 300mg/kg in drinking water, resulting in the SL-M (N=4 litters) and NL-M (N=4 litters), or metformin untreated to have NL-C (N=4 litters) and SL-C (N=4 litters) groups. Two months after the end of treatment, we were able to replicate the obesity model; SL-C animals had significantly heavier body weight (SL-C 28788.23 ± 321.9; NL-C 24687.94 ± 168.2; $p < 0.0001$) and retroperitoneal fat (SL-C 1.98 ± 0.09; NL-C 1.351 ± 0.05; $p < 0.0001$) than NL-C animals. Metformin treatment significantly reduced body weight (SL-M 27313.55 ± 273.4; $p < 0.005$) and retroperitoneal fat in SL-M (1.67 ± 0.08; $p < 0.005$) animals compared to SL-C. Preputial separation is considered a marker of the adolescent onset. SL-C animals had earlier preputial separation (35.5 ± 0.42; $p < 0.0001$) compared to NL-C (39.8 ± 0.38; $p < 0.0001$), while, the SL-M (40.0 ± 1.30; $p < 0.05$) group had normalized preputial separation. We conclude that metformin when administered during adolescence can attenuate</p>
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	<p>MD. Possibly, the reprogramming mechanisms necessarily involve puberty normalization.</p> <p>Metformin, obesity, metabolic dysfunction, adolescence.</p>
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Title	Transcriptomic alterations in gastrocnemius muscle during cancer cachexia-associated sarcopenia under the effects of spontaneous physical activity and leucine-rich diet
Authors	<u>Leisa Lopes-Aguiar</u> ^{1*} , João Paulo Dorta Machado ² , Guilherme Augusto da Silva Nogueira ¹ , Rogério Willians dos Santos ¹ , Laís Rosa Viana ¹ , André Schwambach Vieira ² , Maria Cristina Cintra Gomes-Marcondes ^{1*}
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Session	07 - Nutrição e Metabolismo

Abstract, Ethics Committee Number*, and Keywords	<p>Cachexia and sarcopenia, generally due to cancer and age-related conditions, respectively, were characterised by loss of muscle mass. Current evidence has shown that physical exercise and a leucine-rich diet modulate muscle metabolism in pre-clinical cancer cachexia. However, this role in the process of cancer cachexia-associated sarcopenia is not totally known. Therefore, we evaluated this relationship in ageing Walker 256 tumour-bearing rats under the effects of physical exercise and a leucine-rich diet. Ageing male Wistar rats, with and without access to spontaneous physical activity wheel, were distributed into experimental groups: control diet plus exercised (CE) and sedentary (CS); 3% leucine-rich diet plus exercised (LE) and sedentary (LS); Walker 256 tumour-bearing, control diet plus exercise (WE) and sedentary (WS) and Walker 256 tumour-bearing, 3% leucine-rich diet plus exercise (WLE) and sedentary (WLS). After 21 days or a pre-agonic state, all animals were euthanised for RNA-Seq transcriptomic analysis of the gastrocnemius muscle. The differentially expressed genes (DEGs) were calculated and submitted to Gene Ontology enrichment analysis. The DEGs identified in comparisons between groups were 4,466 (WSvsCS), 4,044 (WLSvsLS) and 44 (WLSvsWS); 3,896 (WEvsCE), 2,574 (WLEvsLE) and 7 (WLEvsWE); 1 (CEvsCS), 41 (WEvsWS) and 2 (WLEvsWLS). Interestingly, upon comparison of the WLEvsWLS groups, the gene <i>Lpal2</i> was upregulated exhibiting the largest log₂FC value (20.98). Additionally, all 7 DEGs in WLEvsWE groups and 43 out of 44 DEGs in WLSvsWS groups were downregulated. The most significantly enriched biological processes in almost all comparisons were ribonucleoprotein complex biogenesis and generation of precursor metabolites and energy. Our findings suggest that, during cancer cachexia-associated sarcopenia, the combination of spontaneous physical activity</p>
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and a leucine-rich diet can modify ribosome and metabolic-related transcripts in the gastrocnemius muscle.

Ethics Committee Number: 5843-1/2021 and 6110-1/2022

Keywords: cancer cachexia; sarcopenia; physical activity; leucine-rich diet; transcriptomic



Title	Metformin in adolescence attenuates metabolic dysfunctions in rats programmed early for obesity
Authors	Rafael Pereria Lopes Scarlet Rodrigues Raposo Letícia Ferreria Barbosa Luis Gustavo Antunes Pessoa Maria Natália Chimirri Peres Ana Carolina Carvalho de Sá Lucas Paulo Jacinto Saavedra Ana Letícia Manso Assakawa Fernanda Sayuri Fuzishima Marcos Vinícius Martins Paulo Cezar de Freitas Matias
Affiliations	1- DOHaD, Maringá, PR.
Session	Section 07- Nutrition and Metabolism

Abstract, Ethics Committee Number*, and Keywords	<p>Obesity is a global public health problem caused by excess body fat, with negative health effects, dyslipidemia and fat accumulation. The concept known as the Developmental Origins of Health and Disease (DOHaD) investigates how early exposures affect long-term health. Our aim is to verify the effects of a short-term treatment with metformin during adolescence on the metabolic parameters of rats programmed for obesity. The experimental protocols will follow the legislation of the National Council for the Control of Animal Experimentation CONCEA: 6943140224 and the ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments). We used male Wistar rats as an experimental model, which were reduced to 9 pups per lactating mother for the control litters (NL) and 3 pups (SL) for the induction of obesity through neonatal overnutrition. The control groups received an intraperitoneal injection of saline solution (NaCl 0.09%) (NL-C and SL-C) and the groups treated with metformin 100mg/kg (NL-M and SL-M) during 35 to 42 days of life and were evaluated at 105 days. Two months after the end of treatment, the SL-C animals were significantly heavier than the NL-C (SL-C 22979 ± 251.2; NL-C 21229 ± 332.3; $p < 0.05$) accumulated perigonadal fat (SL-C 1.5 ± 0.1; NL-C 1.1 ± 0.047; $p < 0.005$) and showed peripheral insulin resistance (SL-C 0.5 ± 0.1; NL-C 2.8 ± 0.1; $p < 0.001$). SL rats treated with metformin for 12 days during puberty reduced weight (SL-M 22662.6 ± 275.8 $p < 0.05$), glucose intolerance (SL-M 10716.5 ± 849.9 $p < 0.05$) and improved peripheral insulin resistance (SL-M 1.9 ± 0.2; NL-M 3.0 ± 0.5 $p < 0.05$). Fat accumulated in the liver of SL-M animals was drastically reduced by metformin treatment (SL-M 36.1 ± 2.6 $p < 0.05$). We conclude that treatment with metformin in adolescence was able to inhibit the programming of metabolic</p>
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dysfunction caused by overnutrition in childhood. It is worth noting that the treatment was of short duration, marked by the preputial separation of the rats.

Keywords: metformin, adolescence and obesity



Title	Consumption of Yerba mate (<i>Ilex paraguariensis</i>) increases the deposition of cardiac collagen and changes its organization: animal experimental study
Authors	Isabelle TiburcioPecin Ferreira ¹ Danilo Silva Martins Santos ¹ Carlos Eduardo Brochini de Paiva ¹ Thaíssa Baptista Brochini de Paiva ¹ Sandra Cristina Genaro ¹ Thaoan Bruno Mariano ¹ Julia Amanda Rodrigues Fracasso ² Lucas Pires Guarnier ³ Francis Lopes Pacagnelli ¹
Affiliations	1 – University of West Paulista, Presidente Prudente, Brazil 2 – University State Paulista, Araçatuba, Brazil 3 – University of Sao Paulo, Ribeirão Preto, Brazil
Session	Nutrition and Metabolism

Abstract, Ethics Committee Number*, andKeywords	<p>Yerba mate (<i>Ilex paraguariensis</i>) is known for its therapeutic properties, including antioxidant, anti-inflammatory, antihypertensive and antiatherosclerotic actions, but concerns persist regarding the caffeine in its composition and its impact on the cardiovascular system. This study investigated the effects of yerba mate consumption on cardiac structural aspects in relation to collagen in rats. This study was approved by the Animal Use Ethics Committee (Protocol 5300) and was conducted following the ARRIVE guidelines. Twenty-four male Wistar rats were divided into two groups: Control (CG, n=12), which received filtered water and a standard diet, and Erva Mate (EM, n=12), which received 6 g of <i>Ilex paraguariensis</i> in 100 ml/water and the same diet for 30 days. After this period, the animals were euthanized and histological sections of the left ventricle were stained with Picrosirius Red to analyze the percentage of collagen, and then subjected to polarized light to evaluate the types of collagen and the fractal dimension was also performed. The Imagem J program was used. The Shapiro Wilk test was used to analyze normality and the t test or Mann Whitney test was used to compare parametric data for non-parametric data ($p < 0.05$). There was an increase in collagen deposition in the left ventricle of animals subjected to yerba mate (CT: $3.53 \pm 0.56\%$; ME: $4.59 \pm 0.64\%$, $p = 0.003$), and also a reduction in size fractal (CT: $1.62 \{1.51- 1.73\}$; MS: $1.42 \{1.30- 1.55\}$; $p = 0.001$). In relation to the types of collagen, consumption of yerba mate did not change collagen type I (CT: 31.73 ± 10.39 ua ME: 26.90 ± 1.04 ua) and type III (CT: 16.88 ± 8.75 vs. EM: 12.21 ± 1.09 ua). Consumption of yerba mate for 30 days promoted cardiac structural changes by increasing collagen deposition and altering the fractal dimension in the left ventricle.</p> <p>Keywords: Tereré, <i>Ilex Paraguariensis</i>, yerba mate, cardiac remodeling, fractal dimension, and hypertrophy.</p>
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Title	Effect of cyclic hyperlipidic diet (HFD) on central control of energy metabolism and glycemic homeostasis in Swiss mice
Authors	1 – Erika Anne de Freitas Robles Roman 2 – Bruna Bombassaro 3 – Letícia da Silva Pires Canevare 4 – Pedro Augusto da Silva Nogueira 5 – Marcela Reymond Simões 6 – Ana Luisa Gallo Ferraz 7 – Licio Augusto Velloso 8 – Márcio Alberto Torsoni
Affiliations	1,2,3,5,6 and 7 – Faculty of Medical Sciences (FCM), University of Campinas (UNICAMP), Campinas-SP, Brazil; 4 – Cell Biology Department, University of Texas Southwestern Medical Center (UTSW), Dallas-Texas, United States; 8 – Nutrition, Sports and Metabolism Science, School of Applied Sciences (FCA), University of Campinas (UNICAMP), Campinas-SP, Brazil.
Session	Nutrição e Metabolismo

Abstract,	<p>According to WHO, obesity is a chronic complex disease defined by excessive fat deposits that can impair health. In most cases, is a multifactorial disease due to obesogenic, psycho-social factors, and genetic variants. Many scientific articles have been showed that a high- fat diet intake is a determinant factor in the gain of weight. As a consequence, to avoid the weight gain process many people adopt restrictive diets However, food is an important factor in each culture and contributes to the peculiar identity of the different countries. With that in mind, the objective of this work was to investigate if the intake of a cyclic high-fat diet promoted the same negative results on energy metabolism and glycemic homeostasis when compared to a continuous intake of a high-fat diet. To answer this question, 6-weeks male mice were divided into three groups: i) High-fat diet (HFD); ii) Chow diet (CD), and iii) Cyclic high-fat diet/chow diet (CHFD), and they were followed by 6 weeks. Food intake, body weight, and body composition, as well as, lipid and hormone profile, ipGTT, ipITT, respirometry, neuropeptide expression, mitochondrial oxygen consumption, and temperature were measured. CHFD and CD group presented the same ponderal evolution, adiposity, leptinemia, insulinemia, fasting glycaemia, glucose tolerance, lipid profile and cumulative food intake. All these parameters are higher on HFD group, except the cumulative food intake; CHFD group increases high-fat diet intake on weekends while compensate during the week decreasing the ingestion of chow diet; There were no difference in the VO₂, VCO₂, EE, Interscapular temperature and BAT activity between the 3 groups; CHFD and CD group presented the same core temperature and lower hypothalamic astrocytes and microglial activation than HFD group suggesting that hypothalamic leptin sensibility is preserved.</p> <p>- CEUA 5512-1/2020 and CEUA 6142-1/2022 - Obesity, leptin, cyclic high-fat diet, energetic metabolism, glycemic homeostasis.</p>
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Title	Hepatoprotective effect of citral against injury caused by metabolic endotoxemia in adult male C57BL/6J mice fed with a high-fat diet
Authors	Mariana Moraes Fioravanti Maycon Tavares Emilio-Silva Felipe Lima Dario Vinicius Peixoto Rodrigues Gabriela Bueno Felipe Leonardo Fagundes Laura Vitória Fortunato de Oliveira Isabela Galende Guidolin Clélia Akiko Hiruma-Lima
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Session	Nutrition and Metabolism - Oral presentation

Abstract	<p>Obesity is a global public problem. Intake of a high-fat diet (HFD) can compromise liver function and promote non-alcoholic fatty liver disease (NAFLD) development. NAFLD has an inflammatory process enhanced by increased systemic lipopolysaccharide (LPS) levels during disease. Citral (CT) is a natural product with proven anti-inflammatory action. Thus, we aimed to evaluate the CT protective effect in NAFLD using obese C57Bl/J6 mice with LPS-induced metabolic endotoxemia. Male mice (5-6 weeks) were separated into 4 groups (n=10) according to diet and treatments, respectively: standard diet (SD) + vehicle (1% Tween 80, 10 mL/kg); HFD + vehicle; HFD + LPS (10 µg/kg; intraperitoneal); and HFD + LPS + CT (300 mg/kg, orally). The metabolic endotoxemia was induced by injection with LPS once a week along the HFD fed. After 10-week, the animals were euthanized and liver samples were used to evaluate the inflammatory activity, lipid peroxidation and antioxidant capacity through biochemistry assay. The statistical analysis was determined by one and two-way ANOVA followed by Tukey's post-test ($p < 0.05$) (CEUA 6702310820). LPS together with the HFD intake caused a significant increase in body weight and adiposity index in relation to the HFD group ($p < 0.05$). It also led to a reduction in liver mass, associated with liver damage, even in the CT-treated group ($p > 0.05$). However, CT reduced myeloperoxidase activity and malondialdehyde levels in the liver compared to the HFD group ($p < 0.05$), demonstrating the anti-inflammatory and protective activity of the monoterpene against liver damage characteristic of NAFLD. The antioxidant capacity of CT was observed by preventing the increase in superoxide dismutase activity caused by HFD ingestion, when compared to the SD group ($p < 0.05$). Thus, CT prevents hepatic alterations in NAFLD caused by HFD ingestion and metabolic endotoxemia, through an anti-inflammatory and antioxidant effect.</p> <p>Keywords: NAFLD; obesity; lipopolysaccharide; inflammation; monoterpene.</p>
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Title	Supplementation with a commercial <i>Agaricus blazei</i> Murril extract did not affect obesity and glucose homeostasis in obese mice
Authors	Edson Júnior Rossi Silva, João Pedro Giannotti Alves, Heloisa de Matos Maillard, Maria Lúcia Bonfleur.
Affiliations	Western Paraná State University, Cascavel, Brazil
Session	Poster

Abstract, Ethics Committee Number*, and Keywords	<p><i>Agaricus blazei</i> Murril (ABM) is a mushroom popularly known for its benefits on weight loss, diabetes, and dyslipidaemia but, the scientific literature is scarce regarding the effects upon obesity. So, we aimed to evaluate the effects of a commercially available ABM extract on obesity and glucose homeostasis in mice fed with a high-fat diet (HFD). The 60-day-old male C57Bl/6 mice were divided into three groups: HFD-CTL, HFD-ABM1 and HFD-ABM2. At 60 days after HFD ingestion, ABM1 and ABM2 received the extract orally by gavage at doses of 8.46 mg and 4.32 mg daily for 88 days. The body weight (BW) and food intake were measured weekly. In the last week of supplementation, the oGTT and ipITT were performed. At euthanasia, tissues were collected and weighed. All experimental procedures were approved by CEUA/Unioeste: 23-24. Total weight gain and food ingestion were not altered between the groups. HFD-CTL, HFD-ABM1 and HFD-ABM2 mice displayed similar fasting glycemia (128 ± 6.3, 134 ± 4.8, and 143 ± 6.9 mg/dL), cholesterolemia (174 ± 16; 124 ± 22, and 146 ± 17 mg/dL), and triglyceridemia (51 ± 7.7, 52 ± 3.9, and 52 ± 3.6 mg/dL) respectively. Total glycemia during GTT did not differ significantly among all groups (32934 ± 1162, 30883 ± 1192, and 31729 ± 1282 mg/dL.min⁻¹, in HFD-CTL, HFD-ABM1 and HFD-ABM2, respectively). Also, the K_{ITT} was similar between HFD-CTL, HFD-ABM1 and HFD-ABM2 (5.3 ± 1.2, 4.5 ± 0.5, and 4.3 ± 0.9 % blood glucose disappearance, respectively). In addition, liver, pancreas, spleen, perigonadal and retroperitoneal fat were similar across all groups. In summary, supplementation with this commercial ABM extract at the concentrations used and over the period studied was not effective in reducing obesity and glucose disturbances in mice fed a HFD.</p> <p>Keywords: high-fat diet, obesity, mushroom</p>
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Title	Effects of acute exposure to a high-fat diet on epigenetic mechanisms that can modulate the expression of the $\alpha 7nAChR$ receptor
Authors	Melissa Santos de Aguiar, Isis de Cássia Martins, Wenicios Ferreira Chaves, Adriana Souza, Marcio Alberto Torsoni
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Session	07 - Nutrition and Metabolism

Abstract, Ethics Committee Number*, and Keywords	<p>The $\alpha 7$ nicotinic acetylcholine receptor ($\alpha 7nAChR$) plays a crucial role in the cholinergic anti-inflammatory response and is now also implicated in regulating energy balance in the hypothalamus. The expression and activity of the $\alpha 7nAChR$ are governed by a variety of cellular mechanisms that can either enhance or suppress the <i>Chrna7</i> gene expression. Factors like methylation and chaperone protein-mediated maturation influence the presence and function of the receptor on the cell membrane. This study explores the impact of short-term exposure to a high-fat diet on epigenetic modifications linked to $\alpha 7nAChR$ in hypothalamic cells. Male mice at the 8th week of life were assigned to either a control group or a group subjected to a high-fat, high-calorie diet for 3 days (DHH; 45% kcal from lipids). At the end of the dietary regimen, the animals were euthanized, and samples from both serum and hypothalamus were collected. The serum was then utilized to treat mHypoA-POMC/GFP cells, and subsequent inflammatory effects on the pre-transcriptional and post-translational regulation of $\alpha 7nAChR$ expression were assessed. Statistical analysis was performed using mean \pm standard error of the mean and assessed using a T-test, with the significance level set at $p < 0.05$. Following acute exposure to the high-fat diet, a reduction in hypothalamic <i>Chrna7</i> expression was observed alongside decreased levels of <i>Ric3</i> and <i>DNMT3A</i>. In vitro experimentation showed increased activity of the transcription factors <i>PPARG</i> and <i>Egr1</i>. These findings suggest a potential involvement of post-translational mechanisms, indicating a possible reduction in chaperone proteins responsible for $\alpha 7nAChR$ functionality. Additionally, the upregulation of <i>PPARG</i> and <i>EGR-1</i> may contribute to the decreased <i>Chrna7</i> mRNA levels through the inhibition of its transcription.</p> <p>CEUA 6292-1/2023 Keywords: $\alpha 7nAChR$; <i>Chrna7</i>; hypothalamus; Epigenetic.</p>
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Title	Branched-chain amino acid (BCAA) leucine supplementation attenuates fatty liver disease in mice
Authors	Thayna dos S. Vieira Natália P. Corte Érique de Castro Álbert S. Peixoto Marina A. A. Honda Bianca F. Leonardi Natália M. Pessoa Érika Vicência M. Pessoa Ana B. Pires William T. Festuccia
Affiliations	Institute of Biomedical Sciences, University of São Paulo – Brazil.
Session	07 - Nutrition and Metabolism

Abstract, Ethics Committee Number*, and Keywords	<p>Introduction: Patients with Metabolic Associated Fatty Liver Disease (MAFLD) show elevated levels of branched-chain amino acids (BCAA) in serum, but the connections between MAFLD and BCAA metabolism and signaling in the liver remain unclear. Objective: We investigated herein the impact of dietary leucine supplementation on MAFLD development and progression with focus on the involvement of mechanistic target of rapamycin complex 1 (mTORC1) as a mediator. Methodology: Mice with hepatocyte deletion of either Pten or Pten+Raptor and control littermates were fed or not with a diet supplemented with leucine for 8 weeks and evaluated for liver morphology and metabolism (CEUA N° 6160250820). Results: Leucine supplementation attenuated the increase in liver mass displayed by mice with hepatocyte deletion of either Pten (2326 ± 263 vs 1634 ± 45.5) or Pten+Raptor (2629 ± 13 vs 2084 ± 195) at 20 weeks of age, but accelerated tumor development in older mice with hepatocyte Pten+Raptor deletion as evidenced by increased tumor number (6.8 ± 1.28 vs 10.80 ± 2.85) and size (2.18 ± 0.48 vs 3.60 ± 0.80) despite reduced liver mass compared to diet control (3176 ± 233 vs 2576 ± 303). Conclusion: Dietary leucine supplementation reduces liver mass independently of mTORC1, but accelerate liver tumor development in mice with hepatocyte Pten+Raptor deficiency.</p> <p>Keywords: Steatosis, BCAA, Leucine, Metabolism, mTORC1.</p>
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Title	Involvement of gut microbiota in the development and progression of non-alcoholic fatty liver disease (NAFLD)
Authors	Natália Pontara Corte Thayna dos Santos Vieira Érique de Castro Álbert Souza Érika Vicência Monteiro Pessoa Luiz Augusto Perandini William T. Festuccia
Affiliations	Institute of Biomedical Sciences, University of São Paulo – Brazil.
Session	Nutrition and Metabolism

Abstract, Ethics Committee Number*, and Keywords	<p>Introduction: Gut microbiota is involved in the development of metabolic associated fatty liver disease (NAFLD), a group of diseases characterized by lipid accumulation in the liver, ranging from simple steatosis (MAFL), to more severe steatopatitis (MASH) and hepatocellular carcinoma (HCC).</p> <p>Objective: To investigate the interrelationship between gut microbiota and development and progression of liver disease induced by Pten deletion in hepatocytes.</p> <p>Methodology: Gut microbiota was depleted by an antibiotic cocktail (ABX, vancomycin 0.5/L, neomycin sulfate 1g/L and ampicillin 1g/L) administered in drinking water to male mice bearing (KO) or not (WT) Pten deletion in hepatocytes for 8 weeks fed with a regular chow diet. Mice were evaluated for weight gain, food intake and masses of liver, brown and inguinal white adipose tissue (BAT and iWAT), gastrocnemius, cecum and colon. Mice procedures were approved by ICB ethic committee for animal usage (CEUA 6160250820). Results are expressed as mean \pm SEM, significance level $p < 0.05$, $n = 5-9$ mice per group. Results: There were no changes in food intake between groups, but antibiotics increased body weight of mice with hepatocyte Pten deletion (WT 27.12 ± 0.55 vs KO 27.10 ± 0.34 vs WT+ABX 27.51 ± 0.52 vs KO+ABX 29.09 ± 0.66). Antibiotics also attenuated the increase in liver weight induced by hepatocyte Pten deletion (WT 1057 ± 35.35 vs KO 2891 ± 259 vs WT+ABX 895.3 ± 48.04 vs KO+ABX 2006 ± 182) increased colon weight in both WT and KO (WT $160,6 \pm 11,08$ vs KO $169,6 \pm 14,58$ vs WT+ABX $283,8 \pm 21,50$ vs KO+ABX $335,9 \pm 15,93$) and reduction in the weight of the cecum in KO mice with treatment compared to WT mice (WT $224,8 \pm 18,98$ vs KO $262,8 \pm 15,73$ vs WT+ABX $180,1 \pm 9,94$ vs KO $187,7 \pm 14,13$)</p> <p>Conclusion: Microbiota depletion attenuate the increase liver mass induced by pten deletion in hepatocyte.</p> <p>Keywords: intestinal microbiota; NAFLD; fibers; lipid metabolism; inflammation</p>
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Title	Effect of a diet based on <i>Attalea phalerata</i> oil standardized in β-carotene on the glycemic profile morphometry of the pancreas of mice
Authors	Daniela de Souza Dahmer Bottle Elaine Cristina by Lara Spada Edgar Willibaldo Allebrandt Neto Wéliton Barreto da Silva Thainara Agnis Dourado Meuquizedek Faziolato da Costa Suélem Aparecida de França Lemes
Affiliations	Biochemistry Laboratory Professor Nair Honda Kawashita, Department of Chemistry, Federal University of Mato Grosso, Cuiabá, Brazil
Session	Poster

Abstract Ethics Committee Number* and Keywords	<p><i>Attalea phalerata</i> Mart. ex Spreng., is a palm tree native to the Pantanal of Mato Grosso, its ripe fruits display a bright orange color and the oil extracted from the pulp has high concentrations of β-carotene. The objective is to investigate the effects of including <i>A. phalerata</i> oil, standardized in β-carotene, in 21-day-old mice (13 to 14g). The fruits used in oil extraction were collected in Várzea-Grande/MT, the exsiccata deposited in the UFMT herbarium no. 44310. The study was approved by the ethics committee CEUA-UFMT n.23108.977342/2018-54 and the animals (31) were separated into three experimental groups: control group (C) fed with AIN93G diet, a group deficient in vitamin A (DVA) fed with an AIN93G diet free of vitamin A (with soybean oil), and the group received <i>A. phalerata</i> oil (OA) standardized in β-carotene added to the AIN93G diet free of vitamin A for 45 days, under controlled light conditions (12-hour light and dark cycle), temperature ($22^{\circ}\text{C}\pm 1$), humidity ($47\%\pm 1$). Glycemic parameters were evaluated using specific kits, body mass Lee Index ($\sqrt[3]{\text{Weight (g)}/\text{length naso-anal}\times 100}$) (One-way ANOVA; $P < 0.0001$) and morphometric analyzes were performed on pancreatic tissue sections stained by hematoxylin and eosin using AxionVisio 4.8 software. The Lee Index was 62% higher in the OA group than DVA and 32% lower in DVA than C. Basal glycemia was 17% and 32% higher in the OA group than C and DVA, respectively, the glycemia of the group DVA was 11% smaller than in C. The size of the pancreas was smaller in the group with DVA (0.36%) compared to groups C (0.56%) and OA (0.64%), the weight of the islets (Islet Area(g)/Pancreas Area \times Pancreas Weight (g)) was higher in the OA group (47.29%) compared to groups C (34.27%) and DVA A (30.85%), there were no changes in islet size distribution. Dietary intake with <i>A. phalerata</i> oil. <i>ex Spreng.</i> induced changes in the analyzed parameters.</p> <p>Keywords: β-carotene; Glycemia; Pancreatic islets</p>
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Title	mTORC2 partly mediates the increase in mass and de novo fatty synthesis induced by constitutive activation of PI3K signaling in brown and white adipocytes
Authors	1- Ana Carolina Pereira Baptista 2- Gustavo Renan Gilio 3- Ériqre Castro 4- Albert Peixoto 5- William T. Festuccia
Affiliations	Department of Physiology and Biophysics, Institute of Biomedical Sciences (ICB), University of São Paulo (USP), São Paulo, São Paulo (SP).
Session	07- Nutrition and Metabolism

Abstract, Ethics Committee Number*, and Keywords	<p>Insulin signaling in the phosphoinositide 3 kinase (PI3K)-Akt pathway, which requires Akt phosphorylation at Ser473 by mechanistic target of rapamycin complex 2 (mTORC2) and at Thr308 by phosphoinositide dependent kinase (PDK), enhances glucose uptake and lipid synthesis in white and brown adipose tissue (WAT and BAT, respectively). We investigated herein the involvement of mTORC2 as a mediator of changes in lipid and glucose metabolism in WAT and BAT induced by activation of PI3K signaling. For this, mice bearing constitutive activation of PI3K signaling due to Pten deletion in adipocytes (PtenKO) associated or not with mTORC2 deficiency due to adipocyte Rictor deletion (PtRicKO) (CEUA, ICB/USP 6160250820) and littermate floxed controls (WT) were fed a standard chow diet for 8 weeks and evaluated for energy balance, tissue mass and de novo lipogenesis. Despite similar weight gain (WT: 1.71 ± 0.16 g vs PtenKO: 2.61 ± 0.36 vs PtRicKO: 2.22 ± 0.34 g) and food intake (WT: 25.23 ± 0.73 vs PtenKO: 26.21 ± 1.35 vs PtRicKO: 24.78 ± 0.62 g), adipocyte Pten+Rictor deletion abolished in BAT (WT: 45.86 ± 2.10 vs PtenKO: 94.33 ± 6.37 vs PtRicKO: 37.14 ± 1.34 mg), attenuated in inguinal WAT (WT: 153.1 ± 10.56 vs PtenKO: 436.8 ± 21.64 vs PtRicKO: 285.2 ± 18.64 mg), and did not affect in epididymal WAT (WT: 266.3 ± 17.4 vs PtenKO: 393.8 ± 30.97 vs PtRicKO: 368.1 ± 16.3 mg) the increase in mass induced by adipocyte Pten deletion. Along with these findings, adipocyte Rictor deletion attenuated in brown adipose tissue (WT: 12.41 ± 3.2 vs PtenKO: 63.10 ± 11.25 vs PtRicKO: 27.23 ± 9.71 nmol/mg ptn) and completely abolished in inguinal WAT (WT: 83.80 ± 17.28 vs PtenKO: 210.4 ± 30.95 vs PtRicKO: 60.33 ± 18.97 nmol/mg ptn) the increase in acetate incorporation in fatty acids from triacylglycerol induced by Pten deletion. In conclusion, mTORC2 mediates in part the increase in mass and de novo fatty synthesis induced by constitutive activation of PI3K signaling in brown and white adipocytes.</p> <p>Keywords: mTORC2, adipose tissue, lipid metabolism</p>
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XXXVIII REUNIÃO ANUAL DA FESBE
XXII REUNIÃO ANUAL DA BRAVO
XVIII CONGRESSO DA SBCAL
III CONGRESSO DOHAD BRASIL
II CONGRESSO DA SBBA
2 A 5 DE JULHO 2024, CAMPINAS/SP
FRONTEIRAS DAS CIÊNCIAS BIOMÉDICAS E SEUS DESAFIOS

Title	LPS activates mTORC1 and 2 in white adipose tissue
Authors	Marina Akemi Abe Honda Bianca Leonardi Érique de Castro Albert Souza Peixoto Thayna dos Santos Vieira Natália Monteiro Pessoa Érika Vicência Monteiro Pessoa Ana Brandão Pires Luciano Pedro da Silva William T. Festuccia
Affiliations	Instituto de Ciências Biomédicas – USP, São Paulo, Brasil
Session	Nutrição e Metabolismo

Abstract, Ethics Committee Number*, and Keywords	<p>Obesity is characterized by an increase in intestinal permeability and absorption of the gram negative membrane component lipopolysaccharide (LPS). Indeed, chronic infusion of LPS in mice induces, through unknown mechanisms, an increase in the number of small adipocytes in subcutaneous adipose tissue. We investigated herein the effect of acute and chronic LPS administration in mass, morphology, and intracellular signaling through mechanistic target of rapamycin complexes 1 and 2 (mTORC1 and 2) in white adipose tissue as well as the involvement of mTORC2/Rictor as a mediator of LPS actions. Experimental procedures were approved by the Ethics Committee on the Use of Mice of the Institute of Biomedical Sciences (CEUA Protocols: 6160250820 and 6071181120). Male C57BL/6J mice with or without Rictor deletion in adipocytes were sacrificed 30 or 120 min after intraperitoneal injection of either sterile PBS or LPS at doses of either 0.1 mg/Kg and evaluated for adipose tissue signaling. Acute LPS increased epididymal adipose tissue mTORC2 and mTORC1 activities (n= 6-7) (PBS 1.11 ± 0.19 vs LPS 2.35 ± 0.93), as evidenced by the increased contents of Serine 473 phosphorylated (p) Akt and pS6 (PBS 0.90 ± 0.21 vs LPS 4.12 ± 1.70), respectively. Chronic LPS (5 mg/Kg, daily during 7 days) also increased mTORC1 activity (n= 4-5) in in epididymal (PBS 3.07 ± 1.45 vs LPS 19.46 ± 1.14) and inguinal (PBS 0.81 ± 0.27 vs LPS 1.24 ± 0.11) adipose tissues. Adipocyte Rictor deletion reduced Ser473 pAkt content in epididymal (WT 1.19 ± 0.26 vs KO 0.16 ± 0.04) and inguinal (WT 0.96 ± 0.25 vs KO 0.04 ± 0.01) and total and pS6 (WT 1.50 ± 0.35 vs KO 0.72 ± 0.04) in epididymal tissue. In conclusion, LPS activates mTORC2 and 1 in white adipose tissue.</p> <p>Keywords: LPS, Adipose Tissue, Obesity, mTORC1, mTORC2</p>
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Title	Retrospective study of dysmagnesemia in patients hospitalized with a diagnosis of Covid-19 in the adult intensive care unit at Ouro Preto Minas Gerais
Authors	Camargos, L.R. ¹ ; Félix, C.A. ¹ ; Souza, R.V. ¹ ; Fragoso, M.T.A. ¹ ; de Souza, A.B.F. ¹ ; Bezerra, F.S. ¹
Affiliations	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.
Session	07 - Nutrição e Metabolismo

Abstract and Keywords	<p>COVID-19 triggered the pandemic in 2020, with varying degrees of severity among patients. Magnesium, essential for metabolic processes, has been studied for its role in controlling the disease. The aim of this study was to analyze the profile of patients hospitalized with a diagnosis of COVID-19 associated with dysmagnesemia in an adult intensive care unit. This is a retrospective study conducted between March 2020 and August 2022 in patients admitted to the adult ICU diagnosed with COVID-19, the study was approved by the Ethics Committee under No. 4.777.491. After signing the informed consent form, 47 patients were included in the study. The outcome variable was magnesium. Explanatory variables were length of stay in days, presence of comorbidities, patient outcome, ventilatory support, length of mechanical ventilation in days, and medications. In general, 59.57% of the patients included in the study were male, with a mean age of 60.72 years. The most common comorbidities were systemic arterial hypertension and diabetes mellitus. 51.06% of patients had normal magnesium levels and 48.94% had hypomagnesemia. 56.25% of patients required invasive ventilatory support, and analysis between patients with hypomagnesemia and normal magnesium levels revealed a higher frequency (69.57%) of invasive ventilatory support in patients with hypomagnesemia. A higher total leukocyte count (mm³) was observed in COVID-19 patients with hypomagnesemia [13700 (10200; 17800)] compared to patients with normal serum magnesium levels [10400 (6550; 13025)]. Potassium levels (mEq/L) were high in patients with hypomagnesemia (4.55 ± 0.51) compared to patients with magnesium levels in the normal range (4.11 ± 0.69). Our data suggest that patients diagnosed with COVID-19 and hypomagnesemia had a greater need for ventilatory support and an inflammatory state characterized by high leukocyte counts, suggesting that these patients had more severe disease.</p> <p>Keywords: COVID-19; Magnesium, Hypomagnesemia, Intensive Care Unit Funding: CNPq, CAPES, UFOP and FAPEMIG.</p>
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Title	Effect of <i>Athalea phalerata</i> pulp oil on the neural metabolism of rats subjected to vitamin A deficiency
Authors	Elaine Cristina de Lara Spada Daniela de Souza Vial Dahmer Edgar Willibaldo Allebrandt Neto Wéilton Barreto da Silva Thainara Agnis Dourado Joaquim Alves de Oliveira Neto Anderson de Oliveira Souza Suélem Aparecida de França Lemes
Affiliations	Department of Chemistry, Federal University of Mato Grosso, Cuiabá-MT, Brazil
Session	07 - Nutrição e Metabolismo

Abstract, Ethics Committee Number*, and Keywords	<p>The pulp oil of <i>Athalea phalerata</i> Mart. ex Spreng is rich in β-carotene, a precursor of vitamin A, it has bioactive components, flavonoids, monounsaturated fatty acids and essential amino acids, with antioxidant potential for the neural system. The goal was to evaluate the oxidative action of <i>A. phalerata</i> pulp oil on the neural system of rats subjected to vitamin A deficiency and recovered with <i>A. phalerata</i> pulp oil. Fruits collected in Várzea-Grande/MT, the fruit pulp oil was cold extracted with Hexane. Study approved by the CEUA-UFMT ethics committee n.23108.977342/2018-54. Male Wistar rats 21 days (~60g) N:4-6, divided into Control Group(C), AIN-93G diet for 75 days and vitamin A deficiency group(VAD), AIN-93G diet without vitamin-A for 45 days. VAD Group was divided into 3 groups for 30 days: VAD Group, Acuri Oil Group(AO), AIN-93G diet with 14.414μg/kg β-carotene in <i>A. phalerata</i> oil; Synthetic β-carotene group(BC), diet-AIN-93G with 14.414μg/kg synthetic β-carotene. By high performance liquid chromatography, a concentration of β-carotene in <i>A. phalerata</i> oil of 308.1μg/mL(\pm0.10). On the 75th day the animals were euthanized for cortex and hippocampus collection in the of the brain to assess the neural oxidative activity of Acetylcholinesterase (ACHE) enzymes in spectrophotometer, in the animal model, VAD group in the hippocampus and cortex was greater than group C (6.8% and 11.5%). Monoamine oxidase (MAO) by fluorescence, in the animal model of the VAD and C group, differentiated in the cortex and hippocampus with 88.7% and 46.9%. In the BC and AO treatment groups of the cortex and hippocampus there was reduction above 80% in MAO-A and MAO-B when compared to the VAD group. The enzymatic lactate test of the BC group in the cortex was 58.6% higher than the VAD. Vitamin A deficiency causes cellular oxidative damage; in the enzymatic assay, in the groups recovered with synthetic β-carotene and <i>A. phalerata</i> oil, there was a possible inhibition of monoamine oxidase.</p> <p>Keywords: <i>Athalea phalerata</i>, Acetylcholinesterase, Monoamine oxidase.</p>
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Title	The impact of ketogenic diet during cancer cachexia: a metabolomic study in a pre-clinical model
Authors	Viana, L.R.; ² Sousa, B.F.P.; ^{1 2} Lopes-Aguiar, L.; ² Scandiuizzi, R.T.; ² Gomes-Marcondes, M.C.; ²
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Session	7- Nutrição e metabolismo

Abstract, Ethics Committee Number*, and Keywords	<p>The cancer cachexia syndrome, mainly related to skeletal muscle and adipose tissue loss could be mitigated by a ketogenic diet, but the effectiveness and molecular mechanisms aren't fully known. In this regard, our aim was to evaluate the effect of a ketogenic diet on the serum and muscle metabolomics profile of a cancer cachexia model, approved by the Institutional Committee for Ethics in Animal Research (34289-1). Beside that, we also perform histologic analysis to compare muscle mass loss in both groups by cross section area. For this purpose, two experimental groups were performed: Tumor bearing rats fed with a control diet (W) and Tumor bearing rats fed with a ketogenic diet (KW). All animals received tumor s.c inoculation with 2.5×10^6 viable cells of Walker 256 carcinosarcoma. After approximately 21 days of tumor inoculation, all animals were euthanized and serum and muscle tissue collected. All data was expressed as mean \pm standard deviation and analyzed by t-test with P value < 0.05 considered as significant. The serum concentration of lactate, histamine and alanine showed a decrease in KW group when compared to W. Despite these changes in serum, these metabolites aren't modulated in the muscle of these animals. In this regard, the gastrocnemius muscle metabolomic profile has shown an increase in muscle concentration of AMP, pyruvate, methionine, ascorbate and biotin, while ATP, IMP, glutamine and acetate decreased in KW in comparison to W group. Despite these alterations, we did not find any changes in the muscle cross section area. These results suggested that a ketogenic diet can ameliorate muscle damages caused by cancer-associated cachexia increasing the concentrations of metabolites, such as ascorbate and biotin which have anti-inflammatory properties.</p> <p>Keywords: Ketogenic diet; cancer-associated cachexia; metabolomics; histology; Walker 256.</p>
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Title	Effects of a leucine-rich diet on histological aspects of the gastrocnemius muscle in Walker 256 tumor-bearing pregnant rats
Authors	Marcelly Rodrigues Tavares Luizeto Igor Fernando dos Santos Prof ^a Maria Cristina Cintra Gomes Marcondes Prof ^a Laís Rosa Viana
Affiliations	1- Pontificia Universidade Católica de Campinas (PUCC) 2- Universidade Estadual de Campinas (UNICAMP) 3- Universidade Estadual de Campinas (UNICAMP) 4- Universidade Estadual de Campinas (UNICAMP)
Session	Nutrition and metabolism

Abstract, Ethics Committee Number*, and Keywords	<p>Cachexia, present in most advanced cancer stages, is characterized by involuntary weight loss, mainly related to skeletal muscle loss. This condition could worsen if cancer is associated with pregnancy. As a possible muscle therapy target, leucine supplementation could be used due to its capacity to increase protein synthesis. So, we investigated the impact of a leucine-rich diet on the skeletal muscle of tumor-bearing pregnant rats. For this purpose, pregnant rats were divided into four experimental groups: (C) healthy pregnant rats; (W) tumor-bearing pregnant rats; (L) healthy pregnant rats fed with a leucine rich-diet; and (LW) tumor-bearing pregnant rats fed with a leucine rich- diet. Animals from W and LW received tumor s.c inoculation with 2.5×10^6 viable cells of Walker 256 carcinosarcoma two days post conception (dpc). After 18 days, all animals were euthanized and muscle tissue was collected. The experimental protocol was approved by the Institutional Committee for Ethics in Animal Research (6136-1/2022). Gastrocnemius muscle was embedded transversely in paraffin for 5 μm thick sections. Subsequently, the slides were stained with hematoxylin and eosin. Images were analyzed and the muscle cross- section area (CSA) was measured using the Image J program. Statistical analyses were performed using Graph Pad Prism 9.0 software (Graph-Pad Software, Inc). Data are expressed as mean \pm standard error of the mean (SEM), analyzed by one-way ANOVA, followed by Tukey's post-hoc test. For all statistical analyses, $P < 0.05$ was considered significant. We did not observe any significant differences ($P < 0.05$) between the experimental groups for the muscle CSA. Additional histological analyses, such as glycogen content through periodic acid- Schiff staining are ongoing and results will also be presented at the congress. Keywords: cancer, leucine, muscle.</p>
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Title	Characterization of fluoroquinolone compounds capable of inducing weight loss and preserving glycemic control in obesity
Authors	Gerson Profeta de Souza ^{1,2*} , Niedson Correia de Lima Junior ^{1*} , Linnéa Bergenholm ³ , Gavin O'Mahony ³ , Jeremie Boucher ³ , Henver Simionato Brunetta ¹ , Jessica Aparecida da Silva Pereira ² , Fernanda Luísa Basei ¹ , João Pedro Mazzi ¹ , Letícia de Souza Figueiredo ¹ , Gabriel Palermo Ruiz ^{1,2} , Deisi Braga Shimo ¹ , Andrea Livia Rocha ⁴ , Raul Gobato Costa ¹ , Kauê de Oliveira Chinaglia ¹ , Marcelo Alves da Silva Mori ¹
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Session	07 - Nutrition and metabolism

Abstract, Ethics Committee Number*, and Keywords	<p>Introduction: Despite the efficacy of lifestyle changes in weight management, obesity rates raise public health concerns. Therefore, it is important to explore new compounds that contribute to obesity treatment. Previously, our group demonstrated the efficacy of enoxacin, a fluoroquinolone antibiotic, in weight control in a pre-clinical model. However, concerns about antibiotic therapy for obesity encouraged us to seek alternative fluoroquinolones with similar metabolic benefits but without antibiotic effects.</p> <p>Methods: We utilized 9W murine white preadipocyte cell lineages for <i>in vitro</i> analyses. Following differentiation, cells underwent 24-hour treatment with compounds before proceeding to assays. Additionally, male young adult mice on a 60% high-fat diet (HFD) were treated with two selected fluoroquinolone compounds to assess <i>in vivo</i> outcomes.</p> <p>Results: Among 208 screened compounds, two met our criteria, i.e increased thermogenic markers, enhanced miRNA biogenesis, and lack of antibiotic activity. Two of them were compatible with <i>in vivo</i> studies based on pharmacokinetics/pharmacodynamics and safety analyzes in mice. Both compounds altered mitochondrial dynamics leading to increased respiratory function and enhanced glycolytic capacity and decreased dependency <i>in vitro</i>. These effects were dependent on cAMP and mitochondrial fission. <i>In vivo</i>, the compounds upregulated thermogenic markers in adipose tissue, attenuated weight gain, and improved glycemic homeostasis of mice on an HFD. Additionally, acute treatment resulted in enhanced carbohydrate utilization. Thermoneutrality and Dicer knockout in adipocytes blunted the metabolic benefits. No side effects were observed in long-term treatment with the compounds.</p> <p>Conclusion: Thus, the tested compounds can alleviate the metabolic impairments resulted from obesity, and the mechanisms are associated with mitochondrial remodeling, cAMP signaling, and miRNA biogenesis in adipocytes, leading to an increase in glucose oxidation.</p> <p>Key words: Obesity; weight loss; adipose tissue; diabetes</p>
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Title	Antiobesogenic effects of botryosphaeran, a (1→3)(1→6)-β-D-glucan, in female rats
Authors	Fabiane, I.Z.; ¹ Netto, D. F. C.; ² Ferreira, F. N.; ² Oliveira, I. P.; ² Junges, S. L. B.; ² Silva, V. C.; ² Bavaresco, P.S.; ² Vilela, A. C.; ³ Sinhorin, V. D. G.; ³ Alegranci, P.; ² Queiroz, E.A.I.F. ¹
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Session	07 - Nutrição e Metabolismo

Abstract, Ethics Committee Number*, and Keywords	<p>Botryosphaeran is a (1→3)(1→6)-β-D-glucan produced by the ascomyceteous fungus, <i>Botryosphaeria rhodina</i>, that promotes significant metabolic effects in male rats, as antiobesogenic and hypoglycemic effects. This study aims to analyze the metabolic effects of botryosphaeran in obese and non obese female rats. The study was approved by the Ethics Committee (nº23108.072920/2023-21). Rats were divided into four groups: Control (C), Control treated with botryosphaeran (CB), Obese (O) and Obese treated with botryosphaeran (OB); n=8/group. Control rats fed a standart diet and water and obese rats fed a high-fat diet and water with sucrose, ad-libitum, for 8 weeks. On 6th week, CB and OB groups received treatment with botryosphaeran (12 mg/kg/day) by gavage for 15 days. After treatment anthropometrics and metabolic parameters were analyzed, as body weight (BW), glucose tolerance test, insulin tolerance test and oxidative stress. Obese rats presented high weight gain, accumulation of adipose tissue, glucose intolerance, insulin resistance, hepatomegaly and high levels of TBARS in the liver, a marker of oxidative stress. Treatment with botryosphaeran reduced significantly the final BW (g)(C: 226.3±14.0 vs. CB: 208.6±14.5; O: 255.3±22.4 vs. OB:235.0±21.6), visceral adipose tissue (g)(C: 7.1±2.0 vs. CB: 5.9±1.0; O: 19.8±5.1 vs. OB:15.3±4.4), retroperitoneal adipose tissue (g)(C: 3.6±1.2 vs. CB: 1.9±0.4; O: 9.4±3.0 vs. OB: 6.6±2.3, P<0.05), liver tissue and corrected the glucose intolerance (P<0.01). Furthermore, botryosphaeran reduzed the levels of TBARS in 45.4% in the liver, reducing the oxidative stress. In addition, in the liver, no difference were observed for carbonyl, SOD, catalase, GPx, GSH, GST and vitamin C between groups. In conclusion, it was observed that botryosphaeran promoted a significant antiobesogenic effect, promoting a expressive weight loss, reduction of adipose tissue, correction of glucose intolerance and promoting an antioxidant effect in female rats.</p> <p>Keywords: obesity; botryosphaeran; glucose intolerance; oxidative stress.</p>
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Title	Metformin treatment exclusively during peripubertal phase mitigates effects of early overfeeding in female rats
Authors	Ana Carolina Carvalho de Sá Scarlett Rodrigues Raposo Giovana Volpato Leticia Ferreira Barbos Maria Natália Chimirri Peres Luiz Gustavo Antunes Ana Leticia Assakawa Rafael Pereira Lopes Douglas Lopes de Almeida Paulo Cesar de Freitas Mathias
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Session	Nutrition and Metabolism

Abstract, Ethics Committee Number*, and Keywords	<p>Puberty is a period of significant physiological changes. Obesity in early life has become a public health risk of great concern. Besides, according to the Developmental Origins of Health and Disease (DOHaD) concept both, lactation and adolescence, are critical periods of development due to metabolic plasticity. Metformin, a hypoglycemic drug, has demonstrated beneficial effects for body weight and body fat weight loss. Therefore, we hypothesized that treatment with metformin exclusively during the peripubertal phase may mitigate the effects of overfeeding during lactation in female offspring Wistar rats. To induce obesity, we reduced the number of pups per dam (SL) to 3, while the control group remained with 9 pups (NL) (CEUA-UEM nº 4831020822). Litters were weaned at 21 days-old and only female rats were used. A set of animals from both groups were treated with metformin from 30 to 60 days-old (300mg/kg in drinking water), resulting in the four groups: NL-C (N=7) and SL-C (N=5) (no metformin), NL-T (N=8) and SL-T (N=4) with metformin. We observed that SL-C had significantly higher body weight compared to NL-C (NL-C 11508.1±264.1; SL-C 12890±346.7; p<0.05) with heavier periovarian fat depot (NL-C 0.99±0.05 vs SL-C 1.39 ±0.08; p <0.0001), demonstrating the reproduction of the obesogenic model in females. Treatment with metformin significantly reduced these parameters in SL-T litters compared to SL-C (SL-C 12890±346.7 vs SL-T 11579.5±298.55; p <0.05). Furthermore, the vaginal opening, considered a marker of the puberty onset in rats, was earlier in the SL-C group compared to the NL-C group (NL-C 40.2±0.38 vs SL-C 36.37±0.75; p <0.05), while the SL-T group had normalized vaginal opening time (SL-C 36.37±0.75 vs SL-T 39.7±0.53; p <0.005). Thus, data shows that metformin administration during</p>
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	<p>the peripubertal period can mitigate the effects caused by overfeeding during lactation in body weight and adiposity and in the puberty onset in female rats. Obesity, Metformin, Peripuberty, Adolescents, Early Overnutrition, DOHaD</p>
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Title	Obesogenic diets worsen the cognitive performance of C57BL/6 mice
Authors	Natalia Pereira da Silva Araujo Amanda Kelly de Lima Andrade Karina Barbosa de Queiroz Allan Jefferson Cruz Calsavara Daniela Caldeira Costa
Affiliations	1 – SBBC
Session	Nutrição e Metabolismo

Abstract, Ethics Committee Number*, and Keywords	<p>The shift in dietary patterns, characterized by increased consumption of saturated fats and refined sugars, has been associated with cognitive decline. This study aimed to investigate cognitive deficits associated with obesogenic diets. C57BL/6 mice were randomly assigned to three groups: Control (C), receiving a standard AIN-93M diet; High Sugar (HS), with 70% sucrose; and High Fat (HF), with 25% lard. After 16 weeks, behavioral tests (Y-maze, object recognition) were conducted. Post-euthanasia, serum metabolic markers and brain/intestinal oxidative damage were assessed. Results were analyzed using ANOVA and Tukey's post-test. Ethics Committee on the Use of Animals (CEUA/UFOP) - 9100220322. The results shows that both HS and HF groups exhibited reduced cognitive performance (Y-maze: C 60.8 ± 5.32 vs. HS 54.02 ± 6.80, $p=0.0433$ and vs. HF 52.88 ± 7.01, $p=0.018$) and (RO: C 0.31 ± 0.14 vs. HS -0.07 ± 0.11 and vs. HF -0.17 ± 0.27, $p<0.0001$). HF showed higher cholesterol and insulin compared to C and HS: Cholesterol (C 113.6 ± 11.9 vs. HF 148.9 ± 19.99, $p=0.001$ and HS 121.5 ± 4.72 vs. HF 148.9 ± 19.99, $p=0.009$); Insulin (C 0.90 ± 0.09 vs. HF 1.25 ± 0.29, $p=0.018$ and HS 0.92 ± 0.28 vs. HF 1.26 ± 0.29, $p=0.027$). Triglycerides decreased in both diets compared to C (C 48.57 ± 18.66 vs. HS 30.82 ± 7.48 $p=0.024$ and C 48.57 ± 18.66 vs. HF 22.96 ± 7.66, $p=0.001$). Lower thiol group levels were observed in HS and HF in the brain (C 209.2 ± 28.72 vs. HS 161.7 ± 22.46, $p=0.001$ and C 209.2 ± 28.72 vs. HF 175.2 ± 17.79, $p=0.014$) and intestine (C 167.1 ± 18.09 vs. HS 145.9 ± 17.58, $p=0.044$ and C 167.1 ± 18.09 vs. HF 140.4 ± 17.13, $p=0.007$). HS showed higher TBARS values in the brain (C 1.72 ± 0.22 vs. HS 2.15 ± 0.40, $p=0.022$). In the intestine, HF showed higher TBARS values (C 0.44 ± 0.098 vs. HF 0.42 ± 0.10, $p=0.011$) and HS exhibited higher carbonyl protein levels (C 7.32 ± 0.63 vs. HS 8.42 ± 0.73, $p=0.035$). In conclusion, diet composition can influence cognition, with obesogenic diets leading to cognitive deficits.</p> <p>Obesogenic diets, oxidative stress, cognitive performance.</p>
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Title	Effects of high sugar and high fat diets on cognitive performance, glycometabolic mediators, and cerebral oxidative stress in mice
Authors	Amanda Kelly de Lima Andrade Natalia Pereira da Silva Araujo Karina Barbosa de Queiroz Allan Jefferson Cruz Calsavara Daniela Caldeira Costa
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Session	Nutrição e Metabolismo

Abstract, Ethics Committee Number*, and Keywords	<p>The consumption of diets high in simple sugars and saturated fats, and deficient in fresh foods, fiber and antioxidants, common in Western countries, these ultra-processed foods, rich in calories and poor in nutrients, has negative consequences for public health. Studies highlight the connection between the consumption of “high sugar” and “high fat” diets, and the emergence of overweight, obesity, chronic diseases, neurocognitive changes and increased oxidative stress. Therefore, the objective of this study was to evaluate cognitive performance, serum metabolic changes and cerebral oxidative stress in C57BL/6 mice submitted to diets rich in sucrose or saturated fat for 8 weeks - Ethics Committee on the Use of Animals (CEUA/UFOP) - 9100220322. To characterize such changes, serum glycometabolite markers (glucose, insulin, total cholesterol, triglycerides), oxidative damage in the brain (TBARS and carbonyl protein) were measured, in addition to neurocognitive tests (Y-maze and new object recognition test). The data presented a normal distribution and were analyzed according to one-way ANOVA, followed by Tukey's post-test, expressed as mean \pm standard deviation. Among the results obtained, a significant worsening in cognitive performance stands out in animals fed a high sugar diet in relation to the control (Y-maze C 60.29 ± 5.5 vs. HS 52.46 ± 8.72 $p=0.0349$; HF 55.81 ± 6.77 $p=0.3063$ and RO C 0.22 ± 0.27 vs. HS -0.03 ± 0.14 $p=0.0063$; HF 0.083 ± 0.11 $p=0.2001$), in addition to a glycemic increase in the HS group in relation to the control (C 205.4 ± 3.05 vs. HS 210 ± 1.27 $p=0.0013$; HF 205.4 ± 0.72 $p=>0.9999$). Regarding lipid peroxidation levels, there was a significant increase in both the HS and HF groups when compared to the control (TBARS C 0.38 ± 0.12 vs HS 0.55 ± 0.1 $p=0.0157$; HF 0.64 ± 0.12 $p=0.0005$). Thus, it can be concluded that the high sugar diet was responsible for inducing more significant changes than the high fat diet in 8 weeks.</p> <p>Obesogenic diets, oxidative stress, cognitive performance.</p>
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Title	Metabolic alterations in the prostate at different stages of development and under androgen deprivation
Authors	Francisco Breno S. Teófilo ¹ ; João Rodolfo Tuckumantel-Valim ¹ ; Adriana L. Santoro ² ; Antônio Thiago P. Campos ³ ; Larissa M. dos Reis ⁴ ; Mariana O. Baratti ¹ ; Leandro Cardoso ¹ ; Silvio R. Consonni ² ; Pedro M. Moraes-Vieira ⁴ ; Hernandes F. Carvalho ¹ .
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Session	07-Nutrição e Metabolismo

Abstract, Ethics Committee Number*, and Keywords	<p>Puberty is a significant stage in prostate development. Starting at the sixth week after birth in line with the increase in testosterone concentration, this period is marked by events such as an increase in the cell proliferation rate, as well as the emergence of secretory activity by epithelium. In this secretion, the high levels of citrate –an intermediate compound of the CAC– stand out in comparison with other biological fluids. The implications of this mechanism lead us to turn our attention to mitochondrial function, where it is suggested that changes are being made to maintain OXPHOS and provide citrate. We have explored this gap here, as it seems reasonable to evaluate morphofunctional aspects and establish relationships with changes in the prostate associated with androgen influence. We determined groups contemplating temporally distinct physiological states (prepubertal and postpubertal), as well as groups induced to androgen deprivation (euthanized on day 3 or 7 after castration). Accordingly, we aimed to: characterize the mitochondrial morphology of epithelial cells through confocal and airyscan microscopy, and their ultrastructure using TEM; measure mitochondrial activity, particularly oxygen consumption and complex activities; and evaluate the metabolome using UPLC-HRMS. Briefly, we observed variations in the mitochondrial phenotype, especially in the postpubertal group, as well changes in respiration rates. Additionally, through untargeted metabolomics, we were able to discriminate the grouping of samples with a considerable separation between the groups and perform the tentative annotation of compounds. Overall, our results pave ways for a better comprehension of prostatic physiology.</p> <p>Keywords: metabolism; mitochondria; prostate development.</p>
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Title	Effects of gestrinone administration on the morphology of brown adipose tissue in rat feed a normolipid and hyperlipidic diet
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Session	7 – Nutrição e Metabolismo

Abstract, Ethics Committee Number*, and Keywords	<p>Gestrinone is a synthetic steroid with androgenic, antiprogesterin and anti-estrogenic actions, used for endometriosis and myoma treatments. In the last years the internet has been noticed that gestrinone implant may regulates fat mass. Herein, we investigated the effects of gestrinone administration on body mass composition of female rats submitted to normo- or high-fat diets. Adult female Wistar rats were fed on a normo- or a high-fat diet (HFD), and received 3 times/week, a gavage of soybean oil containing 0.16% DMSO (CTL and HFD groups), without or with 0.26 (CGES0.26 and HGES0.26 groups), or 0.52 mg gestrinone/kg body weight (BW; CGES0.52 and HGES0.52 groups), for 12 weeks. HFD rats displayed increased BW (318.4 ± 15.5 g), abdominal adiposity (110.4 ± 6.9 mg/g BW), without changes in interscapular brown adipose tissue (BAT) weight (1.69 ± 0.2 mg/g BW), when compared to CTL (255.6 ± 7.5 g, 48.7 ± 7.1 and 1.7 ± 0.3 mg/g BW, respectively). Gestrinone administration prevented HFD effects, since, HGES0.26 and HGES0.52 females exhibited lower BW (253.9 ± 8.5 and 261.9 ± 14.3 g) and visceral adiposity (54.9 ± 2.7 and 54.7 ± 10.7 mg/g BW), when compared to HFD. Also, HFD rats displayed a 46% increase in fat mass, but reductions of 7.8% and 7.5% in lean and water content in their carcasses, in comparison to CTL. Gestrinone administration caused reductions of 20.7% and 27.0% in fat mass, and increases of 6.7% and 7.8% in lean mass of HGES0.26 and HGES0.52 rats, respectively, when compared to HFD. Despite, gestrinone treated rats did not change BAT weight (HGES0.26 = 1.7 ± 0.2, HGES0.52 = 1.8 ± 0.2 mg/g BW), at microscopical level, they exhibited lower brown adipocyte size and lipid vacuoles (HGES0.26 174.5 ± 13.1 and $6.2 \pm 0.7 \mu\text{m}^2$; and HGES0.52 = 210.4 ± 14.3 and $7.2 \pm 0.6 \mu\text{m}^2$), when compared to HFD ($315.0 \pm 20.9 \mu\text{m}^2$ and $12.8 \pm 1.5 \mu\text{m}^2$). Therefore, gestrinone administration prevented obesity induced by HFD, possibly this effect may involves improvement in BAT morphofunction.</p> <p>Keywords: Gestrinone, Obese, Brown Adipose Tissue. High fat diet</p>
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Title	Utilization of grape pomace flour in functional food development: nutritional potential and health implications
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Session	7 – Nutrição e Metabolismo

Abstract, Ethics Committee Number*, and Keywords	<p>Several studies have highlighted the benefits of phenolic compounds, flavonoids, antioxidants, and dietary fibers in mitigating chronic diseases. However, many by-products from food and beverage processing, rich in these compounds, are often wasted, causing significant environmental impact. This study aimed to explore alternatives to integrate grape pomace, derived from juice and wine processing, into the human diet. To this end, grape pomace was processed, milled, and sifted through 60 mm sieves to obtain a flour used in the preparation of functional foods. Whole wheat bread, jam, and ketchup were developed from the flour. The pomace flour underwent bromatological analysis for centesimal and phytochemical determination. The resulting foods were subjected to acceptance evaluation and bioactive compound analysis. Total phenolic compounds in the pomace and foods were determined by the Folin-Ciocalteu method, flavonoids by the aluminum chloride method, and antioxidant activity by DPPH assay. The moisture content was 7 ± 0.09, ash 5.4 ± 0.12, proteins 11 ± 0.12, lipids 9.5 ± 0.08, and carbohydrates 66.7 ± 0.15 g/100g. Phytochemical analysis showed the presence of phenolic compounds, tannins, and flavonoids in both the flour and developed foods, as well as significant antioxidant activity. Public acceptance was approximately 85% for bread, 90% for jam, and 65% for ketchup. The results demonstrated that grape pomace flour has promising potential in the development of functional foods, indicating its utility in preventing non-communicable chronic diseases associated with inflammation, thanks to the bioactive compounds retained in the developed products.</p> <p>Keywords: functional foods; bioactivated compound; antioxidant; inflammation.</p> <p>Acknowledgements: FAPESC n° 54/2022, TO n° 2023TR000885, CNPq Doctoral Scholarship n° 69/2022.</p>
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Title	Histomorphology of perirenal and perigonadal adipose tissue in MSG-obese male and females rats supplemented with Copaiba oil
Authors	Helen de França Kailer Eduarda Felchack Caldas Elizangela Stein Marina Helena Forlin Beatriz Machado Daudt Marianela Diaz Urrutia Zoé Maria Neves de Carvalho Guareschi Ellen Carolina Zawoski Gomes Sabrina Grassioli
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Session	07 - Nutrição e Metabolismo

Abstract, Ethics Committee Number, and Keywords	<p>Copaiba oil (CO) extracted from <i>Copaifera langsdorfii</i> exert anti-inflammatory and anti-obesity effects but its action on adipocyte expansion are unknow. Here we evaluated the effects of oral CO supplementation on adipose content and adipocyte histology in obese male and female rats. Ethics Committee Approval n° 13-20. Male and female Wistar rats were induced to obesity by monosodium glutamate (OB; 4g/Kg). Control (CT) group received equimolar saline. At 21 days, the rats were subdivided in CO supplemented (0.5 mL/Kg; 3 times/week/8 weeks) or non-supplemented (NS). At 90 days, the rats were euthanized, and the number and area of perirenal (PR) and perigonadal (PG) adipose tissue were analysed. Factor (F) obesity, CO and interaction (obesity x CO) effects were obtained in Anova two way. PG content increased in OB-NS male (F=36 p<0.05) and female (F=35 p<0.05) rats, compared to CT-NS; without CO effects. Adipocyte hypertrophy was noted in PG of OB-NS male (F=6.5 p<0.05) and female (F=8.6 p<0.05), related to CT-NS. Interaction effects were observed in adipocyte area of PG in male (F=6.5 p<0.05) and female (F=8.1 p<0.05) groups. Thus, CO abolished differences in adipocyte area between male CT and OB rats, while in females the difference was accentuated. Adipocyte number in PG reduced in OB-NS male (F=22 p<0.05) and female (F=4.6 p<0.05), in relation to CT groups. However, interaction effects (F=29 p<0.05) were observed in PG of female, as both CT and OB-CO had similar adipocyte number. PR content was higher in OB-NS male (F=48 p<0.05) and female (F=21 p<0.05) rats, than CT-NS; without CO effects. PR showed hypertrophy and number reduction in OB-NS male (F=21 p<0.05 and F= 29 p<0.05, respectively) and female (F=13 p<0.05 and F=32 p<0.05, respectively), compared to CT-NS; without CO effects. In conclusion, CO was not able to avoids adipose expansion in OB male and females. However, PG appear more responsive to CO, a response influenced by sex and obesity.</p> <p>Keywords: Obesity, adipocytes, Copaiba Oil.</p>
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Title	Involvement of adipocyte peroxisomes in metabolic actions in mice fed a high-fat diet
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Session	Session: Nutrition and Metabolism

Abstract, Ethics Committee Number*, and Keywords	<p>Introduction: Several studies have shown that mitochondrial dysfunction in adipocytes is associated lipotoxicity, oxidative stress and insulin resistance in mice. Whether dysfunction in peroxisomes, which similarly to mitochondria oxidizes lipids and produces reactive oxygen species, leads to a similar metabolic phenotype is unknown.</p> <p>Objective: Herein, we investigated the impact of adipocyte peroxisomes dysfunction in mice energy balance and glucose homeostasis upon intake of either a chow or a high-fat diet.</p> <p>Methodology: Mice with deletion of Pex5 in adipocytes (KO) and littermate controls (WT) were fed for 8 weeks with either a chow (C) or a high-fat diet (HFD) and evaluated for weight gain, food intake, glucose (GTT) and insulin tolerance (ITT) and tissue masses (CEUA: 6071181120). Results are expressed as mean \pm SEM, ANOVA two way $p < 0.05$, $n = 7-10$ mice per group.</p> <p>Results: There were no changes in food intake between groups (WT+C 102.2\pm2.23 vs WT HFD: 83.7\pm1.72 vs KO+C: 92.3\pm0.65 vs KO+HFD: 94.2\pm2.19 kcal/week), WT and KO mice ingesting the HFD display a similar increase in body weight (WT+C 4.51\pm0.56 vs WT HFD: 9.33\pm0.45 vs KO+C: 3.93\pm0.52 vs KO+HFD: 9.52\pm0.64 g) and inguinal (WT+C 194,9\pm10,83 vs WT HFD: 787,1\pm61,58 vs KO+C: 216,7\pm9,51 vs KO+HFD: 687,7\pm74,18 g) and epididymal (WT+C 287,1\pm14,37 vs WT HFD: 1430\pm128,2 vs KO+C: 234,4\pm12,87 vs KO+HFD: 1194\pm152 g) adipose tissue masses, glucose intolerance (WT+C 21820\pm826,6 vs WT HFD: 31187\pm1576 vs KO+C: 22235\pm797,5 vs KO+HFD: 32882\pm1059 AU) and insulin resistance in comparison to chow fed mice (WT+C 1366\pm0,09 vs WT HFD: 0,8713\pm0,08 vs KO+C: 1316\pm0,12 vs KO+HFD: 0,7439\pm0,08 AU).</p> <p>Conclusion: Peroxisomal dysfunction in adipocytes due to Pex5 deletion does not affect energy balance, adiposity and glucose homeostasis in mice.</p> <p>Keywords: Peroxisomes, obesity, insulin resistance and Peroxin 5</p>
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Title	Suppression of cholesterol synthesis reduces hepatic steatosis and inflammation in mice with constitutive activation of PI3K signaling in hepatocytes
Authors	Natália M. Pessoa ¹ , Érique de Castro ¹ , Érika V. M. Pessoa ¹ , Thayna S. Vieira ¹ , Albert S. Peixoto ¹ , Bianca Franco Leonardi ¹ , Marina A. A. Honda ¹ , Willian T. Festuccia ¹ .
Affiliations	1 Institute of Biomedical Sciences of the University of São Paulo (ICB-USP).
Session	

Abstract, Ethics Committee Number*, and Keywords	<p>Introduction: Lipid accumulation triggers a series of parallel events in liver promoting the progressive development of metabolic associated fatty liver disease (MAFLD). The specific implication of cholesterol, which is mainly synthesized in hepatocytes, in MAFLD development and progression is not well understood. Objective: To investigate the implication of cholesterol in the development and progression of MAFLD. Methods: Male mice (n= 5-12) bearing Pten deletion in hepatocytes (KO) and littermate floxed controls (WT) were treated with vehicle or the cholesterol synthesis inhibitor atorvastatin (ATV, 15 mg/kg/day) for 10 weeks and evaluated for body weight, serum metabolites, glucose homeostasis, liver mass, lipid content, mRNA and protein content, and inflammation (CEUA, 6160250820). Results are mean ± SEM. ANOVA two way, followed by Tukey's post-test, p<0.05. Results: ATV treatment improved liver morphology and reduced liver mass (KO+ATV 2340±95 vs KO 2810±225 mg), fasting cholesterol (KO+ATV 151.09±7.95 vs KO 182.15±7.7 mg/dL), liver cholesterol content (KO+ATV 0.098±0.009 vs KOVEH 0.204±0.018 ug/mg of protein) and TAG KOATV (1.04±0.09) vs KO 1.75±0.12 ug/mg of protein) contents, mRNA levels of ACC (KO+ATV 1.48±0.24 vs KO 2.24±0.28 AU) and SCD1 (KO+ATV 3.95±1.08 vs KO 7.102±1.558 AU) and fibrosis-related proteins collagen 1a1c (KO+ATV 8.20±2.14 KO+VEH 29.10±5.98 AU) and 4a1a (KO+ATV 2.03±0.50 vs KO 5.07±1.18 AU) and proteins composing the NLRP3-inflammasome IL1-beta (KO+ATV 1.18±0.24 vs KO 2.72±0.66 AU), NLRP3 (KO+ATV 0.75±0.16 vs KO 2.43±0.65 AU) and ASC pycard (KO+ATV 1.33±0.212 vs KO 1.99±0.28 AU) in KO mice when compared to vehicle treated KO. No alterations in these parameters were observed by ATV treatment of WT mice. In conclusion, inhibition of cholesterol synthesis with ATV reduced hepatic steatosis, inflammation and fibrosis induced by constitutive PI3K signaling activation.</p> <p>Keywords: Cholesterol, MAFLD and Pten</p>
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Title	Exploring how EFL-1 and DCR-1 interact and respond to calorie restriction to control ageing
Authors	Guilherme Tonon-da-Silva ^{1,2} Willian Goulart Salgueiro ^{1,2,6} Evandro Araújo de-Souza ^{1,2,3,4,5} Marcelo Alves da Silva Morj ^{1,2,3}
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Session	07 - Nutrição e Metabolismo

Abstract, Ethics Committee Number*, and Keywords	<p>The world's population is aging, causing a rise in age-related diseases and their socioeconomic burden. Calorie restriction (CR) promotes longevity and protect against age-associated diseases. However, its challenging adherence and potential adverse effects spur research into alternative strategies through unraveling CR molecular mechanisms. A key regulator of longevity and CR benefits is Dicer, a ribonuclease that controls a critical step in the miRNA processing pathway. Highlighting its pivotal role in aging, Dicer deletion decreases both lifespan and oxidative stress resistance in worms and mice, while its overexpression increases these parameters in worms. Moreover, calorie and methionine restriction rely on <i>dcr-1</i>/Dicer for longevity and metabolic adaptation. Yet, Dicer's transcriptional regulation remains unclear. We identified EFL-1, a transcription factor involved in cell cycle and development, as a <i>dcr-1</i> repressor. There are conflicting reports of either increased or decreased longevity upon <i>efl-1</i> silencing. Accordingly, our data show substantial variability in worm lifespan when <i>efl-1</i> is silenced by RNAi during both whole-life and adult-only. Nevertheless, <i>efl-1</i> loss-of-function(lox) mutant worms live shorter than controls. We underpinned EFL-1 as a repressor of <i>dcr-1</i> by showing that both <i>efl-1</i> RNAi and <i>efl-1</i> mutation(lox) increase <i>dcr-1</i> expression. Finally, we show that the <i>eat-2(ad1116)</i> mutation, a genetic model of CR, relies on <i>efl-1</i> to promote longevity, suggesting that <i>efl-1</i> is a mediator of the beneficial effects of CR. Thus, we present a working model in which EFL-1 regulates longevity by transcriptionally suppressing <i>dcr-1</i> and modulating CR-activated pathways. We hypothesize that the variability in lifespan upon <i>efl-1</i> silencing arises from the erratic balance between the negative effects of reducing <i>efl-1</i> expression <i>per se</i> and the beneficial increase in <i>dcr-1</i> upon <i>efl-1</i> silencing.</p> <p>Keywords: EFL-1; miRNAs; Dicer; caloric restriction; aging</p>
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Title	Interesterified fat alters molecular lipid in hypothalamus and mHypoA neuronal cells
Authors	Josiane Érica Miyamoto ¹ , Beatriz Piatezzi Siqueira ² , Raísa Magno dos Santos ² , João Victor da Silva Domingues ² , Penelope Lacrisio Reis Menta ² , Jeanne Guitton ³ , Adriana Souza Torsoni ² , Letícia Martins Ignacio-Souza ² , Marcio Alberto Torsoni ² , Christophe Magnan ⁴ , Hervé Le Stunff ⁴ , Marcelo Alves da Silva Mori ¹ , Marciane Milanski ²
Affiliations	1 – Instituto de Biologia - UNICAMP, Campinas, Brasil 2 – Faculdade de Ciências Aplicadas - UNICAMP, Campinas, Brasil 3 - Molecular Neuroendocrinology of Food Intake - Université Paris Saclay, França 4 - Regulation of Glycemia by Central Nervous System - Université Paris Cité, França
Session	07 - Nutrição e Metabolismo

Abstract, Ethics Committee Number*, and Keywords	<p>The chemical interesterification of vegetable oils results in an increased placement of saturated fatty acids (SFA), predominantly palmitic (C16:0) and/or stearic (C18:0) acids, in the middle (sn-2) position of the triacylglycerol (TAG). As previously described, interesterified fat intake has been associated with altered body composition and disrupted glucose homeostasis in mice. These data sustain the nutritional and metabolic importance of fatty acid localization within TAGs beyond peripheral tissues since the hypothalamus plays an essential role in controlling energy homeostasis, which can be impaired by excessive consumption of saturated fatty acids leading to a state of lipotoxicity. In the present study, we aimed to investigate the molecular mechanisms of the interesterified fat and its predicted metabolite, named 2-palmitoylglycerol (PG) in the hypothalamus and hypothalamic neuronal cell line (mHypoA). Four-week-old male Swiss mice were submitted to either normolipidic diet containing palm oil (PO) or interesterified palm oil (IPO), or a high-fat diet made by PO (POHF) or IPO (IPOHF) for 8 weeks. Targeted lipidomic assessment from hypothalamic and cortex tissue evidenced altered ceramide composition, indicating that altered TAG stereospecific composition is capable to change the lipid metabolites. <i>In vitro</i> analysis with mHypoA cells evidenced that Palmitate and PG increased cellular stress markers, altered lipid molecular species by increasing levels of total ceramide and diacylglycerol and disrupted insulin signalling. Myriocin, a serine palmitoyltransferase (SPT) inhibitor, rescued Akt phosphorylation indicating the involvement of de novo ceramide synthesis in the driving aspects of IR mediated by Palm and PG. In conclusion, we believe that the modification of TAG structure by interesterification, causing unnatural placement of saturated fats in the sn-2 position of the glycerol backbone, could potentiate the effect of SFA.</p>
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The projects were approved by Comitê de Ética em Pesquisa Animal da Universidade Estadual de Campinas (CEUA: n° 4864-1/2018) and Buffon-Université Paris Cité" Ethics Committee (#2016040414129137, French Ministry of Research).

Key words: Hypothalamus, insulin resistance, obesity, lipidomic, ceramides, diacylglycerol



Title	The effects of maternal obesogenic diet on hypertrophic capacity and fiber area of the skeletal muscle of adult rats.
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Session	Poster

Abstract, Ethics Committee Number*, and Keywords	<p>Can have consequences for the next generation and compromise the individual's health. It has become widely accepted that a high-calorie diet during critical phases in early life can cause metabolic disorders such as type 2 diabetes and obesity. Neonates were obtained by mating male and nulliparous female in a 1:2 ratio. The research project followed the standards of the National Council for Animal Control and Experimentation and was approved by the Animal Experimentation Ethics Committee of UFPE(Process n° 0035/2022). One week pre-mating, the rats (8 rats, being CTRL n=4 and OB n=4) were fed with AIN-93G control diet or obesogenic diet, based on the control diet, which will be rich in energy and highly palatable (43% carbohydrate, 39% lipid and 18% protein) and condensed milk (77.6% sugar, 11.2% fat, 11.2% protein). The pups were weaned on the 22nd postnatal day and fed the standard Nuvilab diet until 90 days of age. On the 83rd DPN, they underwent muscle ablation surgery causing compensatory hypertrophy in the EDL muscle and were euthanized on the 90th DPN. The following experimental groups were established: Control diet (CTRL, n=8) subdivided into contralateral paw (DCMatPC) and hypertrophic paw (DCMatPH); Obesogenic diet (OB, n=8) subdivided into contralateral paw (DOMatPC) and hypertrophic paw (DOMatPH). The wet weight of hypertrophied EDL (mg/cm*1000) was higher in both groups compared to their respective controls (DCMatPC:41.45 vs. DCMatPH:49.43 P:0.0040 / DOMatPC:42.43 vs. DOMatPH:51.58 P:0.0002 CI 95%). The median measurement of muscle fibers of the DOMat:PH group was larger than those of their control DOMat:PC (DOMatPC: 94.76µm vs. DOMatPH: 104.2µm CI 95%).</p>
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It can be concluded that the maternal obesogenic diet was not able to change the wet mass of the EDL, however it contributed to an increase in fiber circumference.

Keywords: Obesogenic Diet. Metabolic Disorders. Skeletal muscle Compensatory Hypertrophy.



Title	Impact of high-fat diet and Bisphenol S on colon morphology in male mice
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Affiliations	1 - Department of Morphology, Morphology and Metabolism Research Center (NUPEMM), Universidade Federal Fluminense, RJ, Brazil. 2 - Laboratory of Experimental Endocrinology (LEEx), Institute of Biomedical Sciences, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil. 3 - Department of Morphology, Laboratory of Teaching and Research in Histology and Comparative Embryology, Universidade Federal Fluminense, RJ, Brazil. 4 - Postgraduate Program in Pathology, Fluminense Federal University (UFF), Niterói, RJ, Brazil 5 - Exercise Science Laboratory (LACE), Fluminense Federal University, RJ, Brazil.
Session	07 - Nutrição e Metabolismo

Abstract, Ethics Committee Number*, and Keywords	<p>Bisphenol is a plasticizer present in the environment that may contribute to the development of obesity, considered an endocrine disruptor (ED). Bisphenol A (BPA) is the best known of these and has been banned after being proven to be an environmental contaminant. Bisphenol S (BPS) also has ED chemical characteristics and is the major substitute for BPA. The gastrointestinal tract (GIT) is the first site of contact after food and water intake, and therefore the gut may be the first site of inflammation induced by diet and EDs. The aim of this study is to demonstrate that exposure to BPS could alter the colon morphology in eutrophic and obese mice. Adult male C57BL/6 mice received standard (SC) or hyperlipidic diet (HF), being exposed or not to BPS at 4 or 50µg/Kg/day for 12 weeks, separated in groups: SC, SCB4, SCB50, HF, HFB4, HFB50 (CEUA nº 1929240521). Body mass was evaluated daily. The colon was collected for histology analysis. Intestinal crypts were measured and goblet cells were quantified. The SCB4 group showed an increase in body mass in relation to the SC and SCB50 from the fifth week of BPS administration BPS (+6,5% SCB4xSC, p<0,05; +7,9% SCB4xSCB50, p<0,01). The HFB4 and HFB50 groups showed a decrease in body mass in relation to HF from the second week of BPS administration (-7,2% HFB4xHF, p<0,05; -9,7% HFB50xHF, p<0,01). The SCB4 and SCB50 groups had larger intestinal crypts than the SC (+14,4% SCB4xSC,</p>
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$p < 0,01$; +50,0% SCB50xSC, $p < 0,01$), and the HFB4 had larger intestinal crypts than the HF (+50,4% HFB4xHF, $p < 0,05$). The SCB4 and SCB50 groups showed a decrease in the percentage of goblet cells compared to the SC (-37,9% SCB4xSC, $p < 0,05$; -48% SCB4xSCB50, $p < 0,05$). The HFB50 group showed a decrease in the percentage of goblet cells compared to HF (-48,07% HFB50xHF, $p < 0,05$). Exposure to BPS causes significant impacts on colon morphology and intestinal health, potentially leading to disturbances in the gastrointestinal tract of both eutrophic and obese mice.



Title	Serum levels of vitamin D, anthropometric parameters, and neuromotor aspects in children
Authors	Ana Beatriz Januário da Silva ^{1,4} ; Maria Eduarda Rodrigues Alves dos Santos ^{1,4} ; Waleska Maria Almeida Barros ^{2,4} ; Sandra Lopes de Souza ¹ ; Ana Patrícia da Silva Souza ^{1,4} ; Karollainy Gomes da Silva ^{1,4} ; Beatriz Machado Silva ³ ; Robson Feliciano da Silva ⁴ ; Mayara Luclécia da Silva ⁴ ; Antonietta Claudia Barbosa da Fonseca Carneiro ^{1,4} ; Priscyla Evelyn da Silva Albuquerque de Melo Rolindo ⁴ ; Aline Ananias de Lima ¹ ; José Maurício Flôrencio da Silva ⁴ ; Williclecia Walkiria Dias Ferreira ⁴ ; Érica Helena Alves da Silva ⁴
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Session	Nutrition e metabolism

Abstract, Ethics Committee Number*, and Keywords	Vitamin D (vit D) is a fat-soluble vitamin. Its metabolic role includes regulating calcium homeostasis and various extra-skeletal actions. Low levels of vit D can impair motor performance and motor coordination development; however, this is not universally agreed upon in the pediatric population. The correlation between vit D levels, anthropometric data, and neuromotor aspects of children aged 7 to 11 years was investigated. This study employed an analytical cross-sectional design, with body composition assessed through measurements such as weight, height, and skinfold thickness. Handgrip strength was measured using a digital handgrip dynamometer, and motor performance was assessed using the Körperkoordination Test für Kinder (KTK). Vit D levels were determined in blood serum and quantified using chemiluminescence. A total of 134 volunteers were evaluated, with 68 being male. Anthropometric data were age-adjusted for weight, height, BMI, and body fat percentage (%BF). The majority of participants exhibited handgrip strength at the 75th percentile in both hemibodies. The mean motor coordination score was 98.08 ± 15.48 points. Correlations were observed between vit D levels and motor coordination in children aged 7 to 9 years ($r = 0.31$, $p = 0.039$) and aged 10 to 11 years ($r = 0.35$, $p = 0.029$), both indicating a weak positive correlation. Additionally, vit D levels were correlated with body weight ($r = -0.34$, $p = 0.031$) and %BF ($r = -0.32$, $p = 0.046$) in children aged 10 to 11 years, suggesting a weak negative correlation. These findings highlight the importance of early detection of vit D deficiency and its association with
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anthropometric parameters and neuromotor function during childhood, with the aim of attenuating impairments in muscular functionality, as motor development may predict overall development.

Committee number: UFPE/CAAE - 44497921.0.0000.5208; 4.848.847.

Keywords: anthropometric; performance neuromotor; motor coordination; vitamin D.



Title	Caloric restriction improves glucose homeostasis via induction of de novo lipogenesis in adipose tissue in mice
Authors	Vinícius Franco de Freitas ¹ Raissa Guimarães Ludwig ¹ Felippe Mousovich Neto ² Niedson Correia de Lima Junior ¹ Josiane Érica Miyamoto ¹ Stepheny Carneiro de Campos Zani ¹ Gisele de Castro ¹ Pedro Manoel Mendes de Moraes Vieira ¹ Marcelo Alves da Silva Mori ¹
Affiliations	1 – UNICAMP, Campinas, Brazil 2 – Weill Cornell Medicine, New York, USA
Session	7 – Nutrition and metabolism

Abstract, Ethics Committee Number*, and Keywords	<p>Food intake has a large impact on organism metabolism wherein an adequate diet can provide prevention for diseases such as diabetes and hepatic steatosis and promote an increase in life quality. Dietary restriction or caloric restriction (CR) creates a negative energy balance allowing for increased longevity, improved insulin sensitivity, and other health benefits. <i>Chrebp</i> is a transcription factor expressed with metabolic dominance in adipose tissue (AT) and activates a wide range of genes involved in many pathways such as insulin signaling, cell cycle, TCA cycle, <i>de novo</i> lipogenesis (DNL), etc. Transcriptomics and proteomics data from the subcutaneous white adipose tissue (sWAT) of mice indicate that CR induces genes and proteins related to DNL. The main objective of this work was to evaluate the role of adipose <i>Chrebp</i> in mice subjected to CR. To this end, the <i>Chrebp</i> gene was knocked out (<i>Chrebp</i>-KO) specifically in adipocytes of male and female mice. The mice were exposed to <i>ad libitum</i> or CR for one or three months and metabolic parameters were evaluated. Genes involved in DNL such as fatty acid synthase and acetyl carboxylase were measured to assess the induction of the pathway. We found that CR requires adipocyte <i>Chrebp</i> to induce DNL related genes in AT and improve glucose tolerance and insulin sensitivity. Incubation of differentiated brown adipocytes with serum of mice under CR did not recapitulate the inductions found in vivo. So far, we conclude that CR increases lipogenic genes in sWAT of mice and this may be required for the improvement of glucose metabolism during CR. Keywords: Caloric restriction; <i>de novo</i> lipogenesis; <i>Chrebp</i>; Adipose tissue</p> <p>Ethics committee number: 6092-1/2022</p>
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Title	Metabolic syndrome and the relationship with the prodrome symptoms of Parkinson's disease
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 Silva ⁴ , José Maurício Lucas da Silva ³ , Mayara Luclécia da Silva ³ , Williclecia Walkiria Dias Ferreira ³ , Érica Helena Alves da Silva ³ , Priscyla Evelyn da Silva Albuquerque ³ , Robson Feliciano da Silva ³ , Georgiane Brandão Rodrigues da Silva ³ , Antonietta Cláudia Barbosa da Fonseca Carneiro ^{1,2,3} , Sandra Lopes de Souza ¹ , Waleska Maria Almeida Barros ^{1,2,3}
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Session	Nutrition and metabolism

Abstract, Ethics Committee Number*, and Keywords	<p>Mechanisms such as neuroinflammation, mitochondrial dysfunction and increased oxidative stress link Metabolic Syndrome (MS) and Parkinson's Disease (PD). The objective was to investigate the frequency of MS in adult patients without PD, and relate it to prodromal symptoms of PD. This is a cross-sectional study, blood collections were carried out to analyze fasting serum levels of glucose, triglycerides, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol. To assess daytime sleepiness, the Epworth sleepiness scale was applied, for symptoms suggestive of Depression the Patient Health Questionnaire-9, with the Brazilian version of the Montreal Cognitive Assessment, cognitive function was assessed. Anthropometry assessment, measurement of systemic blood pressure and Berg balance test. Ethics committee number: 37085720.2.0000.5208 Federal University of Pernambuco. 179 individuals were evaluated, the majority 141 (78.8%) female, with a mean age of 49.64 (± 6.0) years. To allocate groups with and without MS, a sample of 89 volunteers was obtained, among which 33 (71.7%) were obese. Between the prodromal symptoms and MS, significant correlations were found: between the Daytime Sleepiness scores and triglycerides in the group without MS ($n=28$, $r=0.39$, $p=0.048$); between cognitive assessment scores and diastolic blood pressure in the group without MS ($n=30$, $r=0.47$, $p=0.017$); and Waist circumference in the group without MS ($n=30$, $r=-0.4$, $p=0.033$); and glucose in the MS $n=32$ ($r=0.43$, $p=0.019$) and no MS ($n=30$, $r=0.39$, $p=0.037$) groups. The frequency of MS among those evaluated was 51.7% and there was a relationship between its components and the prodromal symptoms of PD, such as excessive daytime sleepiness and mild cognitive impairment, both in individuals with MS and without the syndrome. These components, in addition to representing a cardiovascular risk factor, when added to prodromal symptoms, can signal the onset of a neurodegenerative disease.</p> <p>Keywords: Metabolic syndrome; Parkinson's disease; Prodromal symptoms; daytime sleepiness; depression</p>
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Title	Metabolic influence of <i>Curcuma longa</i> extract in <i>Macrobrachium rosenbergii</i> post-larvae
Authors	Pedro Trabulsi Junqueira Franco Milena Cia Retcheski Daniel Massato Vital Hide Ana Paula Pelinson da Fonseca Luciano Tormen Sílvia Romão Luísa Helena Cazarolli Caroline Cristina Ribeiro Simões de Souza
Affiliations	Universidade Federal da Fronteira Sul
Session	Nutrição e metabolismo

Abstract, Ethics Committee Number*, and Keywords	<p>This work studied the effects of <i>Curcuma longa</i> extract as a metabolic modulator for <i>Macrobrachium rosenbergii</i> post-larvae. Shrimp post-larvae received diets with different concentrations of turmeric extract (0.05, 0.2, 1%) for 60 days. At the end of the supplementation period, weight gain as well as metabolic enzymes activities such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), glutamate dehydrogenase (GLDH) from aminoacidsamino acids metabolism and citrate synthase (CS) from Krebs Cycle were evaluated. Turmeric extract (0.05%) significantly increased weight gain on treated animals. The glutamate dehydrogenase activity in the 0.05% group was also increased by turmeric extract while no changes were observed in the activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Also, a significant increase in citrate synthase activity for all concentrations of turmeric was observed in liver and muscle from <i>M. rosenbergii</i>. The turmeric extract increased weight gain and improved amino acids metabolism and energy production through Krebs Cycle in <i>M. rosenbergii</i> post-larvae demonstrating its potential to be incorporated in the shrimps' diet as a metabolic modulator to improve development.</p> <p>KEYWORDS: turmeric, metabolism, freshwater shrimp, energy production.</p>
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Title	Investigation of the association between skeletal muscle and treatment with fluoroquinolone compounds in the prevention of obesity and its comorbidities
Authors	Niedson Correia de Lima Junior ¹ , João Pedro Mazzi¹ , Gerson Profeta de Souza ^{1 2} , Linnéa Bergenholm ³ , Gavin O'Mahony ³ , Jeremie Boucher ³ , Henvér Simionato Brunetta ¹ , Jessica Aparecida da Silva Pereira ¹ , Fernanda Luísa Basei ¹ , Letícia de Souza Figueiredo ¹ , Gabriel Palermo Ruiz ^{1 2} , Deisi Braga Shimo ¹ , Andrea Livia Rocha ⁴ , Raul Gobato Costa ¹ , Kauê de Oliveira Chinaglia ¹ , Marcelo Alves da Silva Mori ¹
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 4- Clayton Foundation Laboratories for Peptide Biology, Salk Institute for Biological Studies, La Jolla, CA, USA
Session	07 - Nutrition and Metabolism

Abstract and Keywords	<p>Introduction: Obesity is one of the largest public health problems around the world. Overweight and obesity are relevant risk factors for several diseases such as type 2 diabetes. Increased fat accumulation and changes in glycemic homeostasis are closely related to the development of these pathologies. However, the loss of muscle mass is a common issue along the weight loss process. Recent research from our team shows that fluoroquinolone compounds promote weight loss and improve glycemic homeostasis in a mouse model of obesity while preserving muscle mass. Hence, we decided to investigate the effects of these compounds in muscle mass in mice.</p> <p>Methods: To comprehend the relationship between fluoroquinolones and striated skeletal muscle, we utilized C57BL/6J mice (CEUA: 5946-1/2022) acclimated at 21°C and fed a high-fat diet (HFD) for 10 weeks to induce obesity. Subsequently, we administered fluoroquinolone compounds for an additional 10 weeks concomitant to HFD.</p> <p>Results: Treatment of obese mice with two fluoroquinolone compounds that lack antibiotic effect resulted in attenuation of weight gain, maintenance of glycemic homeostasis, increased weight in the gastrocnemius muscle, along with an increase in grip strength. In the gastrocnemius muscle, the administration of the compounds increased gene expression related to mitochondrial biogenesis. There was also an increase in the expression of some glycolytic fiber markers. In addition, genes related to the structure of muscle fibers displayed changes, suggesting modifications in muscle structure and bioenergetics.</p> <p>Conclusion: Taken together, these findings highlight the potential of muscle tissue to mediate the beneficial metabolic effects of fluoroquinolone compounds. Our next steps involve exploring how these compounds interact in conjunction with physical exercise, employing an obese mouse model to unravel the potential metabolic changes arising from this combination.</p> <p>Key words: Obesity, muscle, fluoroquinolone.</p>
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Title	Maternal low-protein diet: impact on sex-specific behavioral changes in aging mouse offspring
Authors	Vinícius Schiavinatto Mariano ¹ Amanda Cristina de Souza ¹ Gabriela Ninin de Carvalho Barros ¹ Isabele Cristina Globo Tibiriçá ¹ Patricia Aline Boer ¹ José Antonio Rocha Gontijo ¹
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Session	07 - Nutrição e Metabolismo

Abstract, Ethics Committee Number*, and Keywords	<p>The gestational low-protein (LP) diet experimental model provides insights into adult neuropsychiatric disorder origins, but its impact on aging remains incompletely elucidated. Prior research from our group indicates LP diet affects various middle-aged male mice behaviors, including learning and memory, exploratory, depressive, and anxiety-like behaviors. However, the impact of this diet on aged mice remains unclear. This study aimed to assess behavior in aged male and female mice subjected to gestational protein restriction. Mated C57BL/6J female mice (CEUA 5776-1/2021; 20-23g, 8 weeks old) were submitted to a normal protein (NP: 17% protein, n=7) or low protein (LP: 6% protein, n=7) diet throughout pregnancy. Following birth, the pups were weaned at 21 postnatal days and raised until they reached 18 months of age for behavior tests. Data was expressed as mean±SD. In the open field test (OF), 18-month-old male LP offspring exhibited a significant increase in locomotor and horizontal exploratory behavior (distance traveled: NP: 8127±4303 vs LP: 11632±1211 mm; velocity: NP: 28±15 vs LP: 41±7 mm/s). Female LP offspring showed no changes in exploratory behavior but spent more time at the borders (NP: 297±2 vs LP: 299±1 sec.) and less time in the center (NP: 1,9±1,6 vs LP: 0,5±0,5) of the OF, indicating thigmotaxis behavior. Memory performance assessed by the novel object recognition test (NOR), social interaction (SI), social memory (SM), and sucrose preference test (SPT) showed no changes in male LP offspring. However, female LP offspring exhibited improved long-term memory in NOR and SM but reduced sucrose preference in SPT (NP: 69±10%, LP: 62±11%), suggesting depressive-like behavior. No differences were observed in the elevated plus maze (EPM) test for either sex. These findings showed that maternal LP diet has distinct effects between genders on aging behavior, showing hyperactivity-like behavior on male and anxiety-like and depressive-like in female.</p> <p>Keywords: Low-protein diet; neuroscience; behavior; aging</p>
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Title	Effects of virtual reality on aspects related to sleep and mental health in adult individuals with metabolic syndrome
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Session	Nutrition and Metabolic

Abstract, Ethics Committee Number, and Keywords	<p>Metabolic syndrome (MS) is a set of metabolic dysregulations, which includes increased abdominal obesity, insulin resistance, dysfunctions in glucose metabolism, atherogenic dyslipidemia, systemic arterial hypertension, which can interfere with aspects of sleep, mental health, executive functions. The objective was to evaluate the effects of virtual reality on aspects related to sleep, symptoms suggestive of depression, anxiety and executive functions in adult individuals with MS. This is a prospective longitudinal study, blood collection was performed to analyze glycemic levels, triglycerides and high- density lipoprotein cholesterol. To assess sleep quality, the Pittsburgh sleep quality index was applied, excessive daytime sleepiness using the Epworth sleepiness scale, symptoms suggestive of depression using the patient health questionnaire, state-trait anxiety inventory, and tower in London to evaluate executive functions, in addition to carrying out anthropometry, measuring systemic blood pressure and applying exergaming in virtual environments using the Xbox 360 Kinect (Ethics committee number: 58145522.3.0000.9430 Federal University of Pernambuco). 76 individuals were evaluated, the majority of whom were female, 71 (93.4%), aged 48-55. To allocate volunteers into the groups without MS Control (C), without MS Intervention (I), MS C and MS I, the classification of the International Diabetes Federation was used. In the SM group I, positive effects with virtual reality were observed for waist circumference, and systolic and diastolic blood pressure. The groups without SM and SM had poor sleep quality in pre- and post-intervention data. In anxiety, the majority presented low and medium levels of symptoms, with an effect being seen on the trait of the SM group I. In the Tower of London test, the effect of virtual reality on the SM I group was demonstrated, with an increase in the score and a decrease in execution time.</p> <p>Keywords: Metabolic syndrome. Anxiety. Depression. Cognition. Sleep. Virtual reality</p>
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XXXVIII REUNIÃO ANUAL DA FESBE
XXII REUNIÃO ANUAL DA BRAVO
XVIII CONGRESSO DA SBCAL
III CONGRESSO DOHAD BRASIL
II CONGRESSO DA SBBA
2 A 5 DE JULHO 2024, CAMPINAS/SP
FRONTEIRAS DAS CIÊNCIAS BIOMÉDICAS E SEUS DESAFIOS

Title	Adrenal gland histomorphometry in obese rats due to lactational hypernutrition subjected to regular swimming
Authors	Elizangela Stein Helen de França Kailer Marina Helena Forlin Eduarda Felchack Caldas Ellen Carolina Zawoski Gomes Sabrina Grassioli
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Session	07 - Nutrição e Metabolismo

Abstract, Ethics Committee Number, and Keywords	<p>The concept Developmental Origins of Health and Disease postulates that nutritional changes during lactation impact at risk to develop chronic diseases throughout life. Lactational hypernutrition results in obesity and changes in pituitary adrenal axis. In this study, we investigated the effects of physical activity on obesity induced by overnutrition in the lactation period and the histomorphometry of adrenal gland. Ethics Committee Approval nº 16-12. To induce overnutrition, at 3 days, male Wistar rats were adjusted to Normal Litters (NL; 9 newborn mice/rat) and Reduced Litters (RL; 3 newborn mice/rat) and maintained until 21 days, when they were weaned and subdivided into exercised (EXE) and sedentary (SED); forming 4 experimental groups (n=4-6 rats/group): NLSSED; NLEXE; RLSED; RLEXE. The physical exercise program consisted of swimming (3 times/week; 30 minutes/day; for 8 weeks). At 90 days, the adrenal glands were removed, weighed, and prepared for histological analysis. Factor (F) litter reduction, exercise (E) and interaction (litter reduction x E) effects were obtained by Anova two way. RLSED adult animals developed obesity and had the worst nuclear pattern of chromaffin cells. The medulla area/adrenal area ratio was affected by litter reduction in EXE groups (F= 6,464 p= 0,0258), with a decrease of 39,7% in the RLEXE group, compared to NLEXE. Early swimming increased 28,57% the adrenal glands weight in the RLEXE group (F= 5,447 p= 0,0220) and improved the nuclear pattern of chromaffin cells, compared to RLSED rats. Higher caloric intake promoted by the litter reduction favored obesity and induced changes in adrenal gland. Physical exercise started early and maintained throughout life increased the gland weight and improved the pattern of chromaffin cells. Probably, physical exercise can promote positive adjustments in the adrenal gland of rats born in NR, reprogramming this organ for health.</p> <p>Keywords: Obesity, Lactation, Exercise.</p>
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Title	Bergamot by-product modulates cardiac biogenic amines and prevents unhealthy diet-induced cardiovascular disease
Authors	Matheus Antônio Filiol Belin - 1 Taynara Aparecida Vieira - 1 Núbia Alves Grandini - 1 Jordanna Cruzeiro - 1 Luis Eduardo Sormani - 1 Thiago Luiz Novaga Palacio - 1 Juliana Silva Siqueira - 1 Murilo Dalarme Tanganini - 1 Cristina Schmitt Gregolin - 1 Dijon Henrique Salomé de Campos - 1 Silmeia Garcia Zanati Bazan - 1 Igor Otávio Minatel - 2 Giancarlo Aldini - 3 Giuseppina Pace Pereira Lima - 2 Camila Renata Correa - 1
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Session	Oral Session

Abstract, Ethics Committee Number*, and Keywords	An unhealthy diet (UD), rich in sugar and fat, generates obesity, inflammation, and oxidative stress, triggering cardiovascular diseases (CVD). The bergamot (<i>Citrus bergamia</i>) by-product (BE) has anti-inflammatory and antioxidant capacity and can modulate CVD markers, such as biogenic amines. This study aimed to evaluate the influence of supplementation with bergamot by-product on CVD induced by an unhealthy diet and on cardiac biogenic amines. Wistar rats (n=54) were randomly distributed into 3 groups: control, UD, and UD +BE for 20 weeks (Ethics Committee: CEUA,1337/2019). UD is rich in sugar and fat, added 25% sucrose in drinking water. BE was diluted in drinking water and administered by gavage (250mg/kg/d). Body weight, daily caloric intake, and adiposity index were assessed. Systolic blood pressure (mmHg) was assessed by tail plethysmography and cardiac structure and function by Doppler Echocardiography. Cardiac biogenic amine levels (µg/g) were assessed by high-performance liquid chromatography (HPLC). Data were submitted to the Tukey test or Dunn's test at 5% significance. UD generated obesity (p<0.001), systolic arterial hypertension (SAH) (p<0.001), cardiac remodeling (p<0.001) and dysfunction (p<0.001), a decrease in cadaverine (3.9 vs. 0.4; p<0.05), agmatine
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(4.3 vs. 1.3; $p < 0.05$), spermidine (20 vs. 15; $p = 0.001$), putrescine (2.5 vs. 1.5; $p < 0.05$) and increase in total serotonin (28 vs. 54; $p < 0.05$) compared to the control group. Supplementation with BE did not prevent obesity, but it prevented SAH ($p = 0.016$), cardiac remodeling ($p < 0.001$) and dysfunction ($p < 0.001$), and the decrease in spermidine (15 vs. 19, $p = 0.025$), putrescine (1.5 vs. 2.0; $p < 0.05$); and increased spermine (12 vs. 17; $p < 0.05$) compared to the UD group. We conclude that supplementation with bergamot by-product prevented CVD induced by an unhealthy diet and modulated levels of cardiac biogenic amines, maintaining spermidine and putrescine, and increasing spermine.

Keywords: cardiac remodeling, cardiac dysfunction, hypertension, spermidine, serotonin.



Title	Elevated BMI relates to increased VLDL particles transporting miR-122 from the liver in humans
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Session	Session 7: Nutrição e Metabolismo

Abstract, Ethics Committee Number*, and Keywords	<p>In the pathogenesis of the metabolic dysfunction-associated steatotic liver disease (MASLD) there is an increase in VLDL content in the bloodstream, responsible for transporting mostly triglycerides to mainly adipose tissue. Recently, a study showed the presence of miRNAs into VLDL particles. A liver-specific miRNA, miR-122 involved in lipogenesis silencing, is modulated in hepatocytes, and has increased circulating rates in the MASLD models.</p> <p>To investigate the miR-122 levels into VLDL, liver and adipose tissue and its association with BMI in human patients, participants were invited to join our research signing the Research Ethics Committee protocol CAAE: 44054421.7.0000.5404. After agreement, liver, visceral and subcutaneous adipose tissues and plasma were collected from patients who underwent a cholecystectomy (to compound Control group-C) or bariatric surgery (for Obesity group-Ob) in Hospital das Clínicas – UNICAMP, Brazil. Ob patients had normal levels of liver injury markers (AST and ALT) and fibrosis indicators (FIB-4 and APRI), and the lipid profile (cholesterol and triglycerides) wasn't different from C (N: C=19, Ob=15). Despite that, hepatic miR-122 levels were increased in Ob group compared with the C group (N: C=5 C, Ob=7; p<0.0001). Although some authors describe miR-122 as liver-specific, its expression were found in subcutaneous (N=4 each group) and visceral adipose tissues (N: C=11,Ob=13), whereas in smaller quantities than the liver (p<0.0001). miR-122 expression was also found in VLDL particles,</p>
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with no differences between groups (N: C=13,Ob=10). miR-122 levels in VLDL and in liver were positively associated with each other and with the BMI ($p<0.05$). In summary, VLDL particles can transport miR-122 from the liver to extrahepatic tissues, and the presence of obesity has been sufficient to significantly increase the expression of miR-122 in the liver. Furthermore, adipose tissue, the main destination of VLDLs, exhibits lower levels of miR-122.

Keywords: MASLD, miR-122, VLDL, Obesity.



Title	Palmitate modulates $\alpha 7$nAChR expression and cause different disruptions in epigenetic mechanisms in microglia and neurons
Authors	<u>Martins, Í.C.A.</u> , Aguiar, M.S., Chaves, W. F., Costa, C. L., Silote, G. P., Torsoni, A.S., Torsoni, M.A.
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Session	07- Nutrição e Metabolismo

Abstract, Ethics Committee Number*, and Keywords	<p>Inflammatory processes can development metabolic disorders in hypothalamus. Alpha7 Nicotinic acetylcholine receptor ($\alpha 7$nAChR) are expressed in the central nervous system and his activation leads to the inhibition of inflammatory cytokines transcription. Short-term high-fat diet impairs the expression of $\alpha 7$nAChR, rendering the hypothalamus more susceptible to inflammatory damage, but the specific cellular type and the mechanisms behind these impairments it is still unknown. These mechanisms can act by inhibiting the <i>Chrna7</i> gene expression or by controlling his presence/activity in the membrane. The mouse-derived microglia and neuronal immortalized cell lines, BV-2 and mHypoA-POMC/GFP, were treated with palmitic acid (PA) at 150uM and 100uM for 16 hours. The gene expression were performed by RTq-PCR. Results are presented as mean and S.E.M. and assessed using a T-test, with the significance level set at $p < 0.05$. After stimulus with PA, in both cell lines we observed an increase in $TNF\alpha$, $IL-1\beta$ and $IL-6$ and a decrease in <i>Chrna7</i> expression. In BV-2, there's a tendency to increase the expression of DNMT1 and in the mHypoA-POMC/GFP an increase in DNMT3a and 3b. Also, an increase in HDAC3 were observed in both cell lines. About the $\alpha 7$nAChR chaperones, there's a decrease in Ric3 and Tmen35A in microglia, and only Ric3 in neuron. The genes related to ubiquitin ligases E1 (<i>Uba 1</i>) and E3 (<i>Uba3a</i> and <i>Ube3b</i>) were increased in microglia, but not in neuron. Our results suggest that PA treatment leads to some different effects in the cell lines. The activation of pre-transcriptional mechanisms by the action of the DNMTs enzymes and HDAC3 in prevent the transcription of the $\alpha 7$nAChR receptor must be active in both cell lines. The post-translational mechanisms must be active in microglia by the ubiquitin ligases and through the decrease of chaperones, responsible for the surface expression of the $\alpha 7$nAChR receptor.</p> <p>Keywords: $\alpha 7$nAChR, cell culture, epigenetic, inflammation. #2020/06757-0</p>
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Title	Accase inhibitor as a treatment protects the development of obesity in Wistar rats treated with a diet rich in carbohydrates
Authors	Silva, M.C ¹ Fratti, A.B.P ¹ Guatelli, E. ¹ Peralta, R.M ¹ Bonfim, P.S ² Constantin, R.P ¹ Dos Santos, W.D ¹
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Session	07 - Nutrição e Metabolismo 27 - Abordagens Pré-clínicas em DOHAD

Abstract, Ethics Committee Number*, and Keywords	<p>Obesity is a constantly growing global problem, affecting all age groups, from children to adults of both sexes. This condition of chronic low-grade inflammation is a concern for overall health as it can result in the accumulation of body fat, release of pro-inflammatory cytokines, and increase the risk of insulin resistance (IR), metabolic syndrome, and other systemic abnormalities. Additionally, adolescence is considered a critical phase of development, known as a sensitive period, making the adolescent vulnerable to insults that can lead to the occurrence of diseases throughout their lives. Based on this principle and the literature, we hypothesize that inhibition of the key enzyme of the ACC lipogenic pathway concomitantly with exposure to a diet rich in carbohydrates would reduce obesity and its consequences in adult life. Approved by CEUA nº 1339190623, male Wistar rats of 25DPN housed in individual boxes with free access to standard food and water for 5 days of adaptation, at 30DPN there is start O protocol for 40 days of treatment with inhibitor diluted in corn oil and diet and posteriorly 40 days of dietary recovery. Housed in 3 animals per box, they were divided into the following groups: (CON) which received a standard diet and vehicle, (CCP) standard diet and inhibitor, group (HC) diet rich in carbohydrates and vehicle, group (HCCP) diet and inhibitor. At 120 days of age, the animals were euthanized to collect tissue and blood samples. Animals treated with a dose of 0.8 mg/kg (HCCP) had an improvement in IR, KiTT ($p < 0.00165$) and TyG ($p < 0.0002$) followed by an improvement in the profile hypercholesterolemic, triglycerides ($p < 0.0002$), LDL ($p < 0.0010$) and VLDL ($p < 0.0001$). The results suggest that the inhibition of ACC is a potential target for the treatment of syndrome metabolic, as well as that this pathway has modulation capacity and the adolescence. It may be an intervention phase.</p> <p>Keywords: Metabolic syndrome, glycemic homeostasis, lipid metabolism.</p>
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Title	Nephroprotective and antioxidant effects on renal ischemia and reperfusion displayed by peptides from hardened common bean (<i>Phaseolus vulgaris</i>)
Authors	Ribeiro, J ¹ ; Carvalho, J ¹ ; Berrio, S ¹ ; Graziani, D ¹ ; Batista, K ² ; Xavier, C ¹
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Session	08- biology and kidney diseases
Abstract, Ethics Committee Number*, and Keywords	<p>Introduction: The impairments resulting from ischemia and reperfusion plays a significant role in the occurrence of acute kidney injury. The main mechanism relies on the production of reactive oxygen species (ROS) during the past ischemia reoxygenation. Since the body is unable to perform a complete ROS elimination, the use of antioxidants to mitigate the potential damages caused by the transient ischemia is required. Antioxidant molecules can be acquired from nutraceutical sources. Hardening process in common beans (<i>Phaseolus vulgaris</i>) may impair their digestibility, but peptides that may be a source antioxidant activity remain preserved. We next assessed whether a peptide fraction (PV3) from common beans can mitigate the effects caused ischemia and subsequent reperfusion in kidneys of rats. Methods: Bean flour underwent ultrafiltration process to separate low molecular weight peptides (<3KDa). In vitro tests were performed to evaluate PV3 antioxidant activity using the DPPH method. In endothelial cell assays, we used fluorescent probes to determine ROS and nitric oxide levels. In vivo testing (CEUA-UFG: 057/22) comprised ischemia and reperfusion to evaluate PV3 antioxidant and nephroprotective effects, followed by assessments of food and fluid ingestions, and metabolic and urinary parameters in metabolic cages. Results: in vitro findings showed that PV3 is antioxidant and has a cytoprotective effect against oxidative stress induced by H₂O₂. PV3 also reduced the damage resulting from the renal ischemia and reperfusion process, as evidenced by improvements in urinary biochemical parameters. We conclude that PV3 can preserve the function of kidneys submitted to ischemia probably through potent antioxidant effects, thus supporting the use of hardened beans as a source of peptides with nutraceutical properties able to attenuate the damages caused oxidative-related ischemic effects.</p> <p>Keywords: kidney injury; ischemia; oxidative stress; common beans; PV3.</p>



Title	Bisphenol S exposure promotes kidney injuries in healthy mice
Authors	Michele Lima Brito ¹ , Karen Salve Coutinho-Wolino ² , Beatriz Gouvêa de Luca ¹ , Kauet de Matos Gama e Souza ³ , Beatriz Alexandre-Santos ^{4,5} , Leandro Miranda-Alves ⁶ , Eliete Dalla Corte Frantz ^{4,5} , Clarice Machado dos Santos ³ , D'angelo Carlo Magliano ^{1,4} , Milena Barcza Stockler-Pinto ^{1,2} .
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Session	Biologia e doenças renais

Abstract, Ethics Committee Number*, and Keywords	<p>Exposure to environmental pollutants promotes several metabolic disorders, mainly through inflammation and oxidative stress. Bisphenol S (BPS), acts as an agonist of the aryl hydrocarbon receptor (AHR), which is a mediator of renal uremic toxicity, and may alter kidney morphology. The aim of this study was to evaluate the impact of BPS exposure on AHR expression, renal function and morphological parameters in the kidneys of healthy mice. C57BL/6 male mice were divided into Control (C) (n=9) and Control+BPS (CBPS) (n=12). The BPS (25µg/kg body mass/day) was administered through drinking water. Body mass was measured weekly. After 12 weeks, animals were euthanized and the kidneys were weighed, and plasma was collected to assess the analyses. The glomerular filtration rate (eGFR) was calculated with a specific formula for rodents. Periodic acid–Schiff (PAS) and Hematoxylin eosin (HE) staining were used for morphological analysis and AHR expression was measured using immunohistochemistry. Ethics approval n° 1929240521. With regard to renal function, plasma creatinine was elevated in CBPS group (0.17±0.06 vs 0.15±0.05 [mg/dL]; p=0.3073), while urea and eGFR were not different compared to C group. The percentage of renal steatosis was higher in CBPS group (8.64±4.54 vs 3.47±1.19 [%]; p=0.0393), as well the quantity of glomeruli per</p>
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area (0.96 ± 0.23 vs 1.23 ± 0.07 [mm^2]; $p=0.0337$). However, there was no increase in glomerular diameter. Immunohistochemistry showed higher staining for AHR in CBPS group compared to C group. Qualitative analysis of the renal tissue photomicrographs revealed that the CBPS group showed an increase in tubular lumen, lipid accumulation, segmentation of glomeruli, as well as thickening of the glomerular basement membrane. In conclusion, exposure to BPS resulted in changes in renal morphology, including increased expression of AHR, tubular and glomerular lesions, and lipid accumulation.

Key words: Bisphenol S, kidney damage, environmental pollutant.



Title	Role of erythrocytes in S1P regulation: insights from plasma analysis in hemodialysis patients
Authors	Yuri Daitschman Ozogovski, Vitor André Brugnolo dos Santos, Júlia Bacarin Monte Alegre, Erika Sousa Dias, Beatriz Akemi Kondo Van Spitzenbergen, Gabriela Bohnen Andrade, Andréa Novais Moreno-Amaral
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Session	8 – Biologia e Doenças Renais

Abstract and Keywords	<p>Sphingosine-1-phosphate (S1P) is a lipid signaling molecule with a particular function as a mediator of pro- or anti-inflammation induced by the cytokine network. In chronic kidney disease (CKD), persistent low-grade inflammation is a substantial factor in its development and progression and has correlated with increased mortality and cardiovascular problems. Red blood cells (RBC) are the largest stores and transporters of S1P in the blood. Since anemia is associated with CKD progression, this research aims to evaluate S1P levels in the chronic kidney disease population on hemodialysis. S1P levels were assessed in plasma and plasma enriched with RBC obtained from HD patients (n=19), juxtaposed with healthy controls (CON, n=7). The measurement of S1P was conducted utilizing the Enzyme-Linked Immunosorbent Assay (ELISA) according to the manufacturer's instructions. In the plasma-only analysis, no significant difference was found between HD and CON groups (111.5 ± 36.75 vs. 151 ± 71.02). However, lower levels of S1P were detected in Plasma + RBC from the HD population (168.4 ± 61.2) compared to the CON (267.1 ± 56.1), suggesting that the reduction of S1P may be directly proportional to the presence of RBCs in the sample. The results of the comparison between groups reinforce the importance of RBC in the storage and release of S1P.</p> <p>Keywords: S1P; Anemia; Erythrocytes; Hemodialysis; Eryptosis</p>
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Title	Probiotic-enriched yogurt (<i>Lactobacillus acidophilus</i> LA-5) consumption does not affect oxidative stress damage parameters in a rat model of chronic kidney disease model.
Authors	<u>Joana R. Araujo</u> ¹ , Karen S. Coutinho-Wolino ² , Michele L. Brito ¹ , Ana Letícia M. Lima ³ , Nathalia S Costa ² , Patricia P Almeida ¹ , Manuela F S Melo ² , Beatriz O Cruz ² , Lis J. Aufran ⁴ , Renato S. Abboud ⁵ , Jonas T. Guimarães ⁶ , D'Angelo Carlo Magliano ⁷ , Adriano G. Cruz ⁸ , Milena B. Stockler-Pinto ^{1,2,3}
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Session	Poster and/or Oral

Abstract, Ethics Committee Number*, and Keywords	Chronic kidney disease (CKD) progression is closely related to oxidative stress. To reduce the damage caused by CKD, probiotics have been considered as a promising alternative; however, their use is still controversial. This study aimed to evaluate the effects of probiotic-enriched yogurt on renal parameters, lipid profile, and oxidative stress parameters in 5/6 Nephrectomy rats. Male <i>Wistar</i> rats (n=18) underwent a two-stage 5/6 nephrectomy (Nx) surgery. After 4 weeks, animals were divided into 3 groups (n=6) and received 4 mL daily supplementation by oral gavage as follows: sterile saline solution (Nx), conventional yogurt (NxY), and probiotic yogurt (NxPY). <i>Lactobacillus acidophilus</i> LA-5 concentration was 10 ⁸ to 10 ⁹ colony-forming units. After 8 weeks, serum biochemical analysis, protein carbonyl in plasma, and thiobarbituric acid reactive substances (TBARS) in plasma, colon, heart, and kidney tissues were assessed. Probiotic yogurt increased the estimated glomerular filtration rate compared to NxY (NxPY: 1450±114.1 vs NxY: 1126±232.60 (mmol/L), p= 0.0084), but was similar to Nx group. There was no significant difference between groups respect the lipid profile. Regarding oxidative stress, serum protein carbonyls did not show significative differences the between groups. Also was not observed significative difference in plasma, colon, heart, and kidney tissues TBARS levels between groups. The consumption of probiotic-enriched yogurt with <i>Lactobacillus acidophilus</i> LA-5 during 8 weeks seemed to improve renal function compared to
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conventional yogurt in 5/6 nephrectomized animals. However, did not affect the lipid profile, protein carbonyl, and lipid peroxidation.

Ethical Committee Number: CEUA/UFF Nº 2304150818

Keywords: Chronic kidney disease, *Lactobacillus acidophilus* LA-5, probiotic yogurt, oxidative stress.



Title	Supplementation with the <i>Lactobacillus acidophilus</i> La-05 probiotic aggravates heart hypertrophy and histomorphometry in a chronic kidney disease model
Authors	Karen S. Coutinho-Wolino ¹ , <u>Joana R. Araujo</u> ² , Michele L. Brito ² , Clara S. do Nascimento ³ , Ana Letícia M. Lima ⁴ , Pricilla C. Trigueira ² , Thaís S. Carvalho ⁵ , Guilherme S. Reis ⁵ , D'Angelo C. Magliano ⁵ , Lis J. Autran ⁶ , Renato S. Abboud ⁶ , Adriano G. Cruz ⁷ , Jonas T. Guimarães ⁸ , Milena B. Stockler-Pinto ^{1,2}
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Session	Poster and/or Oral

Abstract, Ethics Committee Number*, and Keywords	Chronic kidney disease (CKD) leads to several cardiac changes and recently probiotics have emerged as important modulators of cardiovascular health. However, few studies demonstrated the effects on the cardiovascular system in CKD. This study aimed to evaluate probiotic supplementation on heart hypertrophy and histomorphometry parameters in 5/6 Nephrectomy rats. Sixteen male <i>Wistar</i> rats were divided into two groups: the nephrectomized group (Nx, n=8) and the probiotic group (NxP, n=8). The NxP group received 4mL of supplementation with <i>Lactobacillus acidophilus</i> La-05 (10^8 - 10^9 log CFU/mL) daily by gavage over eight weeks and the Nx group received sterile saline. Cardiac hypertrophy was assessed using heart-relative weight and heart weight corrected for the tibia length. The left ventricle (LV) of the heart and the aorta were stained with hematoxylin and eosin to evaluate histomorphometry. It was estimated: that the thickness of the LV wall, the diameter of the LV lumen, the diameter of the cardiomyocytes, the thickness of the tunica intima and tunica media from the aorta, and the diameter of the aortic lumen through the Image Pro Plus program. After the supplementation, a significant increase in relative heart weight and heart weight corrected for tibia length was observed in the NxP
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group, respectively (0.323 ± 0.03 vs 0.282 ± 0.01 , $p= 0.002$ and 0.341 ± 0.02 vs 0.311 ± 0.01 , $p= 0.01$). Also, an increase in LV wall thickness (3.11 ± 0.21 vs 2.82 ± 0.08 , $p= 0.04$) and cardiomyocyte diameter (24.43 ± 1.26 vs 22.13 ± 1.07 , $p= 0.004$) was found in the NxP group. No differences were found in the other parameters analyzed. In conclusion, eight weeks of supplementation with the *Lactobacillus acidophilus* La-05 probiotic in a CKD model worsened cardiac hypertrophy and heart histomorphometry parameters.

Ethical Committee Number: CEUA/UFF N° 2304150818.

Keywords: Chronic kidney disease, heart, probiotic.



Title	Impact of artificial sweetener consumption on behavior and gastrointestinal tract motility of rats with chronic kidney disease
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Session	Pôster

Abstract, Ethics Committee Number*, and Keywords	<p>Artificial sweeteners are often used by people with chronic diseases, such as Chronic Kidney Disease (CKD). Recently studies demonstrated that artificial sweeteners may have controversial effects, such as interference with natural appetite regulation, eating behavior, and gastrointestinal (GIT) environment. The study aims to evaluate the effects of consuming artificial sweeteners on indirect parameters related to GIT motility and behavior in a CKD model. Male <i>Wistar</i> rats (n=53; 90 days), were divided into 7 groups: Sham (surgical stress), Nx (removal of 5/6 of the kidneys), Nx + Sucrose, (NxSac), Nx + Sucralose FDA (NxSF), Nx + Sucralose EFSA (NxSE), Nx + Aspartame FDA (NxAF) and Nx + Aspartame EFSA (NxAE)(CEUA/ UFF N° 9803060520). After 12 weeks, animals were subjected to the elevated cross maze test and the open field test to check anxiety-related behavior. Time of first fecal expulsion, fecal pellet length and fecal water content were also analyzed. The animals were monitored for 12 weeks and euthanasia was performed. Regarding open field test, no significant differences were found in horizontal locomotor activity, rearings, total time inner zone and total spent moving between groups. About the elevated cross maze test, NxSF group showed a significant increase in the occurrence of entry in open arms compared to NxSE group (4.83±0.40 vs 1.28±1.38, $p=0.0231$); NxAF group showed significantly more time spent in open arms and less time spent in close arms compared to NxAE group (9.85±3.07 vs 0.55±0.88,%, $p=0.0265$). The fecal water content was higher in NxSac group compared to Nx group (62.92±3.13 vs 51±1.56,%, $p<0.001$). No significant differences were found in relation to time of first fecal expulsion and fecal pellets length. Consumption of aspartame and sucralose impacted behavioral parameters but did not affect GIT motility in a CKD model.</p> <p>Keywords: Chronic kidney disease, motility, artificial sweeteners, animal behavior</p>
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Title	Synergic effect of physical exercise with iSGLT2 on renal redox parameters in an experimental diabetic kidney disease
Authors	Mateus Teixeira da Rocha Giulia Pedroso Fidelis André Domingos Lass Julia de Bortolo Letícia Bressan Anizelli Paulo André Bispo Machado Júnior Ricardo Aurino de Pinho
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Session	08 - Biologia e Doenças Renais / Biology and Kidney Diseases 11 - Respostas de Treinamento Físico / Physical Training Answers

Abstract, Ethics Committee Number*, and Keywords	<p>Diabetic Kidney Disease (DKD) is a chronic disease derived from Diabetes Mellitus (DM) which affects the kidneys functions. The sodium-glucose co-transporter inhibitor (iSGLT2) and physical has been used as in the treatment of DKD, however, the interplay between these two therapies has not yet been elucidated. Thus this work to evaluate the symergic effect of physical exercise and iSGLT2 on histological, clinical and redox markers in the experimental DKD (EDKD). The committee number 23075.018158/2021-09/UFPR the used C57BL/6J mice were divided into Sham, EDKD with and without exercise and iSGLT2 (n=56). The EDKD animals received a combination of high-fat diet and streptozotocin (STZ) for 16 weeks while the SHAM groups just a standard died. After that, an aerobic physical training program and or iSGLT2 (35 mg/kg) or was employed for 8 weeks. The animals ware euthanized 48 hours after the last exercise session and the kidney was removed for further analysis. The findings revealed that the EDKD group exhibited elevated levels of urinary creatinine and albumin compared to the control group. The effects of physical training together with iSGLT2 decreased the body mass and blood glucose levels. Moreover, the combined use of iSGLT2 and exercise reduced the histopathological alteration caused by EDKD. While the EDKD group displayed changes in lopoperoxidation and protein carbonylation, only iSGLT2 treatment decreased these markers, as well as the H2O2 production. In addition, the synergistic effects of both treatments significantly decreased protein carbonylation levels. Together, while the results did not reavel a clear synergistic effect of physical exercise and iSGLT2 on all assessed markers, this study showed individual impacts of these therapies on histological, clinical, and redox markers. Further research is necessary to understand the synergic effects in treatment approaches of DKD.</p> <p>Keywords: Diabetic Nephropathy; Physical exercise; iSGLT2; Redox regulation</p>
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Title	Study of the effects of D-limonene on glycemia in rats induced to diabetes mellitus by alloxan and the repercussions of this treatment on the cardiorenal system and inflammation
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Session	08 - Biology and Kidney Diseases

Abstract, Ethics Committee Number*, and Keywords	<p>Diabetes Mellitus (DM) is a metabolic condition characterised by high blood glucose levels due to a lack of or resistance to insulin, which can lead to kidney, heart, brain and arterial complications. D-limonene, present in citrus fruits, is recognised for its antioxidant, anti-inflammatory and hypoglycaemic properties. To assess the effects of limonene treatment on body weight and kidney weight in animals with alloxan-induced DM and compare it to untreated animals.</p> <p>Alloxan-induced diabetic Wistar rats were used. Results: Preliminary results confirm the diabetic model. Diabetic animals treated with limonene showed a reduction in weight over the weeks evaluated compared to untreated animals (untreated DM): mean 265.1g vs DM+limonene: mean 251.8g), with a higher delta of weight gain in the first week of treatment (DM untreated: 3.0 vs DM+limonene: 1.7). At the end of the experiment, the DM+limonene animals showed a significant increase in kidney weight compared to the untreated sham and sham+limonene groups ($p < 0.05$). However, there was no reduction in glycemia levels in the diabetic animals treated with limonene compared to the untreated animals.</p> <p>Preliminary data suggest a possible impact of limonene on the body weight and kidney weight of animals with DM, the continuation of this study will evaluate biochemical markers of diabetic kidney disease and the expression of genes related to the onset of kidney disease to verify the possible action of limonene on the kidneys.</p> <p>Keywords: Diabetes Mellitus; Alloxan; Renal system; Cardiovascular system; Inflammation; D-limonene.</p> <p>Animal Ethics Committee Number: 01/2023</p>
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Title	Secretory leukocyte protease inhibitor reduces inflammation in the lung and intestine during acute respiratory distress syndrome in a murine model
Authors	Andressa A. Santos, Thayná D. Oliveira and Stephen F. P. Rodrigues
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Session	9. Biologia e Doenças Respiratórias

Abstract and Keywords	<p>INTRODUCTION: Acute respiratory distress syndrome (ARDS) develops from acute lung injury (ALI) often after a viral or bacterial pneumonia, sepsis, and more recently, COVID-19 disease. It is characterized by an excessive inflammatory response that results in alveolar edema and tissue hypoxia. Distant tissues, such as the intestine, may also play a role in the pathogenesis of ARDS (gut-lung axis). Despite the use of artificial ventilation and corticosteroids as the only strategies to combat ARDS, some patients do not respond to this treatment. In this context, we tested the secretory leukocyte protease inhibitor (SLPI), a non-glycosylated cationic protein expressed on mucosal surfaces, which was demonstrated to reduce inflammation in some animal models of disease.</p> <p>METHODS: ARDS was obtained by an intratracheal (IT) injection of peptidoglycan (PPG) and lipoteichoic acid (LTA) in C57BI/6 mice (CEUA protocol #9737280921), which was repeated twenty four hours later. As a control, distilled water, I.T. injected. Treatment with SLPI (0.2 mg/kg body weight) was carried out also I.T. two and twenty six hours after induction with PPG/LTA.</p> <p>RESULTS: PPG/LTA-injected mice showed a larger number of cells in the BAL (495.1 +/-19.9) compared to the control group (11.1 +/-1.4) (P < 0.0001), these cells consisting mainly of neutrophils, and treatment with SLPI reduced this number (390.9 +/-21.1) (P <0.001, N=6). We observed higher protein concentration in the BAL of animals PPG/LTA-injected compared to the control group, and the SLPI treatment did not reduce this parameter. Leukocyte rolling in intestinal blood vessels was enhanced in PPG/LTA injected mice (10.7 +/-0.42/min) compared to the control group (4.5 +/-0.27/min) (P < 0.0001), and treatment with SLPI reduced this parameter (6.3 +/-0.6/min) (P <0.0001, N=7).</p> <p>CONCLUSION: SLPI treatment reduces leukocyte number in the lung and this may reflect the reduced inflammation in intestine.</p> <p>KEYWORDS: acute lung injury; leukocyte mediators; mice;</p>
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XXXVIII REUNIÃO ANUAL DA FESBE
XXII REUNIÃO ANUAL DA BRAVO
XVIII CONGRESSO DA SBCAL
III CONGRESSO DOHAD BRASIL
II CONGRESSO DA SBBA
2 A 5 DE JULHO 2024, CAMPINAS/SP
FRONTEIRAS DAS CIÊNCIAS BIOMÉDICAS E SEUS DESAFIOS

Title	Luteolim induz alterações morfológicas renais e a resposta ventilatória a hipóxia
Authors	
Affiliations	
Session	

Abstract, Ethics Committee Number, and Keywords	<p>Resumo: O luteolim é um flavonoide antioxidante e anti-inflamatório com efeitos benéficos para o metabolismo, porém seu efeito na ventilação ainda não totalmente esclarecido. AIM: avaliar ação do luteolim no rim, aorta e na ventilação de camundongos e ratos machos. Métodos: Foram usados camundongos machos LepRb-IRES-Cre (18-25g) com marcação <i>tomato</i> em todos os neurônios com ação de leptina e ratos Wistar machos com peso de 260/320g. Os camundongos foram tratados com luteolim (10µg/kg) ou com veículo DMSO (1% - I.P) e mantidos em normóxia ou hipóxia intermitente crônica (HIC) por 7 dias e os parâmetros ventilatórios foram avaliados bem como a morfologia aórtica e renal. Foi administrado agudamente de luteolim (10µg/kg) nos ratos e submetidos a 6 episódios de hipóxia aguda (10%O₂)</p> <p>Resultados: sete dias após o tratamento com luteolim, observou-se redução na resposta ventilatória à hipóxia aguda dos camundongos (n=5) (4,3±0,3 vs lut. 2,2±0,1 mL.kg-1.min-1), redução na expressão genica de HIF-1α no tronco encefálico (n=5) (0,31 ± 0,118 vs 0,12 ± 0,016 mRNA) e proteica de HIF-1α no NTSc (n=5) (16,25 ± 1,73 vs 9,25 ± 1,75 n° de cel.). Observa-se alteração no diâmetro do corpúsculo renal no grupo LUT em normóxia e em HIC (n=5) (49,59 ± 3,31 vs 55,32 ± 2,39 µm / 47,93 ± 0,41 vs 51,95 ± 0,68 µm), espaço glomerular (5,37 ± 0,47 vs 9,17 ± 1,29 µm / 2,21 ± 0,46 vs 3,57 ± 0,04 µm) e do capilar glomerular (2,18 ± 0,04 vs 3,53 ± 0,26 µm / 3,17 ± 0,02 vs 4,19 ± 0,14 µm). A aorta do grupo LUT mostrou aumento de colágeno na túnica média (n=5) (23,84 ± 7,80 vs 42,76 ± 10,01 µm) redução da espessura da túnica média (39,40 ± 1,72 vs 30,84 ± 2,75 µm), área de células musculares lisas (18,06 ± 6,69 vs 6,25 ± 1,76 µm) e espessura das lamelas elásticas (2,73 ± 0,14 vs 2,03 ± 0,13 µm). A administração aguda de LUT (n=5) em ratos reduziu a resposta ventilatória a hipóxia após 20 min (-0,68638 ± 1,573746 vs 62,01292 ± 30,00593 mL.kg-1.min-1) e 60 min (-0,68638 ± 1,573746 vs 70,22584 ± 11,56622 mL.kg-1.min-1). Conclusão: Estes achados sugerem uma ação do luteolim no metabolismo bem como uma influência reduzindo a resposta ventilatória a hipóxia.</p> <p>Apoio Financeiro: CNPq, Capes e FAPESP, Portuguese Foundation for Science: research grant EXPL/MED-NEU/0733/2021 and contract for JS (CEEC IND/02428/2018) and CEECIND/ 04266/ 2017. (CEUA N – 10/2021).</p> <p>Palavras chave: Luteolim, ventilação, renal.</p>
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Title	From pre-COPD to pre-ACO: an Elsa-Brazil - Cohort Study
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Session	Biologia e Doenças Respiratórias.

Abstract, Ethics Committee Number*, and Keywords	<p>Introduction: Asthma-COPD overlap (ACO) is an airway condition characterized by fixed airflow obstruction. We sought to identify demographic, clinical, and biochemical markers associated with ACO. Methods: 174 participants from the ELSA-Brasil cohort study in São Paulo were included from April to November 2022. Divided into four groups: control (n=42), asthma (n=42), pre-COPD (n=49), and pre-ACO (n=41) based on self-report at the beginning of the cohort study. Pre- and post-bronchodilator spirometry parameters were performed by the KOKO spirometer. Quality of life questionnaires were assessed. Additionally, hematologic analysis and interleukins in serum by Luminex were performed. The p-value of <0.05 was used. Results: 56% of participantes were women, with a mean age of 64±9 years. Spirometry showed a decrease in post-bronchodilator (BD) forced expiratory volume in one second (FEV1) (%) in the asthma and pre-ACO groups, as well as a decrease in post-BD FEV1/forced vital capacity (FVC) (L) and (%) in the asthma, pre-COPD, and pre-ACO groups. No significant differences among asthma, COPD, and ACO groups were observed in SF-36 short, CAT, and WHOQOL-bref scores questionnaires. Hematologic analysis showed na increase in platelet Count and IgE levels in the asthma group. Serum cytokine levels in the pre-COPD group exhibited higher levels on TNF-α, IL-4, IL-5, IL-6, IL-8, and IL-13 compared to the asthma and pre-ACO groups.</p>
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Conclusion: The spirometric results are consistent with respiratory disease criteria. The increase in IgE and platelets in the asthma group indicates worsened symptoms and pathological characteristics. The pre-COPD group stands out regarding cytokines, contrary to the literature. Our participants exhibited partial characteristics of the literal diagnosis of ACO, suggesting a pre-ACO state.

Keywords: asthma, COPD, ACO, spirometry, cytokines. Sponsorship: FAPESP(2018/02537-5, 2019/26449-0 and 2022/02510-5), LIM20-HC-FMUSP, CNPq(01 060115.00 SP).



Title	Assessing the effects of isoflurane exposure in healthy adult C57BL/6 GP91 PHOX gene knockout mice
Authors	Silva, G.G.C. ¹ ; Machado-Junior, P.A. ¹ ; Castro, M. L. C. ² ; Costa, D.C. ² ; Russo, R. C. ³ ; Fujiwara, R. T. ⁴ ; Bezerra, F.S. ¹ .
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Session	9 Biologia e Doenças Respiratórias

Abstract and Keywords	<p>The isoflurane is widely used for anesthesia, because it has advantages over other anesthetics. However, studies have reported that isoflurane can promote lung inflammation and oxidative stress by increasing the production of reactive oxygen species (ROS), suggesting the NADPH oxidase enzyme complex pathway as the main source. This study aimed to characterize the pathophysiological mechanisms induced by isoflurane on redox imbalance and lung inflammation in wild-type C57BL/6 mice and GP91 phox^{-/-} knockout mice. This study was approved by the UFMG ethics committee (306/2023). 28 female mice aged between 9 and 10 weeks were used. The wild-type strain and the genetically modified strain (GP91phox^{-/-}) on a C57BL/6 background. The mice were divided into 4 groups (n=7): WT control group (WTC) WT isoflurane (WTI), PHOX^{-/-} control (PHOX^{-/-}C) and PHOX^{-/-} isoflurane (PHOX^{-/-}I). The WTI and IPHOX groups were exposed to 1.9% isoflurane in a flow of 2L/min O₂ (21%) for 3 hours in an inhalation chamber, while the WTC and PHOX^{-/-}C were exposed to room air. 24 hours after the exposition, the animals were euthanized and bronchoalveolar lavage (BAL), blood and lungs were collected for analyses. The influx of total leukocytes (x10⁵/mL) and macrophages from BAL was greater in the WTI (12.42±4.44); (10.19±3.44) compared to the WTC (5.42±1.09) and (4.50±1.01). Superoxide dismutase and catalase activity (U/mg) were higher in the WTI (7.88±1.30); (4.25±1.03) compared to WTC (6.47±0.65);</p>
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(2.28 ± 0.71). The glutathione ratio was lower in **WTI** 3.81 ($3.25-4.26$) compared to **WT** 4.63 ($4.00-5.11$). The expression of iNOS, IL1-B, NFkB and NRF2 genes was higher in the **WTI** (1.68 ± 0.72); (1.98 ± 0.81); (1.10 ± 0.06); (1.36 ± 0.20) compared to the **WTC** (0.68 ± 0.32); (0.92 ± 0.36); (0.94 ± 0.16); (0.85 ± 0.43). No differences were observed for the knockout animals. Preliminary results suggest that the NADPH oxidase enzyme complex regulates inflammation and oxidative stress in the lungs of mice.

Keywords: isoflurane, pulmonary inflammation, redox imbalance, mice

Funding: CNPq, CAPES, UFOP and FAPEMIG.



Title	Implications of obesity on muscle morphological and metabolic changes: a post-mortem study after SARS-CoV-2 infection
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Session	09 - Biologia e Doenças Respiratórias

<p>Abstract, Ethics Committee Number*, and Keywords</p>	<p>Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection triggers a variety of changes in the body, which can result in death. Previous studies have reported that obese patients are more susceptible to the effects of the virus, however, the degree of muscle impairment under such conditions remains inconclusive. This study aimed to verify the post-mortem implications of obesity on histopathological and metabolic alterations in skeletal muscle. A total of 25 unvaccinated patients, who died at a Hospital in Curitiba/PR, were</p>
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investigated (ethics committee number: 4.621.159). Samples from the rectus femoris were extracted by biopsy and immersed in 10% formalin solution or frozen for analysis. The data were stratified according to body mass index (BMI) and participants were classified as non-obese (N-OB n=17) and obese (OB n=8). The results showed histopathological alterations, over 80% of both groups exhibited atrophy, inflammatory infiltrate, myositis, fibrosis, and liposubstitution. In the N-OB group, 58% showed necrotic fibers, while in the OB group, 37%. No significant differences were observed between the groups in the immunostaining of angiotensin-converting enzyme 2 (ACE2), transforming growth factor beta (TGF- β), and collagen types I and III. Similar results were also found for PTEN-induced kinase 1 (PINK1), parkin, and calpain. However, levels of calpastatin were significantly higher ($p=0.0448$) in the OB group, and the calpain/calpastatin ratio was higher ($p=0.012$) in the N-OB group. Out of 65 metabolites observed in muscle tissue, the OB group had lower levels of Histidine ($p=0.005$) and α -ketoisocaproic ($p=0.012$). These results indicate that skeletal muscle underwent histopathological changes independent of obesity, without repercussion on muscle degradation mediators. However, the OB group exhibited stronger inhibition of muscle degradation via the calpain-calpastatin system, with reduced histidine and α -ketoisocaproic levels.

Keywords: COVID-19; SARS-COV-2; metabolism; obesity; skeletal muscle.



Title	Effects of chronic high-dose anabolic steroid administration on breathing in mice
Authors	ROSATTI, Marcelo Rafanelli ¹ GARGAGLIONI, Luciane H. ² DIAS, Mirela Barros ¹
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Session	09 - Biologia e Doenças Respiratórias

Abstract, Ethics Committee Number*, and Keywords	<p>At physiological levels sexual hormones participate in the central control of breathing, and disruption of these hormones, as in the case of hypogonadal men, reduces hypoxic and hypercapnic ventilatory responses, changes that are reversed with testosterone replacement therapy. Acute administration of supra-physiological levels of testosterone also affect breathing by causing an exacerbated response to either CO₂ or O₂, but the effects of the chronic high-dose treatment on breathing were previously unexplored. We aimed to examine the impact of high dose androgen treatment, for 5 weeks, on breathing control. Male adult male mice (C57Bl6) were treated with 25mg/kg/week of Testosterone Cypionate (Deposteron®) or its vehicle (peanut oil). Unanaesthetized mice were submitted to a whole-body plethysmography to register respiratory frequency (fR) and tidal volume (V_T) to obtain the pulmonary ventilation (V_E), along body temperature (T_b) measurements, during room air, 10% O₂ and 7% CO₂. All procedures were approved by the Animal Care and Use Committee for the Institute of Biosciences at Botucatu, Brazil (CEUA – IBB, UNESP; protocol n.º. 3013260423). High-dose testosterone treatment for 5 weeks increased V_E during baseline conditions, compared to the control (V_E = 2521.4 ± 425.4 mL kg⁻¹ min⁻¹, n = 6, versus 1809.5 ± 366.6 mL kg⁻¹ min⁻¹, n = 5; P = 0.0166; unpaired t-test), caused by a reduced fR. During 7% CO₂ exposure treated mice showed a reduced hypercapnic ventilatory response compared to control animals [V_E % change (min20) = 95.1 ± 60.8, n = 4, versus 304.5 ± 87.3 mL kg⁻¹ min⁻¹, n = 4; P= 0.0002; two-way ANOVA], which also was mainly caused by a reduced fR. The ventilatory parameters remained unaltered during hypoxia. We conclude that chronic high-testosterone treatment alters the baseline respiratory rhythm while it inhibits the hypercapnic ventilatory response in mice.</p> <p>Keywords: breathing control; anabolic steroids abuse; testosterone; hypoxia; hypercapnia.</p>
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Title	Hypercapnic and hypoxic ventilatory response in <i>mdx</i> mice during wakefulness and sleep
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Session	09 - Biologia e Doenças Respiratórias

Abstract, Ethics Committee Number*, and Keywords	<p>Duchenne Muscular Dystrophy (DMD) is a disease related to mutations in the dystrophin gene, mostly affecting boys. <i>Mdx</i> mice, like DMD patients, lack dystrophin and have an analogous condition in the diaphragm. The ventilatory response to hypercapnia, but not to hypoxia, has been shown to be altered in these animals, but whether these changes are dependent on the sleep-wake cycle, is unknown. We aimed to evaluate the hypercapnic and hypoxic ventilatory response in <i>mdx</i> mice during wakefulness and sleep. Unanesthetized adult (8-month-old) and old (18-month-old) <i>mdx</i> mice were submitted to the whole-body plethysmograph method to measure pulmonary ventilation (VE), together with body temperature (Tb) during room air, hypercapnia (7% CO₂) and hypoxia (10% O₂). EMG and EEG were recorded only in 8-month-old animals. All procedures were approved by the Ethics Committee of Use of Animals (CEUA - IBB, UNESP, protocol no. 5769310822). 18-month-old <i>mdx</i> mice had no change in the VE during hypercapnia, compared to control mice (VE= 3376,6 ± 384,9; n=3 vs 4035 ± 350 mL g⁻¹ min⁻¹; n=3; P >0.05; Two-way ANOVA), and also during hypoxia (VE= 2747 ± 336; n=3 vs 1915 ± 394 mL g⁻¹ min⁻¹; n=3; P >0.05; Two-way ANOVA). 8-month-old <i>mdx</i> mice had the same CO₂ ventilatory response as control mice, either during wakefulness (VE= 4372,4 ± 524,8; n=4 vs 5542,3 ± 1028,3 mL g⁻¹ min⁻¹; n=4; P >0.05; Two-way ANOVA) or sleep (VE= 4013 ± 41; n=3 vs 5463 ± 599 mL g⁻¹ min⁻¹; n=4; P >0.05; Two-way ANOVA). Also, during hypoxia the responses did not change in awake <i>mdx</i> mice (VE= 2347 ± 384; n=4 vs 2242 ± 259 mL g⁻¹ min⁻¹; n=4; P >0.05) or during sleep (VE=2875,1 ± 412; n=3 vs 2297 ± 209 mL g⁻¹ min⁻¹; n=4; P >0.05). We conclude that there are no differences between the ventilatory responses to hypercapnia and hypoxia in 8-month-old and 18-month-old <i>mdx</i> mice, probably due to compensatory activities of accessory respiratory muscles.</p> <p>Keywords: Duchenne Muscular Dystrophy, <i>mdx</i> mice, hypercapnia, hypoxia, arousal</p>
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Title	Local acute lung injury impairs short-term memory and reduced brain nicotinic receptors expression
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Session	Biology and Respiratory Diseases

Abstract, Ethics Committee Number*, and Keywords	<p>Introduction: Pathophysiology associated with acute respiratory distress syndrome (ARDS) extends beyond acute lung injury (ALI) to the brain causing cognitive impairments in ARDS survivors. Aims: To evaluate whether ALI induced by intratracheal LPS changes behaviour and neuronal nicotinic receptors in mice brain. Material and Methods: All protocols used in the present study were approved by the Animal Use Ethics Committee of Federal University of São Paulo (protocol code n° 2933110321). Male BALB/C mice received intratracheal saline or lipopolysaccharide (LPS, 5mg/Kg). After 24hs or 48hs, short-term memory was assessed by novel object recognition task (NORT). Lung, bronchoalveolar lavage fluid, and brain were collected for evaluation of signs of inflammation and/or nAChR expression. Data were analyzed with t-test with $p < 0.05$ considered significant. Results: LPS induced larger fractional of collapse/hyperinflated and shorter fractional of normal lung area, increased</p>
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wet/dry weight ratio, neutrophils, the levels of IL-6, TNF- α and KC in bronchoalveolar lavage fluid compared to control. In NORT test sessions, LPS-treated mice spent less time exploring the new than the familiar object, indicating a memory deficit. LPS treatment reduced mRNA expression of cortical $\alpha 4$ nAChR and hippocampal $\alpha 7$ and $\alpha 4$ nAChRs. There was no difference in the expression of $\beta 2$ nAChR mRNA, however, there is a trend towards reduction in the LPS groups ($p=0.07$). Conclusion: ALI induced by pulmonary insult leads to an impairment in short-term memory that can be related to reduced nAChR expression in the brain, suggesting that peripheral inflammation *per se* would be a stimulus for the deterioration of cognitive functions.

Keywords: acute respiratory distress syndrome, LPS, nAChR, cognitive



Title	Seed-based functional connectivity changes in elderly subjects playing serious games
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Session	10 Neurobiology

Abstract and Keywords	<p>Serious games (SG) are interactive experiences that allow players to engage in activities aimed at honing skills and attaining goals beyond mere entertainment. SG have been shown to promote cognitive decline prevention. However, there are few studies that have analyzed functional connectivity (FC) changes in elderly subjects who played SG. The aim of this study was to evaluate FC changes in older individuals who played SG, using functional magnetic resonance imaging (fMRI). Seventeen subjects (age range 53-76, 2 men) participated in this study. They played the Active Brain SG on a smartphone, for 1h30min, once a week, for 3-5 months. Resting state fMR images were acquired before (t1) and after (t2) the intervention. Six regions of interest (ROIs) commonly associated with logical reasoning and attention were selected (AAL atlas) for performing a seed-based FC analysis: 5: Frontal Superior Orbital Left, 10: Frontal Middle Orbital Right, 33: Cingulum Middle Left, 34: Cingulum Middle Right, 59: Parietal Superior Left and 61: Parietal Inferior Left. FC maps for each seed were then compared between t1 and t2. We found a decrease in connectivity for ROI 5 with the supplementary motor area (SMA) and ROI 10 with the right (close to the hand) motor area (M1) after the intervention. The orbitofrontal cortex (OFC, which encompasses ROIs 5 and 10) serves as a central hub for integrating sensory information, regulating autonomic responses, and engaging in processes of learning, anticipation, and decision-making related to emotions and rewards. SMA has been attributed the functions of postural stabilization, coordination, and control of voluntary movements. The observed decrease in FC between the OFC and SMA, as well as between the OFC and M1, following the SG intervention, indicates significant alterations in neural networks related to motor control, sensory integration, emotional processing, and reward mechanisms. CAAE: 84395818.7.0000.5404 Keywords: neuroplasticity, serious games, aging, fMRI</p>
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Title	Klotho-hypomorphic mice show sex-based reduced expression of AMPA receptor in the cerebellum
Authors	Geovana Rosa Oliveira dos Santos Isabela Ribeiro Possebom Elisa Mitiko Kawamoto
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Session	10 - Neurobiologia

Abstract, Ethics Committee Number*, and Keywords	<p>Aging is characterized by a functional decline in several physiological systems. Klotho participates in some signaling pathways involved in aging, such as Wnt and p53, and in the regulation of synaptic plasticity in the hippocampus. Klotho-hypomorphic mice (KI^{-/-}) exhibit accelerated aging and cognitive decline. Glutamatergic signaling is important for synaptic plasticity, since N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamatergic receptors participate in long-term potentiation (LTP) and long-term depression (LTD). KI^{-/-} and wild-type (KI^{+/+}) male and female mice (n = 7) were euthanized at 8 weeks of age and cerebellum and hippocampus were collected, after genotyping the animals using conventional polymerase chain reaction (PCR). For total protein extraction, the cerebellum and hippocampus were homogenized using RIPA buffer and protease and phosphatase inhibitors. Western Blotting assays were performed to investigate the protein expression of the subunits GluN1 (NMDA receptor) and GluA1 (AMPA receptor), using beta-actin as an internal control. Data was statistically analyzed by Two-way ANOVA and Tukey's multiple comparisons. Differences are considered significant for p value < 0.05. We demonstrated in the cerebellum that KI^{-/-} male mice show reduced expression of GluA1 compared to KI^{+/+} male (51% of reduction; (F (1,22 = 4.862); p = 0.0082) and KI^{-/-} female (51% of reduction; (F (1,22 = 9.391 for interaction); p = 0.0079)). No differences were observed in the expression of GluA1 in the hippocampus or GluN1 in the cerebellum and hippocampus. Our findings suggest that Klotho could influence the expression of AMPA receptor in the cerebellum in a sex-dependent manner, and these changes may be associated, in part, with the cognitive decline found in these animals. Project approved by the Ethics in the Use of Animals Committee (CEUA 8613021222).</p> <p>Keywords: Klotho; aging; NMDA; AMPA; synaptic plasticity.</p>
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Title	Doxycycline improves cognitive performance in mice with polymicrobial sepsis
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Session	Neurobiologia

Abstract, Ethics Committee Number*, and Keywords	<p>Survivors of sepsis may have neurological sequelae with decreased cognitive function. One of the mechanisms responsible is the increased activity of matrix metalloproteinases (MMPs). Strategies for decreasing MMP activity involve pharmacological inhibition. Doxycycline (DOX) is an antibiotic and a potent inhibitor of MMPs. The aim of this work was to evaluate the effect of doxycycline (a metalloproteinase inhibitor) on the possible cognitive improvement of mice with sepsis. 115 isogenic C57BL/6 male mice, divided into four groups were used: Group 1-white surgery(sham)(n=22); Group 2-cecal ligation and puncture surgery (CLP)(n=38); Group 3-CLP treated with DOX (20 mg/kg/day; n=29); Group 4-CLP treated with DOX (40 mg/kg/day; n=26)(CEUA: 1310110521). The treatments were performed through a daily subcutaneous injection for 5 days. On the fourth day of treatment the animals were submitted to cognitive tests. To analyze cognitive functions, the New Object Recognition (NOR) and the Y-Maze (Y) Test were performed. MMP-9 activity and the levels of cytokines TNF, IL-6, and VEGF were evaluated in the cerebral cortex. One-way ANOVA was performed followed by tukey post-test. Data were presented as mean ± standard error of mean. Doxycycline was effective in reducing brain levels of TNF (TNF: CLP 339.2±6.056 vs. DOX20 291.6± 7.385 p=0.0341; CLP vs. DOX40 279.7± 14.03</p>
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p= 0.0047) and VEGF (CLP 98.74±2.850 vs. DOX20 54.94 ±2.609 p<0.0001; CLP vs. DOX40 78.90 ± 5.662 p= 0.0313), inhibiting MMP9 (CLP 7.254.865± 2.225.298 vs. DOX20 598.225±196.644 p=0.0015; CLP vs. DOX40 1.225.125 ± 307.826 p=0.0032), and improving cognition in septic mice (NOR:CLP - 0.1333±0.03608 vs. DOX20 0.2182 ±0.03770 p<0.0001; CLP vs. DOX40 0.1875±0.06665 P=0.0001)); (Y:CLP 50.00±2.046 vs. DOX20 68.15± 2.454 p<0.0001; CLP vs. DOX40 66.00±3.124 p=0.0002). Therefore, our results suggest that doxycycline is effective in reducing neuroinflammation, inhibiting metalloproteinases and improving cognition in septic mice.

Ethics Committee Number: 1310110521

Funding: FAPEMIG, CNPq, CAPES, FINEP, FAPEMIG APQ-02511-22

Keywords: Sepsis; Doxycycline; Cognition; Neuroinflammation; Oxidative stress; C57BL/6 mice



XXXVIII REUNIÃO ANUAL DA FESBE
XXII REUNIÃO ANUAL DA BRAVO
XVIII CONGRESSO DA SBCAL
III CONGRESSO DOHAD BRASIL
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2 A 5 DE JULHO 2024, CAMPINAS/SP
FRONTEIRAS DAS CIÊNCIAS BIOMÉDICAS E SEUS DESAFIOS

Title	The role of astocyte-like glial cells in <i>C. elegans</i> healthspan
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Session	Neurobiology

Abstract and Keywords	<p>The central nervous system (CNS) coordinates the physiology of different organs in our body. In the context of aging, it has been noted that molecules secreted by neurons modulate organismal lifespan. However, recent findings have highlighted that glial cells, in addition to neurons, also contribute to this type of regulation. Specifically, signals from astrocyte-like glia (CEPsh glia) have been shown to extend lifespan in the nematode <i>Caenorhabditis elegans</i> by regulating proteostasis in peripheral tissues. This motivated us to explore in-depth the function of these glial cells. Interestingly, <i>C. elegans</i> is one of the few organism models where glial cell elimination can be achieved without causing neuronal death. Thus, we decided to investigate the physiological consequences of genetically ablating this cell type, using a glia-ablated strain (AGD2173), which expresses a caspase protein under the regulation of <i>hlh-17p</i> (CEPsh-glia-specific promoter). Using this strain, we are investigating the role of these cells on the worm's development, longevity, and stress resistance. Our preliminary results indicate that the absence of CEPsh glia impacts developmental time (63 ± 1h to obtain 50% of adults in the population for wild type N2 strain; 68.7 ± 0.6h for AGD2173 strain; n=3) in a similar way described to the HLH-17 mutant strain. It also indicates a decrease in survival to acute oxidative stress caused by sodium arsenite 10 mM ($85.3\% \pm 13.7$ N2 survival; $20.9\% \pm 9.1$ AGD2173; n=3) and 15 mM ($56.4\% \pm 29.2$ N2 survival; $25.3\% \pm 17.9$ AGD2173; n=3), which suggests that astrocyte-like cells could be important regulators of systemic stress resistance. We intend to perform transcriptome analysis (RNA-seq) to identify signaling pathways that are non-autonomously regulated by glial cells and control stress resistance in <i>C. elegans</i>. Ultimately, our investigation involving healthspan has the potential to go beyond invertebrates, opening questions to be explored in other animal models.</p> <p>Keywords: <i>C. elegans</i>, glia, Proteostasis, Oxidative stress</p>
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Title	Altered behaviors in male mice prenatally exposed to valproic acid
Authors	Bianca Andrade Rodrigues Josiane do Nascimento Silva Thiago Amorim de Souza Lima Elisa Mitiko Kawamoto
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Session	Neurobiology

Abstract, Ethics Committee Number*, and Keywords	<p>Valproic acid (VPA) is an antiepileptic drug. Prenatal exposure to VPA results in behavioral deficits similar to those observed in autistic patients. Therefore, this drug is related to the development of communication and cognitive deficits and altered behaviors. The objective was to investigate possible altered behaviors in the offspring of male mice exposed in utero to VPA. Pregnant female mice (C57/BL6) were separated into two groups: a control group and the VPA group. VPA or 0.9% saline solution were injected intraperitoneally, in two doses of 300 mg/kg each, on embryonic days 10 and 12. At one month old, the males (n=16 each group) were exposed to the behavioral tests: Elevated Plus Maze, Social Interaction and Inhibitory Avoidance, therefore, anxiety-like behavior, social interaction and memory were evaluated. The statistical analysis was performed using the unpaired Student's T-Test in the GraphPad Prism. Project approved by Ethics in the Use of Animals Committee 2945190521. In the Elevated Plus Maze test, a statistically significant increase in time in closed arms (16% compared to the control, $p=0.0110$; $t=2.707$) and a significant reduction in time in open arms was observed in the group exposed to VPA (36% compared to the control, $p=0.0218$; $t=2,416$), but no significant differences were found in the number of entries into the closed arms. In the Social Interaction test, the VPA group showed a significant reduction in social interaction with the unknown animal (59% compared to the control, $p<0.001$; $t=7.240$), however, there was a significant increase in interaction with the object (54% in relation to the control, $p=0.0010$; $t=3.637$). Finally, in the Inhibitory Avoidance test, a significant reduction in the latency time to the dark field (16% compared to control, $p= 0.0169$; $F(1, 30)=6.396$) was observed in the VPA group. These results suggest that these animals present anxiety-like behavior, in addition to impairments in social interaction and long-term memory.</p> <p>Keywords: Valproic acid; cognition; behavior.</p>
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Title	Excitotoxic lesion in the striatum as an inductor of inflammation, axonal injury and oligodendrocyte impairment in the rat's internal capsule
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Session	Neurobiology

Abstract, Ethics Committee Number*, and Keywords	<p>Excitotoxicity is a phenomenon related to tissue disruption caused by the detrimental effects of excessive concentrations of glutamate in the nervous system, triggered by morphophysiological disorders, leading to structural and physiological changes culminating in cell death. The present study aims to evaluate the axonal and myelin alterations in the internal capsule of the rat following a focal microinjection of N-methyl-D-aspartate (NMDA) into the dorsal striatum at 1, 3, and 7 post-lesion days (PLD) (ID #CEPAE-001-2007). The tissue was processed for immunohistochemistry to evaluate inflammatory response (MBS1, ED1), axonal lesion (βAPP) and pathological oligodendrocytes (Tau). Quantitative analysis of MBS1/ED1 and Tau immunolabeling was determined by cell counts across the internal capsule, while axonal lesion was assessed using densitometric analysis. Neutrophil recruitment was prominent at 1PLD as compared to control (55.1 ± 1.28 and 1.2 ± 0.32 cells/mm², respectively) ($p < 0.05$), while the peak of macrophage activation occurred at 3PLD as compared to control (83.2 ± 1.97 cells and 3.1 ± 0.60 cells/mm², respectively) ($p < 0.05$). Noticeable oligodendrocyte damage was observed at 3PLD, persisting at 7PLD (63.3 ± 1.52 and 26.1 ± 0.97 cells/mm², respectively) as compared to control (0.8 ± 0.25 cells/mm²) ($p < 0.05$). A distinctive pattern of axonal lesion, especially evident at 7PLD, was also identified (control: 0.250 ± 0.007; 1PLD: 0.281 ± 0.009; 3PLD: 0.309 ± 0.014; 7PLD: 0.508 ± 0.02; $p < 0.05$). These findings point out that NMDA-induced acute excitotoxic injury in the striatum can result in disturbance to axons and oligodendrocytes within the internal capsule. Such damage has the potential to disrupt the function of nervous tissue.</p> <p>Keywords: excitotoxicity; inflammation; axonal damage; oligodendrocytes; myelin; internal capsule.</p>
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Title	Chronic intracerebroventricular treatment with irisin reduces hypothalamic neuroinflammation in obese mice
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Session	10 - Neurobiologia

Abstract, Ethics Committee Number*, and Keywords	<p>The saturated fatty acids from hypercaloric diets bind to Toll-Like Receptor 4 (TLR4) and activate the pro-inflammatory downstream signaling. Inflammation causes hypothalamic dysregulation, impairing energy homeostasis and favoring obesity. Irisin is an adipomiotin released during exercise that could mediate responses to decrease inflammation. CEUA-UNICAMP: 5926-1/2021. Male mice C57BL/6J, 4wk, 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 300ng/day. After 13wk in diets, right lateral ventricle cannulation surgery was performed and animals were submitted to ICV treatment for 7d. The Hypothalamus was collected after euthanasia. The normality of the data was verified by the Shapiro-Wilk test and analyzed by Kruskal-Wallis test or One-Way ANOVA, followed by Tukey's post-test ($P < 0.05$). The HFD group presented: higher gene (TLR4: 2.0 ± 0.5, MD2: 8.5 ± 0.6, MyD88: 1.4 ± 0.2, ILβ: 5.9 ± 2.3, IL-6: 3.3 ± 0.08, fold change of CTL) and protein expression (TLR4: 1.6 ± 0.3, MD2: 1.6 ± 0.2, MyD88: 1.3 ± 0.1, fold change of CTL) of inflammatory markers; higher reactivity of IBA⁺ cells (3.2 ± 0.53) and GFAP⁺ (2.7 ± 1.0), compared to CTL (IBA⁺: 1.6 ± 0.4, GFAP⁺: 1.0 ± 0.8). The HFD-Iri group showed lower gene (TLR4: 1.1 ± 0.3, MD2: 1.2 ± 0.2, IL-6: 0.9 ± 0.04, fold change of CTL) and protein expression of inflammatory markers (TLR4: 1.2 ± 0.1, MD2: 0.9 ± 0.3, MyD88: 1.1 ± 0.1, fold changes of CTL); higher gene expression of anti-inflammatory marker (IL4: 1.2 ± 0.3, IL10: 0.9 ± 0.2 fold change of CTL); lower reactivity of IBA⁺ cells (1.9 ± 0.4) and GFAP⁺ (1.9 ± 0.4), compared to HFD. Chronic ICV treatment with irisin reduced hypothalamic inflammation in obese high fat-fed mice by suppressing the TLR4 signaling pathway and decreasing the reactivity of microglia/macrophages and astrocytes.</p> <p>Keywords: Irisin, Obesity, Neuroinflammation, Microglia, Astrocytes.</p>
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Title	Evaluation of the effects of BD-15 in hippocampal neurogenesis
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Session	10 - Neurobiologia

Abstract, Ethics Committee Number*, and Keywords	<p>Neurogenesis is a complex process linked to learning and memory enhancement. Occurs mainly in the subgranular zone of the hippocampal formation and involves the proliferation, migration and differentiation of neural precursor cells (NPCs) into astrocytes, oligodendrocytes or new neurons. Through activation of signaling pathways by Na,K-ATPase cardiotoxic steroids may be capable of modulating neurogenesis. The aim of this study is to verify if a digoxin derivative called 15-benzylidene digoxin (BD-15), selective for α3-Na, K-ATPase, has neurogenesis modulating properties. Hippocampus from neonatal rats were used to prepare NPC culture. NPCs clustered into neurospheres and were dissociated and plated according to the protocol. We performed immunofluorescence assays to characterize cellular composition, measured changes in cell viability through the reduction of MTT and the activity of the cytosolic enzyme lactate dehydrogenase (LDH) released through cell membrane lysis, establishing a concentration of BD-15 for future assays and to measure a possible difference in the total number of cells between concentrations and control, total LDH assays were performed. To measure proliferation, the number of neurospheres in NPCs treated at each concentration was counted for periods of 24, 48 and 72 hours. This project was approved by the Animal Research Ethics Committee, CEUA no. 3272180822. Immunofluorescence results (n=2) showed that 80.21% of the cells were labeled for cells at an early stage of differentiation (DCX) and 81.06% of the cells were labeled for undifferentiated cells (Nest) proving that our NPC culture is undifferentiated. There were no significant differences for MTT (n=6; p=0.1416) and LDH (n=5; p=0.7991) assays when comparing the concentrations tested with the control (0.1% DMSO). Total LDH results (n=2) showed that concentrations of 500nM and 250nM did not change the number of total cells compared to the control (p=0.9444). Proliferation assays (n=4) in cells treated with BD-15 did not show significant results when compared to the control. BD-15 has suggested in previous studies neuroprotective effects. Here we show that BD-15 is not a neurotoxic substance for hippocampal NPCS and does not alter cell viability or death by membrane lysis. BD-15 does not change proliferation rate since none of the established concentrations of BD-15 significantly decrease, increase or alter cell proliferation during the treatment periods defined (24, 48 and 72 hours).</p> <p>Keywords: neurogenesis; Na,K-ATPase, BD-15.</p>
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Title	Effects of reversible inactivation of the ventral hippocampal CA1 region on the recall of aversive memory and anxiety-like behaviors in rats
Authors	1- Victor Hugo da Silva Braga 2- Gabrielle Araujo Pimentel 3- Rodrigo Barbarotti 4- Luiz Henrique Florindo
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Session	10 – Neurobiology

Abstract, Ethics Committee Number*, and Keywords	<p>This experiment aimed to analyze the lateralized reversible inactivation of a specific region of the hippocampus (CA1 of the ventral HPC) regarding the recall of aversive memory and anxiety-like behaviors in rats.</p> <p>For this, 22 animals underwent stereotaxic surgery for bilateral guide cannula implants in the ventral HPC CA1 area and received lidocaine (transient lesions) or vehicle (RINGER) according to their experimental groups: vehicle (VG): n=5; left (EG): n=7; right (DG): n=5; and bilateral (BiG): n=5.</p> <p>Then, they were trained and tested in an elevated plus maze, where the time spent in an aversive arm, the time spent in a non-aversive arm, the frequency of entries into the arms, and other behaviors such as stretched posture and head-dipping were evaluated. All procedures were accepted by the ethics committee under the protocol n°. 226/2020 – CEUA.</p> <p>The results indicated that there were no significant differences between the experimental groups regarding the learning of the discriminative avoidance task. However, all groups showed a preference for the non-aversive arm compared to the aversive one (regarding time spent in each arm ANOVA one-way Tukey $F(7, 36) = 21, 29; P < 0,0001$).</p> <p>In the open field test, the results showed that the animals explored the peripheral quadrants more than the central one, but there were no significant differences between the groups (ANOVA two-way Sidak $F(1, 36) = 100602; P < 0, 0001$).</p> <p>In summary, these results suggest that the inactivation of the CA1 region of the vHPC did not significantly affect the recall of aversive memory or anxiety-like behaviors in rats, but further research is needed to confirm these findings due to the sample size.</p> <p>Financial Support: Fapesp (2021/10704-1)</p> <p>Keywords: functional lateralization, spatial navigation, anxiety behavior.</p>
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Title	The role of ventral hippocampus CA1 in spatial memory in Wistar rats
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Affiliations	1- Graduation Program in Biodiversity, UNESP, São José do Rio Preto, São Paulo, Brazil. 2- Department of Biological Sciences, UNESP, São José do Rio Preto, São Paulo, Brazil. 3- Postgraduate program in physiological sciences (PPGCF), UNESP/UFSCAR, São Carlos, São Paulo, Brazil. 4- Graduation Program in Biodiversity, UNESP, São José do Rio Preto, São Paulo, Brazil. Postgraduate program in physiological sciences (PPGCF), UNESP/UFSCAR, São Carlos, São Paulo, Brazil.
Session	10 - Neurobiology

Abstract and Keywords	<p>Several studies have indicated functional compartmentalization in the hippocampus (HPC), but asymmetric processing and subregions contributions are poorly understood. Therefore, the aim was to assess the function and functional lateralization of the ventral HPC CA1 area in spatial memory recall. Thirty-five Wistar rats (CEUA protocol 226/2020) underwent stereotaxic surgery for bilateral guide cannula implants in the ventral HPC CA1 area. Following post-surgical recovery and drug administration procedure habituation, rats underwent training and testing in the Morris water maze (MWM) and exposure to the open field. Animals received lidocaine (transient lesions) or vehicle (RINGER) according to their experimental group [vehicle (VG): n=9; left (EG): n=9; right (DG): n=10; and bilateral (BiG): n=7] before the last day of MWM testing and open field exposure. Escape latency data collected during MWM training were used to construct a learning curve. A two-way ANOVA (factor 1: treatment, factor 2: test time) followed by Tukey's post-test showed no treatment effect (factor 1 - $F_{(3,31)} = 2.26$; $P=0.1$), but there was a reduction in escape latency on the last training day for all groups (factor 2 - $F_{(1,9, 60)} = 19.54$; $P < 0.0001$). Thus, animals learned the task despite the presence of inserted guide cannulas. In memory testing, a one-way ANOVA showed no differences between groups ($F_{(3,31)} = 2.24$; $P=0.1$) in time spent swimming in the quadrant where the platform was previously located. Despite the results, further sample supplementation is expected. Regarding the open field, frequency of quadrant entries and rearing behavior were analyzed by one-way ANOVA, indicating similarity between groups (entries: VG: 62 ± 7; EG: 42 ± 8; DG: 56 ± 8; BiG: 65 ± 8; $F_{(3, 30)} = 1.28$; $P=0.29$; rearings: VG: 28 ± 3; EG: 22 ± 3; DG: 33 ± 4; BiG: 33 ± 5; $F_{(3, 30)} = 1.46$; $P=0.24$).</p>
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The data show no motor or exploratory impairments in any animals. Financial Support: Capes (88887.817853/2023-00); Fapesp (2021/10704-1).
Keywords: functional lateralization, spatial navigation, transient lesion.



Title	Study of the influence of enteric glia pro-inflammatory activity on intestinal epithelial cells
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Session	10- Neurobiology.

Abstract, Ethics Committee Number*, and Keywords	<p>Background: The enteric nervous system (ENS) is the intrinsic innervation of the gastrointestinal tract. Enteric glia (EG) plays important roles, such as maintaining the intestinal epithelial barrier (IEB). However, its role in the context of inflammation will depend on its state, which can be either "activated," where it maintains homeostasis, or "reactive," where it exhibits a deleterious response to the integrity of the IEB, with increased GFAP and the release of S100β and other cytokines. In inflammatory bowel diseases (IBDs), EG exacerbates the inflammatory condition. This study aims to characterize the EG in its active and reactive states, its pro-inflammatory activity, and its influence on intestinal epithelial cells. Methodology: Investigate in vitro how EG (rat EG cell line, CRL2690) responds to LPS 1μg/ml after 24h, 3 and 6 days, evaluating – by immunocytochemistry, western blotting, ELISA and qRT-PCR - glial markers, inflammatory signaling pathways, and the release of pro-inflammatory cytokines. Evaluate the influence of EG conditioned medium and/or LPS in Caco-2 or RKO intestinal epithelial cells. Results: Our preliminary results indicate that EG presents an increase in S100β release, and GFAP and NFκB-P65 protein levels when treated with LPS 1μg/ml for 72 hours. WB and immunofluorescence assays do not indicate a change in cx43 under different LPS treatment times. LPS causes disorganization of Caco2 tight junctions, which appears to be mitigated in co-culture with EG. Treatment with a conditioned medium from GE-LPS24h or GE-LPS72h and did not alter proliferation of RKO cells. Conclusions: Initial data suggest a different phenotype of EG after short and long exposure times to LPS, with reactivity and pro-inflammatory activity after 3 days of exposure to LPS, which was not observed after 6 days. This study will help us to understand the role of EG in IBDs and how its inhibition should be considered for IBDs treatment.</p> <p>Keywords: ENS. Enteric Glia. Intestinal Epithelial Barrier. Acute Colitis.</p>
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Title	Hyperexpression of the vesicular acetylcholine transporter increases quantal current and frequency of spontaneous release at the mouse neuromuscular junction
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Session	10 - Neurobiologia

Abstract, Ethics Committee Number*, and Keywords	<p>The vesicular acetylcholine transporter (VACHT) is required for filling cholinergic vesicles at the nerve terminal of the vertebrate neuromuscular junction (NMJ). Changing VACHT activity affects neurotransmission and synaptic development. We have shown previously that an increase in VACHT expression enhances the size of the miniature endplate potentials (MEPPs), suggesting that the amount of ACh in each synaptic vesicle is increased. Changes in the muscle fibers can affect the passive properties of the membrane and interfere with the evaluation of the ACh release per vesicle. To better estimate the amount of ACh released per quantum, we performed voltage clamp measurements of the miniature endplate currents (MEPCs), a procedure that eliminates the interference of the passive properties of the membrane.</p> <p>All experiments followed protocols approved by the local ethics committee (CEUA-UFMG - protocol number 106/2015). We used male ChAT-ChR2-EYFP mice (Hyper VACHT) and age-matched wild-type (WT) littermates as control. We recorded about 100 MEPCs from 5 fibers of each animal, holding the membrane potential at -60 mV. We used 5 WT and 4 HyperVACHT mice.</p> <p>Our results show that the size of MEPCs in WT was 2.3 ± 0.6 nA and in 4.7 ± 1.4 nA in HyperVACHT ($p < 0.05$, Student t-test). Surprisingly, we also observed an increased rate of spontaneous release. In WT mice the frequency of MEPC (fMEPCs) was 0.36 ± 0.18 Hz and in HyperVACHT it was 0.79 ± 0.23 Hz ($p < 0.05$, Student t-test).</p> <p>We conclude that hyperexpression of VACHT not only increased the size of quantal currents, presumably reflecting more ACh per vesicle, but also changes the number of quanta released spontaneously, suggesting other, as yet unrecognized, roles for this transporter in synaptic vesicle exocytosis.</p> <p>Neuromuscular junction, Vesicular ACh transporter, Voltage clamp</p>
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FRONTEIRAS DAS CIÊNCIAS BIOMÉDICAS E SEUS DESAFIOS

Title	Effect of statins on metabolism and anxious behavior of rats subjected to chronic sleep deprivation
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Session	10 - Neurobiology

Abstract, Ethics Committee Number*, and Keywords	<p>Chronic sleep deprivation has become increasingly common, with sleep playing a crucial role in cognitive functions and overall well-being. Neuroprotective agents, such as statins, have garnered attention for their effects on the nervous system. This study aimed to investigate effect of rosuvastatin, atorvastatin, and simvastatin on anxiety and metabolism in rats subjected to chronic sleep deprivation. Male Wistar rats (CEUA 1414/2022) were divided into 8 groups (N=15): control (C); sleep deprivation (P); deprivation + rosuvastatin at 2.1mg/kg (R-) and 20mg/kg (R+); deprivation + atorvastatin at 4.2mg/kg (A-) and 20mg/kg (A+); deprivation + simvastatin at 4.2mg/kg (S-) and 20mg/kg (S+). Treatments starting from DPN 75 were concomitant for 45 days. At DPN 98, open field (OF) and light-dark box (LDB) tests were performed. Blood was collected post decapitation for serum levels of cholesterol, triglycerides, HDL, LDL, fructosamine, glucose, CK, ALT, AST, GGT, LDH, ALP, urea, creatinine, albumin, and total serum protein. Compared to group C, in the OF, groups R-, R+, and A- showed more central ambulation. P, S-, S+, A-, and A+ exhibited more peripheral ambulation. P, A-, and A+ showed more rearing behavior. There was no difference in immobility and self-grooming. In the LDB, P, S-, S+, A-, and A+ made more transitions between compartments, while S+ and R+ showed more stretches from the dark compartment to the light. Total time and latency to enter compartments showed no difference. In the biochemical data, S+ showed more creatinine, R+ more ALP, S- and S+ less LDH, P, A-, and A+ more fructosamine, while R+ showed less. All groups had lower serum proteins and cholesterol. S-, A-, A+, and R+ showed less triglycerides. S+, A-, A+, and R- exhibited less LDL. The other tests showed no differences. The results show variations in metabolism according to statin profiles and dosages. Treatments increased overall activity in the animals during the tests and reduced their anxiety.</p> <p>Key-words: stress, pandemic, pharmacology.</p>
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Title	Impacts of food restriction upon different ontogeny phases on the neuronal activation pattern and FOS expression in the hippocampus of adult male Wistar rats
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Session	Session 10 – Neurobiologia

Abstract, Ethics Committee Number, and Keywords	<p>The hippocampus is a structure essential to memory processing and cognition. However, its also notably sensitive to an array of environmental factors, such as diet. Food restriction applied through pregnancy and lactation induces fetal programming that can affect neuronal activation on the hippocampus leading to cognitive and memory impairments in later life stages. In contrast, dietary restriction during adulthood promotes opposite effects. Therefore, the aim of this study is to analyze the neuronal activation pattern by FOS immunoreactivity in the hippocampus of male <i>Wistar</i> rats subjected to fetal and neonatal programming combined with food restriction during adult phase. Pregnant <i>Wistar</i> rats were separated: control programming (CP), fed <i>ad libitum</i> and food-restricted programming (RP) – 50% of daily food intake of CP over gestation and lactation periods. The offspring from both groups were then divided into two other groups, related to food restriction in adulthood: control feeding (CF), <i>ad libitum</i> and food-restricted (RF), 30% restriction of the mean daily consumption by corresponding CF over 155 days. Thus, four experimental groups were studied: CP-CF; CP-RF; RP-CF and RP-RF (CEUA n° 8789260620). Barnes Maze was used to assess memory conditions and neuronal activity in the hippocampal region. After the behavioral test, animals underwent transcardiac perfusion, and their brains were collected. The brain slices went through immunohistochemistry protocol for FOS visualization. The analysis demonstrated a significant effect of programming as there were a greater number of immunoreactive cells to FOS identified in the dentate gyrus region of the RP groups. However, no differences were detected regarding the counting of immunoreactive cells to FOS in total hippocampus extension, neither on CA1 and CA3 regions, separately. It was concluded that food restriction across different life stages modifies neuronal activation patterns in the dentate gyrus.</p> <p>Keywords: Programming; food restriction; neuronal activation; dentate gyrus; FOS.</p>
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FRONTEIRAS DAS CIÊNCIAS BIOMÉDICAS E SEUS DESAFIOS

Title	Effect of fetal programming associated with dietary restriction in adulthood on FOS protein expression and cortical thickness of the entorhinal cortex
Authors	Verônica de Araujo Parducci Gabriela Larissa Lima da Silva José De Anchieta de Castro e Horta Junior
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Session	Neurobiology

Abstract, Ethics Committee Number*, and Keywords	<p>Hunger and malnutrition are still a major concern, not only in Brazil but throughout the world and this problem directly affects learning and memory. The entorhinal cortex (CE) is directly associated with these functions, especially the lateral entorhinal cortex (LEC) and medial entorhinal cortex (MEC). One way to observe its activity is through the expression of the FOS protein. The objective of this study is to analyze neuronal activation and EC thickness in <i>Wistar</i> rats subjected to food restriction in adulthood and during pregnancy and lactation through the quantification of cells that express FOS after memory testing. Pregnant female <i>Wistar</i> rats were divided into 2 groups (CEUA 8789260620): Control Programming (PC), with the normal diet <i>ad libitum</i> and Restricted Programming (PR), with a 50% reduction in diet. The litters were standardized, and the male offspring were divided into two additional groups: Control Feeding (AC), receiving control food <i>ad libitum</i>, and Restricted Feeding at 30% (AR), totaling four experimental groups: PC-AC, PC-AR, PR-AC and PR-AR. Seventy minutes before euthanasia, new object recognition (NORT) and object localization (OLT) tests were performed. After transcardiac perfusion, the brains were collected, fixed and cut into 30µm sections using a freezing microtome. The immunohistochemistry protocol for FOS protein was performed in all cases, images of the EC of each were captured for semiquantitative analysis of cell count, density and cortical thickness. The data were subjected to statistical analysis at a significance level of $\alpha=0.05$. As preliminary results, at level of bregma -6.48mm, it was observed that food restriction during the "Programming" stages of development was very significant ($P<0.01$), leading to a reduction in the thickness of the MEC, specifically between the PR-AC (thinner thickness) and PC-AR (greater thickness) groups. This shows that maternal food restriction affected the development of the offspring's MEC.</p> <p>Keywords: immunohistochemistry, spatial memory, DOHAD, <i>Wistar</i> rat, <i>c-fos</i> gene, food insecurity.</p>
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Title	Functional thermographic evaluation of sensorimotor activation by whole-body vibration exercise in a patient with transmetatarsal amputation: a case report
Authors	<p>José Alexandre Bachur^{1,2} Isabelle Alves Cintra¹ Carolina Maximiano Spirlandeli¹ Marcia Cristina Moura Fernandes² Ana Carolina Coelho-Oliveira² Cynthia Antonia Kallas Bachur¹ Almir Resende Coelho¹ Sinésio Grace Duarte¹ Alessandro dos Santos Pin³ Luiz Carlos de Abreu⁴ Ana Cristina Rodrigues Lacerda⁵ Vanessa Amaral Mendonça⁵ Anelise Sonza⁶ Mario Bernardo-Filho² Danúbia de Sá-Caputo²</p>
Affiliations	<p>1. Universidade de Franca – Unifran, Franca-SP/Brasil. 2. Universidade do Estado do Rio de Janeiro – UERJ, Rio de Janeiro, RJ, Brasil. 3. Centro Universitário de Goiatuba – UniCerrado, Goiatuba, GO, Brasil. 4. Universidade Federal do Espírito Santo – UFES, Vitória-ES, Brasil. 5. Universidade Federal dos Vales do Jequitinhonha e Mucuri, Diamantina, MG, Brasil. 6. Universidade do Estado de Santa Catarina – UDESC, Florianópolis, SC, Brasil.</p>
Session	11 - Respostas de Treinamento Físico

Abstract, Ethics Committee Number*, and Keywords	<p>Introduction: Case report of a 50-year-old patient, with partial lateral transmetatarsal amputation (TMJ) 10 years ago, with insecurity when changing direction to the left and plantar pain on the right in standing position. In the TMJ, a deficit in functionality is observed, which results from sensorimotor interaction. This validates the relationship between reported complaints and the current pattern of neuromuscular control. The ‘Whole Body Vibratory Exercise’ (WBVE) is indicated in sensorimotor re-education due to the cyclic activation of proprioceptors and myocontractile phenomena. Thermal expressions of bodily</p>
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activities are evaluated by infrared thermography. **Objective:** To evaluate the thermal delta (TD) in the lower limbs of patients with left TMJ undergoing late WBVE. **Method:** Thermography of the body regions (BR): mid-distal plantar forefoot (BR1), central plantar hindfoot (BR2), anterior ankle joint (BR3) and central dorsal forefoot (BR4), were performed at the beginning of the week with the patient at rest. 3 times in each of the 7 weeks, the WBVE was performed in 4 sets of 10 minutes each, with increasing intensity and with a predominance of left body support. The TD values of the BR were statistically evaluated. **Results:** In BR 1, 2 and 4, there was a reduction in the differences between the right DTs in relation to the left: from 1.3 to -0.3°C, 1.1 to -0.4°C and 1.2 to 0.0°C. In BR3 there was a reduction from 1.7 to 1.1°C. These reductions occurred amid an increase in the thermal rest pattern also on the right in BR, significantly higher ($p=0.01$) on the left (4.6%). These data corroborate the patient's perception of "improvement in carrying out bodily activities". **Conclusion:** The evolution of the sensorimotor pattern by WBVE can be monitored by Functional Thermographic Assessment. **Ethics Committee:** The patient signed the ICF approved by the Ethics Committee (n°4.050.724). **Keywords:** proprioception, vibration, exercise, function.



Title	Identifying molecules mediating the effects of resistance training on pancreatic beta-cell function
Authors	Gabriela Alves Bronczek Gabriela Moreira Soares Joel Alves da Silva Júnior Antonio Carlos Boschero
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Session	11 - Respostas de Treinamento Físico

Abstract, Ethics Committee Number*, and Keywords	<p>Resistance training benefits beta-cell function and survival in healthy and type 1 diabetic mice, which is mediated by exercise-induced humoral factors released in the bloodstream. Here, we fractionated the serum from trained mice to identify this blood-borne molecule. We used two groups of C57BL/6 mice: control (CON) and resistance training (RT). RT mice performed 1 session/day (8 climbs at 4 different loads), 5 days/week for 10 weeks. We analysed glucose tolerance, insulin secretion, and gene expression of <i>Ins2</i> in the pancreatic islet. Also, a rat pancreatic beta-cell line (INS-1E) was incubated for 24h with medium containing 10% of: full serum (from CON or RT mice) or serum fractions (>100, 50, 30, 10, 3, and <3 kDa), obtained by a nominal molecular mass limit (NMWL) filter. Data are mean \pm SEM, $P \leq 0.05$ (Student's t-test or One-Way ANOVA with Tukey's post-hoc test). Experiments were approved by the Animal Care Committee at UNICAMP, license number 6282-1/2023. RT mice showed improved glucose tolerance, increased insulin secretion, and higher expression of the gene <i>Ins2</i> in the pancreatic islet (CON 23125\pm741.7 AUC; 105.4\pm19.9 ng/μg protein; 0.75\pm0.51 fold change (FC) x RT 20768\pm635.4 AUC; 287.2\pm52.21 ng/μg protein; 3.8\pm1.25 FC). INS-1E cells treated with trained-serum also secreted more insulin and presented higher gene expression of <i>Ins2</i> (Serum CON 219.4\pm21.16 ng/μg protein; 1.0\pm0.16 FC x Serum RT 470\pm104.6 ng/μg protein; 1.9\pm0.28 FC). In cells treated with serum fractions, the only fraction that displayed outcomes similar to the full serum on insulin secretion and <i>Ins2</i> expression was the 50-30 kDa (CON 50-30kDa 271.5\pm37.42 ng/μg protein; 1.0\pm0.10 FC x RT 50-30 kDa 381.8\pm89.32 ng/μg protein; 1.8\pm0.16 FC), suggesting that the blood-borne molecule mediating the benefits of resistance training on beta-cells may be around 50-30 kDa. Our next step is to test whether the molecule we are searching for is a protein or a metabolite.</p> <p>Keywords: exercise, exer kineses, insulin, beta-cell.</p>
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Title	Aerobic exercise training prevents perivascular adipose tissue dysfunction and circulatory inflammation in obese female mice
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Session	Respostas de Treinamento Físico

Abstract and Keywords	<p>Perivascular adipose tissue (PVAT) plays endocrine and paracrine roles and aerobic exercise directly influence its secretory pattern. In obesity, PVAT secretes pro-inflammatory factors linked to oxidative stress, contributing to cardiovascular complications. However, mechanisms underlying PVAT dysfunction in obesity, particularly in females, remains unclear. This study aimed to assess the effects of aerobic exercise on circulatory biomarkers of inflammation and oxidative stress, along with PVAT morphological alterations in high-fat diet-induced obese female mice. Female C57BL6/JUnib mice (4-5 weeks old, CEUA:5849-1/2021) were divided into sedentary (SD), trained (TR), high-fat diet-induced obese sedentary (OB/SD), and high-fat diet-induced obese trained (OB/TR). Obesity was induced with 16-week high-fat diet (6.2 kcal/g). Moderate aerobic exercise on treadmill (50-60% of maximum speed) started after 8 weeks and continued for 8 more weeks (5 days/week, 60 min/session). Serum levels of glucose, 17β-estradiol, tumor necrosis factor-α (TNFα), adiponectin, leptin, and thiobarbituric acid reactive substances (TBARS) were measured. Morphological analysis of thoracic aorta PVAT (tPVAT) was performed. OB/SD exhibited increased body weight, perigonadal fat pad weight, serum glucose, leptin, TNFα and TBARS compared to SD. Exercise training effectively reduced leptin, TNFα and TBARS levels in OB/TR without altering body weight, perigonadal fat pad weight or serum glucose. No changes were observed in 17β-estradiol and adiponectin levels across groups. OB/SD adipocytes from tPVAT showed enlarged lipid droplets compared to SD, while in OB/TR, lipid droplets were smaller. Our findings show significant tPVAT alterations in high-fat diet-induced obese female mice, accompanied by increased systemic inflammatory response and lipid peroxidation. Aerobic exercise training mitigated some obesity-induced alterations in tPVAT and reduced circulatory inflammation and oxidative stress.</p> <p>Key words: aerobic exercise training, female mice, obesity, perivascular adipose tissue, inflammation.</p> <p>Financial Support: CAPES; CNPq; FAPESP (2022/09111-9; 2022/10354-3); FAEPEX (2062/23).</p>
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Title	Eight-week dynamic resistance training promotes strength gain without impacting the respiratory capacity, DNA methylation and morphology of different muscle fiber types
Authors	André Hideaki Quaresma Ueda ¹ Mariana Mendes Silva Reis ¹ Julia Maia Viudes Agostinho ¹ Murillo Silva Cardoso ¹ Gabriel Evangelista dos Santos ¹ André Olean-Oliveira ¹ Gisele Alborghetti Nai ² Marcos Fernando Souza Teixeira ¹ Patricia Rogrigues Lourenço Gomes ³ José Cipolla Neto ³ Patricia Monteiro Seraphim ¹
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Session	Physical training response

Abstract, Ethics Committee Number*, and Keywords	<p>Resistance training causes beneficial changes in the skeletal muscle, but the mechanisms involved are still not completely elucidated. Diverse patterns of responses at different levels can be observed on the muscle fibers types. The study aimed to evaluate the impact of 8-week dynamic resistance training (8wDRT) on the global DNA methylation, functional, respiratory capacity and structural adaptation of different muscle fibers in healthy rats.</p> <p>All interventions were approved by the Ethics Committee on the Use of Animals of the School of Sciences and Technology of the Sao Paulo State University campus of Presidente Prudente, Brazil (protocol no. 01/2020). Twenty-two young adult male Wistar rats were randomly divided into: Control (C) and Trained (T) groups. T performed 8wDRT climbing a vertical ladder, 3 series/day, 3x/week for 8 weeks with a progressive load increment every week. Under anesthesia, the extensor digitorum longus, gastrocnemius and soleus skeletal muscles were removed for global DNA methylation assay, gene expression, oxygen consumption evaluation and morphohistological analysis. Strength was assessed using the Maximum Load Test at three points along the intervention. Unpaired Student's and ANOVA one-way tests were done considering $p < 0.05$ as statistically significant value.</p> <p>8wDRT increased muscle strength at the three evaluations points ($p < 0.0001$), without changing the structural parameters of any muscle fiber. 8wDRT did not change global DNA methylation pattern, but it downregulated Dnmt1 mRNA levels in oxidative and glycolytic muscle fibers. Dnmt3b mRNA levels were upregulated in oxidative fibers. Mixed muscle showed the highest degree of global DNA methylation. Also, 8wDRT did not change mitochondrial respiration rates.</p> <p>We concluded the improvement of strength by 8wDRT did not involve changes in the DNA methylation nor in the morphostructural or even in the respiratory capacity of different muscle fibers.</p> <p>Keywords: Epigenetics; mitochondrial respiration; DNA methylation; skeletal muscle; strength training.</p>
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Title	Relative maximum strength index in rats induced to polycystic ovary syndrome
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Session	Respostas de Treinamento Físico

Abstract, Ethics Committee Number*, and Keywords	<p>Strength training is beneficial and promotes improvements in physical fitness, including in Polycystic Ovary Syndrome (PCOS), a disease that causes endocrine imbalances and influence body mass. The objective was to verify the relative maximum strength index (MRSI) in rats induced to PCOS. Sixty adult Wistar rats were used, kept under controlled temperature and light conditions. Water and food <i>ad libitum</i>. PCOS induction: single dose of estradiol valerate. Groups (n=5): control, control/training, PCOS, and PCOS/training, 3 time points: 30, 45, and 60 days. Strength training consisted of ladder climbing (3x/week) with the load fixed to the animal's tail. Body mass (BM) was analyzed weekly. Maximum voluntary load carried (MVLC) was analyzed (1x/week) by the Hornberger and Farrar protocol. To determine RMSI, the MVLC weight/body weight was used. The data were analyzed using ANOVA and Tukey's test. The results are reported as the means \pm SD ($p < 0.05$). The study was approved by the Animal Use Ethics Committee (CEUA–UNIARA n° 025/16). BM: There were no statistically significant differences between the analyzed groups. MVLC: When comparing the control/training groups at 30 days (372.8 ± 30.2), 45 days (437.4 ± 13.6), and 60 days (652.8 ± 55.2), with the PCOS/training groups at 30 days (339.5 ± 33.3), 45 days (423.8 ± 13.6), and 60 days (597.6 ± 55.2), there was a statistically significant difference only at 60 days, where the control/training group showed higher MVLC. MRSI: There were no statistically significant differences between the analyzed groups. The results suggest that MVLC increased in the control/training group, in a time-dependent manner. MRIS showed no statistically significant difference, suggesting that further studies with longer duration are necessary to understand the effects of PCOS on muscle tissue and its relationship with strength.</p> <p>Keywords: Strength training, Polycystic Ovary Syndrome, Relative maximum strength index, Maximum voluntary load carried</p>
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Title	Impact of resistance training on mitochondrial proteins in the gastrocnemius muscle of obese rats
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Session	Respostas de Treinamento Físico

Abstract, Ethics Committee Number*, and Keywords	<p>Obesity damages mitochondria, crucial for muscle adaptation, whose shape is maintained through fusion and fission mediated by proteins like MFN1 and MFN2. While resistance training and prevent diet-induced obesity, its impact on mitochondrial fusion/mitofusins is underexplored. The study investigated if obesity affects MFN1 and MFN2 proteins in the gastrocnemius muscle and if resistance training mitigates these effects. Twenty-four Wistar rats were divided into four groups: Control (C), Obese (O), Exercised (E), and Obese Exercised (OE). O and OE groups were fed a hypercaloric diet (cafeteria diet) containing standard food and pieces of white chocolate or stuffed biscuits or sausage, and intake of soda and water, accounting for 1200 Kcal/cage with 3 rats in each, while E and OE groups underwent vertical ladder climbing four series a day, three times a week, for 12 weeks. After euthanasia, gastrocnemius muscle was analyzed using Western Blotting. The study was approved by the local Animal Use Ethics Committee, CEUA nº 01/2017, and statistical analyses were conducted using GraphPad Prism 8.0. The O group showed higher weight and fat mass ($p < 0.0001$). The E group exhibited higher gastrocnemius muscle weight proportion compared to O groups ($p < 0.05$). However, absolute muscle weight did not differ between groups. No changes in MFN1 and MFN2 protein expression were observed. These findings suggest that neither hypercaloric diet-induced obesity nor 12-week resistance training affect MFN1 and MFN2 expression in the gastrocnemius muscle, indicating no compromise in mitochondrial fusion in models. Indicating that mitochondrial fusion is not modulated by nor diet-induced obesity and neither resistance training.</p> <p>Keywords: Mitochondrial Biogenesis, Skeletal Muscle, Obesity, Resistance Training, Hypercaloric Diet.</p>
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Title	Can dance modify oxidative balance in elderly women?
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Session	11 Respostas de Treinamento Físico

Abstract, Committee Number*, and Keywords	<p>Dance is an activity that can be used to promote physical and mental well-being, and it could have a good effect on the health of older adults. The present study aims to evaluate the effect of the dance on the oxidative balance in older women. This study was approved by CEP protocol #5.845.351/2023. The volunteers were women in the old group (OG)(n=51) who danced 50 min/day, three days/week for six months, and the young group (YG) (n=9, who did not dance). The peripheral blood was collected to measure oxidative stress at pre-dance – P0 time and post-dance - P1 time (after six months). The oxidative balance was measured by malondialdehyde-MDA, carbonyl-CB, total thiol-SH, and superoxide dismutase-SOD, catalase-CAT and glutathione S-transferase-GST activities. For OG data were applied the paired t-test and nonparametric test for comparison between P0 vs P1 time. An unpaired t-test was used to assess the difference between OG and YO. The results showed that the aging increase of MDA 79.7% (p= 0.0028) and CB 175 times (p<0.0001) content, but no significant effect was observed in SH (p= 0.6669). When compared to the aging effect on the enzyme activity we observed no difference in SOD (p= 0.1224) and GST (p= 0.3385), but decrease CAT 18.85% (p= 0.0125) in older women. After when we compare the dance effect in older women, we observed that the dance decreased by 32% of MDA (p= 0.0024), increased by 106.8% of CB (p<0.0001), but also increased by 86.9% of SH (p<0.0001). The results of the enzymatic antioxidant system show that the dance increased the SOD activity 204.4% (p<0.0001), decreased the CAT 64.89% (p <0.0001), and 39.85% GST (p= 0.0018) activity compared with P0 time. Taken together we demonstrate that aging process induce oxidative</p>
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stress; in addition, we suggest that dance helps against the effect of aging, by positively modulation of antioxidant system in older women's, attenuating the effect of oxidative stress.

KEYWORDS: Dance, Red Blood Cells, REDOX status



title	Inspiratory muscle strength training reverses neurovascular dysfunction, lowers blood pressure, and increases exercise tolerance in long COVID patients: a randomized, double-blind, sham-controlled trial
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Session	Respostas de Treinamento Físico

Abstract, Ethics Committee Number*, and Keywords	<p>Long COVID patients exhibit an increased risk of developing cardiovascular diseases, which seems to be explained, in part, by neurovascular dysfunction and attenuated exercise capacity. Thus, it is urgent to propose rehabilitation strategies to restore the cardiovascular health of these patients. We hypothesized the inspiratory muscle strength training [IMST at 75% of maximal inspiratory pressure (PI_{max})] reduces muscle sympathetic nerve activity (MSNA), improves endothelial function, reduces aortic stiffening and blood pressure (BP), and increases exercise tolerance in long COVID patients. To test this hypothesis, a randomized, double-blind, sham-controlled clinical trial (Identifier: NCT06091384) with 6 weeks of IMST (30 breaths/day, 6 days/week at 75% PI_{max}) vs SHAM (30 breaths/day, 6 days/week at 15%PI_{max}) was conducted. PI_{max} (Manovacuometry), MSNA (Microneurography), brachial artery flow-mediated dilation (BAFMD, Ultrasound-Doppler), femoral-carotid pulse wave velocity (CFPWV, tonometry), systolic and diastolic blood pressure (SBP and DBP, semiautomatic system), heart rate (HR, ECG), and peak oxygen consumption (VO₂peak, cardiopulmonary exercise test) were measured before and after 6 weeks of follow-up. Thirty-two patients completed the study, being 17 in IMST (Age: 48.1±9.96 years and BMI: 31.9±5.2Kg/m²) and 15 in SHAM (Age: 51.7±10.56 years and BMI: 33.4±5.5 Kg/m²). IMST reduced the MSNA frequency and incidence ($p<0.001$), SBP ($p=0.02$), DBP ($p=0.04$), MAP ($p<0.01$) and HR ($p<0.01$) and increased BAFMD ($p<0.05$) and PI_{max} ($p<0.001$). IMST increased load and peak effort time ($p<0.05$), but not VO₂peak ($p>0.05$). Also, IMST did not change CFPWV ($p>0.05$). Our findings revealed that IMST reduces MSNA, improves endothelial function, lowers BP, and improves exercise tolerance, indicating that this exercise paradigm is a promising strategy for restoring cardiovascular health of long COVID patients.</p> <p>Keywords: long COVID, sympathetic activity, endothelium, inspiratory muscle strength training.</p>
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Title	Moderate physical exercise exclusively during the peripubertal phase attenuates the lifelong decline in VO_{2max} in early overfed male rats
Authors	Letícia Ferreira Barbosa Scarlett Rodrigues Raposo Rafael Pereira Lopes Lucas Paulo Jacinto Saavedra Luiz Gustavo Antunes Pessoa Maria Natália Chimirri Peres Fernanda Sayuri Fuzishima Ana Carolina Carvalho de Sá Ana Letícia Manso Assakawa Marcos Vinícius Martins Kérolym Lomes da Cruz Gabriel Kian Guimarães Lopes Veridiana Mota Moreira Lima Douglas Lopes de Almeida Paulo Cezar de Freitas Mathias
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Session	11 - Respostas de Treinamento Físico

Abstract, Ethics Committee Number*, and Keywords	<p>Despite the role of highly processed foods and sedentary lifestyle in increasing rates of obesity, there is also an effect for stress on developmental stages of life (DOHaD, Developmental Origins of Health and Disease) in the etiology of such conditions. Considering childhood and adolescence as plastic phases for development, we hypothesize that aerobic moderate training during peripuberty can reverse childhood obesity-induced preventing the decline of maximal oxygen consumption (VO_{2max}) in male rats. We aim to investigate the effects of aerobic exercise during the peripubertal phase to attenuate the metabolic programming induced by overfeeding during lactation. Wistar rat litters were standardized on 9 (normal litter, NL) or 3 pups (small litter, SL) per dam (CEUA7852011121). At weaning on post-natal day (PND) 21, rats were divided in sedentary (S) or exercised (E), forming NLS (n=5), NLE (n=5), SLS (n=5), SLE (n=4) groups. Exercised rats performed aerobic exercise on treadmill, 3-times-week, for 40min, from PND30 to 60, at 55-60-65% of the maximum running speed (MRS) obtained on maximal effort incremental test. Body weight (BW), VO_{2max} and MRS were evaluated at PND30, 60, 90 and 120. At PND120 animals were euthanized and adipose tissues collected and weighed. The data showed that reducing litter size results in heavier pups (NL:47,23g±1,70 e SL=57,88g±1,76; p=0,0008) on PND21. Overfed during lactation promoted increased BW (34,9%, p<0,0001) and relative adiposity (37,2%, p=0,001), while aerobic exercise promoted a reduction in BW (24,6%, p=0,0002), relative adiposity (19,6%, p=0,0108), increased MRS (50,0%, p=0,0045) and a reduced decline of VO_{2max} on obesity-</p>
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induced exercised offspring compared to SLS (10,1%, $p=0,0279$) at PND120. We suggest that aerobic exercise during the peripubertal phase attenuates the decay of oxygen consumption capacity, increase MRS and reduce BW and adiposity of adult offspring obesity-induced during lactation.

Keywords: DOHaD, obesity, peripuberty, moderate exercise, VO_{2max} .



Title	Effects of the “Living High–Training Low” model on glycogen stores in glycolytic and oxidative muscles in C57BL/6J mice
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Session	Respostas de Treinamento Físico

Abstract, Ethics Committee Number*, and Keywords	<p>Exposure to hypoxia and physical training can boost glycogen stores (GLY-S). However, it is unclear the effect of chronic hypoxia combined with aerobic training (“live high–train low” model, LH-TL) on GLY-S. The study aimed to investigate the effects of the LH-TL model on glycogen content in oxidative (soleus-SO) and glycolytic (gastrocnemius-GA) muscles, comparing the results with the same training in normoxia. C57BL/6J mice (n=40) were divided into 2 environments: normoxia (Nx) and hypoxia (Hx), each split into trained (T) and untrained (N) groups. Hx animals were maintained in a CAT tent (USA) with 14.5% oxygen for 18h/day (~3,000m of altitude) (CEUA Ethical Approval 6054-1/2022). T groups underwent a 6-week training at 80% of the individual critical velocity intensity, 40min/day, 5 days/week in normoxia. After the experimental period (48h after the last training session), mice were euthanized to extraction of the SO and GA muscles. Regarding GLY-S (µg/100mg of tissue), Hx animals had a higher content than Nx animals in both SO (F=7.06; P=0.013) and GA muscles (F=4.70; P=0.037), indicating the effect of environmental condition. An interaction between environment and training in GA was observed, with Hx animals experienced greater training effects than Nx group (F=11.17; P=0.002). According to Fisher LSD post-hoc analysis, soleus GLY-S in Hx- T (44.95±3.9) were higher than in Nx-N (30.43±1.5, P=0.032) and Nx-T (30.38±1.5, P=0.013). Also, for the GA, the Hx-T group (110.32±10.7) was higher than Nx-N (42.09±12.7; P<0.001), Nx-T (48.81±5.8; P<0.001) and Hx-N (59.05±4.9; P<0.001).</p> <p>Our results show that implementing the LH-TL training model for six weeks has a beneficial impact on GLY-S in both glycolytic and oxidative muscles. These findings contribute to a better comprehension of carbohydrate metabolism after LH-TL training program using by high-performance athletes.</p> <p>Key words: Glycogen; Oxidative muscle; Glycolytic muscle; Hypoxia; Aerobic training; Mice.</p>
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Title	Acute treatment with an allosteric SERCA activator enhances submaximal force generation and reduces fatigue resistance in isolated skeletal muscle fibers
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Session	11

Abstract, Ethics Committee Number*, and Keywords	<p>Free intracellular calcium transients are central for excitation-contraction coupling during skeletal muscle stimulation. Following stimulation cessation, free intracellular calcium returns to resting levels primarily due to the action of the sarcoplasmic reticulum calcium ATPase (SERCA), resulting in muscle relaxation. SERCA activity can be decreased in skeletal muscle during fatigue development and in different chronic diseases. Conversely, increased exercise capacity and improved muscle function have been observed in experimental models with enhanced SERCA activity. We investigated the effects of acute treatment with CDN1163, a recently identified specific allosteric activator of SERCA, on force generation and fatigue resistance in isolated skeletal muscle fibers. Fibers bundles, containing ~5 to 10 intact cells, were isolated from male C57BL6J mouse flexor digitorum brevis muscle (approved by the institutional animal care committee CEUA/UNICAMP #6042-1/2022). The isolated fibers were electrically stimulated to generate force-frequency curves and subjected to fatigue-inducing stimulation protocols after incubation with either DMSO (control) or CDN1163. Treatment with CDN1163 significantly increased submaximal force generation at 1, 10 and 40 Hz stimulation frequencies, as well as the maximum rate of force development at 10, 30, 40 and 50 Hz. However, fibers treated with CDN1163 exhibited decreased fatigue resistance compared to control (259.7 ± 53.1 s vs 352.9 ± 48.8 s respectively). These findings likely result from elevated sarcoplasmic reticulum calcium levels and increased ATP consumption induced by an enhanced SERCA activity in the fibers treated with CDN1163. While CDN1163 shows promise as a potential therapeutic tool to improve muscle force generation, caution is warranted given its potential to reduce fatigue resistance.</p> <p>Keywords: Skeletal Muscle; Fatigue; sarcoplasmic reticulum calcium ATPase; SERCA.</p>
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Title	Inspiratory muscle strength training decreases the sympathetic neural and blood pressure reactivity to acute mental stress in long COVID patients: a randomized, double-blind, sham-controlled trial
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Session	11

Abstract, Ethics Committee Number*, and Keywords	<p>Background: Our group recently showed that long COVID patients exhibit an exaggerated increase in muscular sympathetic nerve activity (MSNA) and an attenuated peripheral blood flow response during a stressful challenge, indicating that they may be at increased cardiovascular risk. On the other hand, inspiratory muscle strength training (IMST) reduces MSNA and mean arterial pressure (MAP) and improves vascular function in different populations. Thus, the IMST may be an important strategy to attenuate cardiovascular reactivity to mental stress (MS) in patients living with long COVID. Methods: To test this hypothesis, a randomized, double-blind, sham-controlled clinical trial (Identifier: NCT06091384) with 6 weeks of IMST (30 breaths/day, 6 days/week at 75% P_Imax) vs. SHAM (30 breaths/day, 6 days/week at 15%P_Imax) was conducted. MSNA (Microneurography), brachial artery blood flow (BABF, Ultrasound-Doppler), MAP (Finometer), and heart rate (HR, ECG) were measured during 3 min of MS (Stroop color test) before and after 6 weeks of follow-up.</p> <p>Results: Twenty-eight patients completed the study, 14 in IMST (Age: 48.0±1.6 years and BMI: 32.2±1.2Kg/m²) and 14 in SHAM (Age: 53.0±2.5 years and BMI: 33.6±1.1Kg/m²). IMST decreased the MSNA frequency and incidence responses to MS (pre Δ: 8.2±1.3 bursts/min vs. post Δ: 3.5±1.7 bursts/min, p=0.002 and pre Δ: 12.4±2.6 bursts/100 hb vs post Δ: 5.6±2.2 bursts/100 hb p<0.001, respectively). Also, IMST decreased the MAP to MS (pre Δ: 5.8±1.7 mmHg vs. post Δ: 4.1±1.4 mmHg, p=0.04), but it did not change the HR (pre Δ: 6.5±1.4 bpm vs. post Δ: 3.2±1.7 bpm, p=0.07) and BABF (pre Δ: 27.4±6.1 mL/min vs. post Δ: 25.1±6.2 mL/min, p=0.1). No changes were observed in the studied variables in SHAM.</p> <p>Conclusion: IMST decreases sympathetic neural and BP responses to MS in long COVID patients, indicating that this exercise paradigm is a promising strategy for reducing cardiovascular risk in this population.</p> <p>Keywords: Long COVID, mental stress, sympathetic activity, and blood pressure.</p>
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Title	Impact of aerobic training on the damage caused by variable stress on the endothelial function of male and female Wistar rats
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Session	11- Physical training answer

Abstract, Ethics Committee Number*, and Keywords	<p>Variable stress can increase the risk of endothelial dysfunction, while aerobic training may act as a non-pharmacological strategy with beneficial potential. Study approved by the ethics committee (9518170621) with four groups of Wistar rats: Control (C), Control with training (CT), Stress (S) and Stress with training (ST) (n=8/group/sex). In groups S and ST, the 8 days of variable stress protocol was applied before starting 2 months of treadmill training in CT and ST groups. In the thoracic aorta, ex vivo vascular reactivity was analyzed by concentration-response curve of acetylcholine (ACh) and phenylephrine (FNF) and expression of eNOS and eNOSp (ser1177) were quantified via Western Blot, serum analysis of corticosterone and Nitric Oxide (NO) were realized. Comparison between groups was performed using two-way ANOVA and data were expressed as mean±SEM. Stress was able to increase corticosterone (C:4.65±0.52; S:5.95±0.16, p=0.04) and decrease NO concentration only in females (C:0.36±0.10; CT:0.24±0.05; S:0.15±0.025; ST:0.20±0.04, p=0.006). However, males showed no difference in corticosterone (C:6.60±0.14; S:6.47±0.14 p=0.52) and NO (C:0.26±0.02;</p>
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CT:0.24±0.03; S:0.21±0.02; ST:0.24±0.03 p=0.04). Stress increase contractile response to FNF, while the training reduced it, in both sexes (Females pCE50: C:6.61±0.28; CT:7.25±0.19; S:7.86±0.075; ST:6.87±0.12; p=0.0009 Males: C:6,77±0,11;CT:6,81±0,15;S:7,66±0,10;ST:6,90±0,09; p<0,0001). S decreased ACh-induced maximum relaxation, but training improved vasodilation. (Females C:106.4±3.59; S:81.37±6.76; p=0.0002 Males C:99.90±2.076; S:82.45±3.972; p=0.0007). Lastly, S reduced eNOSp expression (C:1.06±0.10;CT:0.60±0.16;S:0.38±0.086; ST:1.06±0.23; p=0.04) and eNOSp/eNOS ratio in females (S:0.28±0.11; C:1.06±0.139; p=0.04),while no difference were observed in males(C:1.74±0.89; CT:1.89±0.97; S:1.61±1.01; ST:1.67±1.23; p=0.97). Training prevented stress-induced endothelial dysfunction in both sexes, possibly improving eNOS and NO in females.

Keywords: Stress, Vascular reactivity, Sexual dimorphism, Aerobic training, Oxidative stress, Nitric oxide, Corticosterone.



Title	Influence of aerobic exercise training on the heme oxygenase 1 and heme pathways in tumor in animal model of colon cancer
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Session	11 – Exercise Training Responses

Abstract, Ethics Committee Number*, and Keywords	<p>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 Heme (HM) and Heme oxygenase 1 (HO-1). HM supports the synthesis of hemoproteins and in cell signaling and gene regulation processes, and HO-1 is an intracellular enzyme responsible for HM degradation. 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 cancer tumors in animals. Ethical approval: CEUA EEFUSP2021/04. Male BALB/C mice (25-30g) 3mo were assigned into two groups: untrained CT26 (CT26ut, n=5) and trained CT26 (CT26t, n=5). CT26t group underwent AET (treadmill exercise) for 1h, 5d/week, for 30 days. Subsequently, CT26 cells were inoculated into both groups, then CT26t group exercised until the 14th day post-injection, and on the 16th day animals were euthanized and tumors were collected. Tumor tissue gene expression was assessed using qPCR. Statistical analysis: Student's t-test (p<0.05). We observed tendency toward decelerated tumor growth in CT26t group (p=0.06), particularly from the 12th day onwards. No differences were observed in HO-1 downstream pathways, but a significant decrease was observed in mRNAs expression of proteins involved in HM synthesis pathway in tumors of CT26t: ALAS1 (p=0.0003), FECH1 (p=0.0002), HCP1 (p=0.0002), TLR4 (p=0.0140), FLVCR1 (p=0.0029), NRF2 (p=0.0476), and BACH1 (p=0.0476). Our findings indicate that AET reduces the expression of genes associated with tumor</p>
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progression, reinforcing AET as an adjuvant therapy for cancer. Keywords:
Aerobic Exercise Training, HO-1, Heme, Colorectal Cancer.



Title	Physical exercise does not influence brain oxidative damage in the brain in the experimental diabetic nephropathy
Authors	Larissa Cristina Marques André Domingos Lass Paulo André Bispo Machado Júnior Edson Antônio Fiorenza Neto Giulia Pedroso Fidelis Mateus Teixeira da Rocha Letícia Bressan Anizelli Luis Felipe Beltão Marqueze Ricardo Aurino de Pinho
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Session	10 Respostas de treinamento físico

Abstract, Ethics Committee Number*, and Keywords	<p>Diabetes mellitus is a metabolic syndrome responsible for numerous health complications and it may cause kidney disease as Diabetic Kidney Disease (DKD). The kidney-brain axis in the DKD is a significant public health concern because both the brain and kidney share similar metabolic characteristics and risk factors. The pathophysiology of the kidney-brain axis is not completely understood, but physical exercise programs have been recommended to reduce the effects of DKD on both organs. However, the role of physical exercise on this axis is still unclear. In this way, this study aimed to verify induced-brain redox modulation alterations by physical exercise in the DKD experimental (EDKD) with ethics committee number 23075.018158/2021-09/UFPR. C57BL/6J mice were divided into Sham, EDKD-with and without exercise. The EDKD-animals received a combination of a high-fat diet and streptozotocin for 16 weeks while the sham groups just a standard diet. After that, an aerobic physical training program was employed for 8-wk. The animals were euthanized 48 hours after the last exercise session and the brain was removed for further analysis. The results indicate that the EDKD-experimental model resulted in increased levels of creatinine and urinary albumin compared to Sham animals. The physical training did not reduce body mass in the EDKD-group, but it led to a significant decrease in blood glucose values. The EDKD-animals exhibited elevated protein carbonylation levels, while physical training did not influence the oxidative damage markers. In conclusion, the DKD-model induced renal impairment and metabolic disturbances, while the physical training showed promise in improving glucose regulation despite its ineffectiveness on the brain redox system.</p> <p>Keywords: diabetic kidney disease, oxidative stress, physical exercise, kidney brain axis</p>
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Title	Performance in the 400m and 3,000-m running tests, and deoxyhemoglobin in more and less active muscles in Brazilian Paralympic endurance Team
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Session	11- Physical Training Responses

Abstract, Ethics Committee Number*, and Keywords	<p>Near-infrared Spectroscopy (NIRS) is a non-invasive method to determine muscle oxygenation in exercise. This study aimed to compare and correlate the running tests performance (400m and 3,000m) and the deoxyhemoglobin (HHb) in more (vastus lateralis-VL) and less (biceps brachii-BB) active muscles in athletes from Brazilian Paralympic Endurance Team. Twelve male middle and long-distance athletes (30±12 yrs; 65.2±7.7kg; 175.3±5.5cm; 7.2±3.6% body fat), with visual, intellectual, and physical impairments (not affecting running) were evaluated (CAAE 52313721.0.0000.5404). Participants underwent two maximal intensity running tests (T400 and T3000, separated by 24-hr) in an official track, with final time (FT) and mean velocity (V) recorded. Muscle oxygenation measurements (HHb, μM) were obtained at 10Hz by NIRS wearable devices placed on the BB and VL. A two-way ANOVA investigated the effects of distances and local muscles measurements, and Pearson's product-moment identified the correlations between FT, V, and the mean BB-HHb and VL-HHb in both tests ($P \leq 0.05$). The FT and V (T400: 57.3±2.5s, 25.4±1.1km/h; T3000: 621.9±32.4s, 17.6±0.9km/h) exhibited no significant correlation between distances. The mean BB-HHb and VL-HHb were, respectively 6.1±11.2 and 11±7.9 in T400, and 11±12.3 and 20.4±10.1 in T3000, with differences observed between tests ($P=0.02$) and muscles ($P=0.02$). Mean VL-HHb in T400 and T3000 was significantly correlated ($R=0.76$; $P<0.01$), with no statistical correlation between BB-HHb in two</p>
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distances ($R=0.40$; $P=0.20$). Our results suggest greater deoxygenation in the more active muscles in running, particularly in the 3,000m test. Furthermore, despite the distinct metabolic characteristics, a positive correlation between VL-HHb in T400 and T3000 confirm the significance of VL and muscle oxygen utilization in both tests, which was not observed in the less active muscle.

Committee number: CAEE 52313721.0.0000.5404

Keywords: muscle oxygenation, paralympic athletics, running test.



Title	New intermittent hypoxia protocol: Effects on muscle oxygenation in young swimmers
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Session	Respostas de Treinamento Físico

Abstract and Keywords	<p>Normobaric hypoxia models, including intermittent hypoxia (IH) exposure during rest, have been under investigation to benefit athletes. However, the impacts of IH in rest on oxygenation in different muscles were not investigated. This study examined the effects of acute IH in rest on oxygenation in biceps brachii (BB) and intercostalis (I) muscles. Six young swimmers (n=4 males and n=2 females, 18±0 yrs-old) underwent 3 sessions. In the 1st, they underwent iDEXA, provided informed consent, and completed medical history questionnaires (CAAE: 64691620.0.0000.5659). In subsequent sessions, participants were randomly subjected to 6 cycles of 4-minute bouts using a mask and exposed to either IH (FiO₂=10%) or placebo (PLA; FiO₂=20.9%), followed by 2-minute intervals in normoxia (FiO₂=20.9%). Throughout all protocols, wearable near-infrared spectroscopy (NIRS) devices were attached in BB and I to measure tissue saturation index (TSI,%), and a pulse oximeter register peripheral oxygen saturation (SpO₂). The mean values (6 cycles) of SpO₂ and TSI (BB and I) in the IH and PLA sessions were compared (paired Student's t-test) and correlated (Pearson's product-moment test) (P≤0.05). Although SpO₂ presented lower values in IH (97.39±1.15; 73.19±12.55; P<0.01), TSI in BB (66.07±4.08; 63±1.74) and in I (67.75±3.77; 66.11±2.73) was not different in the PLA and IH conditions, without significant correlation between them. TSI in BB was more affected by IH in relation to I (P=0.03), while no statistical difference was observed in PLA. Only in IH, SpO₂ was correlated with TSI in BB (r=0.90; P=0.04) and in I (r=0.87; P=0.05). Our results indicated that IH at rest reduced SpO₂ and only in this condition, there were significant correlations between SpO₂ and TSI%, suggesting an influence of IH on muscle oxygenation. Furthermore, it may be inferred that IH induced greater deoxygenation in BB compared to I, as evidenced by lower TSI% values in this muscle.</p> <p>Keywords: Normobaric hypoxia; oxygen saturation; swimming; near-infrared spectroscopy.</p>
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Title	Intermittent hypoxia as pre-activation stimulus: Effects on mechanical parameters of young swimmers subjected to tethered swimming
Authors	Gabriela Olivo Pereira ¹ Lara Soares de Araujo ¹ Marcelo Papoti ² Figueiredo Foresti ² Claudio Alexandre Gobatto ¹ Fúlvia de Barros Manchado-Gobatto ¹
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Session	Treinamento Físico

Abstract and Keywords	<p>Several acute strategies including different muscle pre-activations (PA) have been conducted to improve sports performance. Similarly, hypoxia models, such as intermittent hypoxia (IH) exposure can be applied. However, to our knowledge, there are still no studies regarding the use of IH as a PA strategy for subsequent acute effort in swimmers. This study aimed contributed with this lack. Six young swimmers (n=4 men and n=2 women, 18±0yrs) were submitted to three protocol sessions (CAAE:64691620.0.0000.5659). In the 1st, they completed the informed consent form, medical questionnaires and were submitted to DEXA scan. In the subsequent sessions, athletes were randomly subjected tethered swimming test (all-out 30-s, AO30) preceded by 6 cycles of 4-minute using a mask, being exposed to IH (FiO₂=10%) or placebo (PLA; FiO₂=20.9%), with two-minute intervals in normoxia (FiO₂=20.9%). A tethered system, composed by a load cell, an elastic cable, a signal amplifier and software for digital acquisition, was used to obtained the following mechanical parameters: relativized mean and peak force (MF and PF, respectively (N.Kg⁻¹)), impulse (N.s) and fatigue index (IF, %). Paired Student's t-test was applied to compare the mechanical swimming results after PLA and IH intervention and Pearson's product-moment test detected significant correlations between these measurements in efforts preceded or not by IH. Our results showed no statistical difference between MF (1.65±0.26 and 1.67±0.27), PF (1.76±0.29, 1.79±0.27), I (3331.7 ±644.1 and 3438.7±529.7) and FI (47.0±29.6, 50.9±25.2), in swimming preceded or not by IH, respectively. Significant correlations were observed between the same parameters in PLA and IH conditions (MF: r=0.98, P<0.01; PF: r=0.95, P<0.01 and I: r=0.97, P<0.01). In summary, IH was not able to increase the mean and peak force, impulse, or attenuate the FI in young swimmers subjected to an AO30 tethered swimming test.</p> <p>Keywords: Normobaric Hypoxia; Warm-Up; Swimming; Performance.</p>
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Title	Maternal obesogenic diet impairs the mitochondrial bioenergetics and hepatic oxidative balance in male offspring: Can moderate training mitigate these effects?
Authors	<ol style="list-style-type: none"> 1. Gizele Santiago Moura Silva 2. Wellington de Almeida Oliveira 3. Deisiane de Araujo Correia 4. Leticia da Silva Pacheco 5. Jose Winglinson de Oliveira 6. Ramon Nascimento da Silva 7. Reginaldo Correia da Silva Filho 8. Osmar Henrique Santos-Junior 9. Jonata Henrique de Santana 10. Claudia Jacques Lagranha 11. Mariana Pinheiro Fernandes.
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Session	Respostas de Treinamento Físico

Abstract and Keywords	<p>Maternal obesogenic diet (MOD) predisposes mitochondrial disorders and liver disease in offspring. The literature has shown that moderate exercise training (MET) improves the oxidative metabolism. Thus, we evaluated the effects of MET on the mitochondrial bioenergetics and hepatic oxidative balance in the offspring of rats fed with MOD. Wistar rats of control group (n=8) received vivarium diet and the Obesogenic group (n=8) received a high-fat and sucrose diet during pregnancy and lactation. The male puppies were subdivided into the</p>
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groups: Sedentary Control (SC), Trained Control (TC), Sedentary Obesogenic (SO), and Trained Obesogenic (TO), (n=8 per group). From the 26th to the 28th day of life, the incremental test took place and at 30 days of life, the MET protocol was performed on the treadmill (60 min/day, 5 days/week, 4 weeks, and at 50% of maximum capacity). At 60 days of life, the euthanasia was performed and the liver was collected and homogenized. The MOD caused in juvenile offspring (SC x SO) a higher NAD⁺/NADH ratio, increased mitochondrial ROS production, greater swelling mitochondrial, greater activity of the SOD antioxidant enzyme, reduction in GSH/GSSG ratio and lower TFAM gene expression. The MET in obesogenic juvenile offspring (SO x TO) increased the citrate synthase activity, reduced the ROS production, prevented mitochondrial swelling, reduced MDA levels, increased SOD, CAT and GST antioxidant enzymes activity and increased TFAM, FIS 1, OPA 1 and SIRT 1 expression. These data suggests that high-fat and sucrose diet during critical periods of development may increase the risk of hepatic dysfunction associated to mitochondrial bioenergetics alteration and oxidative stress in offspring and the MET for 4 weeks may act as a nonpharmacological intervention minimizing or preventing these effects.

Keywords: obesogenic diet, physical training, liver, oxidative stress.



Title	ET-1 signaling pathways in sickle cell disease: implications for hemoglobin S polymerization
Authors	Lyzes Rosa Teixeira Alves ^{1 2} , Eluza Silva de Souza ^{1 4} , Rodrigo Abreu Camacho ^{1 2} , Debora de Sousa Alves ^{1 3} , Clemilson Berto Junior ^{1 2 3 4} .
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Session	Biomembranas, Transportadores e Sinalização

Abstract, Ethics Committee Number*, and Keywords	<p>Abstract: Sickle cell disease (SCD) is a hereditary disease, caused by a single substitution in the gene that encodes the beta sub-unit of hemoglobin, with the exchange of glutamic acid for valine, giving rise to hemoglobin S (HbS). When in hypoxia, HbS polymerizes, the cell loses water and K⁺ giving to erythrocyte the crescent aspect or sickle format. This cell shape leads to vasocclusion, infarcts, stroke, kidney disease and lower life expectancy. In this sense, endothelin-1 (ET-1) is a vasoconstrictor peptide produced by endothelial cells, acting via receptors ETA and/or ETB, with increased expression in SCD. In order to observe a correlation between ET-1 and HbS polymerization, which receptor activated and signaling pathway triggered in erythrocyte, we performed hemoglobin S polymerization assay in a 96 well plate. The project registered in Brazil platform with CAAE number 88140418.5.0000.5699. Experiments were conducted in hypoxia by adding ET-1 (20 pM) with BQ-788 (1 μM/mL) or BQ-3020 (1 nM/mL), SENICAPOC (30 nM/mL), GO6983 (50 nM/mL), WORTMANIN (10 μM/mL) or AICAR (1 μM/mL) with 1% final volume of isolated erythrocytes. The polymerization reading was performed for approximately 30 minutes at 700 nm wavelength in microplate reader. ET-1 alone and BQ-3020 (ETB agonist) alone both increased HbS polymerization when compared to control; BQ-788 (ETB antagonist) with ET-1 did not change polymerization, showing that ET-1 increases polymerization via ETB receptor. The addition of SENICAPOC (Gardos channel inhibitor), Wortmannin (PI3K inhibitor), GO6983 (PKC inhibitor), and AICAR (AMPK activator) along with BQ-3020 all reversed the effects of BQ-3020 to control, demonstrating that all these pathways are involved in ETB increasing</p>
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polymerization. In summary, our data shows for the first time that ET-1 increases HbS polymerization by binding to ETB receptor, activating PKC and PI3K while inhibits AMPK, that in turns activates Gardos channel leading to dehydration of erythrocyte.

Keywords: sickle cell disease, endothelin-1, erythrocyte.



Title	Specific interactions of NOX and V-ATPase complexes in zebrafish and humans - models of distinct repair and regeneration systems
Authors	Livia Maria Pimentel Marques Arnoldo Rocha Façanha
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Session	Oral session

Abstract, Ethics Committee Number*, and Keywords	<p>Injured tissues exhibit diverse responses to damage. In certain animals, repair mechanisms characterized by inflammation and specific extracellular matrix deposition, leading to fibrosis, are activated. Conversely, other animals display tissue-specific responses, triggering a cascade of events that reactivate embryonic developmental signaling pathways, ultimately fully restoring the structure and function of the damaged tissue. Experimental evidence has unveiled the mobilization and activation of key transmembrane electrochemical gradient-generating systems at the injury site, immediately following amputation, with particular emphasis on the NOX (NADPH oxidase) and V-ATPase complexes, recognized as central players in the molecular events driving regeneration. This study aimed to explore the interactions between the proteins comprising the multimeric structure of V-ATPase and elements of the NOX complex in silico, with the goal of elucidating their roles in regenerative processes. Rigorous analyses on computerized platforms for protein-protein interactions (Genemania and String) revealed that in <i>D. rerio</i> (zebrafish), renowned for its remarkable regenerative capacity, the connection between V-ATPase and NOX, involving specific G proteins (e.g., RAB, CDC42, and RHO) (Combined Score: 0.550, 0.452 and 0.517 respectively). In contrast, in humans, whose regenerative capabilities are substantially more limited than those of zebrafish, the interactions between both complexes exhibit greater involvement (58,5 %) of NADPH oxidase with V-ATPase components and the participation of specific isoforms of NOX complex subunits (e.g., NOX5 and DUOX1/2) (17,9; 14,3 e 13,5 respectively - the others had less than 13). This points to targets for in vitro validation, among the molecular events that, orchestrated, involve the two main protein complexes activated by injury, already described in animals that regenerate.</p> <p>Keywords: proton pump, blastem, reactive oxygen species</p>
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Title	Effect of 4-phenylbutyric acid and phenyl acetate treatment on the modulation of activity HK II in cells HUH7
Authors	Kelly Oliveira, A.M 1; Fernandes-Siqueira, L.O. 1; da-Silva WS 1
Affiliations	1 Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro, Rio de Janeiro - RJ, Brasil.
Session	

Abstract, Ethics Committee Number*, and Keywords	<p>During the cancerous transformation of hepatocytes, the enzyme catalyzing the first rate-limiting step of the glycolysis, glucokinase (GCK), is replaced by the higher affinity isoenzyme, type II hexokinase (HK II). Previously, our group showed that treatment of L6 myotubes with 4-phenylbutyric acid (4-PBA) for 96 h was able to increase HK II activity in the soluble and mitochondrial fraction, glucose consumption and protein expression. 4-PBA is a low molecular weight fatty acid known as chemical chaperone due to its ability to attenuate endoplasmic reticulum stress. The 4-PBA is metabolized in the β-oxidation in the liver and converted to phenylacetate (PA), with further conjugates with glutamine to form phenylacetylglutamine (PGA), which is freely excreted in the urine. The objective of this study was to investigate whether treatment with 4-PBA has effects in other cell type, HUH7 cells, a human hepatoma-derived cell line. Furthermore, investigate whether PA participates in the effects observed in treatment with 4-PBA. Methods: HUH7 treated with 1 mM 4-PBA, 0,5 mM or 1 mM PA for 96 h. The cultures were subjected to cell viability, subcellular fractionation and evaluated for hexokinase activity in subcellular fractions. Results: Preliminary data showed that cells HUH7 treated with PA for 96 h showed a decrease in HK activity in the total homogenate (41%), tendency the soluble (41%) and particulate (20%) fractions. While with treatment with 4-pba we observed a tendency towards decreased activity in the total homogenate (33%) and in the particulate fraction (33%). When evaluating MTT, we observed a reduction (20%) in cell viability only with PA treatment, after 48 h of treatment. Conclusion: Taken together, our preliminary results suggest that 4-PBA seems to be metabolized in PA and this conversion decreases HK II activity without altering cell viability in culture HUH7.</p> <p>Keywords: 4-PBA, PA, Hexokinase and HUH7.</p>
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Title	Comparing 2D and 3D models of a human queratinocyte cell line (HaCat)
Authors	Kelly Joseane Morari ¹ , Gabriel Anhê ² , Licio Velloso ¹ , and Eliana Araujo ^{1,3}
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Session	Poster

Abstract, Ethics Committee Number*, and Keywords	<p>Abstract : Most of cell culture experiments are based on two-dimensional (2D) models that fail to fully mimic the complex interactions between the cellular and extracellular environments. For this reason, cells growth in 2D conditions generally exhibit a particular morphology and excessive proliferation. On the other hand, three dimensional (3D) methods have been gaining visibility due to their ability in better simulate the interactions between cells yielding cells with more reliable morphology and growth rate. In this study, we would like to compare qPCR and cell viability data from a two 2D and a 3D human queratinocyte cell line (HaCat) culture.</p> <p>Material and Method: For the 2D experiments, a 96-well plate was seeded with 10×10^4 cells/well. For the 3D experiments, firstly a 96-well plate was recovered with 2% agarose and seeded with 10×10^4 cells/well. Next, both 2D and 3D plates were exposed for 72 hours to the following condition: 1) DMEM with 5.6mM glucose; 2) DMEM with 25mM glucose; 3) DMEM with 50mM Glucose. Cell assign to these three conditions were also treated with either Vehicle, 1% Serum (Fetal Bovine Serum or Calf Serum) or a mixture of the cytokines (0,625ng/μl TNF-α and 1,0ng/μl I11-β). In order to verify cell viability we used the MTT test that reveals cell metabolic activity, proliferation and cytotoxicity. qPCR were used to compare the gene expression of two inflammatory markers (<i>Tgf-β</i> and <i>I11-β</i>).</p> <p>Results: In the 2D cell culture, the serum increased cells viability in 5.6 and 25mM of glucose ($p < 0,05$) and the cytokines increased cells viability in 50mM of glucose ($p < 0,05$). 3D-cultured HaCat cells showed opposite patterns of changes in viability. In this case, cytokine decreased cell viability under 5.6 mM, as the increase in glucose concentration. The qPCR data from 2D-cultured HaCat cells revealed that cytokines treatment lead to an increase in the <i>I11-β</i> and <i>Tgf-β</i> gene expression only in the 50mM of glucose. In the 3D model, the cytokine treatment increased <i>I11-β</i> gene expression in all glucose concentrations when compared to vehicle and to serum ($p < 0,05$). However, the increase in glucose concentration decreased <i>I11-β</i> gene expression ($p < 0,05$). Only 5.6mM of glucose up regulated <i>Tgf-β</i> gene expression ($p < 0,05$).</p> <p>Conclusion: In conclusion, we believe that 3D models of keratinocytes (HaCat) cell culture are more capable to mimic the in vivo context.</p> <p>Keywords: HaCat; 2D, 3D, cell culture.</p>
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Title	Resveratrol as an antioxidant in ferroptosis: a literature review
Authors	Ana Beatriz dos Santos ^{1,2,3} Jaqueline Vieira Carletti ^{1,2,4,5} Carmem Juracy Silveira Gottfried ^{1,2,3,4,5}
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Session	Diferenciação, crescimento e morte celular

Abstract, Ethics Committee Number*, and Keywords	<p>Ferroptosis is an Iron (Fe)-dependent cell death characterized by increase in reactive oxygen species (ROS) production, Fe overload, lipid peroxidation (LP) and depletion of Glutathione (GSH) and Glutathione peroxidase 4 (GPX4). Fe-dependent LP is caused by excessive ROS via the Fenton reaction, leading to cell death facilitated by a deficit in GSH antioxidant defenses. Polyphenols such as Resveratrol (RSV) are antioxidants capable of terminating the Fenton reaction, chelating metal ions and reducing LP. Studies have explored RSV's role in regulating ferroptosis, highlighting the necessity for a study that consolidates the main biomarkers regulated by RSV in ferroptosis due to the lack of existing reviews on this topic. This review compiled studies conducted from the interception of publications through March 2024, aiming to categorize ferroptosis biomarkers modulated by RSV treatment in animal models. The search was conducted in PubMed, CINAHL, EmBase and Scopus databases using the terms ferroptosis, mouse, rat and resveratrol. Publications were included if reported data related to ferroptosis and involved animal models. 56 publications were filtered; those that did not discuss the biomolecular mechanisms of RSV action or were not related to RSV were excluded. A total of 13 publications met the inclusion and exclusion criteria. We observed that an amount of 29 biomarkers dysregulated by ferroptosis were regulated by RSV, with GPX4, LP, NRF2, Fe²⁺, SIRT1 and GSH being the most common. Data suggests that RSV is a potential antioxidant against ferroptosis damage. However, few studies explore RSV's protection against ferroptosis in animal models. Therefore, these results may be explored in animal models which exhibit alterations in biomarkers related to</p>
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ferroptosis that are regulated by RSV. In the meantime, until further studies can be performed, RSV may be a viable molecule in the regulation of ferroptosis. CAPES, CNPq, INCT-NIM, FIPE-HCPA.

Key words: Ferroptosis; Antioxidants; Resveratrol; Animal models.



Title	Parkin is a critical player in the effects of caffeine over mitochondrial quality control pathways during skeletal muscle regeneration in mice
Authors	Marcos Esteca ¹ Isabela Divino ¹ Ana Laura Vieira da Silva ¹ Matheus Severino ² Renata Braga ³ Eduardo Ropelle ³ Fernando Simabuco ^{2,4} Igor Baptista ¹
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Session	Poster Session 2

Abstract, Ethics Committee Number*, and Keywords	<p>Skeletal muscle tissue is highly susceptible to injuries. It is known that mitochondrial quality is essential for maintaining the regenerative process of skeletal muscle and Mitophagy is a key process for this. In this process, Parkin protein plays a critical role as a Ubiquitin ligase. Our research group observed that Parkin knockout mice (Parkin^{-/-}) have impairments in the regeneration of muscle tissue. A more understanding of Parkin in regeneration is important, as well as knowledge of strategies that can optimize the process. Caffeine is the most consumed neurostimulant in the world, and has been reported to modulate pathways involved in mitochondrial quality in skeletal muscle. Therefore, we aimed to investigate the effects of caffeine on pathways associated with mitochondrial quality control during skeletal muscle regeneration, emphasizing the Parkin protein. We used C2C12 myoblasts during differentiation with and without caffeine and the Tibialis anterior muscle of C57BL/6J (WT) and Parkintm1Shn (Parkin^{-/-}) mice injured and regenerated on 3, 10 and 21 days. CEUA: 5344- 1/2019. In C2C12 the caffeine decreased the content of Parkin, leading to an increase in the content of DRP1, a protein involved in mitochondrial fission, and PGC-1α, which involves mitochondrial biogenesis. Parkin^{-/-} mice showed that caffeine ingestion during the regenerative process induces an increase in AMPKα phosphorylation and in the content of PGC-1α and TFAM, changes that were partially dependent on Parkin. Furthermore, the absence of Parkin enhances the ergogenic effect of caffeine, increasing mitochondrial capacity and myotube growth. These effects are related to the increase in ATF4 and the activation of protein synthesis, observed from the increase in 4E-BP1 phosphorylation. Our findings demonstrated that caffeine intake alters mitochondrial quality control during skeletal muscle regeneration and Parkin is a key factor in these mechanisms.</p> <p>Keywords: AMPK, caffeine, mitochondrial respiration, muscle recovery</p>
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Title	Cloning, expression and purification of plasmodium falciparum (PFMCA-IA) metacaspase
Authors	Beatriz Vieira Pereira ¹ Emilly Rolim Leme ¹ João Pedro Martins Silva Costa ¹ Ane Caroline Moreira Duarte ¹ Mariana Nascimento Romero Trujillo ¹ Marcelo Ferreira Marcondes ² Maurício Ferreira Marcondes Machado ¹
Affiliations	1 – Interdisciplinary Center for Biochemical Research, , University of Mogi das Cruzes 2 – Department of Biophysics, UNIFESP
Session	Session 13 – Diferenciação, crescimento e morte celular

Abstract, Ethics Committee Number*, and Keywords	<p>INTRODUCTION: <i>Plasmodium falciparum</i>, the malaria-causing parasite, is one of the most prevalent and impactful diseases globally. Metacaspase 1 (PfmCA-Ia) in this protozoan regulates cellular processes, including programmed cell death. Its cloning, expression, and purification are crucial for understanding properties, structure, and function, potentially contributing to effective antimalarial therapies. METHODOLOGY: The mutant was obtained through PCR amplification and T5 exonuclease DNA assembly (TEDA) cloning, using T5 exonuclease to hybridize DNA. Expression in <i>E. coli</i> BL21 DE3pLysS cells was induced with 0.25 mM isopropil-β-D-thiogalactosidase (IPTG). Purification was performed by nickel-sepharose affinity chromatography with a gradient of imidazole from 50 to 300 mM, evaluating quality by SDS-PAGE. RESULTS: After gene amplification by PCR and evaluation by 1% agarose gel electrophoresis, the T5 exonuclease DNA assembly (TEDA) cloning protocol was successfully applied. Transformed <i>E. coli</i> DH5α cells generated colonies, screened and evaluated by PCR and 1% agarose gel electrophoresis, showing positive bands at 2142 bp. The extracted plasmid DNA was transformed into <i>E. coli</i> BL21(DE3)pLysS for protein expression. After confirming positive colonies, the protein was expressed and purified by SDS-PAGE, revealing bands at 71 kDa. CONCLUSION: The obtaining and purification of metacaspase (PfmCA-Ia) will allow evaluating its behavior at different calcium concentrations and performing biochemical characterization experiments. KEYWORDS: Metacaspase; <i>Plasmodium falciparum</i>; Proteolytic enzymes; Cloning; Expression; Purification.</p>
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Title	Obtaining <i>Saccharomyces cerevisiae</i> metacaspase (YCA1) containing site-directed mutations in its key calcium interaction residues
Authors	Emilly Rolim Leme 1 João Pedro Martins Silva Costa 1 Ane Caroline Moreira Duarte 1 Mariana Nascimento Romero Trujilho 1 Beatriz Vieira Pereira 1 Marcelo Ferreira Marcondes 2 Maurício Ferreira Marcondes Machado 1
Affiliations	1 – Interdisciplinary Center for Biochemical Research, , University of Mogi das Cruzes 2 – Department of Biophysics, UNIFESP
Session	Session 13 – Diferenciação, crescimento e morte celular

Abstract, Ethics Committee Number*, and Keywords	<p>INTRODUCTION: <i>Saccharomyces cerevisiae</i> encodes Yeast Caspase 1 (YCA1) metacaspase, essential in regulating programmed cell death and dependent on calcium ions for its activation. Obtaining YCA1 with site-directed mutations in the calcium interaction residues is crucial to investigate its mechanism of action and identify therapeutic targets for fungal diseases. METHODOLOGY: We used the overlap extension PCR amplification method and T5 Exonuclease DNA Assembly (TEDA) cloning to obtain the mutant. Expression occurred in <i>E. coli</i> BL21 DE3pLysS cells induced with 0.25 mM isopropil-β-D-thiogalactosidase (IPTG). The enzyme was purified by nickel-sepharose affinity chromatography, with an imidazole gradient (50-300 mM), and its quality evaluated by SDS-PAGE electrophoresis. RESULTS: Two-step amplification and mutation insertion were confirmed by 1% agarose gel electrophoresis, showing bands at ~1300 bp. T5 Exonuclease DNA Assembly (TEDA) cloning was successful, verified by transformation into <i>E. coli</i> DH5α and screening via PCR and electrophoresis. After cloning, plasmid DNA was transformed into <i>E. coli</i> BL21(DE3)pLysS for protein expression and purification. Expression was confirmed by SDS-PAGE, as well as purification, where bands were visualized at approximately 55 kDa for the unprocessed form and approximately 43 kDa for the processed form. CONCLUSION: The successful obtaining of the mutant and its purification will allow evaluating the enzyme's behavior at different calcium concentrations and conducting biochemical characterization experiments. KEYWORDS: Metacaspase, <i>Saccharomyces cerevisiae</i>, Genetic mutation, Recombinant protein.</p>
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Title	High concentrations of sulforaphane promote cytotoxicity in neural progenitor cells
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Affiliations	Institute of Biomedical Science, University of São Paulo; São Paulo, Brazil
Session	13 - Diferenciação, crescimento e morte celular

Abstract, Ethics Committee Number*, and Keywords	<p>Neural progenitor cells have the capacity for renewal and differentiation into important cell types in the recomposition of brain tissue. In that context, cell exposure to sub-toxic concentrations of some substances can induce an adaptive cellular response to insults. Sulforaphane (SF) is an antioxidant isothiocyanate derived from cruciferous vegetables with the potential to stimulate cell proliferation. To analyze the potential of SF to act on the proliferation and viability of progenitor cells, MTT (viability), LDH (cytotoxicity), and proliferation (number and diameter of neurospheres) assays were carried out on neural progenitor cells from the hippocampus of C57BL-6 mice up to 4 days old (CEUA 3952300622), treated with concentrations of SF (0.25, 0.5, 1, 2, 5, 10 and 15 μM). The analyses happened at different times after treatment: 24 and 48 hours for viability and cytotoxicity, and up to 3 days after treatment for proliferation. We found out, by LDH, that the cytotoxicity was increased by the 10 μM treatment (24h) and 1, 2, 5, and 15 μM (48h). About the MTT test, only the 15 μM treatment (24h) had a decreased viability. Finally, The number of neurospheres was lower in the 5 to 15 μM treatment (2 and 3 days) and the neurosphere's size was smaller in the 1 to 15 μM treatment (1, 2, and 3 days). Therefore, the most consistent result observed is that 0.25 and 0.5 μM concentrations were the only ones that did not significantly interfere with the cytotoxicity (LDH) or viability (MTT) of the cells after 24h and 48h, nor did they reduce the number and diameter of the neurospheres. In a visual analysis, it can be seen that the cells exposed to concentrations between 0.25 and 2 μM retain a healthy appearance, unlike the concentrations of 5 to 15 μM which show clusters with a necrotic appearance. Our findings suggest that SF in low concentrations (0.25 and 0.5 μM) is more suitable for treatment as it does not induce cell toxicity (viability and cytotoxicity tests).</p> <p>Keywords: Sulforaphane; Proliferation; Viability; Cytotoxicity</p>
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Title	Production and biochemical characterization of <i>Trypanosoma cruzi</i> metacaspase
Authors	Ane Caroline Moreira Duarte 1 João Pedro Martins Silva Costa 1 Mariana Nascimento Romero Trujillo 1 Taiz dos Reis Santos 1 Marcelo Ferreira Marcondes 2 Maurício Ferreira Marcondes Machado 1
Affiliations	1 – Interdisciplinary Center for Biochemical Research, , University of Mogi das Cruzes 2 – Department of Biophysics, UNIFESP
Session	Session 13 – Diferenciação, crescimento e morte celular

Abstract, Ethics Committee Number*, and Keywords	<p>INTRODUCTION: <i>Trypanosoma cruzi</i>, a protozoan parasite causing Chagas disease, is one of the major neglected diseases in Latin America. During its life cycle, metacaspase 3 (TcMCA3) plays a crucial role in regulating cellular processes, including programmed cell death. In this context, the production and biochemical characterization of TcMCA3 are essential to elucidate its properties, structure, and function, with potential for new therapeutic strategies.</p> <p>METHODOLOGY: TcMCA3 was obtained using the T5 exonuclease DNA assembly (TEDA) cloning method and expressed in <i>E. coli</i> BL21 DE3pLysS cells, induced by 0.25 mM IPTG. Purification was performed by nickel-sepharose affinity chromatography, with a gradient of imidazole from 50 to 300 mM, validated by SDS-PAGE electrophoresis and Western Blotting with anti-His(6X) antibodies on PVDF membrane, revealed by the SuperSignal West Pico kit (Thermo). First-order kinetic assays were also conducted to investigate the effect of temperature, pH, CaCl₂, and NaCl, as well as intrinsic fluorescence on enzyme activity.</p> <p>RESULTS: After expression, cell lysis, and purification, the obtained protein was confirmed on SDS-PAGE, with an approximate molecular weight of 38 kDa. Western Blotting revealed distinct fragments, indicating processing. First-order kinetic assays demonstrated that temperature affects its activity, with 25°C being ideal. The optimal concentrations of CaCl₂ and NaCl were 0.5 mM and 75 mM, respectively.</p> <p>CONCLUSION: The production of the enzyme in its active form was successful. Subsequent experiments allow elucidating the importance of calcium ion for the structure and activity of TcMCA3, paving the way to answer questions about its properties and specificity on potential substrates.</p> <p>KEYWORDS: Metacaspase; <i>Trypanosoma cruzi</i>; Cloning; Calcium interaction; Enzymatic kinetics; Substrate.</p>
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Title	Evaluation of the effect of calcium on the autoprocessing of <i>Saccharomyces cerevisiae</i> metacaspase
Authors	Karolaine Stela Siqueira de Moraes Valdivia Ane Caroline Moreira Duarte Mariana Nascimento Romero Trujilho Maurício Ferreira Marcondes Machado
Affiliations	Interdisciplinary Center for Biochemical Research, University of Mogi das Cruzes, Mogi das Cruzes, Brazil
Session	Session 13 – Diferenciação, crescimento e morte celular

Abstract, Ethics Committee Number, and Keywords	<p>Metacaspases are enzymes involved on the programmed cell death in a simple eukariotic cells. These enzymes are totally calcium dependent cause metacaspases need suffer a autoprocessing event to became active. Herein, are showed the effect of calcium on the autoprocessing of <i>S.cerevisiae</i> metacaspase (YCA1). Bacteria was incubated with the antibiotic kanamycin, and protein expression was induced by isopropyl-β-D-thiogalactopyranoside (IPTG) 0.25 mM. The protein was purified by affinity chromatography and size exclusion chromatography. Processing assays were performed with different calcium concentrations, with the results analyzed by SDS-PAGE gel. Intrinsic fluorescence was evaluated in a spectrofluorimeter and dissociation constant of calcium vs YCA1 was determined. We observed the effect of Ca^{2+} on the secondary structure of truncated YCA1, which exhibited two dissociation constants for calcium; one with high affinity ($K_D = 8.2 \mu\text{M}$) and one with low affinity ($K_D = 9.5 \text{mM}$). We found that after the addition of 10 mM of EGTA, the effect of calcium on the structure of YCA1 was completely reversed. Regarding the influence of Ca^{2+} on the autoprocessing of truncated YCA1, we incubated the enzyme at 37°C in different concentrations of CaCl_2 (0, 10 μM, and 10 mM) for 0 and 16 hours. The data demonstrated that incubation with 10 mM of CaCl_2 caused precipitation of truncated YCA1 due to the salt in effect, while incubation with a low concentration of CaCl_2 the enzyme did not undergo autoprocessing. We performed a calcium curve at different concentrations (50 μM – 5 mM), mimicking the data obtained in intrinsic fluorescence. We observed that the autoprocessing of this enzyme is calcium and time-dependent and that over long incubation periods, truncated YCA1 ends up degrading completely. In this work, we demonstrate that YCA1 is completely dependent on calcium for its activation, and this effect is also time-dependent. KEYWORDS: Metacaspase; Cloning; Calcium interaction; Enzymatic kinetics; Substrate.</p>
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Title	Evaluation of the antibacterial potential of jelleine-I against clinical isolates of uropathogenic <i>Escherichia coli</i> resistant to quinolones
Authors	Bárbara Gatti Cardoso ¹ Juliana Carvalhar Saboya Andrade ¹ Simone Odília Antunes Fernandes ¹ Julio César Moreira Brito ² Valbert Nascimento Cardoso ¹
Affiliations	1 – Laboratório de Radioisótopos, Departamento de Análises Clínicas e Toxicológicas, Faculdade de Farmácia, Campus Pampulha, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil. 2 – Fundação Ezequiel Dias, Belo Horizonte, Minas Gerais, Brazil.
Session	14 Biociências Nucleares para a Saúde

Abstract, Ethics Committee Number*, and Keywords	<p>Urinary tract infection (UTI) is one of the most frequent conditions in medical practice, with <i>Escherichia coli</i> uropathogenic (UPEC) as its main pathogen. Due to the excessive use of quinolones and the remarkable ability of this pathogen to develop resistance to antibiotics, it is crucial to seek new approaches to treatment. In this context, the antimicrobial peptide jelleine-I stands out as promising in antimicrobial activity. Therefore, the work aims to research, <i>in vitro</i> and <i>in vivo</i>, the antimicrobial pharmacological potential of jelleine-I against resistant UPECs strains. The antibacterial activity test was evaluated by determining the minimum inhibitory concentration (MIC) using the broth microdilution method. Next, the minimum bactericidal concentration (MBC) was determined by collecting 10 µL from wells free of growth in the MIC assay and dispensed onto the agar to analyze whether or not colonies were growing. For the survival curve, the peptide was added to the bacterial inoculum and incubated at different times to analyze the colony count by time of contact with the peptide using the spread plate technique. The standardization of the surgical model of pyelonephritis in mice was carried out by direct administration of the bacterial inoculum into the kidney (CEUA Protocol 51/2020). Proof of the establishment of infection was carried out by counting the colony-forming unit of renal macerate and urine. The analysis of renal tubular function was performed by evaluating the scintigraphic images after ^{99m}Tc-DMSA administration. <i>In vitro</i> studies revealed promising antibacterial activity of jelleine-I against clinical strains of resistant UPEC (MIC and CFM in the range of 32-256 µg/mL), with rapid (1 h) inhibition of the species. The bacterial load recovered in the kidney macerate and urine, as well as the renal scintigraphic image demonstrate success in standardizing the murine model of surgical pyelonephritis for testing new</p>
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antimicrobials. *In vitro* studies open up prospects for using this peptide for the purpose of treating UTIs, such as pyelonephritis.

Antimicrobial peptides; *Escherichia coli*; pyelonephritis; resistant microorganisms.



Title	[¹⁸F]FDG PET imaging of a Down syndrome animal model across life
Authors	Jean Marques Brizola Larissa Estessi de Souza Chiara Maria Righini Lidia Emmanuela Wiazowski Spelta Daniele de Paula Faria
Affiliations	Laboratory of Nuclear Medicine (LIM-43), Department of Radiology and Oncology, FM/USP, São Paulo, Brasil
Session	14 - Nuclear Biosciences for Health

Abstract, Ethics Committee Number*, and Keywords	<p>Down syndrome (DS) is the most common chromosomal abnormality seen in live births and the most common genetic cause of intellectual disability. People with DS presents a premature ageing characterized by cognitive decline and development of Alzheimer's Disease. The aim of this study was to evaluate the brain metabolism of a mouse model of DS (Ts65Dn) during ageing using positron emission tomography (PET) with [¹⁸F]FDG. Males and females both euploid and trisomic Ts65Dn animals were used (n=9-12; CEUA FMUSP 1292/2019). [¹⁸F]FDG PET was performed to access brain metabolism throughout the ageing process: 2, 5, 14, 20 and 24 months of age. Post-mortem analysis was done by immunohistochemistry with NeuN staining. Seven regions of interest were evaluated by both techniques: whole brain, hippocampus, striatum, cortex, thalamus, cerebellum and basal forebrain. There was an increase in [¹⁸F]FDG uptake in all evaluated regions in the euploid animals from 2 to 5 and to 14 months (p<0.05 or p<0.001 depending on the region). In the trisomic ones, this increase was seen only at 14 months (p<0.05 or p<0.001 depending on the region) except in the hippocampus. However, from 14 to 24 months, there was a significant reduction in the [¹⁸F]FDG uptake in the whole brain and in the hippocampus (p ≤ 0.05 on both). NeuN immunohistochemistry showed a significant lower density of NeuN in the forebrain when compared to the euploid animals (p ≤ 0.05). In the striatum of trisomic animals there was lower NeuN density than euploid animals at 14 (p<0.02), 20 and 24 months (p<0.01). This also happened in the basal forebrain (p<0.05) and cerebellum (p<0.05) at 20 and 24 months and in the cortex at 24 months (p<0.01). he alterations in brain metabolism of the trisomic animals as well as the reduction in NeuN density in all the regions assessed, corroborate the fact that ageing can contribute to CNS dysfunction and neurodegeneration in DS.</p> <p>Ethics Committee Number: CEUA FMUSP 1292/2019</p> <p>Keywords: Molecular imaging, Down Syndrome, Aging, Brain metabolism, Neurodegeneration</p>
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Title	Evaluation of [¹¹C]PK11195 uptake during aging on a transgenic model of Down Syndrome
Authors	Chiara Maria Righini Larissa Estessi de Souza Jean Marques Brizola Lidia Emmanuela Wiazowski Spelta Daniele de Paula Faria
Affiliations	Laboratory of Nuclear Medicine (LIM 43), School of Medicine, University of São Paulo (USP)
Session	14- Nuclear Biosciences for Health

Abstract, Ethics Committee Number*, and Keywords	<p>The population with Down Syndrome (DS) experiences an early and accelerated aging process. Trisomy 21 is related to the overexpression of genes associated with mitochondrial dysfunction and inflammation, which contribute to pathological aging and the development of neurodegenerative diseases. Therefore, the aim of this study was to evaluate the [¹¹C]PK11195 uptake, a TSPO tracer, throughout the aging process in the Ts65Dn animal model of DS in the presence and absence of the trisomy.</p> <p>Males and females both euploid and trisomic Ts65Dn animals were used (CEUA 1292/2019) and evaluated throughout aging at 2, 5, 14, 20 and 24 months of age by [¹¹C]PK11195 positron emission tomography (PET). The standardized uptake value was quantified for the whole brain and for the hippocampus, striatum, cortex, thalamus, cerebellum and basal forebrain. Post-mortem analysis was done by immunohistochemistry with staining for IBA-1, GFAP and TSPO in the same regions.</p> <p>[¹¹C]PK11195 uptake increased from 2 to 5 months in all brain regions, regardless of the genotype. From 14 to 20 and to 24 months, a decreased uptake was observed only in trisomic animals ($p < 0.05$, $p < 0.01$ or $p < 0.001$ depending on the region). Indeed, at 24 months, trisomic animals had a lower uptake than the euploids in all the regions ($p < 0.05$ or $p < 0.01$ depending on the region). [¹¹C]PK11195 uptake was positively correlated with the GFAP density in trisomic ($r = 0.56$, $p = 0.03$) and euploid ($r = 0.66$, $p = 0.007$) animals. No correlation was found for IBA-1. Regarding TSPO, its expression was lower in the hippocampus ($p = 0.049$), cortex ($p = 0.041$) and forebrain ($p = 0.04$) of trisomic animals at 24 months of age.</p> <p>Euploid and trisomic animals had a similar pattern of aging up to 14 months, but at older ages (20 and 24 months) there is a greater drop in TSPO expression in trisomic animals, which justifies the lower [¹¹C]PK11195 uptake at this age, suggesting a mitochondrial dysfunction in those animals.</p> <p>Ethics Committee Number: CEUA FMUSP 1292/2019 Keywords: Molecular imaging, Down Syndrome, aging, animal model, mitochondrial dysfunction</p>
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Title	Age-related brain metabolism changes in a Down syndrome animal model: a longitudinal voxel-based analysis
Authors	Lidia Emmanuela Wiazowski Spelta ¹ Larissa Estessi de Souza ¹ Gabriela Lazzarotto ² Eduardo Rigon Zimmer ² Daniele de Paula Faria ¹
Affiliations	1 – Laboratory of Nuclear Medicine (LIM-43), Department of Radiology and Oncology, FMUSP, São Paulo, Brazil 2 – ZimmerLab, Department of Pharmacology, UFRGS, Porto Alegre, Brazil
Session	14 - Biociências Nucleares para a Saúde

Abstract, Ethics Committee Number*, and Keywords	<p>Introduction: Down syndrome (DS), the most common chromosomal abnormality detected in live births, is associated with premature aging characterized by multiple comorbidities, including cognitive impairment. Positron emission tomography (PET) with [¹⁸F]FDG may be an important tool for understanding the aging process, providing in vivo information on cell loss over time. In this study we aimed to investigate age-related changes in brain glucose metabolism in a DS mouse model (Ts65Dn) during aging.</p> <p>Methods: Euploid (n=7) and trisomic (n=5) Ts65Dn animals including males and females (CEUA FMUSP 1292/2019) were longitudinally evaluated by [¹⁸F]FDG PET imaging, in a dedicated small-animal PET scanner, to assess brain metabolism at 14, 20 and 24 months of age. The minc-tools software was used to analyze PET images that were manually co-registered to a standard mouse histological template. The standardized uptake value ratio (SUVr) was calculated using the pons as the reference region and a voxel-wise t-statistical analysis was performed. The number of neurons was semi-quantified by NeuN immunohistochemistry (n=3) at 24 months of age (final time-point) to correlate with PET SUV data.</p> <p>Results: Voxel-wise t-statistical analysis revealed significant glucose hypometabolism in trisomic animals at 20 and 24 months of age (T scale: 2.2 - 4), when compared to euploid animals at the respective ages, especially in the hippocampus and frontoparietal cortex. A positive correlation between [¹⁸F]FDG uptake and integrated NeuN density was observed (r =0.834, p = 0.038).</p> <p>Conclusion: The hypometabolism observed in older aged trisomic animals may be related to neuronal loss and subsequent neurodegeneration.</p> <p>Keywords: Molecular imaging, [¹⁸F]FDG, Down Syndrome, Neurodegeneration</p>
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XXXVIII REUNIÃO ANUAL DA FESBE
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III CONGRESSO DOHAD BRASIL
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FRONTEIRAS DAS CIÊNCIAS BIOMÉDICAS E SEUS DESAFIOS

Title	Gene and protein regulation of interleukin-6 after photobiomodulation in a model of acute induced arthritis in rats
Authors	Sabrina Zanchetta Lanza ¹ , Bruna Silva Gomes ¹ , Marcelo Augusto Marretto Esquisatto ² , Gaspar de Jesus Lopes Filho ¹ , Fernando Russo Costa do Bomfim ^{1,2}
Affiliations	¹ UNIFESP-EPM ² Centro Universitário da Fundação Hermínio Ometto
Session	14 - Biociências Nucleares para a Saúde

Abstract, Ethics Committee Number*, and Keywords	<p>Rheumatoid arthritis is a systemic autoimmune disease that affects the synovial joints in an inflammatory and destructive manner. Photobiomodulation with low-level laser therapy (LLLT) is a non-invasive treatment that has anti-inflammatory and analgesic effects and promotes an increase of local microcirculation. The aim of this study was to evaluate the effects of LLLT on IL-6 gene and protein expression. After approval by CEUA-FHO (025/2021), thirty-six female Wistar rats were divided into three groups (n=12/group): control (no induction), sham (induced arthritis), and LLLT (induced arthritis and LLLT). Animals were maintained on a 12-hour light/dark cycle with food and water ad libitum. At time zero, after anesthesia (ketamine [0.3mg/kg]-xylazine [0.1mg/kg]), sham and LLLT animals received an intrarticular injection of zymosan (200µg). Twenty-four hours after induction, LLLT treatment was performed ($\lambda=808\text{nm}$, 25mW nominal power, fluence of 20J/cm², beam area of 0.02mm², time of 33s, total energy of 0.825 J and one point application). After three days, animals were euthanized with a combination of ketamine [0.9mg/kg] and xylazine [0.3mg/kg] and cervical dislocation. Knees samples (n=6/group) of the animals were subjected to RNA extraction, cDNA synthesis, and qPCR for β-actin and IL-6 genes evaluated by $2^{-\Delta\Delta\text{Ct}}$ technique. Histologic processing and immunohistochemistry for IL-6 (n=6/group) was evaluated by the Image J program. Statistical analysis (mean\pmstandard deviation) between experimental groups was performed using ANOVA and Tukey's post-test with $p<0.05$. qPCR analysis for IL-6 showed significant differences between LLLT (1.087\pm0.117) and sham (1.125\pm0.065) $p<0.0001$ and protein expression between sham (6.867\pm2.446) and control (1.600\pm0.910) and LLLT (1.333\pm0.516) and sham $p<0.0001$. LLLT was effective to reduce IL-6 gene and protein expression in the acute phase of induced inflammation.</p> <p>Key-words: Photobiomodulation, arthritis, inflammation.</p>
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Title	Evaluation of the stability of [⁶⁸Ga]gallium radiolabeled peptides in biological tissue homogenates and serum
Authors	Giovana Oliveira Alves Santos, ^{1,2} Marcio Ferrarini, ³ Ulisses Lacerda de Figueiredo Sá, ³ Koiti Araki, ¹ Fabio Luiz Navarro Marques, ^{2*}
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Session	

Abstract, Ethics Committee Number*, and Keywords	<p>Introduction: Drug stability is usually evaluated by <i>in vivo</i> determination of its integrity in blood or urine samples or, <i>in vitro</i>, by incubation in the serum. An alternative method is drug incubation in biological tissue homogenates. In this work, we evaluated the tissue <i>in vitro</i> stability of radiolabeled peptides [⁶⁸Ga]Ga-PSMA-11 and [⁶⁸Ga]Ga-DOTATATE, used for imaging diagnostic of prostate and neuroendocrine tumors, respectively. Ethical Committee: FMUSP #1974/23. Material and methods: [⁶⁸Ga]Ga³⁺ was produced in a cyclotron and reacted with peptides containing chelators; radiochemical purity was assessed in HPLC. Mice were anesthetized (5% isoflurane in O₂) and euthanized by heart excision. The liver and kidney were removed, sectioned, and frozen at -80 °C. Serum was obtained from volunteers. Tissue homogenates were prepared at 100 mg/mL in HEPES 20 mM pH 7.4. Radiolabeled peptides were added to tissue homogenates and in 0.25 mL of serum, then incubated for 1, 15, 30, and 60 minutes at 37 °C. Samples were centrifuged at 5,000 rpm; the supernatant was removed and mixed with acetonitrile, followed by a new centrifugation step to separate any protein content. Samples were analyzed by HPLC using an RP-C18 column with H₂O/TFA 0.1 % acetonitrile/TFA 0.1 % gradient mobile phases. Signals were measured in a radiation detector. Results: The HPLC retention time for [⁶⁸Ga]Ga³⁺, [⁶⁸Ga]Ga-PSMA-11, and [⁶⁸Ga]Ga-DOTATATE were 3.5 min, 8 min, and 8.8 min, respectively. Both radiolabeled peptides were stable for 1 h, as confirmed by HPLC chromatograms, in agreement with literature data. Conclusion: The protocol is a practice tool to study the drug's <i>in vitro</i> stability, mimicking <i>in vivo</i> drug interaction with the liver and kidney. Furthermore, this allows for saving animals since the organs from one animal can be used to test drug stability at different times or for different compounds. Apoio: CNPq. Keywords: Radioisotope, Gallium-68; Peptide, Stability, Tissue homogenate</p>
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Title	Evaluation of the interleukin-6 profile in adipose tissue after sodium deoxycholate and associations administration in Wistar rats
Authors	Andreza Milena Vicentin ¹ , Marília Gabriela Scarcella Assunção ¹ , Marina Marcuci Camara ¹ , Fernanda Flores Navarro ¹ , Fernando Russo Costa do Bomfim ¹
Affiliations	¹ Centro Universitário da Fundação Hermínio Ometto
Session	14 - Biociências Nucleares para a Saúde

Abstract, Ethics Committee Number*, and Keywords	<p>The multifactorial etiology of obesity has led to studies on its etiopathogenesis and consequently to different therapeutic approaches and the use of different compounds such as sodium deoxycholate (SD) and substances that increase cellular metabolism. The aim of this study was to evaluate the effects of SD, with or without caffeine and yohimbine, on the expression of interleukin-6 (IL-6) in Wistar rats. This study was approved by CEUA-FHO (18/2022). Wistar rats (n=54) were assigned into three groups (n=18/group): Control (saline), SD (SD 6%), and SDA (SD 6%+caffeine [50mg/mL]+yohimbine [0.2%]). At time 0, animals were anesthetized with ketamine (0.3 mg/kg) and xylazine (0.1 mg/kg) and received 0.2 mL of each compound in the central abdominal region. Samples of thick skin containing adipose tissue were collected 24 hours, 3 days, and 7 days after application by deep anesthesia with ketamine (0.9 mg/kg) and xylazine (0.3 mg/kg) and cervical dislocation. The samples were fixed in buffered formalin, processed and immunohistochemistry was performed for IL-6, and positive cells were counted using Image J program. Statistical analyses between experimental groups were performed using ANOVA and Tukey's post-test with $p < 0.05$. The results (median\pmstandard error) showed significant differences 24 hours after application between SD (520.0\pm72.86) and control (225.0\pm49.89) and SD and SDA (279\pm39.63), $p=0.0162$. After 3 days, differences were observed between SD (536.0\pm59.70) and control (198.0\pm53.39), SDA (830.0\pm26.77) and control and between SDA and SD treatments, $p < 0.0001$. On day 7, differences were observed between control (240.5\pm35.31) and SDA (30\pm3.32), SD (323.5\pm58.12) and SDA, $p=0.0007$. The use of SD alone maintains the inflammatory process for up to seven days, while SDA showed higher levels of IL-6 for up to three days, however analyses of cell death profiles are necessary to define the best therapeutic regimen.</p> <p>Keywords: inflammation, interleukin-6, adipose tissue</p>
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Title	Combination of photobiomodulation, photodynamic therapy and cellulose membrane pressure injuries treatment: 3 clinical cases presentation
Authors	Gabriel Pinto de Oliveira Santos ¹ ; Thais Azevedo Reis ¹ ; Luana Moreira Silva ¹ ; Francine Cristina
Affiliations	¹ Universidade Federal da Bahia, Vitória da Conquista, Bahia – Brasil. ² Instituto de Física de São Carlos - USP (IFSC-USP)
Session	15- Tecnologias Ópticas e Mecânicas para a Saúde

Abstract, Ethics Committee Number*, and Keywords	<p>Pressure injuries (PIs) are tissue injuries resulting from continuous and prolonged pressure, impairing tissue circulation adjacent to the lesion area, often associated with hospitalized patients with low mobility, secondary to neurological problems. Infections associated with this problem have made it a public health concern, due to their detrimental effects on patient survival, as well as the budgetary impact on the healthcare system. The use of new therapeutic approaches has become increasingly relevant. Antimicrobial Photodynamic Therapy (aPDT) involves the use of light associated with a photosensitizing agent that can cause bacterial and deleterious microorganism cell death, commonly found in PIs. Similarly, the use of Photobiomodulation Therapy (PBMT) also involves the use of light, which interacts with the cells of the body's tissue layers, stimulating local metabolic modulation and thus culminating in cell growth and tissue recovery at an early stage. In this study, we report the 45- week follow-up of 3 patients with neurological impairment who developed PIs and were treated with a combination of aPDT, PBMT, and cellulose membrane dressing (CM). aPDT was performed with a 1.5% emulsion curcumin photosensitizer, associated with blue LED at 450nm for 12 minutes, irradiance of 30 mW/cm², fluence of 22 J/cm², twice at the beginning of the study, with a 1-week interval. PBMT was performed with a 660 nm LASER, spot of 0.04 cm², power of 40 mW, fluence of 10 J/cm², irradiance of 1000 mW/cm², twice a week until lesion closure or the end of the study period. All lesions showed a reduction in the wound area and microorganism contamination with the use of aPDT in different proportions. Based on the results obtained, we conclude that the combination of aPDT, PBMT, and CM dressing is a promising treatment for PI healing reducing local contamination and wound healing time. This study was approved by Ethics Committee with CAAE number 36925714.0.0000.5556.</p> <p>Keywords: Antimicrobial Photodynamic Therapy (aPDT), Photobiomodulation Therapy (PBMT), Curcumin, LED, Pressure Injury</p>
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Title	Systemic Vibration Therapy and sleep quality in narcolepsy: case report
Authors	Trindade-Gusmão, LC ^{1*} ; Bahia, CM ² ; Silva-Rodrigues, R ¹ ; Cardoso, ALBD ¹ ; Sá-Caputo, DC ¹ ; Bernardo-Filho, M ¹ .
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Session	Optical and Mechanical Technologies for Healthcare

Abstract, Ethics Committee Number*, and Keywords	<p>Introduction: Narcolepsy is a chronic neurological disorder that influences the ability to control sleep–wake patterns. Individuals affected by this disease present nocturnal sleep disorders (NSD), resulting in reduced sleep quality. Physical exercise acting as an effective non-pharmacological treatment option for disturbed sleep. Systemic vibration therapy (SVT) has been suggested as a complementary intervention to improve sleep quality in individuals with narcolepsy. This case is to describe the effects of the SVT on sleep quality in individuals with narcolepsy. Methods: SVT using Mechanical vibration of 25 Hz, amplitude of 2.5 mm, was performed in 5 series (1 min of work, with 1 min of rest between each serie), 2 times/week, for 6 weeks. The Pittsburgh Sleep Quality Index (PSQI) and actigraphy (7 days) was applied pre and post intervention. Project approved by the Ethics Committee of the State University of Rio de Janeiro - UERJ (CAAE 30649620.1.0000.5259). Results: The results obtained did not demonstrate significant differences between the intervention in PSQI pre and post (15-10) TVS. A slight reduction in the final score was observed after the intervention, although the PSQI index remained above average at the cutoff line. In the subjective sleep quality domain, the data obtained points to an improvement of up to 50% compared to the initial score (2-1). Actigraphy data supported these subjective improvements, an increase in total sleep time was observed when comparing the pre (05:53 ± 01:11 hour) and post (06:26 ± 01:28 hour), (<i>p value</i>=0.311) protocol through statistical analysis using the paired Student's t Test. Conclusion: TVS seems to improve the sleep quality in individuals with narcolepsy. More research and larger-scale studies are needed to validate these findings and explore the long-term effects of SVT in the management of narcolepsy.</p> <p>Key words: Narcolepsy, sleep quality, systemic vibration therapy, case report.</p>
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Title	Effects of whole-body vibration exercise on hepatic and renal functions in diabetic Wistar rats: a preliminary study
Authors	André Luiz Bandeira Dionizio Cardoso ^{1,2} Luiza Carla Trindade-Gusmão ¹ Danúbia da Cunha de Sá-Caputo ¹ Mario Bernardo-Filho ¹ Corresponding author andreloedionizio@gmail.com
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Session	Sessão: 15 – Tecnologias Ópticas e Mecânicas para a Saúde

Abstract, Ethics Committee Number*, and Keywords	<p>Introduction: Type 1 diabetes mellitus (T1DM) is an autoimmune disease marked by hyperglycemia, that can lead to hepatic and renal complications. Exercise, such as whole-body vibration (WBV) exercise, in which subjects are exposed to mechanical vibration (MV) by a vibrating platform, have shown promise in improving metabolic parameters in humans and animals. This study evaluated effects of WBV on hepatic and renal biomarkers, as in organ morphometry, in diabetic rats. Methods: 10 male <i>Wistar</i> rats (250-350g, 2-3months) were allocated into two groups, diabetic control (DC,n=5) and diabetic exposed to MV (D-MV,n=5). For T1DM induction, alloxan (170mg/kg) was used. The D-MV group underwent WBV sessions (MV-50Hz, 0.78mm, at 4 bouts of 30s, separated by 1-min rest period in each session) for 5- weeks. After treatment, blood samples were collected for hepatic (creatinine and urea) and renal (alkaline phosphatase(ALP) and aspartate aminotransferase(AST)) biomarker analysis, and liver and kidneys were collected to weight measurements. This study was approved by the ethics committee CEUA/UERJ-006/2019. T-test for independent samples and Pearson's correlation were used to data analysis by GraphPad Prism 6. Difference was considered at p-value\leq0.05. Results: Animals treated with WBV did not exhibit significant changes ($p>$0.05) in hepatic levels of creatinine (0.62 ± 0.03) and urea (97.8 ± 11) concentrations compared to DC ($0.58\pm 0.04/ 134.2\pm 22$). Likewise, renal biomarkers levels of ALP ($125\pm 19/[DC]140\pm 14$) and AST ($172\pm 26/[DC]157\pm 11$) have not change ($p>$0.05) after WBV treatment. Also, no significant correlation ($p>$0.05) was found between liver ($r=0.058;r=0.222$) and kidneys ($r=0.261;r=0.018$) weight with their biomarkers levels. Conclusion: No effects were observed in WBV treatment on hepatic and renal biomarkers, nor any correlation found with organ morphometry changes in rats. Further research is needed for comprehensive insights into WBV action on diabetes condition.</p> <p>Keywords: Diabetes mellitus, whole-body vibration exercise, hepatic biomarkers, renal biomarkers, animals.</p> <p>Financial support: CAPES, CNPQ and FAPERJ</p>
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Title	Effect of six weeks of systemic vibratory therapy on uric acid/albumin ratio and pulse pressure in adults with obesity
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Session	Optical and Mechanical Technologies for Healthcare

Abstract, Ethics Committee Number*, and Keywords	<p>Introduction: Increased adiposity is linked to higher systolic blood pressure (SBP) and/or diastolic blood pressure (DBP), in addition to changes in plasma biomarkers. Pulse pressure (PP) (SBP-DBP) and uric acid/albumin ratio (UAR) are used to prevent cardiovascular events. Physical exercise (PE) brings beneficial adaptations to the cardiovascular system, however, obese individuals face challenges in adhering to traditional protocols. Systemic vibration therapy (SVT) has been integrated into the management of these individuals, providing effects similar to conventional approaches. Objective: To analyze the effect of six weeks of TVS on AUR and PP in individuals with obesity. Methods: Randomized clinical trial (CAAE 30649620.1.0000.5259). Inclusion criteria: adults (18 to 59 years old) with obesity (Body Mass Index [BMI] $\geq 30\text{kg/m}^2$). The individuals performed TVS on an alternating vibrating platform (PVA), the protocol performed was: frequency of 30 Hz, peak-to-peak displacement of 2.5 mm, 15 sets of 1 minute with static squats, followed by 1 minute of rest, 2 times a week for 6 weeks. UAR and PP were performed before and after 6 weeks of SVT. GraphPad Prism 6 was</p>
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used for the analyzes (paired Student's t test), considering a p-value of ≤ 0.05 and data are presented as mean \pm standard deviation. Results: 9 individuals were evaluated (BMI 34.52 ± 2.96 km/m²; Body mass 99.58 ± 12.64 kg; Height 1.71 ± 0.07 m; Age 41.55 ± 9.13 years). The initial UAR was 1.26 ± 0.26 and the final 1.14 ± 0.26 ($P=0.083$), the initial PP was 34.53 ± 8.13 mmHg and the final 36.84 ± 7.45 mmHg ($p=0.12$). Conclusion: There was no statistically significant difference in the UAR and PP variables. However, a reduction in UAR was observed. This is a preliminary study and with the continuation of the study the effect of SVT on these parameters can be better understood.

Keywords: Whole body vibration exercise; Adiposity; Hypertension; Cardiovascular system

Financing: 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).



Title	Cumulative effect of whole-body vibration exercise on cognition and fall risk in frail and pre-frail elderly: preliminary results
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Session	Optical and Mechanical Technologies for Healthcare

Abstract, Ethics Committee Number*, and Keywords	<p>Introduction: Aging can lead to loss of cognition, favoring a lack of balance and the risk of falls. And the whole body vibration exercise (EVCI) is recommended, as it is considered safe and easy to perform. The Mini Mental State Examination (MMSE) assesses cognition with a maximum score of 30 points. The Downton Fall Risk Scale is an instrument that quantifies the risk of falling and elderly people with scores greater than 3 points are at risk of falling. Objective: To evaluate the TVS-S (sitting group) and TVS-P (standing group) groups on cognition and risk of falling. Methods: Longitudinal clinical study, (CAAE nº 30649620.1.0000.5259). The allocation of participants to standing or sitting position consists of their clinical assessment. 20 TVS sessions were performed, using mechanical vibration with a frequency of 5 to 14 Hz, with peak-to-peak displacement: 2.5; 5 and 7.5 mm, with 1 min of intervention and 1 min of rest. Assessments were carried out before and at the end of 20 sessions. A value of $p \leq 0.05$ was considered. Results: 27 individuals in the TVS-P group participated in the study (14 individuals, aged 67.5 ± 5.57 years; BMI 27.92 ± 4.10 kg/m² and frailty 3.64 ± 1.21). And TVS-S (13 individuals, aged 69.23 ± 6.19 years; and BMI 31.27 ± 5.83 and frailty 3.92 ± 0.75). The initial MMSE score in the TVS-P group was 26.50 ± 2.98 and final 28.43 ± 1.51 ($p=0.0231^*$). The initial Downton score was 3.6 ± 1.05 and final 2.41 ± 0.72 ($p=0.0013^*$). In the TVS-S group, the initial MMSE score was 26.92 ± 3.73 and final 27.31 ± 3.11 ($p=0.0004$) and the initial Downton score was 3.84 ± 2.11 and final 2.07 ± 1.60 ($p=0.04453$). Conclusion: Significant differences ($p > 0.05$) were found in the MMSE and Downton in the TVS-P group, and in Downton in the TVS-S group. Although the MMSE score did not show significance in the TVS-S group, there was a decrease in the score; showing that 20 sessions of TVS increases the cognitive capacity of the elderly and reduces the risk of falling.</p> <p>Keywords: fall risk, exercise, older frail, cognition</p>
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Title	In silico comparison in sickle cell ischemic stroke: endothelial colony-forming cells secretome versus scrna- seq data from brain endothelial cells
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Session	Terapia Gênica e Celular, Biologia Omics

Abstract and Keywords	<p>Ischemic stroke (IS) is a severe complication of sickle cell anemia (SCA). Endothelial activation has a pivotal role in vaso-occlusion prior to ischemia, and in tissue recovery. Endothelial progenitor cells (EPCs) are recruited from the bone-marrow to infarcted areas to repair the endothelium through the replacement of damaged cells and the secretion of pro-angiogenic growth factors. However, these circulating cells do not reside naturally in the brain. Thus, our goal was to identify commonly expressed genes from a transcriptomic-based secretome analysis of EPCs in sickle cell ischemic stroke with brain endothelial cells (BECs) markers obtained by single-cell RNA-Seq.</p> <p>To identify upregulated genes related to potentially secreted proteins (PSPs) we compared our RNA-Seq data of Endothelial-colony forming cells (ECFCs - a type of EPCs) from SCA patients with (n=4) and without (n=4) IS (FDR <0.01 and Log2FC >2)(GSE248760), with the secretome dataset from The Human Protein Atlas (HPA). Next, we determined commonly expressed genes between our PSPs and BECs marker genes (Log2FC >0.5 and FDR <0.05) obtained from a scRNA-Seq study of human tissue (dbGAP phs002624.v2.p1). Finally, functional enrichment was performed in enrichR tool.</p> <p>Of the 2,469 differentially expressed genes, 1,833 were upregulated and 129 related to PSPs. Among them, 8 genes are also expressed in brain endothelial cells: EGFL7, HYAL2, LY6E, AGRN, SERPINE2, GAS6, ADAM15 and HLA-C; in terms such as “Regulation Of Endothelial Cell Proliferation”; “Metallopeptidase Activity”; “Hemostasis” and “Extracellular Matrix Organization”.</p> <p>Our results indicate the presence of PSPs expressed by both EPCs in SCA IS and BECs. These PSPs are essential for cell communication and angiogenesis, mediating endothelial recovery. Thus, these findings highlight the importance of these pathways in post-ischemic brain recovery and suggest new therapeutic targets to improve tissue repair in patients with SCA and IS.</p> <p>Keywords: Sickle cell anemia; Ischemic stroke; Transcriptome-based secretome; single-cell RNA-Seq.</p>
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Title	Influence of variants in the <i>DPYD</i> gene and hand-foot syndrome in patients treated with Capecitabine
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Session	[Terapia Gênica e Celular, Biologia Omics]

Abstract, Ethics Committee Number*, and Keywords	<p>Capecitabine is an oral pro-drug of fluoropyrimidines widely used in the treatment of patients with advanced colorectal, breast, and gastric cancer. Hand-foot Syndrome (HFS) is a common toxicity associated with capecitabine treatment. It may be related to reduced activity of the DPD enzyme, mostly caused by genetic variants in the <i>DPYD</i> gene. Therefore, this work aims to evaluate the influence of the four most relevant <i>DPYD</i> variants (c.1129-5923 C>G (rs75017182); c.1905+1 G>A (rs3918290); c.1679 T>G (rs55886062) and c.2846 A>T (rs67376798)) and their association with the HFS induced by capecitabine in Brazilian patients with gastric and colorectal cancer. To reach this goal, we conducted a retrospective cohort study at the Hospital das Clínicas of the UNICAMP, Brazil. This study was approved by the Ethics Committee (CAAE: 65683517.5.0000.5404). The <i>DPYD</i> variants were genotyped by RT-PCR using TaqMan probes and capecitabine toxicities were evaluated following the CTCAE criteria (version 4.0). We enrolled in this study 99 patients diagnosed with gastric or colorectal carcinomas treated with three cycles of capecitabine. Each cycle included an assessment of adverse reactions. 50 (50,5%) were men and 49 (49,5%) were women. The average age was (58,4± 10,1), 51 (51,5%) was Caucasian and 48 (48,5%) was non-Caucasian. HFS toxicity in any cycle was found in 53 (53.5%) patients. Of these, toxicity grades 1 or 2 of were found in 47 (47,5%) and grades 3 or 4 were found in 3 (3%) patients. Regarding the <i>DPYD</i> variants, only two patients presented heterozygous genotypes for the SNVs rs75017182 and rs67376798 and all the other patients presented Wild Type genotypes. No significant association was found between the <i>DPYD</i> variants and HFS toxicity. We concluded that the HFS toxicity is frequent in patients treated</p>
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with capecitabine and until now, there are no relation with the *DPYD* variants studied in this group of patients.

Keywords: *DPYD* gene, Capecitabine, Hand-Foot Syndrome.



Title	Associations between polymorphism in the <i>GSTP1</i> gene (rs1695) and gastrointestinal adverse reactions in patients with gynecological tumors treated with paclitaxel and carboplatin
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Session	16 - Terapia Gênica e Celular, Biologia Omics

Abstract, Ethics Committee Number*, and Keywords	<p>Gynecological tumors, which include neoplasms affecting the female reproductive system, are among the most prevalent forms of cancer on a global scale. Treatment modalities include chemotherapy with paclitaxel and carboplatin. Nevertheless, both agents are associated with drug-related adverse reactions (ADRs), including the gastrointestinal ones; furthermore, interindividual differences in the frequency of such ADRs are closely related with polymorphisms in key pharmacogenes. Therefore, this study aimed to evaluate potential associations between rs1695 polymorphism in the <i>GSTP1</i> gene and gastrointestinal ADRs induced by paclitaxel-carboplatin chemotherapy in patients with gynecological malignancies. This retrospective study included 481 participants diagnosed with gynecological tumors and treated with paclitaxel and carboplatin. The study was approved by the research ethics committee of the University of Campinas (number: 20406413.6.3001.5404). DNA samples were isolated from peripheral blood leukocytes, and the rs1695 polymorphism was assessed by qPCR. ADR severity was classified according to the Common Terminology Criteria for Adverse Events (CTCAE, version 4.03). The most frequent ADRs were nausea (69,1%), diarrhea (43,9%), constipation (42,7%), and vomiting (40,9%), all of which had a grade 1 predominance (mild). Among the included participants, the genotypes AA, AG, and GG frequencies were respectively 42.02%, 45.09%, and 12.88%. Patients with the GG genotype were 2.5 times less likely to experience nausea (OR: 2.5; $p < 0.05$) but were 2.2 times more likely to experience diarrhea (OR: 2.2; $p < 0.05$) when compared with other genotypes. Meanwhile, those with the AA genotype were 1.6 times more likely to experience nausea (OR: 1.6; $p < 0.05$). These results suggest a possible</p>
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relationship between the AA genotype and nausea, while the G allele seems to reduce this ADR but increases the risk of diarrhea during treatment.

Keywords: gynecological neoplasms; carboplatin; paclitaxel; *GSTP1*; adverse reactions.



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Title	Evaluation of a possible association of CYP2C8 polymorphisms with gastrointestinal adverse reactions in patients with gynecological tumors treated with paclitaxel and carboplatin
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Session	16 - Terapia Gênica e Celular, Biologia Omics

Abstract, Ethics Committee Number*, and Keywords	<p>Gynecological tumors represent one of the main etiologies of cancers that affect women worldwide, being commonly treated with the combination of platinum derivatives and taxane. However, despite the efficiency, it still has limitations due to the occurrence of adverse reactions (ADRs). This study aims to investigate the possible relationship of two <i>CYP2C8</i> gene polymorphisms with gastrointestinal ADRs in patients with gynecological malignancies treated with paclitaxel and carboplatin. It is a retrospective cohort study, in which participants at any clinical stage who received the targeted chemotherapy regimen were included. The study was approved by the research ethics committee of the University of Campinas (CAAE: 20406413.6.3001.5404). DNA samples were isolated from peripheral blood leukocytes and polymorphisms were determined by qPCR. Adverse events were evaluated according to the Common Toxicity Criteria for Adverse Events, version 4.03. Within the 377 patients evaluated for the <i>CYP2C8*3</i> 1196T>C (rs10509681) polymorphism, 5,0% had the CC, 12,5% the CT, and 82,5% the TT genotype. Amongst the 332 patients genotyped for the <i>CYP2C8*1C</i> 370A>C (rs17110453) polymorphism, 0,9% had the CC genotype 16,6% the AC, and 82,5% the AA genotype. The most frequent ADRs were nausea (69,1%), followed by diarrhea (43,9%), constipation (42,7%), and vomiting (40,9%), all with grade 1 predominance (mild). Patients with the TT and CT genotypes of the <i>CYP2C8*3</i> allele had a higher risk of vomiting (OR: 3,74; p<0,05), a higher risk of diarrhea (OR: 1,98; p<0,05) and constipation (OR: 1,98; p<0,05). Patients with the CC and AC genotypes of the <i>CYP2C8*1C</i> polymorphism had a higher risk of constipation (OR: 2,78; p<0,05). The study suggests that the TT and CT genotypes of the rs10509681 SNP may favor the occurrence of gastrointestinal ADRs, as well as the CC and AC genotypes of the rs17110453 polymorphism.</p> <p>Keywords: gynecological neoplasms; carboplatin; paclitaxel; CYP2C8; adverse reactions.</p>
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Title	A comprehensive atlas of human adipose tissue macrophages reveals diverse populations associated with fat depot and obesity
Authors	Fontes-Cal, T. C. M. ^{1,2} , Mogollon-Garcia, H. D. ¹ , Reis, L. M. ³ , Virgílio-Da-Silva, J. V. ³ , Grillo, L.G.P. ¹ , Angelim, M. K. C ³ , Moraes, D. ¹ , Figueiredo, L. S. ¹ , Knittel, T. L. ¹ , Chaim, E. A. ⁴ , Chaim, F. D. M. ⁴ , Moraes-Vieira, P. M. ^{3,*} , Boroni, M. L. ^{5,*} , Mori, M. A. ^{1,2,*} .
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Session	16- Gene and cellular therapy, Omics biology

Abstract, Ethics Committee Number*, and Keywords	Single-cell transcriptomics allows the capturing of multifaceted cell profiles in tissue microenvironment and thus permits to correlate cell signatures with disease states. We characterized myeloid cells from different human fat depots by profiling them using single-nucleus RNA sequencing (snRNA-seq) and established associations between adipose tissue macrophage (ATM) subpopulations and obesity pathophysiology. Abdominal visceral (VAT) and subcutaneous (SAT) adipose tissue biopsies from seven obese (BMI≥35) patients were collected during bariatric surgery. Nuclei were isolated and subjected to snRNA-seq using 10x Genomics-Single-Cell 3'v3. Sequences (Illumina NovaSeq) were processed and analyzed using the Cellranger and Seurat platforms, respectively. Outliers were removed (is.outlier) and MT filtering <20. Summarized pipeline consists of doublets removal
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(DoubletFinder), ambient RNA removal (Decontx and Cellbender), and data integration (scANVI). Six publicly available studies were processed and co-integrated with our datasets to compare myeloid-derived cell populations from our cohort with those found in literature. Our study generated one of the largest human ATM atlases comprising more than 15 thousand cells, clustered into 7 subpopulations. Patients with BMI>30 had a higher proportion of a subpopulation we called Mac3. On the other hand, Mac2 and Mac5 subpopulations were proportionally larger in eutrophic patients. We also showed that Mac1 and Mac5 subpopulations were more abundant in normoglycemic patients and SAT, while Mac6 and Mac7 clusters were higher in type 2 diabetic (T2D) patients. Gene Set Enrichment Analysis indicated distinct functions for these macrophages. Our study contributed to a broader characterization of human ATM heterogeneity and its association with distinct fat depots and metabolic states.

Ethics statement: CAAE: 31528820.0.0000.5404

Keywords: Adipose tissue; Single-nucleus RNA sequencing; Obesity.



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Title	<i>ABCB1</i> polymorphism (rs1128503): influence on adverse reactions and survival in lung cancer patients treated with carboplatin+paclitaxel
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Session	16 - Terapia Gênica e Celular, Biologia Omics

Abstract, Ethics Committee Number*, and Keywords	<p>Lung cancer is a prevalent and lethal disease in Brazil, with chemotherapy being the mainstay of treatment for advanced cases. However, this treatment is associated with adverse drug reactions (ADRs), which limit its therapeutic efficacy. This study aims to investigate the influence of polymorphisms in the <i>ABCB1</i> gene on ADRs and survival in patients treated with carboplatin and paclitaxel. The study was approved by the research Ethics Committee of the University of Campinas (CAAE: 83196318.8.0000.5404). Blood samples were collected from patients with non-small cell lung cancer, prior to and following the initial course of chemotherapy. Polymorphisms were determined by RT-PCR. Adverse events were evaluated according to the Common Toxicity Criteria for Adverse Events, version 4.03. Overall survival was calculated. Among the 113 patients evaluated for <i>ABCB1</i> rs1128503, 45.5% had GG genotype. The most prevalent ADRs were nausea (23.9%), anemia (38.1%), reduced creatinine clearance (25.2%), hypocalcemia (26.3%), and hyponatremia (20.5%), all grade 1. A multivariate logistic analysis revealed that individuals with the AG+AA genotypes for rs1128503 were more likely to experience nausea and vomiting, and exhibited a decreased survival rate. These two genotypes demonstrated to result in increased expression of the <i>ABCB1</i> protein, leading to drug resistance and decreased survival (by decreasing efficacy), as well as increased drug concentration outside the cell, resulting in increased incidence of ADRs. This study suggests that polymorphism in <i>ABCB1</i> may influence gastrointestinal ADRs and survival in patients treated with carboplatin and paclitaxel. Pharmacogenomic studies can predict ADRs and drug resistance, and thus provide more appropriate pharmacotherapy and follow-up for these patients.</p> <p>Keywords: lung cancer; polymorphisms; carboplatin; paclitaxel.</p>
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Title	Nfil3 circadian pattern affects Brown Adipose Tissue thermogenesis
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Abstract, Ethics Committee Number*, and Keywords	<p>Brown adipose tissue (BAT) for thermogenesis is a process through which futile energy expenditure occurs, resulting in heat production and increased body temperature. Circadian rhythm is a 24-hour rhythm based on light that regulates physiological, behavioral, and metabolic processes. Mechanistically, circadian rhythms mainly consist of two interconnected transcriptional and translational feedback loops. In recent years, our understanding of circadian control in thermogenic adipocytes has advanced, but its transcriptional regulation remains elusive. Here, we took advantage of a machine-learning-based approach (IMAGE) to understand the transcriptional regulation of circadian rhythms in brown adipocytes. Our data revealed several transcription factors that play important roles in the circadian rhythm of brown fat. In this study, we focused on Nfil3, which shows increased activity during the nocturnal phase, corresponding to the phase of higher thermogenic activity. IMAGE data demonstrated a complete map of genomic regions containing the binding sites of Nfil3. Pathway enrichment analysis revealed that Nfil3 regulates routes related to metabolic and thermogenic pathways. Next, we investigated the circadian regulation of Nfil3 in brown adipocytes in a cell-autonomous manner. The Nfil3 loss- of-function (Nfil3_KD) assay was performed using short hairpin technology. Notably, Nfil3_KD dramatically affected the gene expression and rhythm of clock genes, such as Cry2, Per1 and Per2. It was also observed that Nfil3_KD reduced the expression of genes related to thermogenic programs, such as Ucp1 and Pgc1-α. Moreover, Nfil3_KD, mediates a decrease in cellular oxygen. Taken together, our data revealed that the transcription factor Nfil3 regulates the circadian control of brown adipocytes, by affecting pathways related to energy metabolism and thermogenesis.</p> <p>Keywords: Energy Metabolism; Transcriptional Regulation; Bioinformatics</p>
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Title	Unveiling the transcription factors related to the circadian control of energy metabolism and thermogenesis in brown adipose tissue.
Authors	Thales Paulilo Scarpato ^{1,3} Carlos Eduardo Leher ^{1,3} Fernando Bladimir Valdivierso Rivera ^{1,3} Flavia Caroline Gan ^{1,3} Vanessa de Oliveira Furino ^{1,3} Licio Augusto Velloso ^{1,3,4} Carlos Henrique Grossi Sponton ^{1,2,3}
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Session	Poster

Abstract, Ethics Committee Number*, and Keywords	<p>Circadian rhythms are oscillatory and self-sustained processes based on a certain stimulus from the environment. In mammals, the circadian rhythm is based on the daylight pattern, which acts as a genetic translational negative feedback loop in the suprachiasmatic nucleus (SCN). Circadian rhythm also affects the biological processes of peripheral tissues such as brown adipose tissue (BAT). Studies have demonstrated that circadian rhythm influences various metabolic processes in BAT, such as thermogenic and lipid oxidation. However, the underlying molecular mechanisms are not yet fully understood. In this study, we aimed to investigate the role of circadian transcription factors in the regulation of energy metabolism in brown adipose tissue (BAT). We performed in silico machine learning (IMAGE) analyses using ATAC-seq and RNA-seq data. We identified the circadian pattern of transcription factor (TF) activity over 24h and its downstream regulated signalling pathways. In this study, we focused on the transcription factor (TF) Nfil3 because of its enhanced activity during the transition from the light to the dark phase, corresponding to the higher thermogenic activity period. Several TF (Creb3, Nr1h3, Klf13, Nfil3, Mtf1, Usf2, Rfx5, Rxrg, Rxra, Tef, Spib, and Arntl) have been identified as drivers of the circadian metabolic activity in BAT. Moreover, our data revealed a complex interaction among circadian TF regulating unique or common metabolic pathways, such as metabolic processes, fatty acid oxidation, and lipid metabolism. In conclusion, our findings showed an intricate connection between circadian rhythm and metabolic activity regulated by TF in BAT.</p> <p>Keywords: Circadian rhythm, transcription factors, brown adipocytes, energy metabolism and bioinformatics.</p>
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Title	The transcription factor GLIS3 is a main driver of the human white adipocyte dedifferentiation
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Session	Poster

Abstract, Ethics Committee Number*, and Keywords	<p>Adipocytes are characterized by a high degree of cellular plasticity in response to physiological or pathophysiological stimuli. These cellular changes occur through dedifferentiation or transdifferentiation. Dedifferentiation is a process in which a terminally differentiated cell reverts to an undifferentiated precursor stage. However, transcription factors (TFs) that regulate this process in adipocytes remain unknown. Therefore, the goal of this study is to identify the TFs related to white adipocyte differentiation. We employed in silico analysis of public single-cell RNA sequencing and single-cell ATAC sequencing data from human visceral adipose tissue. The data were then integrated using the CellOracle tool to generate the gene regulatory network (GRN). A total of 25.000 cells were obtained and further classified into seven adipocyte mature cell clusters (ADIPOQ+) and six adipocyte progenitor cell (ASPCs) clusters (PDGFRa+). The resulting GRN showed ten core TFs for each cluster, and each of these initial targets was perturbed using CellOracle. Analysis of TFs revealed that the in-silico gain- or loss-of-function of the GLIS Family Zinc Finger 3 (GLIS3) caused a shift in cell differentiation dynamics, where mature adipocytes displayed changes to the precursor cell stage (dedifferentiation). Thus, our preliminary data revealed that GLIS3 plays a key role in promoting dedifferentiation of human white adipocytes.</p> <p>Keywords: White adipocytes; Dedifferentiation; Transcription Factors; CellOracle; Gene Regulatory Network.</p>
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FRONTEIRAS DAS CIÊNCIAS BIOMÉDICAS E SEUS DESAFIOS

Title	FOX variants located in the Vitamin D Receptor gene frequency in patients with COVID-19
Authors	Pedro Gamberini Martins ¹ , Carla Regina da Silva Correa da Ronda ¹ , Aline de Souza Nicoletti ² , Marília Berlofa Visacri ³ , Rafael Nogueira de Souza ² , Mauricio Wesley Perroud Junior ^{2,4} , Deise de Souza Ventura ⁴ , Adriana Eguti ⁴ , Leonardo Oliveira Reis ² , Luiz Augusto dos Santos ⁵ , Nelson Durán ² , Wagner José Fávaro ² , Magnun Nueldo Nunes dos Santos ² , Patricia Moriel ¹ , Eder de Carvalho Pincinato ²
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Session	16 - [Terapia Gênica e Celular, Biologia Omics]

Abstract, Ethics Committee Number*, and Keywords	<p>The new coronavirus (SARS-CoV-2), the causative agent of Coronavirus Disease 2019 (COVID-19), has become the most recent global emergency. Vitamin D (Vit D) plays an important immunomodulatory function and may indicate a potential risk factor for severity and worse outcomes in patients with COVID-19 regarding the inflammatory characteristics of the disease. Vit D action is modulated by the Vit. D receptor, encoded by the vitamin D receptor (<i>VDR</i>) gene. Therefore, variants in the <i>VDR</i> can affect the action of the Vit. D receptor and, consequently, may be related to the severity of COVID-19. This study investigates the frequency of the variant FOX (rs2228570) of the <i>VDR</i> gene, in patients with COVID-19 and further relation with the severity of the disease. This is an observational, analytical, retrospective cohort study approved by the CEP (Research Ethics Committee - 83196318.8.0000.5404). The patients were subdivided in 2 groups: severe-critical and mild-moderate illness. Genomic DNA samples were isolated using the Promega® kit and the FOX <i>VDR</i> variant were genotyped by RT-PCR (Rotor-Gene, Qiagen) using TaqMan probes. The study included 361 patients with a mean age of 58,2 ± 22,5 years. The majority of the patients were men (55.3%) and caucasian (67.11%). Of the 361 patients, 211 (58.45%) were included in the severe-critical illness group, while the other 150 (41,55%) were included in the mild-moderate group. Regarding the FOX variant, wild allele frequency is 0.33 and the genotypes found were AA= 39 (10.8%), AG= 162 (44.8%) and GG=</p>
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160 (44.3%). The frequency found in this study for the FOX variant is in accordance with the Brazilian frequency described by the ABRAOM. The relation of the genotypes with the severity of the disease is currently being assessed.

Keywords: COVID-19, polymorphism, Vitamin D.



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FRONTEIRAS DAS CIÊNCIAS BIOMÉDICAS E SEUS DESAFIOS

Title	Allele variants frequency in interferon lambda genes found in patients with COVID-19
Authors	Letícia Rogge Nogueira dos Santos ¹ , Carla Regina da Silva Correa da Ronda ¹ , Aline de Souza Nicoletti ² , Marília Berlofa Visacri ³ , Rafael Nogueira de Souza ² , Mauricio Wesley Perroud Junior ^{2,4} , Deise de Souza Ventura ⁴ , Leonardo Oliveira Reis ² , Luiz Augusto dos Santos ⁵ , Nelson Durán ² , Wagner José Fávaro ² , Marcelo Lancellotti ¹ , Magnun Nueldo Nunes dos Santos ² , Patricia Moriel ¹ , Eder de Carvalho Pincinato ²
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Session	16 - [Terapia Gênica e Celular, Biologia Omics]

Abstract, Ethics Committee Number*, and Keywords	<p>COVID-19 has become the most recent global emergency. It is known that upon the entry of virus into lung epithelial cells there is an active replication and release of the virus resulting in cell pyroptosis with release of molecular patterns associated with cellular damage. These proteins induce the release of interferon lambda (IFNL), establishing a pro-inflammatory feedback loop. Some factors are associated with the worsening of the disease, however, patients with the same clinical conditions may develop different outcomes. A possible explanation is the genetic variation of individuals, such as the presence of variants in <i>IFNL</i> genes given its importance in the inflammatory cascade in the outcome of the disease. Thus, the aim of this work is evaluate the allele frequency of variants in the genes <i>IFNL3</i> (rs12980275 and rs8099917) and <i>IFNL4</i> (rs12979860) and further relation with the severity of COVID-19. To reach this goal, patients with COVID-19 were enrolled in this study after agreement and sign of the Ethics Committee documents (CAAE 36041420.0.000.5404 and CAAE 31049320.7.1001.5404). Genomic DNA samples were isolated using the Promega® kit and the <i>IFNL3</i> and <i>IFNL4</i> variants were genotyped by RT-PCR (Rotor-Gene, Qiagen) using TaqMan probes. We enrolled 138 patients, 52 (37.7%) had wild or moderate disease and 86 (62.3%) had severe or critical disease. The wild allele frequency for the rs12980275 and rs8099917 of <i>IFNL3</i> was 0.73 and 0.83, respectively. The wild allele frequency for the rs12979860 of <i>IFNL4</i> was 0.65. The genotypes found for rs12980275 were AA=18 (52.9%), AG=14 (41.2%) and GG=2 (5.9%), for rs8099917 were TT=23 (67.6%), TG=11 (32.4%) and GG=0 (0.0%) and for</p>
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rs12979860 were CC=15 (45.4%), CT=13 (39.4%) and TT=5 (15.2%). The frequency found in this study is in accordance with the Brazilian frequency described by the ABRAOM and the relation of the genotypes with the severity of the disease is currently being assessed.

Keywords: Interferon lambda, COVID-19, Genetic variants.



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FRONTEIRAS DAS CIÊNCIAS BIOMÉDICAS E SEUS DESAFIOS

Title	BSMI and APA1 polymorphisms of the vitamin D receptor (VDR) gene as possible biomarkers of disease induced by the Sars-CoV-2 virus
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Session	16 - [Terapia Gênica e Celular, Biologia Omics]

Abstract, Ethics Committee Number*, and Keywords	<p>The new coronavirus (SARS-CoV-2), the causative agent of Coronavirus Disease 2019 (COVID-19), has become the most recent global emergency. Several risk factors have already been identified and associated with the disease, such as the plasma concentration of Vitamin D (Vit. D). The action of vitamin D in our body is modulated by the Vit. D receptor, encoded by the vitamin D receptor (VDR) gene. Therefore, polymorphisms in the VDR can affect the action of the Vit. D receptor and, consequently, may be related to the severity of COVID-19. Therefore, the general objective of this work is to evaluate the influence of the BSMI and APA1 polymorphisms of the VDR gene on the severity of COVID-19. To perform patient genotyping, the genomic DNA samples were isolated using the Promega® method and polymorphisms are being determined using Real-Time Polymerase Chain Reaction (qPCR), using the Rotor thermocycler-Gene™ Q (Qiagen) in addition to relevant statistical analyzes. So far 160 patients diagnosed with Covid-19 were included. 37 (52,9%) male patients and 33 (47,1%) female with mild to moderate symptoms; and 52 (57,8%) male and 38 (42,2%) female presented a critic or severe symptoms. According to the allele frequency results obtained for the BSMI polymorphism (0,62), it is possible to conclude that the genotyped population is representative of the Brazilian (0,64) and world (0,65) population, according to the Abraom and PharmGKB genetic bases. To date, only 15 patients have been genotyped for the APA1 polymorphism, which may justify the difference in allele frequency (0,07) in relation to national (0,43) and global (0,45) frequencies. This research project was approved as a scientific initiation scholarship by FAPESP (process 2023/07600-5) and it is contained in a larger project, whose protocol was submitted to the Research Ethics Committee of the Faculty of Medical Sciences of the State University of Campinas (CAAE 36041420.0.000.5404 and CAAE 31049320.7.1001.5404).</p> <p>Keywords: Vitamin D Receptor, COVID-19, Polymorphisms.</p>
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Title	Jagged cells as support for HSPCs
Authors	Mayara Cristina Moreira Silva Nathalia Rocha de Oliveira Marcus Alexandre Finzi Corat
Affiliations	Laboratory for Development of Biological Models - CEMIB- UNICAMP- Campinas - SP - Brazil
Session	Terapia Gênica e Celular, Biologia Omics

Abstract, Ethics Committee Number*, and Keywords	<p>Mimicking an <i>in vitro</i> environment with proliferative and undifferentiated hematopoietic stem and progenitor cells(HSPCs) is a pursued challenge due to its great therapeutic potential. We hypothesize here that the post-transplant marrow recovery environment would encompass such a characteristic. Thus, we promoted a regenerative deleterious process of bone marrow(B.M.) by gamma radiation followed by a transplant of HSPCs marked with green fluorescent protein(GFP) capable of recovering the deleted marrow. In order to observe preferential cellular niches, we used some markers of medullary stromal cells such as Nestin, LepR, and Jag-1. It was observed that instead of particular niches, GFP⁺ regenerative cells were spread throughout the medullary stroma, as were Jagged-1(Jag-1⁺) cells. We then decided to isolate the Jag-1⁺ cells and explored their potential as support cells for hematopoietic progenitors. We performed co-culture of Jag-1⁺ cells with GFP⁺ Lin⁻ c-Kit⁺ hematopoietic progenitors isolated by magnetic beads and analyzed their proliferation after 24h, apoptosis/death after 10 days, and colony formation after 12 days. The data showed a good proliferation index in the first 24h and a dynamic decrease in apoptosis and cell death levels over 10 days of culture. Furthermore, HSPCs cultured with Jag-1⁺ cells were able to form BFU-E and CFU-GM colonies in the hematopoietic differentiation assay in Methacult medium after 12 days of culture, colonies involved in the blood formation process. In an <i>in vivo</i> assay, the marrow transplant of cells cultured with Jag-1⁺ cells demonstrated the ability to participate in the restoration of different hematopoietic lineages of B.M. in the recovery process. Our results were very promising, demonstrating that Jag -1⁺ stromal cells are potentially effective candidates as support for the cultivation and maintenance of HSPCs <i>in vitro</i>. Approved by the ethics committee of UNICAMP n°4584-1/2017.</p> <p>Keywords: HSPCs; Jagged Cells; Co-Cultivation.</p>
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Title	Infection control by coronavirus inactivation by CRISPR/Cas9 technology
Authors	Nathália Rocha de Oliveira ¹ Mayara Cristina Moreira Silva ¹ Clarice Weis Arns ² Marcelo Lancellotti ³ Marcus Alexandre Finzi Corat ¹
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Session	16 - Terapia Gênica e Celular, Biologia Omics

Abstract, Ethics Committee Number*, and Keywords	<p>Type II CRISPR-Cas9 system is the most widely used for genomic editing of eukaryotic cells. Although its classic use for dsDNA editing is more common, evidence suggests that the Cas9 protein can act on RNA molecules. Considering that coronaviruses have ssRNA as their genomic material, we hypothesized that the CRISPR-Cas9 system could effectively control viral proliferation in infected cells. For our approach, we tested the efficacy of the CRISPR/Cas9 system on specific targets (guides) of the MHV-3 genome (murine coronavirus). Guides were designed on Benchling.com, selected by scoring their "on target" and "off target" performance on genes essential for MHV formation and infection, based on the MHV-3 strain reference, and cloned into the pL-CRISPR-EFS-GFP plasmid. Infection and in vitro proliferation of MHV-3 were performed in L929 cells. The titer was calculated by DICT50, with 104.75 viral particles/mL. Doses starting from MOI 0.1 were used, and the transduced cells were transfected using the "jetOPTIMOS® DNA Transfection Reagent" kit. For antiviral activity analysis, L929 cells were cultured in 24-well plates. Serial viral dilutions up to 10⁻⁷ for each group showed a significant decrease in cytopathic effect when added to cells with the CRISPR-Cas9 system, demonstrating reduced viral activity in these cells. This was confirmed with flow cytometry analyses using Thermo Fisher "Live Dead," showing a notable decrease in cell death in cells transfected with the guides. Additionally, quantitative viral RNA analysis through Real-Time RT-PCR (qPCR) corroborated this result, with the NSP3 guide being the most efficient for viral RNA cleavage. Thus, we successfully verified that the CRISPR-Cas9 system had activity in acting on viral RNA molecules, potentially acting as an antiviral by reducing the level of infection and viral replication.</p> <p>Keywords: Coronavirus, CRISPR-Cas9, gene editing.</p>
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Title	Proteomic profiling and gene expression of placenta tissue in tumour-bearing rats and impact of a leucine-rich diet administration
Authors	Igor Fernando dos Santos, Carla de Moraes Salgado, Victor Corasolla Carregari, Rogério Williams dos Santos, Laís Rosa Viana, Maria Cristina C Gomes-Marcondes*
Affiliations	University of Campinas, Campinas, Brazil *Research Advisor
Session	Poster

Abstract, Ethics Committee Number*, and Keywords	<p>Pregnancy imposes various molecular changes, including alterations in gene expression and protein synthesis, being more complex when combined with tumour evolution. The amino acid leucine improves protein synthesis, mitigating the changes caused by cancer. We studied an exploratory non-targeted proteomic approach, via LC/MS, combined with gene expression analysis, via rtPCR, of placental tissue. Pregnant (P) Wistar rats fed a control (C) or leucine-rich diet (L) were distributed into 4 groups: control (PC), tumour-bearing rats (PW), leucine diet (PL) and tumour-bearing rats + leucine diet (PWL). The rtPCR was performed with cDNA from placenta on the 21st day of pregnancy, and genes analysed were related to energy metabolism, such as Hif1a, Glut1, Gys1, Cox5a and Gapdh (with Ywhaz as housekeeping gene). Statistical comparisons were performed on data from technical replicates within each group. Protein identification required the detection of a minimum of two fragmented ions per peptide and two peptides per protein. Selected proteins meet specific criteria, with significance set at $p \leq 0.05$. Comparative analysis revealed changes in protein expression of 817 different proteins, between groups. Comparing all groups with the control (PC), the three most significantly changed proteins found in PW group were Nsf1c (upregulated), Rilpl1 (upregulated) and Anxa2 (downregulated), while in PL group were Idh2 (upregulated), Atp6v1e1 (upregulated) and Hbb-bh1 (downregulated). Finally, comparing PWL with PW the most significant proteins changed were Mtco2 (upregulated), Rab7a (downregulated) and Ywhab (upregulated). The gene expression analysis identified significant changes in the expression of Glut1 and Hif1a. we are now undergoing more investigation to identify the biological pathways changes and interconnect the gene expression and protein synthesis to better understand the effects of cancer development during pregnancy and the modulatory effect of leucine administration.</p> <p>Ethic committee number: 6040-1/2022 e 6136-1/2022</p> <p>Keywords: Proteomics; Gene Expression; Pregnancy; Cancer; Placenta; Leucine.</p>
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Title	Genistein affects pre-implantation embryonic development and drives molecular signatures of in vitro-produced bovine embryo
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Session	16 - Terapia Gênica e Celular, Biologia Omics

Abstract, Ethics Committee Number*, and Keywords	<p>To gain insight about negative impacts of phytoestrogens on reproductive aspects, we assessed the effects of genistein (GEN) on in vitro embryo production using bovine embryo model. Genistein was added during in vitro maturation (IVM) of bovine cumulus-oocyte complexes (COCs; Animal care council #7197) and we investigated whether genistein would affect the outcomes for bovine oocytes and further initial embryo development. The study assessed two concentrations of GEN (100 and 500 $\mu\text{M}/\text{mL}$) during IVM on cleavage rate, blastocyst yield, embryonic survival and quality (Q1: excellent; Q2: good; Q3: poor and Q4: dead or degenerated) and global transcriptional profile of bovine embryos. Presumptive zygotes remained to in vitro culture for seven days. In summary, GEN 500 impairs cleavage rate ($p=0.004$) and blastocyst yield ($p=0.035$) compared to control group, respectively. Moreover, regarding embryonic morphological findings, we figure out that GEN 500 decreases ($p=0.033$) proportion of Q1 (excellent) embryos and increase ($p=0.041$) embryos with poor quality (Q3). Nevertheless, embryonic vitrification and rates of re-expansion at 3h and 24h post-heating was not affected by genistein. Furthermore, hereby, we demonstrated the novel findings regarding 229 genes differentially expressed in GEN 500 group ($P\text{-adjust}<0.05$). When we performed pathway enrichment analysis using Reactome®, we demonstrated that down regulated genes are involved with NODAL signals, gastrulation and regulation of pluripotent stem cells. For up regulated genes, we figure out pathways involved with developmental biology and extracellular matrix organization. Taken together, we concluded that addition of GEN 500 during COCs maturation impairs in vitro embryo development and downregulates a keys gene related to inner cells mass differentiation and embryo implantation.</p> <p>Keywords: isoflavone, endocrine disruptor, embryo development.</p>
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Title	Assessing the influence of growing conditions on the chemical profile and biotechnological potential of <i>Gomphrena celosioides</i> Mart. (Amaranthaceae)
Authors	Dayanna Isabel Araque Gelves Giulia Cristina Andreoli de Souza Marcos Jose Salvador
Affiliations	Universidade de Campinas, Campinas, Brazil
Session	Farmacologia Básica e Clínica, Comunicação oral ou pôster

Abstract and Keywords	<p><i>Gomphrena celosioides</i> Mart, is a plant widely used in traditional medicine in American and African countries, as well as in Australia, Cambodia, among others, for the treatment of diseases: infectious, inflammatory, respiratory, gastrointestinal and sexually transmitted. The above has made it a plant of scientific interest, in this sense various research has been carried out that confirms its pharmacological potential. The objective of the present study is to evaluate the effect of factors such as: genotype, collection location and <i>in vitro</i> cultures on the phytochemical profile and consequently on bioactivity. For this, were realized <i>in vitro</i> micropropagation techniques, callogenesis, preparation of hexane and ethanol extracts, UHPLC-UV/DAD-ESI-MS analysis, and <i>in vitro</i> assays for the evaluation of antioxidant, antimicrobial (bacteria, yeast) and antiproliferative activity in tumor lines (PC3, SK-MEL-103) and non-tumorous (Hakat, 3T3) and effect on psoriasis-type inflammatory processes (induction with IMQ). As results, it was obtained that there are differences in the phytochemical profile and bioactivity depending on the genotype, place of collection and growth conditions (<i>in vitro</i>, <i>in natura</i>). Regarding the biotechnological potential, it was observed that the hexane extracts of the plants <i>in natura</i> presented the best results for antimicrobial activity, the callus extract in the prevention of inflammatory processes of the psoriasis type, but none of the extracts presented relevant antioxidant and cytotoxic activity. From this research it can be concluded that the production of bioactive compounds in plants is affected by growth conditions as well as intrinsic factors (genotype, age, etc), therefore the use of techniques such as micropropagation and callogenesis could be used as an alternative, likewise it can be stated that the extracts derived from this plant have relevant biological activity.</p> <p>Cell culture, Bioactive compounds, psoriasis-like assay, biotechnology.</p>
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Title	Aflavinin Alkaloids from <i>Aspergillus</i> sp.: Antibacterial Activity and In Silico Analysis on Cytochrome P450-BM3 ROS Effects
Authors	João Victor Silva-Silva ¹ , André de Oliveira Feitosa ² , Thiago Henrique Doring ^{1,3} , Luciano Almeida Watanabe ² , Patrícia Santana Barbosa Marinho ² , Andrey Moacir do Rosario Marinho ² , Adriano D. Andricopulo ¹
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Session	Basic and Clinical Pharmacology

Abstract and Keywords	<p>Over the years, new challenges have emerged related to the search for compounds with antimicrobial activities due the high capacity of bacteria acquiring resistance to drugs available. In the present work, we have studied extracts of the soil fungus <i>Aspergillus</i> sp. as an important source of new lead compounds for drug discovery research. From the mycelium methanolic extracts, the compounds 14-epi-14-hydroxy-10,23-dihydro-24,25-dihydroaflavinin (1) and 10,23-dihydro-24,25-dihydroaflavinin (2) were isolated by classical chromatography techniques and High-Performance Liquid Chromatography (HPLC). The compounds were identified by Nuclear Magnetic Resonance (NMR) and Mass Spectrometry (MS) data and then subjected to microbroth dilution assays against <i>Escherichia coli</i>, <i>Pseudomonas aeruginosa</i>, <i>Bacillus subtilis</i>, <i>Staphylococcus aureus</i> and <i>Salmonella typhimurium</i> bacteria. Molecular docking simulations were employed to assess the intermolecular interactions of the compounds with the cytochrome P450-BM3 enzyme (PDB ID 4DUE). Global reactivity descriptors were calculated using the Density Functional Theory (DFT) approach. The compound 1, possessing only a single hydroxyl group at C-14 position compared to compound 2, exhibited higher antibacterial activity against <i>B. subtilis</i> and <i>S. aureus</i>. The energetic scores of molecular coupling of alkaloid 1 with cytochrome P450-BM3 were in harmony with the experimental results. DFT analysis demonstrated a more likely electron-donor character for the studied compounds (1 and 2) compared to the penicillin and tetracycline standards. Thus, aflavinin alkaloids exhibited inhibitory properties against gram-positive bacteria, with potential insights into their antimicrobial mechanisms through cytochrome P450-BM3 interactions.</p> <p>Keywords: alkaloid, <i>Aspergillus</i>, antibacterial activity, cytochrome P450-BM3, molecular docking, density functional theory</p>
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Title	Exploring hydrogen sulfide donor, GYY-4137, as a therapeutic potential for interstitial cystitis/bladder pain syndrome
Authors	Santos, LG ¹ Teixeira SA ¹ de Oliveira MG ² Dallazen JL ¹ Whiteman M ³ Muscará MN ¹ Mónica FT ² Antunes E ² Costa SKP ¹
Affiliations	1 – Dept of Pharmacology, São Paulo University, SP, Brazil 2 – Dept of Pharmacology, University of Campinas, SP, Brazil 3 - University of Exeter, England
Session	17 Farmacologia Básica e Clínica

Abstract, Ethics Committee Number*, and Keywords	<p>Interstitial cystitis/bladder pain syndrome (IC/BPS) is a chronic inflammatory disease of the lower urinary tract that remains poorly understood. Characterized by bladder pain, increased urinary frequency, urgency and nocturia, IC/BPS predominantly affects women and poses challenges in treatment. Notably, hydrogen sulfide (H₂S), an endogenous mediator, plays a role in normal bladder and micturition function, and studies with H₂S donors have demonstrated its beneficial effects in several inflammatory conditions. This study aimed to investigate the therapeutic potential of GYY-4137, a slow-releasing H₂S donor, in a murine model of IC/BPS. Female C57BL/6 mice (8 weeks) received an injection of cyclophosphamide (CYP; ip; 300 mg/kg) or saline (Control; 10 ml/kg). One hour post-cystitis induction, GYY-4137 treatment (37.5, 50, 75 mg/kg; sc; n=6) or saline (10 ml/kg) was administered and then micturition activity was evaluated by void spot assay in two periods: initial (0-3h post-GYY) and final (21-24h post-CYP). Mechanical allodynia was assessed at 1, 2, 4, 6, 8 and 24 hours post-cystitis induction using von Frey test. After the final measurement, the mice were euthanized and bladders were removed for contractility assessment using KCl (80 mM) or carbachol (0.001–30 µM; n=6) in an organ bath. Data were analyzed using one- or two-way ANOVA tests followed by post-hoc Tukey's tests (p≤0.05 taken as significant). The results showed that GYY-4137 treatment, at all doses, improved voiding dysfunction during the initial period, but not in the final stage. Moreover, it reduced CYP-induced mechanical allodynia by 47-52% compared to the untreated IC/BPS group. The intermediate dose of GYY-4137 (50 mg/kg) also prevented CYP-induced bladder contractility reduction. In conclusion, GYY-4137 demonstrates a promising protective effect, ameliorating both pain and bladder function (<i>in vivo</i> and <i>in vitro</i>). These findings reveal H₂S donors potential as a therapeutic intervention for IC/BPS.</p> <p>Keywords: Interstitial cystitis; Hydrogen sulfide; Bladder Pain. ETHICS COMMITTEE ON ANIMAL USE: CEUA-ICB/USP, Protocol no. 2055050819</p>
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Title	New insights the role the advanced glycation end products into the diabetic bladder dysfunction
Authors	Akila Lara de Oliveira Matheus Leite de Medeiros Edson Antunes
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Session	17 Farmacologia Básica e Clínica

Abstract, Ethics Committee Number*, and Keywords	<p>Glycolytic overload in diabetes leads to a significant accumulation of the reactive dicarbonyl compound methylglyoxal (MGO) and advanced glycation end products (AGEs), that interact with their receptor (RAGE), contributing to diabetes-associated macrovascular complications. Increased serum AGEs correlating with lower urinary tract symptoms (LUTS) and bladder dysfunction, and significantly impair patients' quality of life, highlighting the necessity of identifying new pharmacological pathways to address this dysfunction. Alagebrium is capable of non-enzymatically breaking the covalent bonds formed in cross-linked proteins, restoring protein function. Here, we aimed to investigate the role of the MGO-AGEs-RAGE pathway in bladder dysfunction of diabetic male and female ob/ob mice compared with wild-type (WT) lean mice. WT and ob/ob mice at 5 weeks of age were administered alagebrium (1mg/kg) for 8 weeks, WT groups received only filtered water for the same period. Serum levels of MGO and AGEs were quantified using fluorescence. The AGEs, MG-H1, RAGE, and collagen content in bladder tissues were measured, and void spot assays in filter paper were conducted. Compared with WT animals, ob/ob mice showed marked hyperglycemia and insulin resistance, whereas fluid intake remained unaltered. Levels of total AGEs, MGO-derived hydroimidazolone 1, RAGE and collagen content in bladder tissues, as well as fluorescent AGEs in serum, were significantly elevated in ob/ob mice of either sex. Void spot assays in filter paper in conscious mice revealed significant increases in total void volume and volume per void in ob/ob mice with no alterations of spot number. Treatment with ALT-711 significantly reduced the levels of MGO, AGEs, RAGE, and collagen content in ob/ob mice. In addition, ALT-711 treatment normalized the volume per void in ob/ob mice. Activation of AGEs-RAGE pathways by MGO in the bladder wall may contribute to the pathogenesis of diabetes-associated bladder dysfunction.</p> <p>Keyword: alagebrium; collagen; glyoxalase; diabetic bladder dysfunction.</p>
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Title	Untargeted proteomics analysis in preeclampsia with severe and non-severe features
Authors	Caroline C. Pinto-Souza Julyane N. S. Kaihara Bruna Cavecci-Mendonça Moises H. Mastella Bruno C. Rossini Ricardo C. Cavalli Lucilene D. dos Santos Valeria C. Sandrim
Affiliations	1 Department of Biophysics and Pharmacology, Institute of Biosciences of Botucatu (IBB), Sao Paulo State University (UNESP), Botucatu, Brazil; 2 Biotechnology Institute (IBTEC), Sao Paulo State University (UNESP), Botucatu, Brazil; 3 Department of Gynecology and Obstetrics, Faculty of Medicine of Ribeirao Preto, University of Sao Paulo (USP), Ribeirao Preto, Brazil.
Session	[Session: 1]

Abstract, Ethics Committee Number*, and Keywords	<p>Preeclampsia (PE) is a hypertension pregnancy disorder that presents a high maternal-fetal morbidity and mortality rate worldwide. Patients are classified as with (PE+) and without severe features (PE-) according to the clinical outcomes. Through the proteomic strategy, we intended to 1) compare the profile of plasma proteins expressed in 7 PE+ and 7 PE- with 10 healthy pregnancies (HP); 2) investigate which proteins are differentially expressed between the groups and 3) find out which signaling pathways are altered among them and whether they are associated with the severity of this cardiovascular disease. The study followed the Declaration of Helsinki and was approved by the Research Ethics Committee of the Ribeirao Preto Medical School, University of Sao Paulo (FMRP-USP, CAAE-37738620.0.0000.5440). We performed plasma protein quantification using mass spectrometry and the obtained data underwent bioinformatics analyses based on Uniprot, PatternLab for Proteomics, String and MetaboAnalyst softwares. Considering a fold change (FC) of 2.0, when comparing HP versus PE- there was only 1 protein differentially expressed: the hemoglobin subunit beta (FC: 2.1), which was upregulated. Between HP and PE+, apolipoprotein 1 was upregulated (FC: 2.3). In PE- versus PE+, the serum amyloid P component was downregulated (FC: 0.5) and the pregnancy-specific beta-1-glycoprotein 1, upregulated (FC: 4.1). These findings help to understand the divergence of pathophysiological mechanisms between the two subgroups of the disease, since the downregulated circulating proteins play roles on blood pressure homeostasis, acting on lipid metabolism and at the immune system. Thus, demonstrating the pathogenesis of PE related to changes in inflammatory and defensive contexts.</p>
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In view of this, giving insights about the complexity and clinical consequences of this hypertensive disorder.

Keywords: preeclampsia; hypertension; severe features; plasma proteomics.



Title	Acute lung injury potentialized by methylglyoxal on mice: the protective effect of Alagebrium (ALT-711)
Authors	Melanie Dieguez de Mesquita Matheus Leite de Medeiros Akila Lara de Oliveira Edson Antunes
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Session	17 - Farmacologia Básica e Clínica

Abstract, Ethics Committee Number*, and Keywords	<p>Acute lung injury (ALI) is characterized by widespread pulmonary inflammation that may arise directly from pneumonia or indirectly from sepsis. In this process, T helper 1 (Th1) immune cells are involved, and neutrophil accumulation in the upper airways serves as a primary biomarker for ALI. Notably, obesity exacerbates the severity of ALI. Individuals who are diabetic, pre-diabetic, or obese exhibit elevated levels of methylglyoxal (MGO), a highly reactive dicarbonyl compound. MGO forms advanced glycation end products (AGEs) through its interaction with proteins, which leads to a loss of function. The activation of the receptor for AGEs (RAGE) by MGO and its AGEs initiates inflammatory changes. Alagebrium chloride (ALT-711) is a molecule designed to non-enzymatically break covalent bonds in cross-linked proteins (AGEs), thereby restoring protein function. Furthermore, ALT-711 can directly inactivate MGO, which may help reduce the effects of AGEs. However, despite existing studies linking MGO to other comorbidities associated with diabetes and obesity, research on the role of the MGO-AGEs pathway in ALI remains absent. In this study, we evaluated the protective effects of ALT-711 (administered at 1 mg/kg for 8 weeks) in mice that received an instillation of lipopolysaccharide (LPS). Bronchoalveolar lavage (BAL) and lung lobes were collected for analysis, including cell count, inflammatory cytokines, and histological examination. Our findings demonstrate that eight weeks of treatment with ALT-711 significantly reduced the number of inflammatory cells and neutrophils in the BAL fluid ($p < 0.05$ compared to the control group). Additionally, we observed that serum MGO levels were higher in animals that received LPS instillation in the BAL ($p < 0.05$ compared to the control group). These results indicate the therapeutic potential of ALT-711 in modulating the inflammatory response associated with LPS instillation in the lungs of mice.</p> <p>Keywords: Acute lung injury, methylglyoxal, alagebrium Ethics committee number: 6197-1/2023</p>
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Title	Characterization of four strains of <i>Schistosoma mansoni</i> with different drug susceptibility to praziquantel
Authors	Marília Bergamini Valentini; Tiago Manuel Fernandes Mendes; <u>Silmara Marques Allegretti</u>
Affiliations	Universidade Estadual de Campinas, Instituto de Biologia, Departamento de Biologia Animal, Laboratório de Helmintologia, Campinas, São Paulo, Brasil
Session	Farmacologia Básica e Clínica

Abstract, Ethics Committee Number*, and Keywords	<p>Different <i>Schistosoma mansoni</i> strains may present differences in pathology and drug susceptibility. These differences may have implications in the parasite distribution, control measures and in the search for new therapeutic alternatives for schistosomiasis. In this work we use four different <i>S. mansoni</i> strains, Belo Horizonte (BH), Bahia (BA), Sergipe (SE) and São José dos Campos (SJ). To understand differences in pathology and praziquantel (PZQ) treatment response in the vertebrate host, BALB/c mice were infected with each one of the strains and 45 days post infection treated with a sub curative dosage of 50 mg/kg or 150 mg/kg or 300 mg/kg in three independent experiments. Fecal egg count was performed weekly starting at 30 dpi. Euthanasia was performed 60 dpi. BH and BA strains presented higher infection rates, with a larger number of worms recovered from the mesenteric veins. No differences were found in granuloma size between BH and SJ strains, however granuloma from the SE strain were significantly smaller than those from the SJ strain. Furthermore, BH strain had a higher number of granuloma, indicating that this strain might be more pathogenic. With the exception of SE infected mice, in groups treated with 150 or 300 mg/kg of PZQ we found a significant reduction in parasite burden and fecal egg count SE strain, at either dosage, did not show significant differences between treated and untreated groups (number of worms, fecal egg count). Our results suggest that BH strain produces/retains higher number of eggs which would have implications in the pathology, SJ strain produced the least amount of eggs (fecal egg count), BA strain presented similar results to BH strain but did not show any particularity compared with the other strains, while SE strain appears to present characteristics of PZQ resistance/tolerance <i>in vivo</i>. CEUA/UNICAMP, 5520-1/2020 e 5520-1(A)/2020</p> <p>Keywords: <i>Schistosoma mansoni</i>, strains, pathology, susceptibility to praziquantel.</p>
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Title	Pharmacological characterization of probenecid in the urinary bladder of rodents and non-rodents
Authors	Guilherme Ruiz Leonardi ¹ Gabriela Reolon Passos ¹ Mariana Burille Moretti ¹ Fabiola Taufic Mónica Iglesias ¹
Affiliations	1 – Department of Translation Medicine (Pharmacology), Faculty of Medical Sciences, University of Campinas, Campinas, Brazil.
Session	Basic and Clinical Pharmacology

Abstract and Keywords	<p>Lower urinary tract symptoms (LUTS) involve issues with storage, micturition, and post-micturition. In Brazil, 75% of people over 40 years old experience these symptoms, with rates of 69% in men and 82% in women. LUTS significantly impact social, sexual, and financial quality of life. Drug repurposing redirects approved medications for new therapeutic uses. This project focuses on probenecid, known for inhibiting multidrug resistance proteins (MRPs), organic acid transporters (OATs) and pannexin-1 and for activating transient receptor potential cation channel (TRPV2). Experiments were conducted on isolated bladders from pig and rats. Concentration-response curves (CRCs) were carried out and the potency (pEC₅₀) and maximal response values (E_{max}) were determined. All protocols were approved by the Ethic Committee (Protocol at CEUA/UNICAMP: 6369-1/2023). Probenecid relaxed pig's bladder with an E_{max} between 50-56%. This response was significantly reduced in the presence of inhibitors of adenylate cyclase (SQ 22,536, 10 μM, 37.7%±11.7; n=9, P<0.05) or phosphodiesterases (IBMX, 100 μM, 34.3%±11.6; n=6, P<0.05). Furthermore, a leftward shift in the relaxing response induced by tadalafil (pEC₅₀: 4.43±0.44 vs 5.07±0.31; n=7) were observed in the presence of probenecid (1 mM; 30 min). In the rat bladder, probenecid did not induce any relaxation, however, in the presence of probenecid (1 mM, 30 min) the relaxation induced by isoprenaline (pEC₅₀: 6.48±0.19 vs 7.24±0.19; E_{max}: 48.9%±5.95 vs 66.6%±6.9; n=3, P<0.05) was significantly potentiated. Similar findings were observed in the relaxation induced by adenosine (53.3±12.8 vs 71.4±4.5; n=4). In conclusion, probenecid relaxes the bladder and enhances responses to key relaxants like tadalafil and isoprenaline, possibly through different pathways. Further studies are needed to elucidate these mechanisms.</p> <p>Keywords: probenecid, bladder, pannexin-1, overactive bladder, multidrug resistance proteins.</p>
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Title	Kv1.3 channel blockage by prednisolone and its inactive form prednisone
Authors	Pedro Henrique Cardoso de Castro ¹ Arthur Schettino ¹ Jader Santos Cruz ² Alessandra Matavel ¹
Affiliations	1 – Research and Development Division, Ezequiel Dias Foundation, Belo Horizonte, Brazil 2 – Department of Biochemistry and Immunology, Federal University of Minas Gerais, Belo Horizonte, Brazil
Session	Farmacologia Básica e Clínica

Abstract, Ethics Committee Number*, and Keywords	<p>Autoimmune diseases are pathologies largely associated with T lymphocytes, caused by the loss of immunologic tolerance to self-antigens. In T lymphocytes, potassium efflux through Kv1.3 and K_{Ca}3.1 is essential to maintain the membrane potential, enabling efficient Ca²⁺ signaling and lymphocyte activation. Because Kv1.3 channels are highly expressed in these cells, they are considered promising targets in modulating the immune response mediated by T lymphocytes, for the treatment of autoimmune diseases. The goal of this study was to elucidate the electrophysiological changes caused by prednisone and prednisolone on Kv1.3 channels in acute and prolonged exposure, and the influence of these drugs on immunosuppressive mechanisms. Electrophysiology experiments using the whole cell voltage clamp technique were used to verify the modulation of potassium currents in HEK293 cells transfected with Kv1.3 channels by the corticosteroids prednisone and prednisolone. The results show that both prednisone and prednisolone rapidly and reversibly blocked Kv1.3 channels. They accelerate the kinetics of inactivation, as the current blockage was stronger at the end of the pulse test, suggesting that corticosteroids favor the inactivated state of the channel. Treatment of the cells with the drugs during 24 to 30 hours did not statistically change the current, suggesting a non-genomic modulation. Prednisone is more efficient than prednisolone in blocking Kv1.3 channels, which was not expected since prednisone behaves like a prodrug, inert in the body before biotransformation. Thus, the blockade of Kv1.3 by prednisone and prednisolone suggests a direct interaction drug-channel.</p> <p>Keywords: Autoimmune disease, Kv1.3, corticosteroid, electrophysiology</p>
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Title	Gefitinib plasma concentration: Influence on dermatological adverse drug reactions (ADRs) in lung cancer patients
Authors	Ana Laura de Andrade Rodrigues ¹ , Mariana Vieira Morau ¹ , Cecília Souto Seguin ¹ , Giovana Fernanda Santos Fidelis ¹ , Aristóteles Souza Barbeiro ¹ , Lair Zambon ¹ , Mauricio Wesley Perroud Jr. ¹ , Éder Pincinato ¹ , Patricia Moriel ²
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Session	17 - Farmacologia Básica e Clínica

Abstract, Ethics Committee Number*, and Keywords	<p>Lung cancer is the most prevalent malignant disease worldwide, ranking first in incidence among men and third among women. Gefitinib is the first drug approved for non-small cell lung cancer (NSCLC) patients with mutations. One of the problems with this treatment is dermatologic adverse drug reactions (ADRs). This study investigates the possible relationship of gefitinib plasma concentrations with dermatological ADRs (skin rash, maculopapular rash, and hyperpigmentation) in patients with NSCLC. This is a clinical and observational study approved by the CEP (Research Ethics Committee - 17328619.90000.5404). ADRs were evaluated according to the Common Toxicity Criteria for Adverse Events, version 4.03. Plasma samples were collected from the patients for analysis of the plasma concentration of gefitinib and its metabolite O-desmethyl-gefitinib using high-performance liquid chromatography. The study included 36 patients with a mean age of 63.1 ± 8.5 years. The majority of the patients were women (69.4%), caucasian (86.1%), and non-smokers (52.8%), and the most common stages were T4 (55.6%), N3 (47.2%), and M1c (44.4%). Among the 36 patients in the study, ADR frequencies were: skin rash (41.6%), maculopapular rash (33.3%), and hyperpigmentation (50.0%). The mean duration of drug use in these patients was 13.2 ± 9.9 months, and the mean plasma concentration of gefitinib was 325.7 ± 155.6 ng/mL. Additionally, the mean concentration of the metabolite was 249.5 ± 225.0 ng/mL. Female patients showed a higher plasma concentration of gefitinib ($p=0.005$) and its metabolite ($p=0.013$). Patients who manifested maculopapular rash ADR had a higher plasma concentration of gefitinib (420.0 ± 189.2 ng/mL) compared to individuals who did not present this ADR (280.9 ± 116.9 ng/mL; $p=0.049$). Therefore, the frequency of dermatologic ADRs in patients treated with gefitinib is high, and the plasma</p>
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concentration of the drug may be associated with the presence of rash-macular ADRs.

Keywords: Non-small cell lung cancer, gefitinib, dermatological adverse drug reactions, EGFR, EGFR-TKI.



Title	Endogenous 6-nitrodopamine as a central modulator of urinary bladder
Authors	Jéssica Mariana Dias ¹ , José Britto-Junior ² , Gilberto De Nucci ² , Mariana Gonçalves de Oliveira ¹
Affiliations	¹ Department of Pharmacology, Faculty of Medical Sciences, State University of Campinas (UNICAMP), Campinas, São Paulo, Brazil. ² Laboratory of Pharmacology, Sao Francisco University (USF), Bragança Paulista, SP, Brazil
Session	17 - Farmacologia Básica e Clínica

Abstract, Ethics Committee Number*, and Keywords	<p>6-Nitrodopamine (6-ND) is a recently described catecholamine that has been extensively investigated in the cardiovascular system; however, its effects on lower urinary tract organs remain unclear. We investigated the basal release of 6-ND, dopamine, noradrenaline, and adrenaline as well as 6-ND effects on isolated bladder smooth muscle reactivity. Experimental protocols were approved by the Ethics Committee for Animal Use of UNICAMP (5959-1/2022). Experiments were conducted in adult male and female mice euthanized by isoflurane overdose. Isolated bladders were suspended in a 3-mL organ bath containing Krebs-Henseleit's solution (37 °C; 95%:5%, O₂:CO₂) with ascorbic acid, in the presence or absence of the sodium channel blocker tetrodotoxin. After 30 min, 2 mL was collected for catecholamines determination by liquid chromatography coupled to tandem mass spectrometry (LC-MS). A pool of 4 bladders was used to constitute each experimental n (limit of quantification 0.1 ng/mL). For functional studies, concentration-responses curves to 6-ND were generated in isolated bladders mounted in organ bath, under either basal tension or after pre-contraction with the muscarinic agonist carbachol (10 µM). Investigation of isolated urinary bladders from both male and female mice revealed 6-ND release comparable to that of dopamine and adrenaline, whereas noradrenaline was not detected in mouse bladders (n=5). Incubation with tetrodotoxin resulted in a significant decrease in 6-ND release (p=0.049). Addition of 6-ND in non-pre-contracted bladders caused a small decrease in the basal tonus, whereas in carbachol pre-contracted bladders, 6-ND produced marked relaxation, by about 56%, with potency (pEC₅₀) of 11.6 ± 1.9. These results demonstrate that 6-ND is constitutively released by mouse urinary bladder, its formation is partially dependent on nerve function, and it is more potent than other catecholamines, supporting its role as a prominent endogenous modulator of bladder relaxation.</p> <p>Keywords: Liquid chromatography, nitrocatecholamines, L-NAME, endothelial nitric oxide synthase, detrusor smooth muscle, voiding.</p>
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Title	Myrcene and limonene: comparison in cell differentiation through modulation of adipogenic factors in 3T3-L1 cells
Authors	Assunção, Renata 1 Pinto, Thais Silva 2 Andrade, Amanda Fantini de Camargo 2 Emílio-Silva, Maycon Tavares1 Zambuzzi, William Fernando 2 Hiruma-Lima, Clelia Akiko1
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Session	17 - Basic and Clinical Pharmacology - Oral presentation

Abstract, Ethics Committee Number*, and Keywords	<p>In Brazil, 26.8% of the adults are obese, so treatments are necessary for this condition. Myrcene (M) and limonene (L) are monoterpenes with similar structures and anti-inflammatory activity. This study aimed to compare the activity of M and L in pre-adipogenic mouse cells (3T3-L1) and evaluate both as potential treatments for obesity. 3T3-L1 cells were subjected to treatments with M and L and evaluated at different concentrations (0.39-100 µg/mL) using 1% Dimethyl Sulfoxide as a vehicle. The in vitro assay was carried in sextuplicate. The treatment was carried out along with differentiation (three days) and then with a culture medium containing only insulin (four days). To assess cytotoxicity and define the treatment concentration of M and L, the MTT cell viability assay was done. After seven days of differentiation, mRNA was extracted for gene expression by RT-qPCR to detect genes related to cell survival (Src), cell cycle (Cdk2/Cdk4/p15), differentiation (Leptin/PPAR-γ), and inflammation (IL-1β/TNF-α). One-way ANOVA followed by Tukey's post-hoc was used for statistical analysis and the minimum level of significance adopted was p<0.05. MTT assay showed that the L and M are not toxic to the cells, with higher cell viability at 25 µg/mL for M and 15 µg/mL for L. Treatment with M induced a significant increase in IL-1β and TNF-α gene expression, indicating increased inflammation compared to L. In addition, treating cells with M increase PPAR-γ and leptin, indicating an increase in the differentiation process compared to L. Treatment of cells with M increased the expression of proliferation gene SRC and cell cycle genes Cdk-2, Cdk-4, and P15, when compared to the L group. Our results show that M acts in increasing pro-inflammatory cytokines and cell differentiation regulators in 3T3 cells, when compared to its action with L. Both monoterpenes, although similar</p>
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in structure, had different actions. However, more studies are needed to better elucidate their effects.

Keywords: Limone, Myrcene, 3T3-L1



Title	The therapeutic potential of empagliflozin in attenuating pulmonary allergic inflammation associated with methylglyoxal in an animal model
Authors	Matheus L. Medeiros ¹ , Akila L. Oliveira ² , Melanie D. Mesquita ³ and Edson Antunes ⁴
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Session	Basic and Clinical Pharmacology

<p>Ethics Committee Number*, and Keywords</p>	<p>Asthma is a chronic inflammatory condition of 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 inflammation in male C57BL6/Junib mice aged 4 weeks. 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 (4mg/kg by gavage) for 15 days. The statistical analysis was performed using ANOVA, followed by Tukey's post-hoc test. We used bronchoalveolar lavage (BAL) to analyze total and differential inflammatory cells, as well as interleukins. The lungs were evaluated using H&E, Masson, and PAS techniques, and MGO levels were measured by ELISA and immunohistochemistry. Mice treated with MGO showed exacerbation of inflammatory cells in BAL, with eosinophilia, as well as increased IL-4 and IL-5 cytokines ($p < 0.05$). Histological analysis demonstrated a significant increase in peribronchiolar 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 BAL of MGO-treated and OVA-challenged animals ($p < 0.05$). However, upon introduction of EMP treatment, we observed inhibition of MGO-induced exacerbations, both in the inflammatory process and in lung morphological changes ($p < 0.05$), as well as reduction in MGO levels ($p < 0.05$). Thus, our results suggest that changes caused by MGO in allergic pulmonary inflammation can be reversed by pharmacological intervention with EMP.</p> <p>Keywords: Empagliflozin, Pulmonary inflammation, Allergic inflammation and Methylglyoxal</p>
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Title	Impact of endocrine disruptors on the development of preeclampsia: analysis of the clinical profile of pregnant women
Authors	Bárbara Campos Jorge ¹ ; Julia Polotto da Silva ² ; Sara Tawany Caetano dos Santos ¹ ; José Carlos Peraçoli ² ; Valéria Cristina Sandrim ¹ ; Arielle Cristina Arena ¹
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Session	Basic and Clinical Pharmacology

Abstract, Ethics Committee Number*, and Keywords	<p>Endocrine-disrupting chemicals (EDCs) are exogenous substances that interfere with endocrine functions and pose health risks. Pregnant women are particularly vulnerable to these compounds, and exposure to EDCs has been linked to the development of preeclampsia (PE). PE affects 4-5% of pregnancies worldwide and leads to serious maternal and neonatal complications. This study aimed to establish a clinical profile of pregnant women with PE in Botucatu, Brazil, to analyze the relationship between EDC exposure and PE development. The study included 100 pregnant women with suspected or diagnosed PE at the Maternity Hospital of The Medical School Botucatu. Of these, 75 completed the study, and the medical records of 30 patients were analyzed to establish a clinical profile. All the patients have been informed and agree with the purpose of this study and with the access to their medical data, signed in the informed consent form. The average age of the pregnant women was 28.46 years, and 47% were married, 33% were in a stable union and 20% were single. With regard to the number of pregnancies per patient, the average was 2.1 pregnancies per woman, with 0.8 births and 0.3 abortions. The average gestational age of the patients was 36 weeks, with 21 full-term deliveries and 9 premature. Of the 30 pregnant women, 3 were classified as having gestational hypertension, 7 with chronic arterial hypertension (CAH) + PE and 20 with PE. Of the women diagnosed with CAH + PE and PE, 63% were considered mild and 37% were severe. The average blood pressure of these patients was 15.3 x 9.7. Thus, we can concluded that two-thirds of the patients selected have a diagnosis of PE. The analysis of the other medical records is underway, as is the quantification of biological materials to establish the relationship between EDCs and the development of PE. Financial support: FAPESP (2023/06651-5 and 2021/12010-7)</p> <p>Keywords: preeclampsia, gestation, clinical profile.</p> <p>Ethics committee: 6677222 FMB/UNESP</p>
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Title	Diapedesis is not altered after intravenous administration of ranelate modified gold nanoparticles
Authors	Daniel Ramos Carvalho ¹ Douglas Santana Franciscato ² Henrique Eisi Toma ² Stephen Fernandes de Paula Rodrigues ¹
Affiliations	¹ Vascular Nanopharmacology Laboratory, Department of Pharmacology, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil. ² Supramolecular Chemistry and Nanotechnology Laboratory, Chemistry Institute, University of Sao Paulo, Sao Paulo, Brazil.
Session	17 - Farmacologia Básica e Clínica

Abstract, Ethics Committee Number*, and Keywords	<p>Despite the many advantages of nanoparticles, including gold nanoparticles which has been successfully used to treat different diseases in animal models, their instability results in aggregation, thus affecting their viability and functionality. In this context, ranelate-modified gold nanoparticles (AuNP@Ran), a highly stable gold nanoparticle, were synthesized. Previous demonstrations showed that AuNP@Ran did not cause toxic effects in a macrophage cell culture lineage, although <i>in vivo</i> studies are still lacking. Diapedesis is an important biological phenomenon that allows the body's defense cells to combat endogenous or exogenous harm. The influence of new compounds on diapedesis must be verified. Thus, we aimed to evaluate the effect of AuNP@Ran intravenous injection on diapedesis, <i>in vivo</i>. To do this, 12 nanometer size and -31 ± 2 mV zeta potential AuNP@Ran were synthesized and acutely intravenously injected in C57Bl/6 male mice (CEUA n° 2697230223) in a dose of 0,79 mg/kg Au/4,1 mg/kg Ran. Cremaster muscle was evaluated for 50 minutes through intravital microscopy. Furthermore, we performed hematological analyses. We observed (mean +/- standard deviation) that AuNP@Ran injection did not alter leukocyte rolling (112.2 +/- 29.8 AuNP@Ran vs. 96 +/- 45.6 distilled water vehicle control solution vs. 78.5 +/- 15.4 saline control solution; N = 9, 7, 8 respectively), adhesion (103.1 +/- 212.6 AuNP@Ran vs. 186.9 +/- 205 distilled water vs. 58.2 +/- 75.3 saline solution; N = 8, 7, 7 respectively) or migration (0.37 +/- 0.74 AuNP@Ran vs. 0 +/- 0 distilled water vs. 1.2 +/- 1.6 saline solution; N = 8, 8, 6 respectively) 50 min after injection. Similar results were observed 5, 20 and 35 minutes after injection. No changes were observed in the hematological analyses compared to control groups. Thus, AuNP@Ran does not alter diapedesis. Financial support: FAPESP #2023/05115-2, #2023/10035-8.</p> <p>Keywords: nanoparticle; cell-biomaterial; mice; biocompatibility; microcirculation.</p>
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Title	Effects of isoflurane exposure on mouse macrophages (J774A1) and human fibroblasts (MRC-5)
Authors	Camargos, L.R. ¹ ; Machado-Junior, P.A. ¹ ; Vieira, R.G. ² ; Malta, W.C. ³ ; Oliveira, L.A.M. ² ; Silva, B.M. ³ ; Bezerra, F.S. ¹ .
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Session	17 Farmacologia Básica e Clínica

Abstract and Keywords	<p>Isoflurane is used in medical practice to temporarily suppress pain and consciousness during surgical procedures. Studies have associated increased production of reactive oxygen species (ROS), redox imbalance and an increase in pro-inflammatory cytokines with this anaesthetic. Some researches have described the potential of isoflurane to increase the production of ROS, such as superoxide anion, through the enzyme complex NADPH oxidase. This study aimed to evaluate the effects of isoflurane exposure on inflammatory mediators and redox imbalance in J774A1 and MRC-5 cells. The cells were kept in 96 (2.5 × 10⁴ cells per well) or 24-well plates (2.0 × 10⁵ cells per well), then the plates were divided into 2 groups for each treatment time: exposure to isoflurane (ISO group) (cells incubated with isoflurane at a flow rate of 2L/min O₂ (21%)) for 3 and 6 hours in a modular incubator chamber in an oven at 37°C); control cells (Control) (cells incubated in ambient air in an oven at 37°C for 3 and 6 hours). At the end of the each exposition, the cells were used to carry out the tests. A decrease in cell viability (%) was observed at 3 hours (88.30±4.75); 98.26 (92.24-101.10) and 6 hours (86.85±6.29); (87.38±4.38) for J774A1 and MRC-5 cells to ISO when compared to the control (103,10±8.92);104.7(98,8-105.00) and (101.30±6.07);(103,10±9.14), respectively. J774A1 and MRC-5 cells in the ISO showed an increase in total ROS (fluorescence) (by 2,7-dichlorofluorescein diacetate) production at 3 hours (308.70±44.89); (319.80±25.64) compared to the control (250.40±21.99); (272.20±48.56), respectively. NRF2 activity by the enzyme luciferase was higher in MRC-5 cells in the ISO for 3 hours (1.35±0.07) compared to the control (0.71±0.38), and in J77A1 cells exposed for 6 hours (1.11±0.23) compared to the control (2.90±0.82). Preliminary results showed</p>
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that isoflurane altered the cellular metabolism and increased ROS production in normoxic cells.

Keywords: isoflurane, reactive oxygen species, redox imbalance, cells

Funding: CNPq, CAPES, UFOP and FAPEMIG.



Title	Physicochemical, pharmacokinetic and toxicological evaluation of spermedine: an <i>in silico</i> study
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Session	Farmacologia Básica e Clínica

Abstract, Ethics Committee Number*, and Keywords	<p>Spermidine (SPD) is a polyamine found in various tissues and organisms. Studies have demonstrated that SPD has several pharmacological functions; however, there are not pharmacokinetic and toxicological studies of SPD. Thus, the aim of was to evaluate the physicochemical, pharmacokinetic, and toxicological parameters of SPD <i>in silico</i>. For this, the physicochemical and pharmacokinetic parameters were assessed using the SWISSADME tool. Toxicological parameters were evaluated using the PROTOX-II toll. Prediction of biological activity spectra was determined using the PASS software, and prediction of interaction profiles with pharmacological targets was conducted using the Swiss Target Prediction tool. Regarding the physicochemical parameters, SPD had a molecular weight of 145.25 g/mol, a partition coefficient of 1.85, 7 rotatable bonds, 3 hydrogen bond acceptors and donors each, and a polar surface area of 64.07 Å². Based on these parameters, SPD did not violate the guidelines recommended by Lipinski, Veber, and Egan. In terms of pharmacokinetic behavior, SPD demonstrates high gastrointestinal absorption, does not cross the blood-brain barrier, is not a substrate of P-glycoprotein, and does not inhibit any of the major cytochrome P450 enzymes. The LD₅₀ founded was 820 mg/kg, classifying it as toxicity class 4, indicating its tolerability if ingested. Regarding toxicity on specific targets, SPD was inactive in terms of hepatotoxicity, carcinogenicity, immunotoxicity, and mutagenicity. SPD stood out as a potential inhibitor of polyamine oxidase, deacetylase inhibitor, and spermine antagonist. Finally, SPD has affinity with various isoforms of carbonic anhydrase, as well as interactions with caspase-2 and the neuronal acetylcholine receptor. These findings highlight the SPD with good biodisponibility and low toxicity. Moreover, it presents itself as multifunctionality and therapeutic potential substance in various physiological and pathological conditions.</p> <p>Keywords: Spermidine; <i>in silico</i>; Polyamine; Pharmacokinetics; Toxicity</p>
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Title	Mouse SEMG1 impairs mouse sperm motility and hyperactivation: a role of EPPIN binding
Authors	Natália Calixto Miranda Santos (Santos, N.C.M.); Noemia Aparecida Partelli Mariani (Mariani, N.A.P.); Beatriz Rezende Santos (Santos, B.R.); Giulia Calderaro (Calderaro G.); Juliana de Jesus Andrade (Andrade J. J.); Ana Clara Ferrari Themer (Themer A. C. F.); Hélio Kushima (Kushima, H.); Erick José Ramo da Silva (Silva, E.J.R).
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Session	Farmacologia Básica e Clínica

Abstract, Ethics Committee Number*, and Keywords	<p>Semenogelin (SEMG1), the main protein of the seminal plasma, is crucial for male fertility. After ejaculation, SEMG1 binds to the sperm surface, leading to inhibition of motility and promoting sperm viability in the uterus. However, the mechanism underlying SEMG1's effects on sperm function remains obscure. The sperm-bound protein EPPIN emerged as a SEMG1-binding partner in human and mouse spermatozoa. Herein, we tested the hypothesis that SEMG1 inhibits mouse sperm motility via EPPIN binding. The protein-protein interaction assay AlphaScreen was employed to determine the binding between recombinant GST-tagged mouse EPPIN (full-length, P22-T134; fl-mEPPIN) and recombinant mouse 6xHis-tagged SEMG1 (full-length, Q32-G375; fl-mSEMG1 and fragments Q32-V118, R98-G375, and Y221-G375). Additionally, computer-assisted sperm analysis was used to evaluate the effects of recombinant mSEMG1 (full-length and fragments, 0.3-25 μM) on motility of spermatozoa isolated from the cauda epididymis of Swiss mice (90 days old; CEUA 5219150420). We confirmed the binding between fl-mSEMG1 to fl-mEPPIN; only mSEMG1^{R98-G375} showed a similar binding pattern to mEPPIN compared to fl-mSEMG1, indicating that the sequence R98-G375 contains the major EPPIN-binding site. fl-mSEMG1 inhibited progressive (IC₅₀ 13.5 μM, 95% confidence interval 0.94-194.2) and hyperactivated (IC₅₀ 6.8 μM, 1.4-32.9) motilities. At 25 μM, mSEMG1^{R98-G375} promoted a similar effect on sperm motility compared to fl-mSEMG1. Despite displaying the low EPPIN-binding capacity, mSEMG1^{Q32-V118}, but not mSEMG1^{Y221-G375}, also inhibited mouse sperm motility. In conclusion, EPPIN acts as an SEMG1 docking site on mouse sperm, and EPPIN binding is involved, but not exclusively, in SEMG1-induced inhibition of sperm motility. Our study sheds new light on the mechanisms by which SEMG1 modulates sperm function and male fertility. FAPESP-CNPq-CAPES. Keywords: spermatozoa, progressive motility, hyperactivation, seminal plasma, male fertility.</p>
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Title	Expression profile and androgenic regulation of Whey-acidic protein four disulfide core domain 5 (<i>Wfdc5</i>) in mice
Authors	Giulia Calderaro (Calderaro, G.); Alexandre Dorth de Andrade (Andrade, A. D.); Hélio Kushima (Kushima, H.); Isabela Andrade de Camargo (Camargo, I. A.); Célio Junior da Costa Fernandes (Fernandes, C. J. C.); Erick José Ramo da Silva (Silva, E. J. R.).
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Session	17 – Basic and Clinical Pharmacology.

Abstract, Ethics Committee Number*, and Keywords	<p>The members of the Whey-acidic protein (WAP) four disulfide core (WFDC) family are protease inhibitors with multifunctional roles in immune and reproductive functions. These genes are predominantly in the male reproductive tract, particularly in the epididymis, an organ critical for post-testicular sperm maturation. Within this cluster, the <i>Wfdc5</i> stands out as a relatively under-explored gene despite its potent antimicrobial activity <i>in vitro</i>. Here, we characterized the expression profile of the <i>Wfdc5</i> gene (mRNA and protein) and investigated its regulation by androgens in mice. A panel of 11 reproductive and 32 non-reproductive tissues from adult male and female C57BL/6 mice (90 days old; N = 2; CEUA-IBB n° 7574270522) were collected and processed for RT-PCR and Western blot analysis. The androgenic regulation of <i>Wfdc5</i> in the epididymis was investigated by (i) sexual maturation and (ii) orchiectomy with or not testosterone (T) replacement (8 mg/kg body weight, s.c.) (N = 4-5). Among male and female reproductive tissues, <i>Wfdc5</i> transcript was specifically expressed in the cauda epididymis and uterus, respectively. Moreover, <i>Wfdc5</i> was differentially expressed in the stomach of male and the trachea of female mice. Western blot analysis identified immunoreactive bands corresponding to WFDC5 as monomers (17-22 kDa) and multimers (~44, ~75 kDa) in all these tissues. Expression of <i>Wfdc5</i> was androgen dependent in the cauda epididymis since its transcript levels (i) became evident at 60 days of age, and (ii) were downregulated after 1, 3, and 10 days after orchiectomy, an effect partially prevented by T replacement. Our findings show that <i>Wfdc5</i> gene expression is restricted and sexually dimorphic in mice, and androgen-dependent in the cauda epididymis. Our study opens routes to investigate the roles of <i>Wfdc5</i> in reproduction.</p> <p>Keywords: epididymis, WFDC, androgens, sexual dimorphism, reproduction. Financial support: PIBIC-CNPq/UNESP, FAPESP.</p>
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Title	Evolution of chalcones, flavonols and carbenes derivatives gold N-heterocyclics (i) as possible inhibitors of cysteine proteases of <i>Leishmania mexicana</i>
Authors	Robson Augusto Massaria Martins Junior ¹ Dênis Pires de Lima ² Camilla Abbehausen ³ Wagner Alves de Souza Júdice ¹
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Session	17- Farmacologia basica e clinica

Abstract, Ethics Committee Number*, and Keywords	<p>INTRODUCTION: Leishmaniasis, caused by the protozoan <i>Leishmania spp.</i>, stands out as a neglected pathology. These parasites rely on various cysteine proteases (CP) for their survival in the host. In this context, investigating compounds that suppress the activity of the enzymes rCPB 2.8, rCPB 3.0, and rH84Y becomes crucial, aiming to inhibit the pathological process and its symptoms. METHODOLOGY: Enzymatic assays were conducted in a buffer containing 100 mM sodium acetate, 20% glycerol, 0.01% Triton X-100, 5 mM DTT, pH 5.5 at 37°C, with pre-activation for 5 min. Enzymatic activity was assessed using an RF6000 Shimadzu spectrofluorometer, utilizing the substrate Z-FR-MCA. The inhibitory potential was determined with increasing concentrations of compounds, calculating the IC₅₀ by nonlinear regression with the GraphPad Prism 5.0 software (GraphPad Software Inc.). Thirteen compounds were tested, including 6 chalcone derivatives, 3 flavonoids derivatives, and 4 dimethyl-imidazole derivatives. RESULTS: N-heterocyclic Au(I) derivatives showed efficacy in inhibiting the rCPB2.8 enzyme but were not effective against rCPB 3.0 and rH84Y. Flavonoid and chalcone derivatives inhibited all three enzymes, standing out for having the lowest IC₅₀ values. CONCLUSION: Flavonoid and chalcone compounds proved to be more effective in inhibiting the cysteine protease enzymes of <i>Leishmania mexicana</i> than N-heterocyclic Au(I) derivatives. The flavonoid compound EM-F12A stood out, inhibiting all three enzymes with the lowest IC₅₀ values, especially rH84Y. The next step is to define the mechanisms of interactions between the enzymes and the tested compounds.</p> <p>Keywords: Chalcones, Flavonols, N-heterocyclic gold carbenes (I), Cysteine protease, <i>Leishmania mexicana</i></p>
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Title	Analysis of hybrid analogs of hydrazones and quinolines as potential therapeutic agents against schistosomiasis: an experimental approach
Authors	Thainá R Teixeira ¹ Thais FA Pavani ^{2,3} Maria E Cirino ¹ Daniela G.G. Rando ² Josué de Moraes ¹
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Session	Oral presentation

Abstract, Ethics Committee Number*, and Keywords	<p>Schistosomiasis, a neglected disease caused by flatworms of the genus <i>Schistosoma</i>, affects over 240 million people globally, with treatment mainly relying on praziquantel. Urgent need exists for discovering new compounds. Chemical classes such as semicarbazones, thiosemicarbazones, and hydrazones have demonstrated potential as antiparasitic agents. These compounds are promising because they can inhibit the <i>Schistosoma mansoni</i> enzyme cathepsin B1 (SmCB1), which is crucial for the worm's nutrition.</p> <p>This study synthesized 22 new hybrid analogs of hydrazones and quinolines, subjecting them to phenotypic screening against <i>S. mansoni</i> adult worms. Five compounds exhibited significant schistosomicidal activity in vitro, notably GPQF-8Q10, causing worm mortality within 24 hours at 25 µM concentration. In <i>S. mansoni</i>-infected mice, a single oral dose of GPQF-8Q8 (400 mg/kg) showed promise, significantly reducing egg excretion in feces (52.8%) and intestinal tissue (45.8%).</p> <p>In addition, the compounds showed low cytotoxicity in Vero cells and the <i>Caenorhabditis elegans</i> model, highlighting the relevance of the research. The results demonstrate the significant potential of hybrid analogs as promising candidates for new therapies against schistosomiasis and highlight the importance of experimental biology in the search for innovative therapeutic solutions. However, further investigation is required to understand the specific mechanisms of action. This study was conducted in accordance with ethical principles and was approved by the Animal Experimentation Ethics Committee (Guarulhos, SP, Brazil; protocol ID 47/20).</p>
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Keywords: schistosomiasis, antiparasitic, semicarbazones, thiosemicarbazones, quinolines.



Title	Evaluation of the antiproliferative potential of <i>Psidium cattleianum</i> extracts in cervical cancer (HeLa) cells
Authors	Vitória Navarro do Amaral Almeida Milena França Longue Vinicius Davila Bitencourt Pascoal (advisor) Aislan Cristina Rheder Fagundes Pascoal (advisor)
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Session	Basic and clinical pharmacology; Cancer Signalling and Therapeutics; Natural Products

Abstract, Ethics Committee Number*, and Keywords	<p>Cervical cancer is the fourth most common cancer in women, according to the World Health Organization (OMS). It is caused by HPV persistent infections with a high number of cases, and deaths, annually. The high mortality level, added to the resistance against drugs currently in the market, reveals the urgency of developing new secure and effective treatments for this disease. This study aimed to evaluate the antiproliferative activity of <i>Psidium cattleianum</i>, a Brazilian species in the Myrtaceae family, in cervical cancer (HeLa) cells. The IC50 values of three samples made of the <i>P. cattleianum</i> extract with hexane (PCH), dichloromethane (PCD), and ethyl acetate (PCA) in HeLa and NIH-3T3 cells were determined using the MTT viability assay. The selectivity index was obtained by dividing the IC50 in non-tumor cells (NIH-3T3) by the IC50 in tumor cells (HeLa). Carboplatin or doxorubicin were used as positive controls. Results were obtained from at least three independent experiments. PCH, PCD, and PCA had IC50 values of 38.5, 64.2, and 50.3 µg/mL, respectively. Carboplatin had an IC50 of 16.1 µg/mL. PCH, PCD, PCA, Carboplatin, and Doxorubicin, were tested in NIH-3T3 cells, with selectivity indices (SI) of 3.58, 0.91, and 1.84, for the samples, and SI of 2.96 for Carboplatin. Therefore, it was concluded that the samples, mainly the PCH sample, present antiproliferative potential and good selectivity in HeLa cells. Future studies and experiments, such as quantification of total phenols and flavonoids, wound-healing assay and mass cytometry, might provide more information about the antiproliferative activity of <i>Psidium cattleianum</i> extracts.</p> <p>Keywords: Cervical cancer; <i>Psidium cattleianum</i>; Antiproliferative potential; Natural Products.</p>
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Title	Excess free heme reduces detrusor smooth muscle contraction: implication for voiding dysfunction in sickle cell anemia
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Session	17 - Basic and Clinical Pharmacology

Abstract, Ethics Committee Number*, and Keywords	<p>Sickle cell anemia (SCA) patients exhibit symptoms of voiding dysfunction. However, research on the pathophysiological mechanisms involved in this dysfunction in SCA is limited. In SCA, the accumulation of plasma-free heme results from intravascular hemolysis. One of the byproducts of heme metabolism by heme oxygenase (HO) is carbon monoxide, which can promote smooth muscle relaxation via the soluble guanylate cyclase (sGC)-cyclic guanosine monophosphate (cGMP) pathway. We hypothesize that the excess free heme caused by intravascular hemolysis contributes to voiding dysfunction in SCA patients. Therefore, this study aims to investigate the effect of heme in vitro on the contractile and relaxing mechanisms of the detrusor smooth muscle in the bladders of healthy C57BL/6 mice. For this purpose, two detrusor strips were isolated from each animal and mounted on a myograph for functional testing. The experimental protocols were approved by CEUA of the University of San Francisco (002.03.2021). Pre-incubation with heme (100 μM) in the detrusors reduced ($p < 0.05$) the maximum response (E_{max}) induced by carbachol and KCl compared to the control group, as well as the contractions induced by electrical field stimulation (EFS). In vitro, heme produced a concentration-dependent relaxation in detrusors. These tests were also repeated in tissues pre-incubated with ODQ (10 μM, sGC inhibitor) or 1J (100 μM, non-selective HO inhibitor), which abolished the effect of heme. cGMP levels were higher ($p < 0.05$) in tissues treated with heme, and pre-incubation with 1J and ODQ decreased these levels, indicating that heme metabolism and sGC stimulation are necessary for its pharmacological effects. In summary, heme decreased contraction and promoted relaxation of the detrusor smooth muscle, suggesting that excess heme contributes to the pathophysiology of voiding dysfunction in SCD.</p> <p>Keywords: Bladder. Cyclic guanosine monophosphate. Heme oxygenase.</p>
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Title	Basal release of 6-cyanodopamine from rat isolated vas deferens and its role on the tissue contractility
Authors	Caroline Fernanda Sanches Dal Pozzo João Felipe Agostini Badin José Britto-Júnior Adriano Fregonesi Gilberto de Nucci
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Session	17 - Farmacologia Básica e Clínica

Abstract, Ethics Committee Number*, and Keywords	<p>Endothelial catecholamines are non-neurogenic catecholamines produced by endothelial cells, such as dopamine and 6-nitrodopamine. These cells possess tyrosine hydroxylase, which is an essential enzyme for the biosynthesis of catecholamines. 6-Cyanodopamine is a novel endothelial catecholamine released from isolated rabbit heart. However, its biological activity remains unknown. This study evaluated whether 6-cyanodopamine is released from rat isolated vas deferens and its effect on this tissue's contractility. The basal release of 6-bromodopamine, 6-nitrodopa, 6-nitrodopamine, 6-cyanodopamine, and 6-nitroadrenaline from the vas deferens was quantified using LC-MS/MS. Electric-field stimulation (EFS) and concentration-response curves to noradrenaline, adrenaline, and dopamine of the rat isolated epididymal vas deferens (RIEVD) were performed in the absence and presence of 6-cyanodopamine. Expression of tyrosine hydroxylase and S100 (a neural marker) was assessed by immunohistochemistry. The rat isolated vas deferens released both 6-cyanodopamine and 6-nitrodopamine. The endothelial cells of the vas deferens were positive for immunostaining for tyrosine hydroxylase. Pre-incubation with tetrodotoxin had no effect on the release of 6-cyanodopamine but virtually abolished the release of 6-nitrodopamine. 6-Cyanodopamine contracted RIEVD only at 1 mM but significantly potentiated the contractions induced by both noradrenaline and EFS at 1 nM. At 10 and 100 nM, 6-cyanodopamine also significantly potentiated the RIEVD contractions induced by adrenaline and dopamine. The potentiation of both noradrenaline and adrenaline contractions by 6-cyanodopamine persisted in tetrodotoxin-pretreated tissues. The identification of epithelium-derived 6-cyanodopamine and its remarkable synergism with catecholamines indicate that epithelial cells may regulate smooth muscle contractility.</p> <p>Ethics committee number: CEUA- Unicamp, Protocol No. 5987-1/2022</p> <p>Keywords: endothelial catecholamines, 6-cyanodopamine, synergism, vas deferens, tandem mass spectrometry, high performance liquid chromatography</p>
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Title:	Effects of <i>O</i>-glycosylation on the gelatinolytic activity of matrix metalloproteinase (MMP)-2 in aortas exposed to glucosamine
Authors:	<u>Evellin Karina Pires Bueno</u> ¹ ; Viviano Gomes de Oliveira Neves ¹ , Marcela Maria Blascke de Mello ¹ ; Glaucio Monteiro Ferreira ² , Rita de Cassia Tostes Passaglia ¹ , Michele Mazzaron de Castro ¹ .
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Session:	17 – Basic and clinical pharmacology

Abstract, Ethics Committee Number, Keywords	<p><i>O</i>-glycosylation (<i>O</i>-GlcNAc) is a post-translational modification modulated by enzymes <i>O</i>-GlcNAc transferase and <i>O</i>-GlcNAcase. Matrix metalloproteinase (MMP)-2 is a protease expressed by vascular smooth muscle cells which contributes to the degradation of extracellular matrix. In hypertension, increase in the gelatinolytic activity of MMP-2 in the arteries may cause hypertrophic remodeling and dysfunction. MMP-2 may be activated by post-translational modifications such as phosphorylation and S-glutathiolation, but it is not known yet whether <i>O</i>-glycosylation also activates MMP-2. Thus, the aim of this study is to evaluate the effects of <i>O</i>-Glycosylation on the activity of MMP-2 in aortas from rats incubated <i>ex vivo</i> with hyperglycemic stimulus, glucosamine. To answer this, the prediction of MMP-2 <i>O</i>-glycosylation sites was made by <i>in silico</i> analysis. Thoracic aortas of Sprague Dawley male rats were incubated <i>ex vivo</i> with glucosamine for 24 hours (CEUA-USP, 1078/2022). Aortas were used for gel and <i>in situ</i> zymography, and Western Blot. The culture medium was used only for the gel zymography. Statistical analysis was performed by One-way ANOVA, followed by Bonferroni correction ($p < 0,05$). Prediction of MMP-2 glycosylation sites suggests its occurrence at the catalytic site, and in the fibronectin-II-like and hemopexin-like domains. The activity of 72 kDa MMP-2 increased significantly in aortas incubated with glucosamine at the concentration of 10^{-12} mol/L ($3,911 \pm 0,2977$ vs. $2,818 \pm 0,2515$; $p < 0,05$; $n=5$), while 64 kDa MMP-2 had a significant increase in the culture medium from aortas incubated with glucosamine at the concentration of 10^{-12} mol/L ($3,493 \pm 0,5748$ vs. $1,904 \pm 0,2177$ vs. $1,536 \pm 0,1623$ vs. $1.508 \pm 0,1852$; $p < 0,05$; $n=5$). <i>O</i>-GlcNAc trended to increase, while MMP-2 and calponin-1 had no statistical difference. In summary, our preliminary data concluded that the MMP-2 is susceptible to <i>O</i>-glycosylation and that glucosamine may increase its gelatinolytic activity in <i>ex vivo</i> aortas.</p> <p>Keywords: matrix metalloproteinase; glycosylation; glucosamine</p>
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Title	Dopamine receptor antagonists modulate the antidyskinetic effects of PDE10A inhibition in L-DOPA-induced dyskinesias
Authors	Aline da Silva Danilo Leandro Ribeiro Hugo Bononi Costa Fernando E. Padovan-Neto
Affiliations	Department of Psychology, Faculty of Philosophy, Sciences and Letters of Ribeirão Preto, Ribeirão Preto, SP, Brazil
Session	Poster

Abstract, Ethics Committee Number*, and Keywords	<p>Parkinson's disease (PD) is the second leading chronic and progressive neurodegenerative disease of the central nervous system, affecting the dopaminergic pathways, especially at the level of D1 and D2 dopamine receptors in the striatum. L-DOPA is the main treatment for PD, but its chronic use leads to L-DOPA-induced dyskinesias (LIDs). LIDs occur as a result of prolonged administration of L-DOPA, which hyperstimulates D1 and D2 dopamine receptors in striatal medium spiny neurons (MSNs). Phosphodiesterase (PDE) enzymes are potential therapeutic targets in the treatment of LIDs. The PDE10A isoform is particularly relevant due to its high expression in striatal MSNs. This study utilized an animal model of parkinsonism induced by 6-hydroxydopamine (6-OHDA) to assess the involvement of D1 and D2 receptors in the antidyskinetic effects achieved with a PDE10A inhibitor. The D1 dopamine receptor antagonist SCH-23390 and the D2 dopamine receptor antagonist eticlopride demonstrated dose-dependent antidyskinetic effects. Notably, the antidyskinetic effects induced by PDE10A inhibition were attenuated by eticlopride, whereas SCH-23390 enhanced the antidyskinetic effects of PDE10A inhibition. In conclusion, this study demonstrated that the antidyskinetic effects of PDE10A inhibition are modulated by D1 or D2 receptor antagonism, highlighting the potential for multi-target manipulation of striatal output pathways in LIDs therapy. All described experimental procedures received approval from the Ethics in Animal Experimentation Committee (CEUA) of the Faculty of Philosophy, Science, and Letters of Ribeirão Preto (FFCLRP) under protocol number 21.1.1065.59.1.</p> <p>Keywords: Parkinson's disease; L-DOPA-induced dyskinesias; phosphodiesterase 10A; medium spiny neurons; 6-hydroxydopamine.</p>
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Title	<i>Banisteriopsis caapi</i> and Parkinson's disease: behavioral effects on a non-motor symptoms rodent model
Authors	Daniela Correa Areias; Carolina Ribeiro Ferraz; Isabella Bacci Busteli; Santhiago Calvelo Graça; Alessandra Linardi; Ariadiny Lima Caetano.
Affiliations	Santa Casa de São Paulo School of Medical Science
Session	Poster and oral presentation

Abstract, Ethics Committee Number*, and Keywords	<p>Parkinson's disease (PD) is caused by the loss of dopaminergic neurons in the Substantia Nigra (SN), leading to reduced dopamine (DA) levels in the nigrostriatal pathway. <i>Banisteriopsis caapi</i> extract, a compound used in Ayahuasca tea, contains beta-carbolines that inhibit monoamine oxidase, potentially boosting DA levels in affected areas. This study examines <i>B. caapi</i>'s impact on spatial memory and motor function in animals subjected to the PD model. PD-like lesions were induced with 6-hydroxydopamine (6-OHDA) injections (24µg/µl) into the caudate-putamen (CPu) in rats (CEUA:2023/01; SisGen:A168D72). After, the animals were treated twice a week for 4 weeks (1.5 ml/kg of <i>B. caapi</i> extract) followed PD induction. Behavioral tests (elevated plus maze, Morris water maze, rotarod) were conducted. Brains were analyzed for lesions by tyrosine hydroxylase (TH) immunostaining in the SN. Bilateral injection of 6-OHDA in the CPu elicited reduction of catecholaminergic neurons in the SN compared to controls (CTRL+VEH:606.50±43.33 vs. DP+VEH: 243.25±1.79; p=0.0028; vs. DP+EXT: 287.75±29.18; p=0.0076), and between control receiving the extract and vehicle-treated PD groups (CTRL+EXT:480.90±82.10 vs. DP+VEH: 243.25±1.79; p=0.0359). In the MWM, there was a difference between time (p<0.0001), group (p=0.0002), and an interaction between the two factors (p=0.0017). Comparing the first training day with the fourth day, there was a reduction in latency to find the platform (day 1: ctrl+vei: 78.37±7.12; ctrl+ext: 68.6±4.76; DP+vei: 88.94±9.63; DP+ext:86.35±3.38 vs. day 4: ctrl+vei:16.3±5.14; ctrl+ext: 13.95±3.35; DP+vei: 25.69±3.64; DP+ext:18.07±1.37). Extract-treated PD animals performed better than non-treated PD on the second training day (DP+vei: 68.44±8.31 vs. DP+ext:34.7±5.74). Here we observed that the treated DP group had a better performance in MWM, then suggesting that the extract could cause an improvement in retention and retrieved spatial memory.</p> <p>Keywords: Parkinson's disease, Ayahuasca, Spatial memory</p>
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Title	Effects of CB2 cannabinoid receptor ligands on cocaine-induced hyperlocomotion
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Affiliations	Federal University of Minas Gerais, Belo Horizonte, Brazil
Session	18 - Neuropsychopharmacology

Abstract, Ethics Committee Number*, and Keywords	<p>Activation of the CB2 receptor inhibits dopamine release, representing a possible approach to the treatment of substance use disorders. The aim of this study was to test the hypothesis that CB2 receptor agonists or positive allosteric modulators (PAMs) reduce cocaine-induced hyperlocomotion. Male and female C57BL/6J mice (n12, 9 weeks old) were administered CB2 agonists, JWH133 (0.3; 1; and 3mg/kg ip) or beta-caryophyllene (25, 50 and 100mg/kg ip), or MAP, EC21a (1, 3 and 10mg/kg ip), prior to cocaine (15mg/kg ip). The animals were tested in the arena for 20 minutes and the distance covered was quantified using Any-Maze® (CEUA/UFMG: 139/2023). Statistical analysis was carried out using One-Way ANOVA followed by Tukey's test ($p < 0.05$), using GraphPad Prism 8 software. JWH133 at a dose of 1mg/kg reduced the hyperlocomotion effect induced by cocaine [Mean\pmSEM vei=17.00\pm1.214; coc=89.61\pm6.459; 0.3mg/kg+coc=81.66\pm9.632; $p=0.9746$; 1mg/kg+coc=62.07\pm8.646; $p=0.02$; 3mg/kg+coc=105.2\pm11.71; $p=0.7090$; $F(2.610, 26.10)=17.83$]. EC21a [Mean\pmSEM vei=20.33\pm1.559; coc=124.0\pm14.64; 1mg/kg+coc=129.5\pm17.71; $p=0.9948$; 3mg/kg+coc=138.6\pm18.84; $p=0.9152$; 10mg/kg+coc=111.4\pm13.24; $p=0.9819$; $F(2.744; 30.19)=12.54$] and beta-caryophyllene [Mean\pmSEM vei=14.81\pm1.994; coc=93.21\pm13.54; 25mg/kg+coc=108.7\pm15.42; $p=0.9293$; 50mg/kg+coc=103.7\pm7.392; $p=0.9396$; 100mg/kg+coc=139.2\pm20.25; $p=0.2999$; $F(2.462; 26.47)=12.93$] showed no significant effects. These results show that activation of the CB2 receptor can significantly reduce cocaine-induced hyperlocomotion, depending on the type of ligand and the dose used. Further studies are needed to better understand the action of different CB2 receptor ligands on cocaine-induced hyperlocomotion.</p> <p>Keywords: endocannabinoid system, substance use disorder, CB2 receptor.</p> <p>Financial support: CAPES, CNPq and FAPEMIG.</p>
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Title	Effects of maternal melatonin deficiency on the hippocampal cholinergic muscarinic neurotransmission in the offspring
Authors	Jeferson Rubens Mamona da Silva ¹ Diana Zulas Andreotti ¹ Ana Luiza Barreto Aliaga ² Paulo Henrique Vieira Lima ² Caio Henrique Tida Oliveira ² Maria Regina Lopes Sandoval ² Fernando Mauricio Francis Abdalla ² José Cipolla Neto ³ Fernanda Gaspar Amaral ⁴ Solange Castro Afeche ² Elisa Mitiko Kawamoto ¹
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Session	Neuropsychopharmacology

Abstract, Ethics Committee Number*, and Keywords	<p>Studies have shown that the absence of maternal melatonin during pregnancy and lactation can generate changes in neural development, such as deficiencies in learning and spatial memory. On the other hand, the administration of melatonin after birth increases cell proliferation and differentiation as well as the survival of new neurons in the hippocampus. The muscarinic cholinergic system is involved in the regulation of different processes in the central nervous system. We investigated the role of maternal melatonin on the expression of hippocampal cholinergic muscarinic receptors (mAChRs) of male adult rats offspring in which gestation and lactation occurred under conditions of hypomelatoninemia. Three experimental groups were made: 12 maternal rats were pinealectomized (PINX), 6 maternal rats were also pinealectomized, but melatonin was replaced (PINX+MEL) and 6 maternal rats were used as control (CTR). Melatonin was replaced in drinking water, during the dark period, for the PINX+MEL group, after surgery and during pregnancy and lactation, at a dose of 0.1mg/kg of body weight being adjusted weekly. The offspring of ninety-day-old male rats were euthanized at ZT2 (2h after the beginning of the light period). [³H]QNB and polyclonal primary antibodies specific for each of the mAChRs subtypes were used in order to evaluate M₁ to M₅ (protocol n° 9805191021). The absence of maternal pineal melatonin during pregnancy and lactation (PINX), induced an increase in the expression of the M₁ subtype and a reduction in the expression of the M₂ subtype of mAChRs when compared to the control group. The melatonin hormone replacement reversed the changes induced by maternal melatonin deficiency. Our results showed that the absence of maternal melatonin during pregnancy and lactation influences hippocampal cholinergic synaptic transmission in the adult offspring, through the modulation of the expression of M₁ and M₂ subtypes of mAChRs.</p> <p>Keywords: melatonin; fetal programming; cholinergic transmission.</p>
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Title	Influence of Chronic Treatment with <i>Banisteriopsis caapi</i> on Alzheimer's Disease: Impact on the Formation of Amyloid Plaques and Hippocampal Neuroinflammation
Authors	Isabella Bacci Bustelli Santhiago Calvelo Graça Érica Victória dos Santos Livia Clemente Motta Teixeira Alessandra Linardi Ariadiny de Lima Caetano
Affiliations	Santa Casa de São Paulo School of Medical Sciences - Department of Physiological Sciences
Session	Oral and Poster

Abstract, Ethics Committee Number*, and Keywords	<p>Alzheimer's disease (AD) is characterized by the deposition of β-amyloid protein (βA), forming amyloid plaques that increase neuroinflammation, mainly in the hippocampus (HPC). One of the substances obtained from the Ayahuasca tea, <i>Banisteriopsis caapi</i> (<i>B.caapi</i>), has an inhibitory effect on monoamine oxidase (MAO). Thus, the present work investigated the chronic effects of <i>B.caapi</i> in AD, as it could increase catecholaminergic levels and reestablish local homeostasis. Transgenic (PDGFB-APPswInd-TG) and non-transgenic (APPswInd-WT) male mice, aged 15-18 months, received <i>B.caapi</i> extract (1.5ml/kg) or vehicle 2x/week for 30 days, orally (CEUA -FCMSCSP 006/22; SisGenA969D82). At the end, the following tests were carried out: Open Field, Object Location (LO) and Rotarod. To quantify βA plaques and microglia, staining with Thioflavin-S and immunofluorescence for Iba-1 were performed, respectively. WT+<i>B.caapi</i> animals interacted less with the moving object between 90' and 24h (entries 90':29,30\pm4,04 vs. 24h:15,60\pm1,79; p=0.0024; time 90':38,43\pm7,23 vs. 24h:21,51\pm3,27; p=0.0220). There was an increased number of βA plaques in TG+<i>B.caapi</i> animals (TG+VEH:37.52\pm5.21 vs. TG+<i>B.caapi</i>:58.62\pm6.89; p<0.0001). The treatment increased the number of microglia (WT+VEH:93.50\pm1.50 vs. WT+<i>B.caapi</i>:256.00\pm4.00, p=0.0002; TG+<i>B.caapi</i>:166.12 \pm6.69; p=0.0096) and decreased the number (WT+VEH:3.95\pm0.15 vs. TG+VEH:2.11\pm0.14 vs. WT+<i>B.caapi</i>:1, 54\pm0.00 vs. TG+<i>B.caapi</i>:1.98\pm0.16, p=0.0002) and size (WT+VEH:230.36\pm10.36 vs. TG+VEH:98.57 \pm4.13 vs. WT+<i>B.caapi</i>:99.76\pm0.03 vs. TG+<i>B.caapi</i>:90.44\pm5.66, p<0.0001) of their branches. In this work, we observed that <i>B.caapi</i> chronic treatment lead to microglial activation and increased βA plaques of TG animals. Furthermore, the extract also altered the spatial memory and activated hippocampal microglia in the WT group. Taken together, these results suggest</p>
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that chronic use of the extract in aged animals, both WT and TG, was not beneficial.

Keywords: Alzheimer's Disease, *Banisteriopsis caapi*, Neuroinflammation, β -amyloid, Hippocampus.



Title	<i>Banisteriopsis caapi</i> chronic treatment promotes changes in spatial memory in a Locus coeruleus neurodegeneration model
Authors	Carolina Ribeiro Ferraz Daniela Correa Areias Isabella Bacci Bustelli Santhiago Calvelo Graça Alessandra Linardi Ariadiny de Lima Caetano
Affiliations	Santa Casa de São Paulo School of Medical Sciences
Session	Poster and oral presentation

Abstract, Ethics Committee Number*, and Keywords	<p>Parkinson's disease (PD) is characterized by neurodegeneration of the substantia nigra (SN), leading to dopamine deficiency. The literature suggests that dopaminergic neuronal loss occurs primarily in the Locus coeruleus (LC) and after in SN. The present work explore the effects of <i>Banisteriopsis caapi</i> (<i>B.caapi</i>) – one of the compounds of Ayahuasca tea – in the neurochemical and behavioral changes of animals with selective bilateral lesion of the LC and/or SN. Male Wistar rats were submitted to selective injury in the LC (24ug/0.5uL) and/or in the CPu (24ug/uL) of 6-hydroxydopamine. After, the animals received <i>B.caapi</i> extract (1.5ml/kg) 2x/week for 4 weeks (CEUA 2023/03; SisGen:A168D72). Behavioral tests were performed using the Elevated plus Maze, Morris Water Maze (MWM) and Rotarod. Brains were analyzed for lesions by tyrosine hydroxylase (TH) immunostaining in the LC and/or SN. There was a reduction in the number of TH+ neurons in the SN and LC in the groups lesioned in CPu (CTRL+VEH:606.50±43.33 vs. SN/LC+VEH:259.00 p=0.0054±18.26; vs. SN/LC+EXT:240.16±8.37; p=0.0012) and/or LC (CTRL+VEH:336.00±30.43 vs. LC+VEH:44.25 ±10.76, p=0.0071; vs. LC+EXT:94.83±10.55, p=0.0205; =0.0185). Thus, a decrease in the SN neurons between the SN/LC group and CTRL that received the extract was observed (CTRL+EXT:480.90±82.103 vs.</p>
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SN/LC+EXT:240.16±8.37; p=0.0325). Furthermore, an increase in TH+ in the SN among animals treated with LC injury (LC+VEH:400.25±115.82 vs. LC+EXT: 621±17.38; p=0.0465). This difference also occurred between the control group, LC and SN/LC treated (CTRL+EXT:312.20±67.25 vs. LC+EXT:94.83±10.55, p=0.0129; vs.SN/LC+EXT:84.00± 29.84, p=0.0123). In the MWM, the latency was reduced between the 1st and the 4th day (day1:LC+VEH:91.66±9.05; LC+EXT:92.03±8.03; SN/LC+VEH:76.54±9.48; LC+EXT:102.91±7.41 vs. day4:LC+VEH:35.50±17.55; LC+EXT:54.16±15.29). These results suggest a neuroprotective effect and an improvement in retention and retrieval in spatial memory.

Keywords: Parkinson's Disease; Locus coeruleus; Banisteriopsis caapi; Memory.



Title	Cannabidiol as a neuroprotectant in prenatal hypoxia-ischemia: impact on hippocampal neurons and microglia in Wistar rats
Authors	Manuely Perez Lage Antunes Cassiana Thayara do Nascimento Balduci Gama Milena Lima Moreira Lucas de Oliveira Correa Guilherme Carneiro Montes Penha Cristina Barradas Daltro Santos Marta Cristina Da Cunha Rodrigues
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Session	Neuropsicofarmacologia

Abstract, Ethics Committee Number*, and Keywords	<p>Perinatal hypoxia-ischemia (HI), a major cause of neurological issues and mortality, leads to lasting cognitive and motor damages. Hippocampus often undergoes neuroinflammation and neuronal loss due to HI. This study explores the effects of cannabidiol (CBD) as a suggested neuroprotectant on hippocampal neurons and microglia in a prenatal systemic HI model in Wistar rats. On the 18th day of gestation, rats underwent surgery to either expose the uterine horns (sham groups-SH) or occlude uterine arteries (HI groups) for 45 minutes (CEUA/UERJ 036/2023). Post-surgery, they orally received CBD-rich Cannabis extract (20mg/Kg) or honey (vehicle-VEH) for 14 days. The female offspring were perfused-fixed, and their hippocampal CA1/CA3 analyzed by immunohistochemistry for NeuN and Iba-1. No difference in maternal glycemia values was found after 6 and 14 days of treatment. At P2, the HI groups had reduced mass compared to the SH, which reversed at P10. CBD treatment contributed to weight gain at P10. The VEHHI group had fewer, more dispersed neurons in CA1 and CA3 pyramidal layers, with reduced thickness in CA1 compared to VEHS. In contrast, CBDSH pyramidal layer was thicker with denser cell bodies, although fewer neurons were noted in the radiate layers of CA1/CA3 in relation to VEHS. CBDHI exhibited robust staining and increased layer thickness over VEHHI in CA1. Differential microglial morphologies were noted across each region. VEHHI presented an intense immunostaining, a larger concentration of microglial cells around vessels in CA1 radiatum layer and more ameboid-like cells in oriens layer than CBDHI and VEHS. On the other hand, CBDHI had larger microglial cell bodies, with finer branches than VEHHI in CA3 pyramidal and radiatum layers. Moreover, CBDSH showed greater immunostaining and branched microglia than VEHS in pyramidal layer. We</p>
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suggest CBD potential in addressing developmental neuroinflammation supporting further research as a therapy for HI brain injury.

Keywords: Prenatal Hypoxia-Ischemia; Hippocampus; Cannabidiol; Microglia



Title	Behavioral evaluation of the activity of the NeuroVAL peptide from social wasp venom as a potential treatment for autism using an experimental model to evaluate social behavior in Swiss mice in the three-chamber sociability test
Authors	Letícia França Pereira, Jéssica Weschenfelder Ferreira, Letícia Germino Veras, Dra. Márcia Renata Mortari
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Session	18 - Neuropsicofarmacologia 35 - Neurociência

Abstract, Ethics Committee Number*, and Keywords	<p>Autism spectrum disorder (ASD) is a neurodevelopmental disorder and its neurophysiology is not fully elucidated, but exposure of mothers to chemical substances such as Valproic Acid (VPA) during pregnancy can cause biochemical changes in their children, such as behavioral changes that resemble those observed in ASD, as well as in experimental rodent models. Treatment for ASD is limited, leading to the search for new therapeutic options, such as peptides found in social wasp venoms, including NeuroVAL, which has been tested for the treatment of pain and has proven neuroactive potential due to its structure, with better antinociceptive activity. The research aims to evaluate the effect of the NeuroVAL peptide in the treatment of repetitive behaviors associated with ASD, using an animal model of prenatal exposure to VPA (Ethics Committee on Animal Use - CEUA of the University of Brasília, case number 23106.147095/2018-8). VPA was administered on the 12th day of gestation to mice, followed by treatment with the peptide intranasally in the offspring for 15 days after birth. The effects were evaluated using the Three-Chamber Sociability Test, where the mice had the option of choosing between three compartments of the box. One of the compartments contained an unfamiliar animal in a cylinder, another was empty and the third contained a previously habituated mouse. The time they spent in each compartment and their interactions were recorded for later analysis. In the control group and VPA group, the minimum number of animals was 8. The intranasal treatment options were: 0.5 mg/kg; 2 mg/kg and 4 mg/kg of NeuroVAL (diluted in Saline + 20% Dimethyl sulfoxide - DMSO); and use of 0.9% sodium chloride solution with 20% DMSO, in equivalent volume (5 µL). The results showed that animals of both sexes in the control group and those exposed to VPA had a longer</p>
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interaction time with strange animals when treated with NeuroVAL, showing that it can be a potential therapy for ASD.

Keywords: Autism spectrum disorder; ASD; NeuroVAL; VPA model; peptide



Title	Neuroprotective and neuroactivating effects of Rosuvastatin on the entorhinal cortex of rats subjected to chronic sleep restriction
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Session	Neuropsychopharmacology

Abstract, Ethics Committee Number*, and Keywords	<p>Sleeping is essential for cognition, but in a capitalist society, It is sacrificed for productivity, making sleep deprivation a common scenario. The project in question aims to observe the probable neuroprotective and neuroactivating effects of Rosuvastatin, a hydrophilic statin, against the state of chronic sleep deprivation, in the entorhinal cortex. For this, male Wistar rats (CEUA 1414/2022) were used, which were divided into 4 groups (N=15): control (C); sleep deprivation (P); deprivation+rosuvastatin at 4.2mg/kg (R-) and deprivation+rosuvastatin at 20mg/kg (R+). Deprivation was carried out for 45 consecutive days, using an adapted multiple platform. Pharmacological administration was carried out orally, using whipped cream powder and distilled water. Behavioral assessments were carried out after the 23rd day of treatment, using the Barnes Maze, which allows the assessment of working memory. For euthanasia, the animals were subjected to anesthesia and then transcardiac perfusion, performed on 7 animals from each group. Then, the brains were collected and cut in the coronal plane into 30 micrometers thick sections by microtomy. To study FOS immunoreactivity, an immunohistochemistry protocol was performed, and then the sections were transferred to a gelatin solution and mounted on slides. As for the forms of analysis, the count of immunoreactive cells will be used to study the immunoreactivity of FOS. The results of all quantitative variables will be analyzed statistically, using a computer program, with a significance of $\alpha = 0.05$. Therefore, it is possible to expect, in relation to the deprivation groups, that those administered the drug tend to present greater expression of FOS, symbolizing greater neuronal activation. In this sense, it may be possible to conclude on the neuroprotective potential of rosuvastatin in the scenario of chronic sleep deprivation.</p> <p>Keywords: sleep deprivation; cognition; memory; immunohistochemistry; statins; FOS protein; Wistar rat</p>
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Title	Exploring Azaphilone: Impact of this new colorant on oxidative stress biomarkers in rat model
Authors	Talita Cristina Mena Segato Camila de Cunha Frattes Rafaella de Barros Mott Erika Leão Ajala Caetano Natasha Lien de Almeida Ibanez Caio de Azevedo Lima Valéria de Carvalhos Santos Ebinuma Denise Grotto
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Session	Toxicologia
Abstract and Keywords	<p>Colorants are widely used to enhance the visual appeal, attractiveness, and marketability of various products. Synthetic dyes are the most widely used, but they have harmful effects on health, such allergic reactions, as well as carcinogenesis and disruptions in hormonal balance. The fungus <i>Talaromyces Amistolkiae</i> is capable of biosynthesizing a new natural red colorant, named azaphilone. However, it is not yet known how this colorant acts, and whether it could interfere with the redox state of those who consume it. Male and female <i>Wistar</i> rats were exposed subchronically (90 days) to concentrations of 12.5%, 25% and 50% (n=6) of the new colorant azaphilone. The study was approved by the Ethics Committee (CEUA-Uniso 213/2022). To assess oxidative stress, the concentrations of reduced glutathione (GSH) and the activities of the enzymes catalase and glutathione peroxidase (GPx), in whole blood, were analyzed by spectrophotometry. The data were expressed as mean and standard deviation and analyzed using one-way ANOVA, and the Duncan follow-up test was applied. The concentration of GSH in males exposed to a 50% concentration of the colorant increased compared to all the other groups, including the control ($p \leq 0.05$). In relation to catalase and GPx, activity remained constant among the groups, with only a significant increase in females exposed to 25% compared to those exposed to 12.5%. The microbial colorant is safe in relation to oxidative stress and it has antioxidant properties.</p> <p>Keywords: <i>Talaromyces amistolkae</i>. Natural colorant. Oxidative stress.</p>



Title	Mitigating lead damage in maternity: the protective power of <i>Agaricus bisporus</i>
Authors	Natasha Lien de Almeida Ibanez Erika Leão Ajala Caetano Talita Cristina Segato Thaísa Borim Pickler Angela Faustino Jozala Denise Grotto
Affiliations	University of Sorocaba, São Paulo, Brazil.
Session	Toxicology

Abstract and Keywords	<p>Lead (Pb) is a toxic pollutant that particularly affects children, the elderly, and pregnant women through ingestion, dermal absorption, or inhalation. In 2022, UNICEF-Innocenti highlighted high levels of Pb exposure worldwide due to improper waste management. According to the World Health Organization, Pb accounts for nearly 1.5% of global annual deaths. Meanwhile, <i>Agaricus bisporus</i> (Ab), a nutritionally rich edible mushroom, may assist in disease prevention and treatment. Therefore, this study aimed to evaluate the protective capacity of Ab against maternal Pb exposure, <i>in vivo</i>. Offspring from female rats exposed to Pb and co-exposed to Ab during gestation were evaluated. Ethical approval was obtained from the Committee of Ethics in Animal Use (CEUA-UNISO, protocol number 175/2020). Healthy female Wistar rats were randomly divided into four groups (n = 5/group): I - Control; II - Ab 100 mg/kg; III - Pb 100 mg/L; IV - Ab+Pb (100 mg/kg + 100 mg/L). Exposure to Pb (via drinking water) and Ab (by gavage) were performed until the 19th day. At the end, the animals were euthanized, and reproductive parameters were assessed. Pb exposure reduced the reproductive capacity of the rats, evidenced by the decreased offspring vitality and numbers of litters (2), as well as the increased pre (54.0 ± 38.8) and postnatal (41.4 ± 53.5) losses, and decrease in the number of fetuses (17), compared to groups I (54) and II (51). However, co-administration with Ab improved all related parameters, such as pre (6.2 ± 9.1) and postnatal (11.2 ± 10.9) outcomes, the number of fetuses (48), numbers of litters (5), and offspring vitality, suggesting that co-exposure may offer protection against the toxic effects of Pb. Additionally, it is worth noting that the weight of the fetuses remained similar in all groups. This finding suggests that Ab could be used as a nutraceutical and natural chelator to prevent these adverse effects.</p> <p>Keywords: Lead; <i>Agaricus bisporus</i>; Reproductive capacity; Nutraceutical</p>
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Title	Innovation in natural dyes: experimental study in rats and assessment of renal and hepatic biomarkers
Authors	Rafaella de Barros Mott Talita Cristina Mena Segato Erika Leão Ajala Caetano Natasha Lien de Almeida Ibanez Caio de Azevedo Lima Valéria de Carvalho Santos Ebinuma Denise Grotto
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Session	Toxicologia

Abstract and Keywords	<p>Dyes are a fundamental element in the food industry, responsible for making food more appealing. Due to market demand, synthetic dyes are the most widely used due to their low cost and high availability. However, studies indicate harmful effects on health such as renal and hepatic impairment. For this reason, the search for healthier options has increased, focusing on microbial sources since some microorganisms have the ability to synthesize dyes as a byproduct of their own metabolism. The fungus <i>Talaromyces amestolkiae</i>, capable of synthesizing the red pigment called Azaphilone, has low toxicity <i>in vitro tests</i>, making it a possibility for replacing synthetic dyes of this pigment. The study evaluated Azaphilone toxicity focusing on metabolic and excretory target organs, liver and kidney respectively. It was a preclinical trial, approved by Ethic Committee (CEUA-Uniso 213/2022). Using male and female Wistar rats, doses of 12.5%, 25%, and 50% of Azaphilone were administered via gavage, for 90 days. After euthanasia, peripheral blood was collected for parameter analysis: alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) as hepatic biomarkers; creatinine, urea, albumin, and total proteins as renal biomarkers. Data were analyzed using mean and standard deviation applying Duncan's post hoc test. The ALP enzyme showed reduced activity proportional to the increase in the Azafilone dose, although only the group that received the 50% dose showed a statistically significant difference. Furthermore, this change occurred only in male rats. Other hepatic parameters showed no differences compared to the control, both in males and females. The renal parameters did not show statistical differences among the dye doses. This, it is possible to suggest that the consumption of the biodye, considering the biomarkers related to target organs, is recommended as safe in doses lower than 50%.</p>
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Title	High dose of anabolic agent promotes oxidative damage and histomorphometric changes in male Wistar rats submandibular glands
Authors	Larissa Victorino Sampaio Renan José Barzotti Rayara Nogueira de Freitas Guilherme Eduardo Rocha Silva José Vitor Furuya de Lima Antonio Hernandes Chaves Neto
Affiliations	Department of Basic Sciences, São Paulo State University (UNESP), School of Dentistry, Araçatuba
Session	Toxicology

Abstract and Keywords	<p>The abusive use of anabolic-androgenic steroids (AAS) is a serious health problem worldwide. Although numerous harmful effects of this abusive use have been described, the potential impacts on salivary gland function are still unknown. Considering that AAS abuse alters the salivary composition of rats, the aim of the study was to analyze the effects of a high dose of AAS on the redox state and histomorphometry of the submandibular glands of male Wistar rats. Twenty 12-week-old Wistar rats were divided into two groups (n=10): control group and AAS group, which received Deposteron®, 20 mg/kg IM, once a week, for 6 weeks. After treatment, the animals were anesthetized, euthanized by cardiac puncture, and the submandibular glands were collected for redox state parameters: total oxidant capacity (TOC), lipid peroxidation (TBARs), protein carbonyl content (PC), reduced glutathione (GSH), total antioxidant capacity, uric acid (UA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Additionally, the glands were fixed and processed for histomorphometric analysis of acini area, ducts, convoluted granular tubules, and stroma (CEUA FOA/UNESP n° 0373-2022). The results were analyzed using unpaired Student's <i>t</i>-test ($p < 0.05$). Oxidative stress was characterized by increased TOC ($p < 0.001$), TBARs ($p < 0.01$), and PC ($p < 0.05$) in the AAS group. Imbalance in antioxidant defense was characterized by higher GSH concentration ($p < 0.001$) in the AAS group, while total antioxidant capacity and UA were similar between groups. On the other hand, there was increased activity of SOD ($p < 0.01$), CAT ($p < 0.05$), and reduced GPx ($p < 0.05$) enzymes. An increase in convoluted granular tubules area ($p < 0.01$) and a decrease in acinar area ($p < 0.05$) were evidenced, while ducts and stroma area were similar between groups. Therefore, it is concluded that treatment with a high dose of AAS causes oxidative damage and histomorphometric changes in rat submandibular glands.</p> <p>Keywords: Salivary glands; Oxidative stress; Testosterone Cypionate</p>
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Title	Atmospheric particulate matter impairs the gills morpho-functional responses of Nile tilapia (<i>Oreochromis niloticus</i>) to swimming effort
Authors	1- De Angelis, C. F. ¹ ; 2- Soares, M. P. ¹ ; 3- Milani, J.A. ² ; 4- Cardoso, I. L. ¹ ; 5- Fragoso-Moura, E. N. ² ; 6- Wunderlin ³ , D. A.; 7- Monferrán ³ , M. V.; 8- Fernandes, M.N. ¹ ; 9- Leite, C.A.C ¹
Affiliations	1 – Department of Physiological Sciences, Federal University of São Carlos, São Carlos, SP, Brazil. 2- Departament of hydrobiology, Federal University of São Carlos, São Carlos, SP, Brazil. 3- ICYTAC: Instituto de Ciencia y Tecnología de Alimentos Córdoba, CONICET and Departamento de Química Orgánica, Universidad Nacional de Córdoba, Bv. Medina Allende s/n, Córdoba, Argentina
Session	Toxicology

Abstract, Ethics Committee Number*, and Keywords	Although experimental evidence indicate settleable atmospheric particulate matter (SePM) produced by metallurgic activity (e.g., Vitoria City Brasil) may be sublethal to healthy adult fish at rest, it has been reported to impose hard limitations on its aerobic performance. We evaluated the effect of SePM contamination on gill morpho-functional changes after maximal swimming effort, highlighting gill structure and ionic balance in a fish experimental model, the Nile tilapia. After swimming, control fish had a 25% reduction in epithelial filament thickness (EFT, $32.16 \pm 2.10 \mu\text{m}$), a 21% increase in distance between lamellae (DL, $33.91 \pm 2.55 \mu\text{m}$), hypertrophy of epithelial pavement cells (PC), and lamellar epithelial lifting (LL). They also had plasmatic Na^+ reduced by 5% ($149.86 \pm 3.04 \text{ mEqL}^{-1}$). SePM exposure (96h) at rest increased EFT by 11 % ($48.79 \pm 2.60 \mu\text{m}$), reduced the DL by 30 % ($18.50 \pm 1.57 \mu\text{m}$), and reduced the lamellar height by 18 % (LH, $87.36 \pm 4.89 \mu\text{m}$), decreased plasmatic Na^+ by a 4% ($151 \pm 3.73 \text{ mEqL}^{-1}$), and increased Cl^- and K^+ by 8 % and 20 % (143 ± 2.90 and $3.02 \pm 0.15 \text{ mEqL}^{-1}$). Swimming SePM-contaminated fish affected gills restructuring and caused a 40 % decrease in EFT ($29.0 \pm 2.62 \mu\text{m}$), a 37 %
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increase in DL ($29.47 \pm 1.81 \mu\text{m}$), a 20 % increase in LH ($109.20 \pm 6.02 \mu\text{m}$), hypertrophy of PC and ionocytes proliferation. Na^+ and Cl^- concentrations were reduced by 9 % and 18 %, respectively (138 ± 5.00 , $117.73 \pm 1.94 \text{ mEqL}^{-1}$), while initial K^+ alteration was maintained. These relevant alterations in gill structure and ionoregulation is problematic to whole body maintenance, which was attested by observed lethality (30%) after swimming.

Committee number: 8105110718

Keywords: iron processing; industry; metal/metalloid; nanoparticle; gill remodeling; ion imbalance; performance.



Title	Maternal toxic effects of graphene oxide nanoparticles administration during pregnancy in rats
Authors	Ailys Karoline Sérgio ¹ Beatriz Digigov Santana Rondon ¹ Gisele Rabelo Alves ¹ João Vitor Alves Damacena ¹ Alliny Alves de Araujo ¹ Larissa Lopes da Cruz ¹ Rafaianne Queiroz de Moraes Souza ¹ Jackson Antônio Lamounier Camargos Resende ² Débora Cristina Damasceno ³ Gustavo Tadeu Volpato ¹
Affiliations	1 – Laboratory of System Physiology and Reproductive Toxicology, Institute of Biological and Health Sciences, UFMT, Brazil 2 – Institute of Exact and Earth Sciences, UFMT, Brazil 3 - Postgraduate Course on Tocogynecology, Laboratory of Experimental Research on Gynecology and Obstetrics - UNESP, Brazil
Session	Poster

Abstract and Keywords	<p>The potential of nanoparticles (NP) in biological and non-biological areas is vast, with new applications constantly being discovered. Graphene oxide (OG) is an example of NP with many applications, which due to its physicochemical properties has demonstrated enormous potential for biomedical applications. Studies of human exposure to NP have advanced rapidly, but information about their safety has not advanced at the same speed. Therefore, the objective of this study is to evaluate the maternal toxicity of OG administration during pregnancy in rats. The local Animal Ethics Committee approved all procedures (23108.022228/2019-76). Wistar rats were mated and randomized into three experimental groups (n=12 rats/group): rats received water (C) or GO at a dose of 2.5 (GO 2.5) or 5.0 (GO 5.0) mg/Kg. Administration of GO was performed daily orally during pregnancy (days 0 to 21). During pregnancy, body weight and water/food consumption were monitored daily. On day 21 of pregnancy, the rats were anesthetized and laparotomy was performed. Blood samples were collected to biochemical analysis. Maternal organs were weighed and the uterine horns were removed to obtain data on maternal reproductive performance. Administration of GO did not change water and food consumption, maternal body weight, relative weight of organs (heart, liver, spleen and kidneys) and biochemical parameters (total protein, alanine aminotransferase, aspartate</p>
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aminotransferase, urea and creatinine). Furthermore, there was no significant difference in pre (C=5.9%; GO 2.5=8.3%; GO 5.0=6.9%) and postimplantation (C=7.7%; GO 2.5=6.2%; GO 5.0=6.8%) losses between the experimental groups. In conclusion, oral administration of GO during pregnancy, at doses of 2.5 and 5.0 mg/kg, do not present maternal toxicity. This indicates that, under these conditions, its use is safe during pregnancy.

Keywords: nanoparticles, graphene oxide, pregnancy, toxicity, reproductive performance.



Title	Effect of temperature on acute sublethal contamination by atmospheric particulate matter in Nile tilapia (<i>Oreochromis niloticus</i>)
Authors	^a Israel Luz Cardoso ^a Michelly Pereira Soares Carol ^a Renato Filogonio ^a Carol Fernandes De Angelis ^a Beatriz Helena Montanari ^a Marisa Narciso Fernandes ^a Cléo Alcântara Costa Leite
Affiliations	^a Department of Physiological Sciences Department, Federal University of São Carlos, Rod Washington Luis km 235, 13565-905 S~ao Carlos, SP, Brazil
Session	19. Toxicology

Abstract, Ethics Committee Number*, and Keywords	<p>The emission of atmospheric particulate matter (PM) by industrial processes containing mixtures of metals, metalloids and metallic nanoparticles is a source of air-to-water cross-contamination with significant effects on aquatic biota. Metals emitted by the siderurgical complex disperses and settles (SePM) in the Bay of Vitória and Santa Cruz (ES-BR). SePM causes relevant sublethal impairments in fish gills, limits the oxygen-carrying capacity of the blood, and increase stress. The affected estuary is subject to temperature variation with the tidal cycles, and that may change metals solubility and their toxic potential to aquatic organisms. We investigated the effects of an environmentally relevant level of SePM (control:C) and (exposed:S) contamination at different temperatures 30 and 20 °C on fundamental aspects of physiological performance in Nile tilapia (<i>Oreochromis niloticus</i>). We assessed the swimming capacity and aerobic performance of fish submitted to progressive swimming (CEUA 8147160123). We results demonstred that SePM limts both swimming capacity and aerobic performance. Short term exposure (96h) reduced critical swimming speed C=5.79±0.85 and S=4.12±0.35. The temperature promoted change in the maximum metabolic rate (30°C: 22.4±4.7; 20°C: 6.8±4.1), metabolic cost of swimming (30°C: 1.41±1.2; 20°C: 0.93±0.6) and swimming efficiency (30°C:3.59±0.70; 20°C:2.11±0.33). There is an interaction between these two factors. The observed reduction in standard metabolic rate at 30°C (C= 8.20±4.17; S=5.93±4.6), together with the decrease in aerobic scope at 20°C (C=6.53±3.1; S=2.81±1.4), indicates that thermal temperature is intensifying the effects of SePM on swimming ability and aerobic performance revealing a complex relationship between SePM contamination and the thermal gradient. Therefore, SePM contamination across a thermal gradient can be exacerbated and thus, critical sublethal contamination can reveal obvious deleterious effects on individuals.</p> <p>Keywords: atmospheric particulate matter, metals/metalloids, nanometals, swim, temperature</p>
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Title	Toxicological evaluation of subchronic oxandrolone administration in female rats undergoing strength training
Authors	Estéfani Marin Nicolas Guimarães dos Santos Shanda Cattani Nice Vilar Torres Maria Manoela Rezende Severo Bruna Haendchen Sant'Ana Mirna Bainy Leal Rosane Gomez Solange Garcia Marcelo Dutra Arbo Bruno Dutra Arbo
Affiliations	Universidade Federal do Rio Grande do Sul, Porto Alegre, Brasil
Session	Poster presentation

Abstract, Ethics Committee Number*, and Keywords	<p>Oxandrolone is an analog of testosterone with potent anabolic and weak androgenic activity. It ranks among the preferred anabolic steroids for female athletes and high-performance physical activity practitioners. Thus, the aim of this study was to evaluate the anabolic effects of oxandrolone and its toxicity in young female rats subjected to an anaerobic exercise protocol. The experimental procedures were approved by the Ethics Committee on Animal Use (CEUA) of UFRGS (#41635). Female Wistar rats (aged 60 days) were randomly divided into two groups (n= 12/group): one group received oxandrolone (1.77 mg/kg/day), while the other received only the vehicle (corn oil). All treatments were administered by gavage. Thirty minutes after treatments, all animals underwent anaerobic training, performed through a protocol of climbing on an inclined ladder, consisting of 6 climbs (2 at each workload, equivalent to 50%, 75% and 100% of the maximum load supported by each animal). They were trained 3 times a week for 28 days, with daily monitoring of the estrous cycle. On the 29th day of the experiment, the animals were anesthetized and euthanized. Subsequently, the effect of oxandrolone on body weight, relative organ and muscle weight, as well as its hematological and biochemical effects, was analyzed. All the investigators were blind during the experiment and data analysis. It was observed that oxandrolone did not affect body mass or the relative weight of organs and muscles. Oxandrolone caused a statistically significant alteration in mean corpuscular volume (MCV) and eosinophil count. No changes were observed in any of the other hematological parameters. The treatment also altered blood urea levels, however, there were no differences in glucose levels, serum and urinary creatinine, total proteins, triglycerides, lactate, LDH, CKMB, FALC, TGO, TGP, and GGT. It is concluded, therefore, that oxandrolone did not exert toxic effects on most of the analyzed parameters.</p> <p>Anabolic Androgenic Steroids; Exercise; Toxicity.</p>
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XXXVIII REUNIÃO ANUAL DA FESBE
XXII REUNIÃO ANUAL DA BRAVO
XVIII CONGRESSO DA SBCAL
III CONGRESSO DOHAD BRASIL
II CONGRESSO DA SBBA
2 A 5 DE JULHO 2024, CAMPINAS/SP
FRONTEIRAS DAS CIÊNCIAS BIOMÉDICAS E SEUS DESAFIOS

Title	Effects of cyanobacteria <i>Arthrospira (Spirulina) platensis (AP)</i> on liver tumor evolution in a diethylnitrosamine induced mice model
Authors	Luis Manuel Sarmiento-Machado ^{1*} Guilherme Ribeiro Romualdo ^{1,2} Luis Fernando Barbisan ¹
Affiliations	¹ Department of Structural and Functional Biology, Botucatu Biosciences Institute, São Paulo State University (UNESP), Botucatu-SP, Brazil. ² Department of Pathology, Botucatu Medical School, São Paulo State University (UNESP), Botucatu-SP, Brazil.
Session	19- Toxicologia

Abstract, Ethics Committee Number*, and Keywords	<p>Liver cancer is the second cause of death among cancers worldwide, and hepatocellular carcinoma (HCC) is the most common subtype (75-90%). <i>Arthrospira (Spirulina) platensis (AP)</i> is a cyanobacteria known for its anti-inflammatory and antioxidant properties observed in previous preclinical studies. Thus, we assessed whether dietary AP attenuates liver tumor development in a diethylnitrosamine (DEN) model. For four weeks, fifteen-day-old female mice C3H/HeJ were fed with standard chow and received four intraperitoneal injections of DEN (25 mg/kg) 0.9% NaCl vehicle (control) once a week. Then, mice were randomly allocated into four groups: (G1) DEN (n=28), (G2) DEN+5% AP (n= 28), (G3) control (n=10), and (G4) control+5% AP (n= 10). After the DEN regimen, G2 and G4 groups received standard chow with 5% AP (w/w) for 43 weeks. Afterward, all animals were euthanized (CEUA 3210170322). Liver tumors were collected for histopathological and RNA-Seq. Data were compared using one-way ANOVA or Kruskal-Wallis tests (<i>post hoc</i> Tukey) while two-way ANOVA was used for food consumption. Chi-square and Mann-Whitney tests were used for tumor incidence and multiplicity analysis, respectively. For DEN (G1) and DEN+5% AP (G2) groups, values of food consumption (5.1 vs 5.2 g/day, p = 0.079) and final body weight (25.94 g vs 26.41 g, p = 0.837) were similar. Incidence of hepatocellular adenomas (59% vs 48%, p = 0.416, p = 0.874) and HCC (26% vs 20% p = 0.612) did not differ between DEN (G1) and DEN+5% AP (G2), as well as tumor multiplicity and immunohistochemistry for cell proliferation (Ki-67) were also similar (p> 0.05). RNA-seq showed that DEN+5% AP (G2) had a discrete effect on tumor transcriptomic compared to the DEN-only group (G1). In summary, AP does not exert a significant protective effect against liver tumor development.</p> <p>Keywords: hepatocarcinogenesis; chemoprevention; RNA-Seq; <i>Arthrospira</i></p>
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Title	Effect of temperature on histopathological damages caused by Settleable Atmospheric Particulate Matter (SePM) in tilapia, <i>Oreochromis niloticus</i>
Authors	1- De Paula, J.H.C. ² 2- Fragoso-Moura, E. N. ² 3- De Angelis, C. F. ¹ 4- Filogonio, R. ¹ 5- Israel, L.C. ¹ 6- Soares, M.P. ¹ 7- Montanari, B.H. ¹ 8- Fernandes, M.N. ¹ 9- Leite, C.A.C. ¹
Affiliations	1- Department of Physiological Sciences, Federal University of São Carlos, São Carlos, SP, Brazil. 2- Departament of Hydrobiology, Federal University of São Carlos, São Carlos, SP, Brazil.
Session	Toxicology

Abstract, Ethics Committee Number*, and Keywords	<p>The Tubarão complex (ES-BR) has an industrial complex that has a significant impact on the emission of particulate matter into the atmosphere (PM). This PM is composed of diverse metals that can settle (SePM) and affect the local water beds and biota. The main affected site is the local estuary, which is subject to natural variations in diverse physical and chemical parameters, including temperature. We analyzed the effect of temperature variation (15, 20, and 30 °C) on histopathological damages caused by an ecologically relevant concentration of SePM (simulated by 1g of raw SePM/L, 96h) in relevant organs (liver and kidney) of Nile tilapia. Potential pathologies were assessed based on severity and frequency of occurrence, and a Histopathological Alteration Index (IAH) was calculated. The results revealed sublethal damages after SePM exposition. In the control (C) and exposed (E) groups, alterations were identified in the hepatic tissue, such as dilation of the sinusoidal capillaries, nuclear atypia, and a sparser parenchyma in terms of the presence of nuclei in the hepatocytes (15 °C, C: 3.1 ± 0.30, E: 4.8 ± 0.30) (20 °C, C: 2.3 ± 0.33, E: 4.6 ± 0.33) (30 °C, C: 3.0 ± 0.36, E: 4.3 ± 0.21). In the renal tissue we</p>
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	<p>observed disorganizations in tubules and glomeruli, tubular degeneration and dilation of glomerular capillaries (15 °C, C: 2.8 ± 0.30, E: 7 ± 2.00) (20 °C, C: 3 ± 0.25, E: 7.5 ± 2.39) (30 °C, C: 3.5 ± 0.42, E: 5.6 ± 1.8). Temperature did not influence the extent of histopathological damages caused by SePM. This result indicates a continuity of damages even in the face of daily thermal oscillations related to the tides. The histopathological damage observed can trigger a series of physiological dysfunctions, impairing organ's performance in their vital functions.</p> <p>Committee number: 9046240523</p> <p>Keywords: Metals; Nanoparticles; Fish; Histopathology; Ecotoxicology; SePM</p>
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Title	Analysis of the myogenic reaction to lung stretching in mice induced with asthma and contaminated with particulate matter (PM)
Authors	1- Thalia Giovana Guimarães Ribeiro 1 - Luciano Rivaroli
Affiliations	1 – Departament of Natural Sciences /Federal University of São João Del-Rei, São João del Rei, MG, Brasil.
Session	Toxicology, oral poster.

Abstract, Ethics Committee Number*, and Keywords	<p>Particulate Matter (PM) is a major pollutant from natural and artificial sources such as biomass burning, industrial activities, and soil dust resuspension. It poses a threat in environmental studies, impacting urban air quality and worsening respiratory diseases. Assessing PM's impact on lung function is vital for protecting exposed populations. Our previous results shows decreased lung tissue compliance due to PM exposure. This study examines differences on the contractile response observed during stretch, possibly influenced by immune activity. Using 32 adult male Balb/C mice (56 days old), distributed into four groups: Control (C), Induced Asthma (IA), Contaminated with Particulate Matter (PM), and Asthma Induced and Contaminated with Particulate Matter (IAPM), according to protocol CEUA 4277220523/UFSJ. Asthma induction involved intraperitoneal and nasal instillation of ovalbumin (OVA), followed by exposure to particulate matter. Left lung samples underwent tension analysis in response to stretching using an organ bath and force transducer (Adinstruments). Tissues were stretched by 1mm using a micromanipulator, with tension recorded by LabChart software –AdInstruments. Data analysis utilized Graphpad/Prism software, employing one-way ANOVA, Kolmogorov-Smirnov test, and Student's t-test for significance ($p < 0.05$). All groups exhibited increased peak tension followed the stretches. Mean peak tensions after each stretch were significantly higher in the order $C < IA < PM < IAPM$, with peaks at 7mm stretch: 14.38 ± 10.8; 25.47 ± 13.47; 29.61 ± 20.22; 70.59 ± 56.9 gf.g⁻¹, respectively. Trend line coefficients (2.7; 3.5; 5.58; 13.8) and R² values (0.91; 0.95; 0.92; 0.93) corroborate these findings. Asthmatic mice exposed to Particulate Matter showed increased smooth muscle response, indicating heightened muscle responsiveness possibly due to immune activity and asthmatic model sensitization. Ongoing histological analyses aim to further elucidate these initial results.</p> <p>Keywords: Lung, Particulate Matter, tissue mechanical properties.</p>
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Title	Histological analysis of the Chorioallantoic Membrane (CAM) used in the evaluation of topical anesthetics toxicity
Authors	Isabella França Quadrado, Arthur Antunes Costa Bezerra, Michelle Franz Montan Braga Leite
Affiliations	Faculdade de Odontologia de Piracicaba, Universidade Estadual de Campinas – UNICAMP, Piracicaba, Brazil
Session	19 – Toxicologia (Modalidade de apresentação: pôster)

Abstract, Ethics Committee Number*, and Keywords	<p>The chorioallantoic membrane (CAM) model has been used to assess the toxicity of chemical substances based on the analysis of macroscopic irritating effects found on the membrane after treatments. Additional information could complement the analysis by the CAM model, such as evaluating histological aspects observed in the membrane. For this study, Ethics Committee approval is not applicable as it involves an internationally recognized alternative method to animal testing. Formulations of topical anesthetics (Benzotop®, Lidocaine®, EMLA®, and Labcaine®) and controls (positive – sodium hydroxide; negative – saline solution) were evaluated according to the DB-ALM protocol No. 96 - Hen's Egg Test on the Chorioallantoic Membrane (HET-CAM), from the European Centre for the Validation of Alternative Methods (ECVAM). After euthanizing the embryos, histological slides of 3 CAMs subjected to each treatment were prepared, following the traditional steps: material collection, fixation in 10% buffered formaldehyde, cleavage, processing, embedding in paraffin block, microtomy, and staining. A descriptive analysis of the histological slides was performed, evaluating factors such as the number and integrity of blood vessels, the presence of inflammatory cells, and the integrity of CAM epithelia. The negative control did not cause changes in membrane morphology, while the Benzotop®, Lidocaine®, and Labcaine® formulations caused slight extravasation of erythrocytes from vessels. However, the positive control and EMLA® caused discontinuity of membrane epithelia, as well as vessel rupture, resulting in extensive areas of hemorrhage and tissue necrosis. Histological analysis of the CAM proved to be an important complementary tool to the protocol used by distinguishing the tested formulations.</p> <p>Keywords: Chorioallantoic membrane. Toxicity. Topical anesthetics. Dentistry.</p>
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Title	Histopathological markers in gills of juvenile shrimp (<i>Macrobrachium rosenbergii</i>) after contamination by settleable atmospheric particulate matter
Authors	Montanari, Beatriz Helena ¹ Silva, Ludmila Mendes ¹ De Angelis, Carolina Fernandes ¹ Soares, Michelly Pereira ¹ Cardoso, Israel Luz ¹ Paula, Julia Helena da Cruz de ¹ Fernandes, Marisa Narciso ¹ Leite, Cleo Alcantara Costa ¹
Affiliations	1- Federal University of São Carlos, São Carlos – SP, Brazil
Session	19 – Toxicologia

Abstract, Ethics Committee Number*, and Keywords	<p>Part of atmospheric particulate matter produced by the steel industrial complex near Vitória - Brazil may settle (SePM) and cause an air-to-water cross contamination. SePM is highly complex contamination due to the number of metals in its composition. It was observed to affect the aquatic biota at sublethal level in most cases but was lethal to shrimp larvae. Our study investigated the effect of acute contamination (96 hours) by SePM (0; 0.01; 0.1; and 1.0 g.L⁻¹ - raw SePM) on shrimp gills (<i>Macrobrachium rosenbergii</i>). 100 juvenile shrimps (0.443±0.16g, 3.6±0.43 cm) were set in 5 experimental groups with 5 individuals each. Gill samples were collected, and histopathological analysis was conducted following the referenced histopathological Index (IOrg). SePM contamination resulted in sublethal damage to gill structures with number of pathologies and their severity increasing progressively with contaminant concentration. The observed changes included hemocyte agglomeration, squamous cell hypertrophy of the epithelium, lamellar cuticle peeling, and gill structure cellular disorganization, and IOrg values were different among control group (0.91±0.06) and each SePM concentrations 0.01, 0.01, and 1.0 (1.81±0.08; 3.41±0.02; and 4.59±0.04, respectively). The observed damage denotes significantly impact in gill function. These changes relates to consistent aerobic limitations. Therefore, SePM may be considered a real substantial threat to general animal performance, reducing populations fitness in a group that lies in the basis of environmental trophic chain. Based on the present results, we recommend a newer risk assessment to address SePM issues.</p> <p>Keywords: Toxicology; histopathology; crustacean; atmospheric particulate matter; metal</p>
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Title	Biopesticide Spinosad causes a reduction in the activity of the antioxidant enzyme catalase in human placental cells, HTR-8/SVneo
Authors	Letícia Pazin Bomfim ¹ Maria Stacy dos Santos Silva ¹ Débora Hipólito Quadrelli ² Idessania Nazareth Costa ² Glaura Scantamburlo Alves Fernandes ¹
Affiliations	1 – Department of General Biology, State University of Londrina, Londrina, Brasil 2 – Department of Parasitology, Immunology and General Pathology, State University of Londrina, Londrina, Brasil
Session	Toxicology

Abstract, Ethics Committee Number*, and Keywords	<p>The biopesticide Spinosad has been used in several countries because it is a promising larvicide and insecticide for controlling insects that cause less impact on public health, including being effective against mosquitoes and larvae of the genus <i>Aedes</i>, <i>Culex</i> and <i>Anopheles</i>. However, it is questionable whether it can be characterized as an endocrine disruptor since studies indicate its toxic effects on non-target species. Endocrine disruptors are characterized as exogenous chemical substances, or a mixture of these substances, that can interfere with any aspect of animal hormonal action and are found in pesticides. Thus, the reproductive system, including the placenta, is largely affected by these molecules, as it is made up of steroid-dependent organs. For this reason, we sought to evaluate the effects of spinosad on HTR-8/SVneo cells, a human trophoblastic cell line. To this end, we evaluated cytotoxicity using the Resazurin test after 24 hours exposure of trophoblastic cells to Spinosad at concentrations of 1, 10, 20, 80 and 100 μM, in triplicates. With this, we obtained the CC50 of the cells (38,9 μM), used to carry out the oxidative profile analyses, where the samples were normalized and, in triplicates (n=6), lipid peroxidation, catalase and GST activity, GSH, GT, GSSG concentration and oxidative stress index were assessed in the cells exposed to Spinosad for 24 hours. The data was subjected to the Shapiro-Wilk test, T-test for parametric tests, and the non-parametric Mann-Whitney test. The difference was considered significant when $p < 0,05$. The results showed a concentration-dependent reduction in cell viability from the 10 μM concentration onwards. Analysis of the oxidative profile showed a reduction in the activity of catalase in cells exposed to Spinosad. The other analyses showed no significant difference. We conclude that Spinosad can cause cell damage and directly influence at gestational factors, requiring further studies into its cytotoxic effects.</p> <p>Keywords: Placenta. Reproduction. Toxicology. Oxidative stress.</p>
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Title	Effects of different doses of chronic glyphosate on cardiac inflammatory and apoptotic aspects in rats
Authors	Lucas Martins Peruque ¹ Ana Carolina Biscola Catucci ¹ Camille Angélica Pereira da Silva ¹ Gisele Correia da Silva ¹ Maria Carolina Pereira Ramos ² Miranda Matos Foltran ¹ Natália Rici Rocha ¹ Allice Santos Cruz Veras ² Giovana Rampazzo Teixeira ² Raissa Mantovani de Oliveira ¹ Francis Lopes Pacagnelli ¹
Affiliations	1 – Universidade do Oeste Paulista, Presidente Prudente, Brazil 2 – Universidade Estadual Paulista, Unesp, Presidente Prudente, Brazil
Session	Toxicology

Abstract and Keywords	<p>Introduction: Glyphosate is a widely used herbicide in agriculture and acts by preventing the growth of weeds. Its main means of contamination is through inhalation during spraying and can cause acute cardiotoxic effects such as cardiogenic shock and cardiac arrhythmias. However, long-term effects on the heart need to be further elucidated. The objective of this study was to evaluate inflammatory and apoptotic markers in the hearts of rats chronically exposed to glyphosate at different concentrations. Methodology: 30 Wistar rats were used (CEUA/UNOESTE protocol n°: 5684), exposed via inhalation, distributed into three experimental groups (n=10 animals/group), control group (CG) exposed to nebulization of distilled water containing 10 ml; low concentration group (GLC) exposed to 3.71x10⁻³ grams of active ingredient per hectare (g.i.a./ha) and high concentration group (GHC) exposed to 9.28x10⁻³ (g.i.a./ha). The animals were exposed for 6 months and, after this period, they were euthanized. The heart was dissected, and the left ventricle analyzed for inflammatory and apoptotic markers using the immunohistochemistry technique. Data normality was assessed using the Shapiro Wilk test, and after ANOVA with Tukey's post test (p<0.05). Results: The antiapoptotic protein Bcl2 showed a decrease in the high concentration group in relation to the control and low concentration groups (GC- 4.60±0.39 nmol/mg vs. GBC- 4.08±0.41 nmol/mg vs. GAC-3.32±0.20 nmol/mg, GC vs. GAC, p=0.023). TNF- alfa, an inflammatory marker, showed a decrease in the exposed groups in relation to the control group (GC- 5.34±0.32 nmol/mg vs. GBC- 4.16±0.13 nmol/mg vs. GAC- 4.20 ± 0.35 nmol/mg; GC vs. GBC, p =0.0014; GC vs. GAC p=0.010). Conclusion: Chronic inhalation exposure to glyphosate at different doses promoted inhibition of the inflammatory and antiapoptotic response in Wistar rats.</p> <p>Keywords: Pesticides, heart, inflammation</p>
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Title	Use of ImageJ software in the analysis of anesthetics toxicity in a HET- CAM model
Authors	Allana Domingues Ferracioli, Arthur Antunes Costa Bezerra, Michelle Franz Montan Braga Leite
Affiliations	Faculdade de Odontologia de Piracicaba, Universidade Estadual de Campinas – UNICAMP, Piracicaba, Brazil
Session	19 – Toxicologia (Modalidade de apresentação: pôster)

Abstract, Ethics Committee Number*, and Keywords	<p>The chorioallantoic membrane (CAM) model of the chicken embryo is used to evaluate the toxicity of chemical substances, but exhibits subjectivity in the results. Therefore, the development of a quantitative analysis method using software becomes necessary to reduce or eliminate the subjectivity of the obtained results. For this study, Ethics Committee approval is not applicable as it involves an internationally recognized alternative method to animal testing. The toxicity of commercial formulations of topical anesthetics (Benzotop®, Lidocaine®, EMLA®, and Labcaine®), as well as positive (sodium hydroxide) and negative (saline solution) controls, was evaluated using the DB-ALM protocol No. 96 - Hen's Egg Test on the Chorioallantoic Membrane (HET-CAM) from the European Centre for the Validation of Alternative Methods (ECVAM). Subsequently, 8 post-treatment images of the CAM were obtained for each test substance and processed using ImageJ, involving: 1) ImageJ calibration; 2) selection of a smaller area for analysis; 3) image format conversion and analysis using the IsoPhotContour2 plugin; and 4) measurement of the number of extravascular clots. A evaluator performed visual clot counting on the same images. A simple linear regression analysis was conducted to compare the number of clots identified by the operator with the quantity measured by ImageJ. Generalized mixed model analysis was used to compare the mean number of clots identified by ImageJ after treatments. The number of clots quantified by ImageJ was a good predictor of visually identified clots (coefficient of determination $R^2 = 0.81$). The positive control and EMLA® showed a higher number of clots compared to other formulations ($p < 0.05$), indicating greater toxicity. Processing CAM images using ImageJ allowed for a quantitative analysis of extravascular clots, facilitating a better distinction of the toxicity of the evaluated substances.</p> <p>Keywords: Toxicity. Chorioallantoic membrane. Topical anesthetics. Dentistry.</p>
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Title	N-nitrosodimethylamine: toxicological and reproductive effects of low dose exposure of male and female Wistar rats
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Session	Sessão 19: Toxicologia

Abstract, Ethics Committee Number*, and Keywords	<p>N-nitrosodimethylamine (NDMA) is one of the most predominantly found N-nitrosamines in water, food, drugs, cosmetics and cigarettes. These compounds have potential mutagenic, genotoxic and carcinogenic effects that have been studied before in high doses of exposure. Studies investigating systemic and reproductive effects of NDMA are scarce, especially on low doses, considered acceptable by regulatory agencies. Thus, this study aims to evaluate toxicological and reproductive consequences of low dose NDMA exposure during adult life in male and female rats. Male and female Wistar rats (n=20/group/sex) were exposed to distilled water or 7.2 ng/kg/day orally (gavage). Animals were exposed during the preconceptional period, postnatal day (PND) 60 to 90, and mating period, PND 90 to 104. Females were also exposed during the gestational/lactational period, gestational day 0 to lactational day 21. The animals were euthanized after the exposure periods to collect organs and blood. In the male exposed group, there was an increase in the platelet count and in the relative weight of the prostate, when compared to the control group. Also, in the sperm count analysis, there was a significant delay in transit time in the caput-corporis region and total transit time. In the female group, there was a significant reduction in mean corpuscular volume and mean corpuscular hemoglobin. So far, it is possible to infer that NDMA is capable of altering normal reproductive parameters, evidenced by changes in the prostate relative weight and in the sperm count analysis, and could potentially alter normal systemic functions, as seen in altered blood parameters in both male and female exposed groups. Nonetheless, these findings are</p>
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	<p>based on initial results, and continuous analysis is crucial to confirm this</p> <p>Keywords: reproductive toxicology, Nitrosodimethylamine, low dose exposure.</p> <p>Acknowledgments: FAPESP(2022/15364-7; 2023/04536-4); CAPES(88887.806581/2023-00)</p>
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Title	NDMA impacts in female offspring: toxicological and developmental parameters
Authors	Stein, J. ¹ Nagaoka, L. T. ¹ Castro, R.L.M. ¹ Alves, J.T. ¹ Aquino, J. V. F. ¹ Rosalem, G.F. ¹ Manoel, B. M. ¹ Marraschi, G. K. ¹ Vitale, O. G. ¹ Magalhães, L. ¹ Jorge, B. C. ¹² Arena, A. C. ¹³
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Session	19 - Toxicologia

Abstract, Ethics Committee Number*, and Keywords	Nitrosodimethylamine (NDMA), a pervasive pollutant in water, food, and medicines, poses mutagenic, genotoxic, and carcinogenic effects. However, reproductive and developmental studies are limited. Critical windows of development are more susceptible to negative effects of exogenous substances, thus NDMA impacts in DOHaD and POHaD must be studied. We aimed to evaluate the impacts of paternal, maternal, and combined exposure, added to postnatal direct exposure on female rat offspring. F0 generation was exposed (gavage) in preconception (postnatal day - PND 60-90), mating, gestation, and lactation period. Males and females were divided in control group (deionized water) and exposed to NDMA (7.2 ng/kg/day) and paired during mating: Control (control males and females); maternal NDMA (NDMA females X control males); paternal NDMA (control females X NDMA males); and combined NDMA (NDMA females and males). Female offspring had body weight and anogenital distance (AGD) (PND 1, 13 and 22) evaluated. 1 female/litter was kept without intervention until PND 60 for organ/blood collection. 2 females/litter were re-exposed from PND 22-60, one exposed to control and other to NDMA, regardless of parental treatment. Puberty onset, estrous cycle, sexual behavior, fertility, and hematological parameters were evaluated. Body weight of paternal group was increased at PND1 compared to control. AGD was reduced on PND 13 in maternal group. At PND 60, there were no changes in body/organ weights, but hematological analysis had reduced mean corpuscular volume of paternal and
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combined groups compared to control. No alterations were observed in puberty onset, estrous cycle, sexual behavior, fertility, or hematological parameters. NDMA indirectly impacted female offspring's developmental and systemic health, but direct exposure didn't add further injuries.

Keywords: Nitrosamines; developmental; toxicology

FAPESP (2022/15849-0; 2022/15364-7); CAPES (88887.809658/2023-00)

Ethics Committee: 470426042



Title	Different doses of Bisphenol S induce cardiac remodeling and might modulate the renin-angiotensin system in male mice
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Session	Pôster

Abstract, Ethics Committee Number*, and Keywords	<p>Bisphenol S (BPS) might be related to cardiovascular diseases (CVD), but if it modulates the renin-angiotensin system (RAS), a key mechanism in CVD, is unknown. 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 (mg) and wall thickness (mm), cholesterol level (mg/dL), cardiac remodeling mediators and RAS components were assessed. Statistical analyses were performed by one way ANOVA and Holm-Sidak pos hoc. 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) were increased in B4 and B25 compared to C and B50. Cholesterol level was elevated in B4 compared to C (+15,0%, p<0,05) and B50 (+53,7%, p<0,0001), and in B25 compared to C (+39,3%, p<0,0001), B4 (+18,0%, p<0,05) and B50 (-81,5%, p<0,0001). B50 cholesterol level was lower (-23,2%, p<0,01) than C. 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. IL-6 protein expression was higher in B4 (+63,9%, p<0,05 vs C; +124,0%, p<0,001 vs B50) and B25 (+224,9%, p<0,0001 vs C; +344,8%, p<0,0001 vs B50) than C and B50. All groups had higher AT1R (B4: +55,9%, p<0,05; B25: +73,6%, p<0,05; B50: +61,8%, p<0,05) protein expression than C, while MasR protein expression was reduced (B4: -58,4%, p<0,05; B25: -31,6%, p<0,05; B50: -30,4%, p<0,05). B4 had lower MasR protein expression than B25 (-39,2%, p<0,05) and B50 (-40,5%, p<0,05). BPS-induced cardiac</p>
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remodeling is dose-dependent, and BPS might shift the balance towards the classical axis of RAS.

Ethical approval n. 1929240521.

Keywords: Bisphenol S; Endocrine disruptor; Renin-Angiotensin System; Cardiac remodeling.



Title	Modulation of paracetamol-induced hepatotoxicity by acute and chronic ethanol consumption in mice
Authors	Allan Cristian Gonçalves; Julio César da Silva Batista; Pedro Alves Machado Júnior; Frank Silva Bezerra; Daniela Caldeira Costa.
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Session	19 – Toxicology

Abstract, Ethics Committee Number*, and Keywords	<p>Paracetamol (APAP) is an analgesic/antipyretic agent capable of causing liver damage in cases of overdose. Paracetamol overdose toxicity is caused by increased metabolism by cytochrome P450, especially CYP2E1, forming a toxic metabolite NAPQI. The use of ethanol diverts APAP metabolism to the cytochrome P450 pathway, reducing hepatic protective thiol stores. The objective of the study was to evaluate the effect of acute and chronic ethanol consumption on APAP overdose hepatotoxicity. Adult male isogenic C57BL/6 mice were used, distributed among the groups: Control (C; n=12), water 2x/day/7days; Paracetamol (APAP; n=15) same as previous, plus a single dose of APAP (500 mg/kg); Acute Ethanol (AE; n=15), single dose of ethanol (10 ml/kg), and single dose of APAP (500 mg/kg) one hour later; Chronic Ethanol (CE; n=15) ethanol (10 ml/kg) 2x/day/7 days; and a single dose of APAP (500 mg/kg). Water and drugs were administered by gavage. Euthanasia was performed 12 hours after the APAP overdose. Serum ALT and AST were analyzed. Histopathological analyzes and glutathione quantification were carried out in the liver. The APAP and AE groups showed higher serum activity of ALT ($p < 0.0001$) and AST (APAP, $p = 0.0017$; AE, $p = 0.0442$). Morphometric analyzes showed a higher level of injury in the APAP and AE groups by counting inflammatory nuclei (APAP, $p < 0.0001$; AE, $p = 0.0006$), binucleated hepatocytes (APAP, $p < 0.0001$; AE, $p = 0.0005$); and steatosis (APAP, $p = 0.0309$; AE, $p = 0.0152$). The APAP group presented a larger area of necrosis ($p = 0.0073$). The APAP and AE groups exhibited a lower GSH/GSSG ratio ($p < 0.0001$) and higher levels of oxidized glutathione ($p < 0.0001$). Moreover, lower levels of total glutathione (GSH) ($p < 0.0001$) and reduced glutathione ($p < 0.0001$) were observed in the AE group. The CE group was statistically equal to the control group in all parameters evaluated ($p > 0.05$). Therefore, acute ethanol consumption, unlike in the CE group, maintained a liver injury profile similar to APAP overdose.</p> <p>Ethics Committee Number: 2899150322</p> <p>Keywords: Paracetamol; ethanol; oxidative stress; hepatotoxicity, liver damage.</p>
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Title	Morphological analysis in the thyroid of obese male mice fed a westernized diet and exposed to glyphosate and 2,4-D herbicides
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Session	19 - Toxicologia

Abstract, Ethics Committee Number*, and Keywords	<p>The intake of a Westernized diet (WD) - enriched in saturated fats and simple sugars - is associated with overweight, obesity, dyslipidemia, and other diseases. In addition, crops and dairy products frequently display pesticide residues, including the herbicides glyphosate and 2,4-D. The concomitant exposure to WD and herbicides is not fully investigated mainly in thyroid disorders. Thus, we evaluated the effects of glyphosate (Gly) and 2,4-D, alone or in their mixture on the thyroid in WD-induced obesity. Male C57BL6J mice were submitted to WD (chow containing 20% lard, 0.2% cholesterol, 20% sucrose, and high sugar solution with 23.1 and 18.9 g/L of D-fructose and D-glucose in drinking water) for 6 months. Concomitantly to WD, the animals received glyphosate (0.05, 5, or 50 mg/kg/day), 2,4-D (0.02, 2 or 20 mg/kg/day) or their mixture (0,05 + 0.02, 5 + 2, or 50 + 20 mg/kg/day) by gavage (5×/week). All the experimental procedures were approved by the Local Ethics Committee on the Use of Animals (CEUA 1344/2020). After six months, the animals were euthanized under anesthesia, blood was collected by cardiac puncture for serum biochemical analysis and the thyroid was collected and processed for histological and morphometric analysis. Data were compared among groups using the ANOVA or Kruskal-Wallis tests. The chronic WD intake induced obesity and glucose intolerance, increasing body weight and fat and cholesterol serum levels but without any influence of herbicide treatments. The measures of thyroid follicles (area and diameter) were similar among the WD groups, independently of Gly and 2,4-D exposure (mean follicular area ~345.52 μm^2 and mean follicular diameter ~ 17.92 μm, $p > 0.05$). Thus, the findings indicate that glyphosate and 2,4-D herbicides (doses/time) used did not alter thyroid follicle morphology in obese male mice.</p> <p>Keywords: glyphosate; 2,4-D; Westernized diet; obesity; thyroid</p>
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Title	Inhibitory effect of the extracts from <i>Aesculus hippocastanum</i> and <i>Melilotus officinalis</i> on the proteolytic and coagulant activity of <i>B. jararaca</i>, <i>B. jararacussu</i> and <i>B. neuwiedi</i> snake venom
Authors	Roberto da Costa Gonçalves ¹ Camila de Castro Pinheiro ¹ Brenda Bairral Queiroz Ornellas ¹ Eladio Oswaldo Flores Sanchez ² Stanislav Sukhikh ³ Olga Babich ³ Svetlana Ivanova ⁴ André Lopes Fuly ¹
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Session	Poster presentation

Abstract,	<p>Snakebite envenomation (SBE) is a neglected tropical disease with 2.7 million incidents, 138,000 deaths, and 400,000 amputations or deformities reported annually. In Brazil, the genus <i>Bothrops</i> is the largest; responsible for 90% of SBE, and the species <i>B. jararaca</i>, <i>B. jararacussu</i>, and <i>B. neuwiedi</i> are of medical interest. The symptoms of SBE are multiple, including pain, edema, muscle damage, hemorrhage, and death. Antivenoms prevent death, but are not so effective against local effects, leading to disabilities of the affected limb. Thus, seeking for new treatment is of great importance. The aim of this study was to analyze the antivenom potential of the plants <i>Aesculus hippocastanum</i> (AH) and <i>Melilotus officinalis</i> (MO) against the <i>in vitro</i> proteolytic and coagulant activities of <i>Bothrops jararaca</i>, <i>B. jararacussu</i>, and <i>B. neuwiedi</i>. The proteolytic activity of <i>B. jararaca</i>, <i>B. jararacussu</i>, and <i>B. neuwiedi</i> was evaluated using azocasein, as substrate. Each snake venom was incubated with saline or 0,9% of dimethylsulfoxide (DMSO) or with the extracts from plants for 30 min at 37°C, followed by the proteolytic test. For the coagulant activity, the extracts were incubated with solvents or with venoms for 30 min at 37°C. Then, aliquot of each mixture was added to plasma and coagulation was monitored in seconds in a digital coagulometer. As controls, the extracts were assessed in the absence of venoms. As result, AH inhibited around 50% the proteolytic activity of <i>B. jararaca</i> and <i>B. neuwiedi</i> venom, and 20% the proteolysis of <i>B. jararacussu</i> venom, as well as inhibited plasma coagulation caused by all the venoms. Moreover, MO inhibited around 75% proteolysis of <i>B. jararaca</i> and <i>B. jararacussu</i>, and 20% that caused by <i>B. neuwiedi</i> venom. Neither AH nor MO interfered on the proteolytic and coagulant assays. Therefore, the extracts from <i>A. hippocastanum</i> and <i>M. officinalis</i> possess antivenom potential against <i>Bothrops</i> spp. venom.</p> <p>Key words: Snakebite; <i>Bothrops</i>; Antivenom; Natural products.</p>
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Title	Chronic exposure to metalliferous atmospheric particulate matter induces changes in gills, liver and kidneys of <i>Oreochromis niloticus</i>
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Session	19- Toxicologia

Abstract, Ethics Committee Number*, and Keywords	<p>Seattleable atmospheric particulate matter (SePM) emission is increasing in the world. SePM is composed of a mixture of metals and metallic nanoparticles that can dissociate in water and become bioavailable. The effects of long-term exposure periods in different fish organs need to be elucidated. Biomarkers are important tools to identify the effects and understand the underlying toxic mechanisms. <i>Oreochromis niloticus</i>, Nile tilapia, a fish species with high economical relevance worldwide, is a model to evaluate effects of metalliferous SePM contamination in aquatic biota by assessing morphological alterations in gills, kidney and liver, alongside biochemical biomarkers. SePM was collected surrounding an iron ore processing and steel industrial complex in Vitória city (Espírito Santo, Brazil). Fish was exposed to 1.0 g.L⁻¹ of SePM for 30 days. Physicochemical and metal analyses in experimental water were done every day. Every 6-day, water sample for metal analyses and fish were anesthetized and killed. Gills, kidneys, and liver were removed and sampled for determination the metallothionein (MT) level in each organ and histological analysis. Gill sections were stained with toluidine blue and kidney and liver sections with toluidine blue and basic fuchsin (Protocol. CEUA UFSCar N° 6082080518). MT concentration did not alter except in the liver at the 18th day of exposure. Histopathological index</p>
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(HI) was higher in the liver from 6th day and 12th for the gill and kidney. The most frequent histologic alterations were cell hypertrophy, atypical cell contour, loss of regular structure. Chronic exposure to SePM induces sublethal effects to fish, promoting structural changes as adjustments to cope with the contamination. Although most histopathology was considered as reversible, they still compromise the organ normal functionality. Morphological adjustments imply in energetic costs and may pose a risk to the animal survival as it shifts from their routine energetic scope.

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Keywords: Metal contamination; metallothionein; histopathology



Title	1,2,3-Triazoles as inhibitors of proteolytic, coagulant and phospholipase activities caused by <i>B. jararaca</i> and <i>B. neuwiedi</i> snake venom
Authors	Brenda Bairral Queiroz Ornellas ¹ ; Camila de Castro Pinheiro ¹ ; Andresa Noberto de Lima Silva ¹ ; Eladio Flores Sanchez ² ; Vitor Francisco Ferreira ³ ; Fernando de Carvalho da Silva ³ ; Daniel T. G. Gonzaga ⁴ ; André Lopes Fuly ¹
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Session	Poster/Banner

Abstract, Ethics Committee Number*, and Keywords	<p>In Brazil, Viperidae family is responsible for 98% of snakebite accidents and the genus <i>Bothrops</i> accounts for 87% of them. Snake venom is a mixture of active peptides and proteins that cause systemic and local toxic effects. Antivenom is the official treatment, but is ineffectiveness in inhibiting the local effects, leading to disabilities. Therefore, seeking alternative or complement is of great importance. Molecules derived of organic synthesis have been used by industries to develop medicines, and triazole has been described as antifungal, anticancer, as well as antivenom. Through, we assessed the effect of sixteen triazole derivatives against the proteolytic, coagulant, and phospholipase A₂ (PLA₂) <i>in vitro</i> activities of <i>B. jararaca</i> (BJ) and <i>B. neuwiedi</i> (BN) venom. The venoms were incubated with saline or dimethylsulfoxide or with the derivatives at 37°C for 30 min, followed by proteolytic (using azocasein as substrate), coagulant (using human plasma), and PLA₂ (using egg yolk, as substrate) activities. Overall, the derivatives inhibited all the toxic activities in a concentration-dependent manner, and with different percentages. The derivatives 7d, 7e, and 7f achieved the lowest IC50 at the proteolysis of BN venom, 50 µg/mg. At coagulation assay, the derivatives 6c, 6d, and 6e inhibited more efficiently BJ venom, while 6c, 6d, and 6e were the most active against BN venom. On the other hand, the derivatives inhibited PLA₂ activity of BJ venom approximately of 60%, and 7a inhibited with the lowest inhibitory percentages, 20%. Therefore, a new series of 1,2,3 triazole was synthesized and able to inhibit the most important group of enzymes of venom, as SVMPs, and PLA₂s with different efficacies. This difference was expected, since snake venom is a complex mixture of molecules with inter and intraspecific variations. It also be concluded that triazoles may be a promising tool for the development of antivenom molecules.</p> <p>Keywords: Venom, Triazole, derivatives, Bothrops, antivenom</p>
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Title	Human health risk assessment of metals in wild and laboratory-exposed Nile tilapia (<i>Oreochromis niloticus</i>) to industrial metallic particulate matter
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Session	19 - Toxicology

Abstract, Ethics Committee Number*, and Keywords	<p>Industrial areas, mainly those with metallurgical and steelwork operations, are significant contributors to atmospheric pollution, often producing settleable particulate matter (SePM) containing metals which contaminate hydric bodies. This study aims to measure the metal concentrations in the muscle of <i>Oreochromis niloticus</i> and assess the human health risks from consuming fish exposed to SePM, both from laboratory and field settings. Muscle from wild fish were taken in two lagoons near Metropolitan Region of Vitória, Espírito Santo, Brazil: Carapebus and Maembá (P1 and P2) and Alegre at 120 km away, in 2021 and 2022. In laboratory, <i>O. niloticus</i> were 96-h exposed to 0.0, 0.01, 0.1 and 1.0 mg/L SePM. Metals in water and fish muscle were analyzed using ICP-MS, and human health risks were assessed by the Estimated Daily Intake (EDI), Target Hazard Quotient (THQ), Hazard Index (HI), and Target Cancer Risk (TCR). Twenty and three metals were quantified in muscle of <i>O. niloticus</i> exposed to SePM and twenty and seven were metals quantified in the four spots of the lagoons. The EDI of Cr was higher than acceptable limits for children in the control group, and Se exceeded permissible levels in Maembá P2 in 2021 and Maembá P1 in 2022. The HI in fish from Maembá P1 (2022) indicated non-carcinogenic risks (HI = 1.16). The TCR exceeded the limits established by USEPA in fish from control group, in all the lagoons (2021) and Alegre, Carapaebus and Maembá P2 lagoons (2022), suggesting carcinogenic risks. These findings suggest that metals from SePM can bioaccumulate in fish muscle, leading to potential risks to human health, both carcinogenic and non-carcinogenic. This study underscores the importance of risk assessment for human consumption to understand the broader public health implications of environmental pollution. (UFSCar-CEUA: CEUA nº 4649170619, 8972160123) Financial support: FAPESP Proc 2019/08491-0, 2023/01356-5, 2023/09739-3; CAPES, Brazil; ANPCYT, FONCYT/PICT-1597, Argentina</p> <p>Keywords: Hazard index, metals, metallurgical industries, food safety</p> <p>Financial support: FAPESP Proc 2019/08491-0, 2023/01356-5, 2023/09739-3;</p>
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Title	Liver biomarker responses in <i>Oreochromis niloticus</i> exposed to glyphosate and polyethylene microplastics individually and combined
Authors	Marco Aurélio Miranda Soares ¹ Ericsson Rubens Rodrigues Ferreira ² Gilzelle Gilzelle Maria da Luz Silva ² Sandro Estevan Moron ² Marisa Narciso Fernandes ¹ Marcelo Gustavo Paulino ³
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Session	19. Toxicology
Abstract, Ethics Committee Number*, and Keywords	<p>Glyphosate-based herbicides (GBH) and polyethylene microplastics (PE-MP) are among the major contaminants in the freshwater systems. In Brazilian rivers, both contaminants were found in elevated concentrations, leading to a high probability of their association, which can alter their individual effects and potentially intensify their toxicity. This present work evaluated the isolated and combined effects of PE-MP (< 500 µm) and GBH in environmental relevant concentrations on <i>Oreochromis niloticus</i>, using multi-biomarkers of toxicity. The fish were exposed for a period of 96-hour, with concentrations set based either isolated, PE-MPs group (5 mg L⁻¹), GBH group (5 mg L⁻¹), or in a group of associated contaminants (GAC), PE-MP + GBH (5 mg L⁻¹ + 5 mg L⁻¹). The toxic effects were evaluated using biochemical, and histopathological biomarkers. The differences between groups were analysed with ANOVA one-way ($p \leq 0.05$). The present work was approved by the Animal Ethics Committee (CEUA) of the Federal University of Tocantins (Proc. N. 23.101.002.470/2020-17). We observed significant changes ($p \leq 0,05$) in the exposed groups. Decrease in total protein content in GBH compared to GAC and GC. Decrease in glycogen in GAC compared to GC and GMP. Histopathological markers indicate a alteration in the blood flow in GMP, indicated by hyperemia. Nevertheless, the organ pathological index showed no morphofunctional impact after exposure. Therefore, our study indicates that PE-MPs and GBHs can induce energy expenditure in <i>O. niloticus</i>, potentially due the activation of detox system. These effects in <i>O. niloticus</i>, at the concentrations used, were intensified by the association of contaminants. Thus, the use of multi-biomarkers proved to be a valuable tool for assessing toxicity, providing data for investigating high levels of contaminant mixture toxicity in aquatic environments.</p> <p>Keywords: Contaminants Interaction; Freshwater contamination; Energetic impairment;</p>



Title	Bisphenol A exposure exacerbates prostate remodeling and promotes lesions in aged female gerbils
Authors	Lorena Gabriela de Souza ¹ Gervásio Evangelista Brito Filho ¹ Luara Jesus Ferrato ¹ Thalles Fernando Rocha Ruiz ² Sebastião Roberto Taboga ¹
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Session	19 - Toxicologia

Abstract, Ethics Committee Number*, and Keywords	<p>Exposure to exogenous compounds affects endocrine pathways and homeostasis. Bisphenol A (BPA), a xenoestrogen, acts through estrogenic pathways and alters morphophysiological characteristics of prostate. Three experimental groups (N=15) were used: control (water), vehicle (0.1 mL, corn oil), and BPA (50 µg/kg/day). Exposure occurred during gestation/lactation and these females were analyzed during aging (18-mo age). Histopathological analysis were performed for analysis of epithelial and stromal changes. Statistical analysis was carried out using the ANOVA or Kruskal-Wallis test ($p < 0.05$). In the BPA group, the incidence of lesions and epithelial changes was increased, such as papillary atypia ($4.51\% \pm 1.04$), hyperplasia ($4.77\% \pm 0.29$), <i>high-grade intraepithelial neoplasia</i> ($0.66\% \pm 0.18$) and budding ($8.1\% \pm 0.63$). The control group showed an increase in atrophic changes ($11.89\% \pm 0.83$). In relation to the epithelium, there was an increase in the expression of epithelial and stromal phospho-histone H3 (PHH3) ($75.26\% \pm 6.94$ and $68.99\% \pm 1.94$, respectively) and activated caspase-3 ($63.80\% \pm 1$ and $21.77\% \pm 1.55$, respectively). In addition, the presence of basal cells (p63) also increased ($34.45\% \pm 0.66$) compared to the control group ($25,92\% \pm 0,52$), as well as for the α-smooth muscle actin (SMA), collagen IV and laminin ($48.19\% \pm 2.81$; $35.60\% \pm 1.75$ and $29.17\% \pm 1.20$, respectively). As well as metalloprotease (MMP) -2, -3, -9 and -13 ($24.60\% \pm 2.56$; $11\% \pm 0.56$; $32.79\% \pm 2.17$ and $25.86\% \pm 1.71$, respectively). The opposite was observed for TIMP-1, in which there was a decrease in the BPA group ($7.44\% \pm 0.65$) compared to the control group ($10,57\% \pm 0,92$). The results showed that BPA acted to favor the development of proliferative lesions, by the pro-proliferative imbalance. Also, in the stroma an hypertrophic tendency were increased, indicating a tissue remodelling.</p> <p>CEUA 217/2019.</p> <p>Keywords: <i>prostate, dysregulation, gerbil.</i></p>
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FRONTEIRAS DAS CIÊNCIAS BIOMÉDICAS E SEUS DESAFIOS

Title	Use of hybrid triazole and naphtoquinone to inhibit coagulation caused by <i>Bothrops jararacussu</i> venom
Authors	Andresa Noberto de Lima Silva ¹ ; Brenda Bairral Queiroz Ornellas ¹ ; Eladio Flores Sanchez ² ; Vitor Francisco Ferreira ³ ; Fernando de Carvalho da Silva ³ ; Ruan Carlos Busquet Ribeiro ³ ; André Lopes Fuly ¹
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Session	Pôster/Banner

Abstract, Ethics Committee Number*, and Keywords	<p>Envenomation by venomous snakes is considered by the World Health Organization (WHO) a neglected tropical disease. The venom contains organic and inorganic molecules, in which active peptides and proteins are responsible for toxic effects, including hemorrhage, edema, tissue necrosis, renal, cardiac, pulmonary failure, and death. WHO recommends antivenom to treat snakebite envenoming. However, antivenom has unwanted effects, as anaphylactic shock and fever, as well as is ineffectiveness to block tissue necrosis, leading to disabilities of the affected limb. Therefore, the search for additional or innovative medicines is of great value. Synthetic derivatives have been gaining ground in the industry, and literature has described their pharmacological properties to benefit human health, as anti-inflammatory and antimicrobial. However, antivenom potential of synthetic molecules has not been fully investigated. Therefore, in this work, the inhibitory effect of twelve hybrid triazole and naphtoquinone derivatives (RC29 to RC40) on the coagulation activity caused by <i>Bothrops jararacussu</i> (BJU) venom was assessed. BJU venom was incubated with saline, dimethylsulfoxide or with the derivatives for 30 min at 37°C, then, an aliquot of each mixture was added to plasma or fibrinogen and coagulation was monitored in a digital coagulometer. The toxicity of the derivatives was evaluated through the in vitro hemotoxicity test. As result, the twelve derivatives did not lyze red blood cells, and thus, are devoid of toxicity. The derivatives inhibited plasma coagulation of BJU venom, and the derivatives RC32, RC36, RC39, and RC40 had the greatest efficacy. On the hand, the fibrinogen-induced coagulation by BJU venom was fully inhibited by RC33 to RC40. In conclusion, the hybrid of triazole and naphtoquinone is a new series of promising derivatives as antivenom molecules, since coagulation disorder caused by BJU venom is a crucial symptom in the envenoming caused by this specie.</p> <p>Keywords: antivenom, triazole, coagulation, naphtoquinone, <i>Bothrops jararacussu</i></p>
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Title	Histopathological study of Wistar rat spleens treated with nanostructured biogel containing piezoelectric particles in non-critical bone defects
Authors	Anna Beatriz Modesto Pires ¹ , Nathanael Vieira Medrado ² , Bruno Henrique Costa ¹ , Beatriz Pimental de Oliveira Andrade ¹ , Michele Munk Pereira ³ , Erika Costa de Alvarenga ^{1,2}
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Session	19 - Toxicologia

Abstract, Ethics Committee Number*, and Keywords	<p>Bone tissue is a rigid mineralized connective tissue composed of an extracellular matrix mainly consisting of type I collagen and hydroxyapatite crystals. This tissue serves multiple functions such as structural support, protection of internal organs, and mineral storage. The occurrence of injuries and/or fractures caused by excessive loads and pathologies such as osteoporosis cause pain and instability to the patient, reducing quality and life expectancy. Therefore, the prompt return of the individual to their daily life activities is essential, leading to a reduction in public spending. In this regard, the use of Barium Titanate can promote adequate bone repair due to its biocompatibility and piezoelectric properties. Thus, our research group developed type I collagen biogels associated with piezoelectric nanoparticles as an alternative to improve bone repair. However, it is essential to evaluate the biological toxicity regarding the use of nanomaterials. The spleen is a secondary immunological organ of the body that is a major target for changes when there is toxicity. Therefore, the present study aimed to evaluate the toxicity of the developed biogel in the spleen of Wistar rats subjected to bone defect in the tibia, approved by the CEUA with protocol number 009/2018. Spleen morphology and histomorphometry were evaluated through H&E stained histological sections, which demonstrated the preservation of the architecture of the red and white pulp, with the presence of well-demarcated lymphoid follicles by the marginal zone, indicating an absence of systemic response to toxicity. The results suggest the maintenance of the number of lymphoid follicles at $\pm 9.10^6$ per total analyzed area and no significant difference in follicular area when compared to the control group treated with saline. Thus, it is concluded that the biomaterial did not cause splenic toxicity and presents good compatibility with the organism, suggesting it is a promising material for bone repair.</p> <p>Keywords: Barium Titanate, Biogel, Bone Repair</p>
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Title	Determination of environmental biomonitoring in the São Lourenço de Jaciara/MT River using the <i>Allium cepa</i> L. test system
Authors	Vanderson Ferreira da Silva Rayane Fabricio dos Santos Eduardo Oliveira Souza George Laylson da Silva Oliveira
Affiliations	Campus São Vicente - IFMT, Jaciara-MT, Brasil
Session	Toxicologia

Abstract, Ethics Committee Number*, and Keywords	<p>River pollution occurs mainly due to tourist activity, rainwater that may or may not come from drainage systems and the disposal of untreated domestic sewage. The research group LEPTOX-F (Teaching and Research Laboratory in Toxicology and Pharmacology) highlights that the objective of the present study is to carry out environmental biomonitoring (2024/rainy period) through the collection of water/sediment samples at various points in the São Lourenço River in the stretches that cross the cities of Don Aquino-MT, called Point 1 (15°37'10.765"S and 054°57'52.269"W), Point 2 (15°48'55.652"S and 054°57'29.482"W), São Pedro da Cipa-MT called Point 3 (15°59'52.669"S and 054°55'17.285"W) and Juscimeira-MT, called Point 4 (16°05'51.655"S and 054 °56'19.773"W). For this, the <i>Allium cepa</i> L. test system (exposure of 48 and 72 hours) was used and parameters such as temperature, turbidity, pH, BOD (Biochemical Oxygen Demand, mg/L), COD (Chemical Oxygen Demand, mg/L) were analyzed, electrical conductivity, EC – $\mu\text{S}/\text{cm}$), sulfate, fluoride, nitrate/nitrite, chromium and total iron, in addition to microbiological analysis of <i>Escherichia coli</i> (NMP/100mL) and total coliforms (NMP/100mL). The results of the <i>A. cepa</i> L. test were expressed as mean \pm S.E.M (standard error of the mean), using <i>one-way ANOVA</i> followed by the <i>Newman-Keuls</i> test as post hoc ($p < 0.05$, GraphPad Prism 8.01). The results of the physical-chemical tests demonstrated no changes in accordance with the standards specified in CONAMA Resolution No. 357, of March 17, 2005, for Class II freshwater. Additionally, the absence of cytotoxicity, mutagenicity and/or genotoxicity was evidenced in the water samples from each point after exposure for 48 and 72 hours. However, the analysis of <i>E. coli</i> at Point 3 indicated the presence of 1.100 MPN/100mL, higher than the maximum allowed by CONAMA Resolution n° 357/2005.</p> <p>Keywords: Cytotoxicity, microbiological analysis, physicochemical tests.</p>
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Title	Effect of fucoidans from brown seaweed <i>Undaria pinnatifida</i> and <i>Fucus vesiculosus</i> against coagulant, proteolytic, and phospholipase A₂ activities of <i>Lachesis muta</i> venom
Authors	¹ Camila de Castro-Pinheiro ¹ Luiz Carlos Simas Pereira Júnior ² Eladio Flores Sanchez ³ Corinna A. Dwan ³ Samuel S. Karpinić ⁴ Alan Trevor Critchley ¹ André Lopes Fuly
Affiliations	¹ Universidade Federal Fluminense, Niterói, Brazil ² Fundação Ezequiel Dias, Belo Horizonte, Brazil ³ Marinova Pty, Ltd., Cambridge, Australia ⁴ Independent Researcher, The Evangeline Trail, Paradise, NS B0S 1R0, Canada
Session	Oral or poster presentation

Abstract, Ethics Committee Number*, and Keywords	<p>According to the World Health Organization (WHO), snake bite envenoming (SBE) is a neglected tropical disease. <i>Viperidae</i> is the most important medically family in Brazil, in which <i>Lachesis muta</i> (LM) incidents have the highest lethality index. LM venom has a complex mixture of proteins that produce systemic and local toxic effects in victims, including hemorrhage, tissue necrosis, coagulation disturbs, and death. Antivenom is recommended by WHO as treatment for SBE. It prevents death, but it is not so efficient to block tissue necrosis, leading to amputation. Thus, seeking new therapies to overcome limitations of serotherapy is of interest to society. The aim of this work was to evaluate antivenom effect of sulfated polysaccharides from the brown seaweed <i>Fucus vesiculosus</i> (FVF) and <i>Undaria pinnatifida</i> (UPF) against coagulant and phospholipase A₂ activities of LM venom and a purified thrombin-like enzyme (TLE). LM venom or TLE was incubated with saline or with fucoidans for 10 min at 37°C, and an aliquot of the mixture was added to plasma or fibrinogen, and coagulation was monitored in a digital coagulometer. The effect of polysaccharides was assessed using a chromogenic substrate for thrombin-like enzymes, S-2238. Anti-phospholipase A₂ and antiproteolytic activity was measured using fluorescent substrate and azocasein, respectively. As results, LM venom-induced coagulation of plasma was not inhibited by FVF and UPF; but they inhibited coagulation of plasma or fibrinogen induced by TLE. FVF and UPF inhibited clotting caused by LM venom. FVF and UPF inhibited the hydrolysis of S-2238 of LM and TLE. FVF and UPF inhibited 65% proteolysis of LM venom, and 46% and 58%, respectively the PLA₂ activity. In conclusion, FVF and UPF inhibited proteolysis and PLA₂ activity of LM venom, as well as coagulation of a purified enzyme. These activities are responsible for the major toxic effects of SBE of LM, and, thus, FVF and UPF are promising molecules against LM venom.</p> <p>Keywords: <i>Lachesis muta</i>; Antivenom; Polysaccharides; <i>Fucus vesiculosus</i>; <i>Undaria pinnatifida</i>; Brown seaweed</p>
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Title	Effect of amazonian <i>Siparunas</i> spp extracts against coagulation and proteolysis caused by <i>Bothrops jararacussu</i> venom
Authors	¹ Camila de Castro-Pinheiro ² Diégina Araújo Fernandes ¹ Brenda Bairral Queiroz Ornellas ² Suzana Guimarães Leitão ³ Eládio Flores Sanchez ² Gilda Guimarães Leitão ¹ André Lopes Fuly
Affiliations	¹ Universidade Federal Fluminense, Niterói, Brazil ² Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil ³ Fundação Ezequiel Dias, Belo Horizonte, Brazil
Session	Oral or poster presentation

Abstract, Ethics Committee Number*, and Keywords	<p>Antivenom serotherapy is the gold standard treatment for snake bite envenoming (SBE). However, antivenom is not so effective to reverse local tissue damage caused by SBE, leading to amputation or disabilities. <i>Bothrops</i> genus venom is rich in proteases that are involved in coagulation disorders, edema, tissue necrosis, and hemorrhage. Thus, seeking alternatives antivenom therapies able to reverse local effects of SBE deserves attention by researchers and health authorities. The aim of this work was to evaluate antivenom property of amazonian <i>Siparunas</i> spp extracts against coagulant and proteolytic activities of <i>B. jararacussu</i> (BJU) snake venom. BJU venom was incubated for 30 min at 37 °C with saline, dimethylsulfoxide, or with crude ethanolic (C) extract and fractions prepared in hexane (H), dichloromethane (D), ethyl acetate (E), and n-butanol (B) from <i>S. cymosa</i>, <i>S. decipiens</i>, <i>S. ficolides</i>, <i>S. glycyarpa</i>, and <i>S. reginae</i>. Then, aliquot of each mixture was tested on the coagulation and proteolysis of BJU venom, in which plasma coagulation was monitored in a digital coagulometer and proteolytic activity using azocasein, as substrate. As result, overall all the species of <i>Siparuna</i> double the coagulation time when compared to BJU incubated with solvents; however the species of <i>S. cymosa</i> and <i>S. reginae</i> was the most efficient to inhibit such a toxic effect. On the other hand, the extracts B and H from <i>S. glycyarpa</i> inhibited proteolysis of BJU venom around 15%, and extracts D and B from <i>S. decipiens</i> or <i>S. reginae</i> inhibited 25% and 10%, respectively. In conclusion, the non-polar extracts from the species <i>Siparunas</i> have antivenom potential, due to inhibition of the major toxic activities of snake venom and may be used as an adjuvant to serotherapy against <i>B. jararacussu</i> venom.</p> <p>Keywords: Snake bite; <i>B. Jararacussu</i>; Antivenom; <i>Siparunas</i>; Natural products</p>
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Title	Can the gut microbiota modulated by cesarean section alter the vulnerability of adult male rats to phthalates?: A behavioral analysis
Authors	Lais Nogueira da Silva ¹ Marcella da Silva Araujo Santiago ¹ Luan Araújo Monteiro ^{2,3} Alessandra Mussi Ribeiro ² Juliana Elaine Perobelli ¹
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Session	19 - Toxicologia

Abstract, Ethics Committee Number*, and Keywords	<p>Gut microbiota (GM) is the group of microorganisms that inhabit the gastrointestinal tract of mammals. It is known that initial establishment and composition of the GM are influenced by the birth pathway: vaginal delivery or C-section. The GM can modulate the physiology of several systems of the host due to the communication axes established between them, like the gut-brain axis. Also, GM acts upon the metabolism of xenobiotics, such as the phthalate DEHP, altering its half-life, bioavailability, and toxicity. This study aims to investigate whether the GM, altered by C-section, can influence the pattern of neurotoxicity exerted by prolonged oral exposure to DEHP, altering behavioral aspects of adult male rats. For this, male Wistar rats born via vaginal delivery (VD) or via vaginal delivery or C-section followed by cross-fostering (groups VDCF and CSCF) were used. Furthermore, each of the 3 groups was subdivided into control and exposed to DEHP (n=10/group). The exposed groups received DEHP at a dose of 48 mg/kg/day diluted in olive oil, orally, by gavage, for 65 days. Control groups received only the vehicle. At PND 83, the animals were submitted to behavioral tests to assess motricity and anxiety-like behavior, and memory and learning (open-field test and object recognition test, respectively). The experimental procedures were approved by CEUA-UNIFESP (process 2428081021). Behavioral tests showed no significant differences between experimental groups, despite an anxiety-like behavior tendency in the VD exposed group (p=0,06) and a memory deficit tendency in the CSCF exposed group (p=0,07). The preliminary results suggest that GM modulated by C-section and/or DEHP exposure didn't interfere with behavioral aspects of animals. Further analysis will be carried out, such as immunohistochemistry</p>
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	<p>assays for claudin-1 and 8-OHDG and determination of serum levels of ACTH, FSH, and LH.</p> <p>Keywords: gut microbiota; dysbiosis; birth pathway; gut-brain axis; phthalates; DEHP</p>
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Title	Effects of orally administrated capsaicin on the intestinal mucositis induced by the chemotherapy drug irinotecan hydrochloride (CPT-11) in C57BL/6J mice
Authors	Daniela Kuniyoshi ¹ Gabriel Bacil ¹ Guilherme Ribeiro Romualdo ² Luis Fernando Barbisan ¹
Affiliations	1 - São Paulo State University (UNESP), Institute of Biosciences, Department of Structural and Functional Biology, Botucatu, Brazil 2 - São Paulo State University (UNESP), Botucatu Medical School, Department of Pathology - Experimental Research Unit (UNIPLEX) – Botucatu Medical School (FMB), Botucatu, Brazil
Session	Poster session 1

Abstract, Ethics Committee Number*, and Keywords	<p>Irinotecan (IRT) is a systemic chemotherapeutic drug used to treat several types of solid cancers but with a common side effect on the gastrointestinal tract, especially intestinal mucositis (IM). Capsaicin (CAP) is a bioactive compound found in peppers and widely consumed by worldwide, presenting anti-inflammatory and antioxidant properties that might exert beneficial effects on gut health. We sought to assess if the oral administration of CAP modulates IRT-induced IM. Male C57BL/6J mice received intraperitoneal injections of IRT [75mg/kg/body weight (b.w.), G3-G5] or vehicle (G1/G2) for 6 days. Additionally, mice received intragastric doses of CAP (12.5 or 25.0 mg/Kg of b.w., G4 and G2-G5, respectively) for 6 days. CAP doses were based on a daily estimate intake by individuals of 100-200 mg/Kg of b.w., with a translational approach of Human Equivalent Dose. 4 hours after the last IRT dose, peripheral blood cells samples were collected for comet assay. At the euthanasia, serum and SI, liver and kidney samples were collected for biochemical and morphological analyses, respectively, according to the animal care guidelines (CEUA/IBB: N°8546150223). Data were analysed using ANOVA or Kruskal-Wallis, with Tukey or Dunn's post hoc tests. There were no effects of G4/G5 groups on the body weight, DNA damage levels in peripheral leucocytes, the alanine aminotransferase, urea, and creatinine serum levels or liver and kidney weight and morphology when compared to the G3 group. The IM score analysis revealed that only the G5 group reduced the intestinal damage caused by IRT ($p=0.0005$) when compared to the G3 group. The histopathological analysis showed that the IRT treatment induced a reduction in villi height ($p=0.0001$) and crypt depth ($p<0.0001$) and an increase in muscular thickness ($p=0.0003$). CAP interventions did not reestablish these adverse effects. Considering our findings,</p>
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	<p>concomitant CAP treatment may alleviate some adverse effects induced by IRT on the SI.</p> <p>Keyword: irinotecan; capsaicin; intestinal muositis; small intestine; adverse effects</p>
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Title	Investigation of the effects of inhalation exposure to polyethylene particles on the redox state of male Wistar rats
Authors	Manuela Vitória Bonfim Alcantara ¹ Jardeson Henrique Bianchini ¹ Mariana Simões Garcia ¹ Rafael Herling Lambertucci ² Juliana Elaine Perobelli ¹
Affiliations	1-Laboratório de Toxicologia Experimental(LaToEx), Departamento de Ciências do Mar, Universidade Federal de São Paulo(UNIFESP), Santos, Brazil. 2- Instituto de Saúde e Sociedade, Universidade Federal de São Paulo(UNIFESP), Santos, Brazil.
Session	19-Toxicologia

Abstract, Ethics Committee Number*, and Keywords	<p>Microplastics (MPs) are particles smaller than 5mm and have been linked to adverse effects on human and animal health, as they can penetrate organs and cross cell membranes. This study aims to investigate the effects of acute exposure to increasing doses of polyethylene (PE) particles via nasal instillation on parameters of oxidative stress and damage in the lungs and liver of adult male Wistar rats. The animals were distributed into five experimental groups: saline control, vehicle control (isopropyl alcohol), low dose (100µg PE/day/animal), medium dose (1000µg PE/day/animal), and high dose (10,000µg PE/day/animal), with n=6-8/group, CEUA-UNIFESP process nº 5779050422. The animals received daily treatments for 15 days via intranasal instillation (final volume 20µL/day). The PE particles were obtained from Sigma Aldrich (CAS number: 9002-88-4). On postnatal day 107, the rats were euthanized, and the liver and lungs were collected and homogenized with PBS. For the evaluation of Total Antioxidant Status (TAS), a solution of Fe²⁺-o-dianisidine with hydrogen peroxide was used; for Total Oxidative Status (TOS), H₂SO₂ was used, both reacting with the tissue samples. The results were evaluated by the Omnibus test for TAS, TOS, and Oxidative Stress Index (OSI) of the lungs and liver, comparing all groups. No significant differences were observed between the groups (p<0.05). For effect size comparisons, a metric of 0.610 was obtained for lung OSI between the saline group and the medium dose group, and a metric of 0.789 for lung TOS between the vehicle control and saline groups compared to the medium dose group, values considered moderate effects. The next steps of this study include the analysis of antioxidant enzyme activity and the assessment of oxidative damage, allowing for a broader understanding of the effects resulting from PE exposure.</p> <p>Keywords: nanoplastics, adverse effects, rats, oxidative stress.</p>
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Title	Evaluation of the antinociceptive effect of <i>Abarema cochliacarpus</i> extract in an animal model of pain
Authors	Vitória Macario Filipe Eloi Alves de Carvalho Bismarck Rezende Cristina Viana Henriques Roberto Carlos Campos Martins Penha Cristina Barradas Daltro Santos Guilherme Carneiro Montes
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Session	Pain and Inflammation

Abstract and Keywords	<p>The plant <i>Abarema cochliacarpus</i> is used by the population to treat festering wounds, as a healing agent and analgesic. Our proposal is to evaluate the antinociceptive effect of <i>Abarema cochliacarpus</i> extract in animal model of pain. The experiments were approved by CEUA/UERJ 014/22. Swiss mice, male and female were used for the formalin test. The aqueous extract of the barks (AEB) and ethanolic extract of the barks (EEB) of <i>Abarema cochliacarpus</i>, at different doses and saline, were administered intraperitoneally (i.p.). After 30 min, 20 µL formalin solution (2.5%) was injected into the right paw. The animals were evaluated through the time of licking, biting or constant paw movements. The two phases of the test were observed: phase 1 (neurogenic) and phase 2 (inflammatory). Phase 1 consists of time 0 to 5 min after formalin administration, from 15 min after formalin administration, phase 2 begins. The GraphPad Prisma software was used for statistical analysis and the comparison was made between saline groups with extracts, results were significant when $p < 0.05$. During phase 1, the formalin reactivity of male mice treated with saline was 59.6 ± 4.9 (s); animals treated with 30 mg/kg EEB decreased to 32.5 ± 7.8 (s). In relation to females treated with saline, formalin reactivity was 73 ± 10.0 (s) and animals treated with 30 mg/kg EEB decreased to 32.1 ± 5.2 (s). During phase 2, the formalin reactivity of male mice treated with saline was 128.5 ± 19.5 (s); animals treated with EEB and AEB decreased to 27.3 ± 12.5 and 51.7 ± 18.4 (s) at dose of 30 and 100 mg/kg, respectively. The formalin reactivity in females was 182 ± 13.1 (s) and animals treated with EEB decreased to 0.80 ± 0.5 (s) at 30 mg/kg dose. The extract of <i>Abarema cochliacarpus</i> shows antinociceptive action in pain animal model.</p> <p>Keywords: <i>Abarema Cochliacarpus</i>, Antinociceptive effect, pain animal model.</p>
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Title	Secosterols produced from ozonized cholesterol active neutrophils
Authors	Wellika Dorta de Oliveira Kris Simone Tranches Dias Maísa Ribeiro Pereira Lima Brigagão
Affiliations	Universidade Federal de Alfenas, Alfenas, Brasil.
Session	Pain and inflammation

Abstract, Ethics Committee Number*, and Keywords	<p>Since the last years, the use of ozone (O₃) in medicine (ozone therapy) is widely used worldwide. This therapy is based on a large number of publications that have exposed the studies carried out demonstrating the biological properties and effects that its action produces in the body. However, growing recent scientific evidence indicates that ozone hemotherapy can lead to damage to the body through the oxidation of blood carbohydrates, proteins and lipids. To study the effect of compounds from cholesterol ozonation on polymorphonuclear neutrophils (PMN), secosterol-A (3β-hydroxy-5-oxo-5,6-secocholestan-6-al) and secosterol-B (3β-hydroxy-5β-hydroxy-B-norcholestane-6β-carboxaldehyde) were synthesized by pumping ozone into cholesterol (10 mM) in chloroform (10 hs). The sample was purified by flash column chromatography using silica gel 60 and monitored by thin layer chromatography after 2,4-dinitrophenylhydrazine derivatization. Samples were subjected to HPLC and infrared analysis and the isolated secosterols in deuterium chloroform (CDCl₃) were analyzed by NMR using a Bruker AC-300 instrument, confirming the presence of both secosterols A and B. Human PMN were isolated from heparinized venous blood samples (Ethics Committee authorization CAAE 70875223.7.0000.5142) by centrifugation gradient. The PMN NADPH oxidase complex (Nox2) activity, responsible for production of superoxide anion (O₂^{•-}), the first radical species released by activated phagocytes, was monitored by the reduction of cytochrome c (λ_{550 nm}). PMN stimulated with PMA (200 ng) released 1.4±0,08x10⁻⁹ O₂^{•-}/min/10⁶ cells, while phagocytes secosteols A and B-triggered (120 ng) released about twice as much O₂^{•-}. This study shows that secosterols A and B formed from the ozonation of cholesterol are potent activators of Nox2, whose overproduction of oxidizing species are directly associated with the deleterious actions arise when PMN escapes from homeostasis control.</p> <p>Secosterol, superoxide anion, neutrophil</p>
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Title	Sexual dimorphism in mice modulates the involvement of glial cells in acute and chronic muscle hyperalgesia
Authors	Beatriz Botasso Gomes Hayla Lourenço Rodrigues Emanuele de Oliveira Dorta Isabelle Caetano Maria Cláudia Gonçalves de Oliveira
Affiliations	Laboratory of Pain and Inflammation Research - FCA/ UNICAMP, Limeira, Brazil
Session	Pain and Inflammation

Abstract, Ethics Committee Number*, and Keywords	<p>Glial cells, mainly astrocytes and microglia, are associated with chronic pain signalling. However, it is unknown whether the involvement of these cells in chronic pain signalling are modulated by sexual dimorphism. Objective: To evaluate the involvement of microglia and astrocytes from dorsal horn of spinal cord in the development of acute and chronic muscle hyperalgesia of inflammatory origin in male and female mice. Methods: It was used C57BL/6 male and female mice (2 months old) from CEMIB/UNICAMP, with the ethics committee approval number 6014-1/2022. Carrageenan (Cg, 100µg) was injected into gastrocnemius muscle to induce acute muscle hyperalgesia and, 10 days later, PGE₂ (1µg) was injected at the same local to evidence the chronic muscle hyperalgesia. Mechanical muscle thresholds were quantified by Randall Selitto test in different time points of the acute and chronic period. The involvement of microglia and astrocytes were evaluated by intrathecal pretreatment with minocycline (10µg, 3 days before Cg) and fluorocitrate (1nmol, 30 minutes before Cg), respectively. Statistics were performed by One Way ANOVA with Tukey's post test from the AUC and the significance level was set at p<0.05. Results: Pretreatment with minocycline reduced acute (n=5, 155.4±7.5, 80.32±6.04, p<0.01) and chronic (n=5, 431.9±32.09, 195.9±21.33, p<0.01) mechanical muscle hyperalgesia in male mice, but in females, mechanical muscle hyperalgesia was reduced only in chronic phase (n=5, 269±25, 171.9±11.83, p<0.01). Pretreatment with fluorocitrate reduced acute (155.4±7.5, 103.8±7.4, p<0.01) and chronic (431.9±32.09, 184.5±19.43, p<0.01), but in females only in the chronic phase (n=5, 269±25, 151.7±13.08, p<0.01). Conclusion: We suggest that, in male mice, microglia and astrocytes from dorsal horn of spinal cord are involved in acute and chronic muscle pain. While in females, they are involved only in the chronic phase.</p> <p>Keywords: Glial cells, muscle pain, sexual dimorphism</p>
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Title	Oral supplementation with caffeine modulates the development of chronic muscle pain
Authors	Dorta, E. O ¹ , Rodrigues, H. L ¹ , Gomes, B. B ¹ , Oliveira, M. C. G ¹
Affiliations	1- Laboratory of Pain and Inflammation Research, School of Applied Sciences, Universidade Estadual de Campinas, Limeira-SP.
Session	20 – Pain and Inflammation

Abstract, Ethics Committee Number*, and Keywords	<p>Chronic pain is a public health problem with great negative impacts. Its treatment consists of the prescription of analgesics and regular physical exercise. However, in patients with chronic muscle pain, the exercise may cause kinesiophobia. Caffeine supplementation, due to its analgesic role, may be an interesting strategy to reduce pain in chronic conditions. Considering we have previously shown that chronic muscle pain is prevented by regular physical exercise through modulation of PPARγ receptors and that there are evidences that caffeine induces expression of PPARγ coactivator-1α (PGC-1α), we aimed to investigate whether oral supplementation with caffeine would be able to reduce chronic muscle pain. Male C57BL/6 mice, 6-week-old, from CEMIB (ethics committee number: 6331-1/2023) were used. Acute muscle hyperalgesia was induced by Carrageenan (Cg, 100μg) injection into the gastrocnemius muscle and, 10 days later, PGE₂ (1μg) was injected into the same site to reveal the chronic muscle hyperalgesia. Caffeine was administered by gavage (3.5 mg/kg of body weight) during the 17 days of the development of chronic muscle pain. Muscle hyperalgesia was quantified by Randall Selitto test at different times of chronic phase. Area Under the Curve and One-Way ANOVA with Tukey's post test were used. The significance level was set at $p < 0.05$. Caffeine reduced the development of chronic muscle hyperalgesia ($84.24 \pm 7.81, p < 0.05, n = 5$) when compared to the control group (gavage with water, $109.7 \pm 5.14, p < 0.05, n = 5$). In conclusion, we suggest that, similar to regular physical exercise, the oral supplementation with caffeine is an efficient strategy to reduce chronic muscle hyperalgesia in male mice.</p> <p>Keywords: chronic pain, Caffeine, muscle.</p>
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Title	Sexual dimorphism modulates the involvement IL-1β in chronic muscle pain
Authors	Hayla Lourenço Rodrigues Beatriz Botasso Gomes Emanuelle Oliveira Dorta Maria Cláudia Gonçalves Oliveira
Affiliations	Laboratory of Pain and Inflammation Research – LABEDI, FCA-UNICAMP
Session	Pain and Inflammation

Abstract, Ethics Committee Number*, and Keywords	<p>Recently, our research group demonstrated, in a model of transition from acute to chronic inflammatory muscle pain, that there are a local increase in pro-inflammatory macrophages during the acute and chronic phases. However, the local increase in IL-1β seems to be only in the acute phase. Considering these findings, we aimed to assay whether IL-1β release in muscle or in dorsal horn of spinal cord are involved in development and maintenance of chronic muscle pain and whether sexual dimorphism modulates these processes. C57BL/6 mice, 6-7 weeks, from CEMIB-UNICAMP (ethics committee number: 5973-1/2022), were used. Carrageenan (Cg, 100μg) was injected into the gastrocnemius muscle to induce acute muscle hyperalgesia, and 10 days later, PGE2 (1μg) was injected at the same site to reveal the state of chronic hyperalgesia. The antagonist of IL-1β receptor, IL-1ra, was injected 48h after Cg into gastrocnemius muscle (500ng) or intrathecally (100 pg). Mechanical muscle hyperalgesia was quantified by the Randall Selitto test at different time points of chronic phase. Statistical analysis were carried out AUC and One Way ANOVA, with a significance level set at $p < 0.05$. Treatment with IL-1ra into gastrocnemius muscle reduced chronic muscle hyperalgesia in male ($n=5$, 103 ± 19, $p < 0.0001$) and female mice ($n=5$, 115.7 ± 21.3) when compared to male ($n=4$, 431.9 ± 32.09, $p < 0.0001$) and female control group ($n=6$, 269 ± 25, $p < 0.0001$). Intratecal IL-1ra reduced chronic muscle hyperalgesia in male ($n=5$, 123.8 ± 14.82, $p < 0.0001$), but not in female ($n=5$, 227.0 ± 15.9) when compared to male ($n=4$, 431.9 ± 32.09, $p < 0.0001$) and female control group ($n=6$, 269.0 ± 25.0, $p > 0.05$). In conclusion, we suggest that IL-1 β is involved in the development of chronic inflammatory muscle pain in male and female mice at a peripheral level. However, at a central level, IL-1 β is involved in development of chronic inflammatory muscle pain only in male mice.</p> <p>Keywords: Hyperalgesia; Cytokine IL-1 β; Muscle Pain.</p>
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Title	Effect entourage of the full spectrum cannabidiol-rich oil on sodium channel involved in pain
Authors	Samuel Gonçalves de Castro Silva ¹ Samuel José Dornelas Ferreira ² Micheline Donato ³ Alessandra Matavel ¹
Affiliations	1- Research of Development Division, Ezequiel Dias Foundation, Belo Horizonte, Brazil. 2 – School of Medicine, Federal University of Minas Gerais, Belo Horizonte, Brazil. 3 - Laboratory of Medical Cannabis and Psychedelic Science; Federal University of Latin American Integration, Foz do Iguaçu, Brazil.
Session	Dor e Inflamação

Abstract, Ethics Committee Number*, and Keywords	<p>Cannabidiol (CBD) is one of the main non-psychoactive phytocannabinoids in Cannabis. It modulates a wide variety of proteins and has applications in several treatments, including inflammatory and chronic pain. Although the effect of CBD on blocking sodium channels involved in pain has already been described in the literature, the entourage effect of CBD-rich oil is little explored. Therefore, the present study aims to verify whether the other phytocannabinoids present in CBD-rich oil have a synergistic effect on blocking Nav1.7 channels. For this, electrophysiology experiments using the whole cell voltage clamp technique were used to verify the blocking of sodium currents in CHO cells permanently expressing Nav1.7 channels by CBD-rich oil. The oil was dissolved in DMSO and used at 15 µg/mL of final concentration. The results showed that the oil rich in CBD blocks the channels partially (42%) and irreversibly. The analysis of the steady state of inactivation showed a significant shift of the curve to hyperpolarized values (9.3 mV), showing that the transition of the channel from the closed state to the inactivated state is being favored by CBD. The inactivation recovery curve suggests that the CBD-rich oil inhibited the transition from the inactivated state to the channel-closed state, making recovery from the inactivated state difficult. The data suggest that the molecular mechanism of the entourage effect of CBD-rich oil in the partial blockade of the Nav1.7 sodium channel is due to the stabilization of the inactivated state of the channel, reducing its availability for opening, which explains the partial blockage observed. Since Nav1.7 is central in transmitting the pain signal, CBD could be a strong candidate for an analgesic drug.</p> <p>Keywords: Cannabidiol, Nav1.7, inactivation, pain</p>
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Title	Anti-inflammatory and antioxidant effects of polysaccharides from <i>Libidibia ferrea</i> associated with rivaroxaban in zymosan-induced peritonitis in rats
Authors	Carlos Jorge Maciel Uchoa Gadelha ¹ Rafael Aires Lessa ² Adriane Sampaio Cavalcante ¹ Isabely Alencar Barreto ¹ Ana Carollyne Carvalho Lima ¹ Ana Maria Sampaio Assreuy ¹ Maria Gonçalves Pereira ^{1,2}
Affiliations	1 – State University of Ceara, ISCB, Fortaleza, Brazil 2 – State University of Ceara, FECLESC, Quixadá, Brazil
Session	[Pain and inflammation]

Abstract and Keywords	<p>Previous studies had demonstrated antithrombotic effect of polysaccharides from <i>Libidibia ferrea</i> (PE-Lf). Besides, the association of these polysaccharides with a low dose (0,1 mg/kg) of rivaroxaban, a direct inhibitor of coagulation factor Xa, potentiated the antithrombotic effect without increasing bleeding risk. Regarding the close relation between hemostasis and inflammation, the aim of present study was to evaluate the association between PE-Lf and RIVA in the rat peritonitis model induced by zymosan. Female Wistar rats (n=5; 150-200g; CEUA-UECE: 11053402/2022) were treated (<i>p.o.</i>) with saline, PE-Lf (5 or 10 mg/kg), RIVA (0.1 or 0.2 mg/kg) or with the association PE-Lf+RIVA. After 90 minutes the peritonitis was induced by zymosan (1 mg/kg; <i>i.p.</i>), and 4 hours later the animals were euthanized and the peritoneal fluid collected for quantification of the parameters: number of total (Neubauer chamber; Turk's) and differential (panoptic stain) leukocytes; proteins (A595 nm); oxidative stress markers (malondialdeyde-MDA at A535 nm and reduced glutathione-GSH at A412 nm). Leukocyte migration was significantly reduced in the peritoneal fluid of animals treated either with PE-Lf alone or in combination with RIVA (0.2 mg/kg), by 67% and 60%, respectively. The differential count revealed a reduction in the number of neutrophils by PE-Lf at 10 mg/kg alone (57%) or in combination with RIVA (42%). Both groups treated with PE-Lf showed a reduction in total proteins by 22% (PE-Lf) and 15% (PE-Lf+RIVA) compared to zymosan. Assessment of the pro-oxidative MDA in peritoneal fluid indicated a reduction by PE-Lf at 10 mg/kg alone (29%) or in combination with RIVA (39%). The antioxidative GSH was significantly increased by EP-Lf alone (2,74x) or in combination with RIVA (2,68x). In conclusion, PE-Lf alone or associated with RIVA protects the acute inflammation induced by zymosan in rats.</p> <p>Keywords: Inflammation; Jucá ou pau-ferro; plant polysaccharides.</p>
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Title	Study of NLRP3 inflammasome activation in C2C12 myoblasts induced by pro-inflammatory cytokines TNF-α and IL-6
Authors	Marcela Bego Nering de Sousa ¹ Angela Maria Alvarez ² Cristiane Damas Gil ³ Vanessa Moreira ¹
Affiliations	1 – Departamento de Farmacologia, Disciplina de Farmacologia Celular - Escola Paulista de Medicina -Universidade Federal de São Paulo – Campus São Paulo. 2 – Reproduction Group. Pharmacy Department, School of Pharmaceutical and Food Sciences. University of Antioquia - UdeA. Medellín, Colombia 3 - Departamento de Histologia - Universidade Federal de São Paulo – Campus São Paulo.
Session	20 - Dor e Inflamação

Abstract, Ethics Committee Number*, and Keywords	<p>Pro-inflammatory cytokines IL-6 and TNF-α, produced by myoblastic cells, influence cellular functions through immune-related pathways. Exploring their effect on NLRP3 inflammasome activation would provide a new perspective on the regulatory mechanisms in muscle degeneration and regeneration, particularly in cytokine-associated myopathies. C2C12 myoblast cells were cultured in DMEM with 10% fetal bovine serum, 2mM glutamine, and 1% penicillin/streptomycin, at 37°C/5% CO₂. A density of 1x10⁴cells/mL was subcultured in 96- and 6-well plates pre-coated with 2% gelatin. Myoblasts (n=4) were incubated with lipopolysaccharide (LPS) (500ng/mL), TNF-α (50ng/mL), or IL-6 (50 ng/mL). After 4h, nigericin (NIG) (5μM) was added. Cell viability was evaluated 6 and 24h post-NIG addition by measuring mitochondrial metabolic activity. Additionally, the expression of apoptosis-associated speck-like protein containing a caspase recruitment domain-ASC and NLRP3 was analyzed using high content screening analysis via confocal microscopy and RT-PCR. The results showed that mitochondrial metabolic activity significantly decreased (p<0.05) in LPS+NIG (inflammasome activation control) (55%), TNF-α+NIG (60%), and IL-6+NIG (45%) cells compared to cells without treatment (100% viability). The quantification of total specks per cell revealed a significant increase (p<0.05) in cytoplasmic ASC expression in cells treated with LPS+NIG (8.7\pm1.3), TNF-α+NIG (7.9\pm0.9), and IL-6+NIG (10.3\pm1.8) compared to the control (2.6\pm0.1). In addition, cytoplasmatic and nuclear expression of NLRP3 was increased</p>
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($p < 0.05$) in C2C12 with TNF- α +NIG compared to control cells. Similarly, gene expression of ASC and NLRP3 was significantly elevated ($p < 0.05$) in cells treated with TNF- α +NIG and IL-6+NIG, but not with LPS+NIG, when compared to control cells. For the first time, it has been demonstrated that IL-6 and TNF- α may act as initial signals in the activation and modulation of the NLRP3 inflammasome in myogenic cells.

Keywords: inflammasome, myogenic cells, cytokines



Title	Antinociceptive action of Citral through the CB2 receptor is more pronounced in obese adult male C57BL/6J mice
Authors	Felipe Lima Dario Maria Luiza Alves Aquino Felipe Leonardo Fagundes Maycon Tavares Emílio-Silva Laura Vitória Fortunato de Oliveira Isabela Galende Guidolin Gabriela Bueno Mariana Moraes Fioravanti Renata Assunção Vinícius Peixoto Rodrigues Lúcia Regina Machado da Rocha Clelia Akiko Hiruma-Lima
Affiliations	São Paulo State University (UNESP), Institute of Biosciences, Department of Structural and Functional Biology, Botucatu, São Paulo, Brazil.
Session	Pain and Inflammation – Oral presentation

Abstract, Ethics Committee Number*, and Keywords	<p>Obesity is a prevalent chronic disease with a high correlation with pain, which profoundly interferes with patients' quality of life. Citral (CT) is a monoterpene with anti-inflammatory and antinociceptive effects. The objective of this study was to characterize the effects of CT on nociception in obese adult male C57BL/6J mice and to investigate the mechanisms of action involved. Therefore, mice (n=232) were fed a standard (SD) or a high-fat diet (HFD) for 12 weeks. They were then subjected to an oral glucose tolerance test to ensure that the HFD animals had metabolic alterations. Subsequently, the formalin-induced nociception test (1% formaldehyde, 20 μL, intraplantar) was conducted to assess the state of nociception and the effect of CT (100 and 300 mg/kg, orally) on neurogenic (phase I) and inflammatory (phase II) pain. Tests were carried out with antagonists for 5-HT_{2A} (ketanserin, 1 mg/kg, intraperitoneally [i.p.]) and CB₂ (AM630, 1 mg/kg, i.p.) receptors. At the end of each experiment, the animals were euthanized to collect adipose tissue, which to determine the adiposity index (CEUA-IBB no. 6856030723). The data were subjected to the Mann-Whitney and two-way ANOVA tests, followed by Tukey test, with $p < 0.05$. The induction of obesity was successful, as evidenced by the higher values observed in the HFD group for body mass, adiposity index, and glycemia in all periods evaluated. In both phases of the formalin test, C300 reduced nociception in SD and HFD animals compared to vehicle (1% Tween 80, 10 mL/kg, orally) groups and were more intense in the HFD group. Ketanserin did not reverse antinociceptive effects in any phase. AM630 reversed the antinociceptive effects in the HFD animals in both phases of the formalin test, but not in SD animals in phase II. Consequently, CT exerts a more pronounced antinociceptive effect in obese animals, reducing neurogenic and inflammatory pain and acting as a CB₂ receptor agonist. Funding: FAPESP 2023/13172-6.</p> <p>Keywords: citral; nociception; obesity; mice; CB₂R.</p>
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Title	Body inflammatory myositis and Parkinson's disease: modeling the illness with Phospholipase A₂
Authors	Vanessa Moreira Gabriel Gutiérrez Ospina
Affiliations	1-Departament of Pharmacology, Laboratory of Pharmacology of Inflammation, Universidade Federal de Sao Paulo (UNIFESP) –Escola Paulista de Medicina, Sao Paulo, Brazil; 2 – Department of Cell Biology and Physiology, Universidad Nacional Autonoma de Mexico (UNAM), Ciudad de Mexico, Mexico
Session	Dor e Inflamação

Abstract, Ethics Committee Number*, and Keywords	<p>An experimental model was designed to evaluate the hypothesis that Parkinson's disease (PD) is not primarily a neurological disorder but a secondary disease that develops after a chronic process of inflammation in the paraxial skeletal muscle. Distinct groups of male Swiss mice (n=5) (6 weeks-CEUA3192280923) received phospholipaseA₂ (PLA₂) (37.5mg/kg/25µL), isolated from a viperid snake venom, directly into paraspinal muscles (i.m.) or 0.9% saline solution (control) every 15 days. Footprint tests were conducted at 16 or 46 days post-initial i.m. injection. Then, animals were euthanized, and blood (plasma), paravertebral muscles, and brain were collected for myokine quantification, including interleukin (IL)-6, IL-15, irisin (IRI), myostatin (MYO), osteonectin (OST), and leukemia inhibitory factor (LIF) using a commercial kit. No significant discomfort or motor impairments were observed during the experimental period. Anatomical assessment of the paravertebral muscles showed a minor decrease in muscle volume and a translucent fibrotic response along the paravertebral longitudinal axis. Despite these, mice injected with saline or PLA₂ did not exhibit gait alterations. Despite no significant changes in plasma myokine levels observed during the study, muscle tissue exhibited a substantial increase (1000-333%) (p<0.05) in pro-inflammatory and tissue-regeneration-modulating myokines, including IL-6, LIF, and OST, compared to control groups. Conversely, levels of neuroprotective, neurogenic, and brain metabolic function myokines in the brain, such as IL-15, MYO, IRI, LIF, and OST, significantly decreased (p<0.05) from 50 to 25% compared to controls. Our results indicate that chronic inflammatory degeneration of the paraxial skeletal muscle may remain relatively asymptomatic. This chronic condition, however, could lead to a gradual reduction in body-brain trophism, affecting the health of the central nervous system, contributing, later in life, to neurodegeneration.</p> <p>Keywords: neurodegeneration, chronic inflammation, muscle degeneration</p>
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Title	Evaluation of antinociceptive and anti-inflammatory effects of citral on obese adult male C57BL/6J mice
Authors	Maria Luiza Alves Aquino Felipe Lima Dario Felipe Leonardo Fagundes Maycon Tavares Emilio-Silva Laura Vitória Fortunato de Oliveira Isabela Galende Guidolin Gabriela Bueno Mariana Moraes Fioravanti Renata Assunção Vinícius Peixoto Rodrigues Lúcia Regina Machado da Rocha Clelia Akiko Hiruma-Lima
Affiliations	São Paulo State University (UNESP), Institute of Biosciences, Department of Structural and Functional Biology, Botucatu, São Paulo, Brazil.
Session	Pain and Inflammation – Poster presentation

Abstract, Ethics Committee Number*, and Keywords	<p>Obesity is a growing concern due to its increasing prevalence and association with chronic pain and inflammatory diseases. Citral (CT) is a monoterpene with analgesic and anti-inflammatory properties. This study aimed to evaluate the effects of CT on pain and inflammation in obese adult male C57BL/6J mice. Animals (n=116) were fed either standard (SD) or high-fat diet (HFD) for 12 weeks, body weight (g) was monitored, and a glucose tolerance test (GTT) was conducted to assure metabolic changes. The formalin test was used to assess nociception in mice treated with CT (100 or 300 mg/kg, orally - p.o), vehicle (1% Tween 80, 10 mL/kg p.o), or morphine (M, 5 mg/kg, subcutaneously) during the neurogenic and inflammatory pain phases of formalin-induced licking, with time measured in seconds. To assess edema, mice were treated with both doses of CT, vehicle, or piroxicam (30 mg/kg p.o) and injected 20 µL of 5% carrageenan on right hind paw and 20 µL sterile saline 0.9% into the left hind paw. Paw volume (µL) was measured using a digital plethysmometer at 1, 2, 3, 4 and 6h after injection. The mice were euthanized and tissues were collected (CEUA no. 6856030723). Data was analyzed using two-way ANOVA test followed by Bonferroni or Tukey tests with p<0.05. Intake of the HFD increased body weight and adiposity index compared to SD. GTT showed higher glycemic levels (mg/dL) in the HFD group in comparison to SD at all time points. Both diet groups showed significantly reduced nociception when treated with M and CT300 in both formalin test phases, and CT300 had an improved antinociceptive effect in the HFD group compared to the SD group. In the paw edema test, treatment with CT did not show any significant anti-edematogenic effects. In conclusion, CT produced an antinociceptive effect in both neurogenic and inflammatory pain phases of formalin-induced licking in a diet-dependent manner but did not affect inflammation-induced edema.</p> <p>Keywords: mice; high-fat diet; nociception; inflammation; citral</p>
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Title	Novel Rho kinase inhibitor in diabetic neuropathic pain
Authors	Aimeé Diogenes Santos ¹ Tadeu Lima Montagnoli ¹ Bruno Eduardo Dematté ² Heber Victor Tolomeu ¹ Carlos Alberto Manssour Fraga ¹ Gisele Zapata-Sudo ^{1,2} (Orientadora)
Affiliations	1 – Programa de Pós-Graduação em Farmacologia e Química Medicinal – UFRJ, Rio de Janeiro, Brazil. 2 – Pós-Graduação em Cardiologia – UFRJ, Rio de Janeiro, Brazil.
Session	20 - Dor e inflamação

Abstract, Ethics Committee Number*, and Keywords	<p>Diabetes mellitus (DM) is a chronic disease associated with cardiovascular and neurological complications. Diabetic painful neuropathy (DPN) remains poorly addressed, even under glycemic control. Neuroprotective actions of Rho kinase inhibitor (iROCK) may alleviate progression of DPN because by regulatating synaptic plasticity and may be associated with CNS remodeling. New iROCK was investigated in rats with DPN.</p> <p>Protocols (CEUA-UFRJ 103/21) were approved by Animal Care and Use Committee at Universidade Federal do Rio de Janeiro. DM was induced by intravenous administration of streptozotocin (60 mg/kg) in male Wistar rats (250-300 g, n= 15). After 4 weeks, animals were treated orally with iROCK (30 mg/kg/day, n= 9) or DMSO (n= 6) during 14 days. Non-diabetic (Non-DM) rats treated with DMSO were control group (n= 6). After 1, 7, 10 and 14 days of treatment, thermal hyperalgesia and mechanical allodynia were evaluated. Swiss mice (25-32 g) were submitted to hot plate test after i.p. treatment with either DMSO, morphine (10 mg/kg) or iROCK (10, 30, and 100 mg/kg).</p> <p>Thermal and mechanical thresholds in DM rats were significantly reduced from 14.5 ± 0.8 to 6.9 ± 0.5 s and from 55.2 ± 2.6 to 34.3 ± 3.3 g, respectively. iROCK increased paw withdrawal latency to 11.9 ± 1.7, 12.9 ± 0.9 and 11.5 ± 0.9 after 1, 7 and 14 days ($p < 0.05$). Allodynia was reversed by iROCK after 14 days, with threshold of 40.5 ± 4.5 g compared to 27.7 ± 3.0 g ($p < 0.05$) in DPN+DMSO group. Weight body or plasma glucose were not alter by iROCK.</p> <p>Administration of a single dose of iROCK did not produce antinociception in mice compared to morphine, which induced 72.1 ± 10.0 % of analgesic activity. iROCK improved thermal and mechanical thresholds in DM rat model, indicating as an alternative treatment of DPN. However, iROCK did not produced antinociception in an acute pain model, suggesting the dependence on structural changes on the sensory nerves caused by DM.</p> <p>Financial support: CNPq; CAPES; FAPERJ; INCT-Inofar; Cristália Produtos Químicos e Farmacêuticos Ltda</p> <p>Keywords: Diabetes Mellitus; Diabetic neuropathy; ROCK; RHO KINASE; PAIN.</p>
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Title	Effects of omega-3 fatty acid treatment on local and systemic-myotoxic effects induced by Bothrops asper snake venom
Authors	Renan Oliveira Lima Guilherme Lemos Fabiano Beraldi Calmasini Vanessa Moreira
Affiliations	Federal University of São Paulo - Paulista School of Medicine
Session	Pain and inflammation

Abstract, Ethics Committee Number*, and Keywords	<p>Accidents caused by <i>Bothrops asper</i> snake venom (Bav) result in both local and systemic damage, such as inflammation and myotoxicity, respectively. While serum therapy is effective against systemic effects, it is ineffective against local effects, highlighting the need for new therapeutic approaches. Omega-3 fatty acids, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) are cardioprotective and anti-inflammatory and may be effective as supportive treatment for both effects caused by Bav. The aim was to evaluate the effect of EPA and DHA in fish oil Nanoemulsion (FON) on Bav-induced systemic and local effects as peritoneal leukocyte influx, cardiac muscle protein kinase activity and functional bladder detrusor muscle (BMD). Male Swiss mice (8 weeks-CEUA3995011223) received Bav intraperitoneally (i.p.) (2.5mg/kg/500µL) or 0.9% saline (Sal). After 1h, groups of animals received FON (i.p.) (6.0µg/g/DHA and 10.2µg/g/EPA/500µL/animal) or 500µL of Sal. After 7 and 14 days, mice were sacrificed for collection of peritoneal exudate, blood, and bladder. Leukocyte counting in the peritoneal exudate was done in Neubauer chamber (Turk-1:20v/v). Cardiac kinase activity (CK-MB) was determined in plasma (commercial kit) and in vitro BDM contractions were evaluated in response to carbachol (CCh, 1nM-10µM). Bav+FON group showed reduction in leukocytes ($1.1 \pm 0.1 \times 10^6$ cells/mL, $p < 0.05$) in peritoneal exudate, compared to Bav+Sal ($2.7 \pm 0.3 \times 10^6$ cells/mL). Regarding CK-MB, Bav+Sal animals showed increase ($p < 0.05$) in activity (1769 ± 39U/L), compared to Sal+Sal (687 ± 39U/L), and Bav+FON animals showed reduction (1120 ± 49.2U/L, $p < 0.05$) compared to Bav+Sal. After 14d, Bav+Sal groups presented a 65% decrease ($p < 0.05$) in CCh-induced BDM contractility compared to Sal+Sal. Bav+FON animals exhibited 26% increase in CCh-induced BDM contractility ($p < 0.05$) compared to Bav+Sal. Omega-3 fatty acids may be adjunctive therapy to mitigate both leukocyte influx and systemic myotoxic effects induced by Bav.</p> <p>Keywords: Toxicity, snakebite accidente, omega-3 fatty acid</p>
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Title	Elevation of CLEC-2 expression and its correlation with laboratory markers in sickle cell disease
Authors	Ivanio Teixeira Borba-Junior ¹ Mayck da Silva Barbosa ¹ Carla R. P. Moraes ¹ Letícia Queiroz da Silva ¹ Bruno Benites ¹ Fernando Ferreira Costa ¹ Erich Vinicius de Paula ¹
Affiliations	1 – State University of Campinas, Campinas, Brazil
Session	Dor e Inflamação

Abstract, Ethics Committee Number*, and Keywords	<p>Hypercoagulability is an important characteristic of sickle cell disease (SCD), and its pathophysiology is related to the concomitant activation of hemostasis and innate immunity, in a process known as immunothrombosis. Recently, studies highlight the podoplanin/CLEC-2 pathway as important in the process of platelet activation and thrombosis. The aim of the study was to evaluate the expression of podoplanin (PDPN) and CLEC-2 in patients with SCD. 31 patients with SCD (SS genotype=23; SC genotype=8) were included, in addition to 10 healthy individuals, with ethical approval (CAAE: 53121421.0.0000.5404). The evaluation of PDPN and CLEC-2 expression was carried out by flow cytometry, using anti-CD45 (leukocytes), Anti-CD41 (platelets), Anti-CD62 (Platelet activation), Anti-PDPN and Anti-CLEC- antibodies. FSC (forward scatter) and SSC (side scatter) were used to separate cell populations. PDPN expression in monocytes showed no difference between groups, Kruskal-Wallis test [$X^2 (2) = 3.69$; $p=0.156$], despite there being a tendency towards greater expression of PDPN in SCD-SS patients when compared to healthy individuals ($p=0.06$, Bonferroni, post-hoc). In granulocytic cells there was no difference in PDPN expression between groups. The expression of CLEC2 in monocytes showed a difference between the groups, Kruskal-Wallis test [$x^2 (2) = 19.82$; $p < 0.0001$]. There was also a difference between groups in the expression of CLEC-2 in granulocytes, Kruskal-Wallis test [$x^2 (2) = 10.11$; $p=0.006$]. There was a correlation of CLEC2+ monocytes with PDPN+ Monocytes ($R= 0.646$), CD62+ Monocytes ($R=0.823$), CD41+ Monocytes ($R=0.720$), CLEC2+ Granulocytes ($R= 0.884$), Hemoglobin ($R= - 0.533$), Platelets ($R= 0.415$), reticulocytes ($R=0.563$) and D-dimer ($R=0.560$). Currently, no work has evaluated the PDPN/CLEC-2 pathway in SCD. Therefore, despite being preliminary, our results suggest that the pathway may be relevant in SCD, as it correlated with laboratory markers.</p> <p>Key-words: imunothrombosis, CLEC-2, hemostasis.</p>
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XXXVIII REUNIÃO ANUAL DA FESBE
XXII REUNIÃO ANUAL DA BRAVO
XVIII CONGRESSO DA SBCAL
III CONGRESSO DOHAD BRASIL
II CONGRESSO DA SBBA
2 A 5 DE JULHO 2024, CAMPINAS/SP
FRONTEIRAS DAS CIÊNCIAS BIOMÉDICAS E SEUS DESAFIOS

Title	Effects of 3,4-metilenodioxichalcone on neuropathic pain induced by chronic constriction of the sciatic nerve in mice
Authors	Evelynn Dalila do Nascimento Melo 1; Luiza Leite Santos 1; Beatriz Rohr Decothé Fonseca 1; Mirella da Costa Botinhão 1; João Vitor da Rocha Reis 1 André Gustavo Calvano Bonavita 1; Michelle Frazão Muzitano 1; Paula Lima do Carmo 1
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Session	Pain and Inflammation

Abstract, Ethics Committee Number*, and Keywords	<p>Neuropathic pain (NP) is important for research since the available pharmacological treatments have low to moderate efficacy. The present work aims to evaluate the antinociceptive potential of 3,4-methylenedioxichalcone (LC5) in the NP model induced by chronic sciatic nerve constriction (CCI); since its anti-inflammatory and antinociceptive activities, in protocols of acute pain, had already been demonstrated by our research group. For that, male Swiss mice (n: 8 per group; 20-22 g) were anesthetized intraperitoneally (I.P.) with ketamine (100 mg/kg) and xylazine (10 mg/kg). Then, an incision in the thigh was performed, to performed three ligatures of the common sciatic nerve, and, the skin was sutured. Eight days after surgery, NP was confirmed, and the animals received the treatment via I.P. with LC5 (10 mg/kg) or DMSO (vehicle) for 14 consecutive days. A false-operated control group (SHAM) was also performed. Evaluations of allodynia and mechanical hyperalgesia by the von Frey test and thermal hyperalgesia by the hot plate test were performed before NP induction, 8 days after surgery, and during an evaluation of the acute and subchronic effects (CEUA/UFRJ, protocol 065/22). The results were: 1) Mechanical allodynia: in the acute assessment, LC5, at 1, 3, 5 and 24 h, increased the nociceptive threshold from 0.98 ± 0.31 to 2.39 ± 0.26 g; from 1.01 ± 0.24 to 2.97 ± 0.30 g, from 1.17 ± 0.28 to 3.62 ± 0.10 g and from 0.78 ± 0.14 to 3.51 ± 0.21 g ($P < 0.05$) respectively. In the subchronic evaluation, LC5, at 3, 7, and 14 days, increased the nociceptive threshold from 0.91 ± 0.11 to 3.37 ± 0.59 g; from 1.00 ± 0.11 to 3.45 ± 0.50 g, from 1.17 ± 0.18 to 3.03 ± 0.66 g ($P < 0.05$) respectively. 2) Thermal hyperalgesia: LC5 was able to increase the animals' latency 3 h (acute effect) and 14 days (subchronic effect) after the start of treatment, from 3.0 ± 0.3 to 6.8 ± 0.4 s and from 1.6 ± 0.2 to $7.6 \pm 1,8$ s ($P < 0.05$), respectively. Then, LC5 is a promising compound in the study of new pharmacological therapies for NP.</p> <p>Keywords: neuropathic pain, chalcone, antinociception</p>
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Title	Investigation of the antiedematogenic effect of galactomannan from <i>Delonix regia</i> in rats induced by carrageenan
Authors	Carlos Jorge Maciel Uchoa Gadelha ¹ Adriane Sampaio Cavalcante ¹ Anny Beatriz Silveira Peixoto ¹ Jessica Kelly Alves Ferreira ¹ Vitória Gabriela Sanxo de Azevedo ¹ Ana Maria Sampaio Assreuy ¹ Alana de Freitas Pires ¹ Rondinelle Ribeiro Castro ²
Affiliations	1 – State University of Ceará, ISCB, Fortaleza, Brazil 2 – State University of Ceará, FAFIDAM, Limoeiro do Norte, Brazil
Session	Dor e Inflamação

Abstract, Ethics Committee Number*, and Keywords	<p>Previous studies by our group demonstrated benefit effects of <i>Delonix regia</i> galactomannan (GM-DR) on osteoarthritis and pancreatitis models, as well from healing of excisional wounds, but actions on classical inflammatory models are yet to be investigated. Thus, the study aims to investigate the mechanisms of the anti-edematogenic effect of GM-DR on carrageenan-induced paw edema in rats. Female Wistar rats were used (age 8-10 weeks, 150-200g, n=6/group), experimental protocols were approved by the UECE Ethics Committee for Animal Use (CEUA/UECE nº 07562055/2022). Animals were pretreated with GM-DR (10 mg/kg i.v), 30 min before intraplantar administration of Cg (300 µg) or inflammatory mediators of the early phase (histamine and serotonin) or late phase (PGE-2, TNF-α and L-arginine). Paw volume (in µL) was measured since baseline values until the time edema ceased. The results were expressed as mean ± standard error of the mean and significant differences were verified by two-way ANOVA followed by the Bonferroni test (p<0.05). GM-DR inhibited paw edema induced by carrageenan (0-2h: 73%56 ± 15 and 3-5h: 53%; 110 ± 54). In the initial phase (histamine: 65%; 47 ± 16 and serotonin : 73%; 204 ± 76) and late phase (TNF-α: 68%; 259 ± 84 and L-arginine: 81%; 220 ± 41), but not by PGE-2 (4425 ± 3525). Galactomannan from <i>Delonix regia</i> has an anti-edematogenic effect expressed in both phases of the inflammatory process.</p> <p>Keywords: Inflammation; Polysaccharide; Edema; Mediator.</p>
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Title	Antinociceptive effect of Cannabigerol (CBG) in pain animal model
Authors	Barbara Oliveira Vitória Macario Filipe Eloi Alves de Carvalho Yure Bazílio dos Santos Bismarck Rezende Guilherme Carneiro Montes
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Session	Pain and Inflammation

Abstract and Keywords	<p>Exploring the non-psychoactive properties of CBG, this study aims to assess its efficacy in pain relief. To achieve this, we will evaluate the antinociceptive effects of a cannabis extract rich in CBG content in pain animal model, emphasizing its benefits.</p> <p>The experiments were approved by CEUA/UERJ 018/23. Male and female Swiss mice were used. The animals were treated with CBG at doses 1, 10 and 30 mg/kg orally, which were diluted in dimethyl sulfoxide (DMSO), used as a control group. After 30 min of administration, 20 μL of formalin solution (2.5%) was injected into the right paw to evaluate peripheral nociception in the animals. The nociception of the animals were studied through the time of licking, biting or constant paw movements. Two phases of the test were observed: phase 1 (neurogenic) and phase 2 (inflammatory). Phase 1 consists of the time from 0 to 5 minutes after formalin administration, from 15 minutes after formalin administration phase 2 begins. The GraphPad Prism software was used for statistical analysis and the comparison was made between groups using one-way ANOVA, the results were significant when $p < 0.05$.</p> <p>During phase 1, the formalin reactivity of male mice treated with DMSO was 64.8 ± 7.4 s; and animals treated with doses of 10 and 30 mg/kg CBG decreased to 38.2 ± 10.2; 41.5 ± 2.7 s, respectively. The females treated with DMSO, formalin reactivity was 72.5 ± 3.5 s and animals treated with doses of 10 and 30 mg/kg CBG decreased to 53.1 ± 4.8; 41 ± 6.6 s, respectively.</p> <p>During phase 2, the formalin reactivity of male mice treated with DMSO was 136.3 ± 27.1 s; and animals treated with doses of 10 and 30 mg/kg CBG decreased to 88 ± 26.3; 57.8 ± 15.3 s at dose of 10 and 30 mg/kg, respectively. Phase 2 there is no significant difference to females any doses. 1 mg/kg CBG showed no effect.</p> <p>The CBG extract exhibits antinociceptive action in the pain animal model, with potential hormonal influences on females during the inflammatory phase.</p> <p>Acknowledgments: Apepi, FAPERJ, CNPq and Capes.</p> <p>Keywords: Cannabis, Cannabigerol, Antinociceptive Activity, animal model</p>
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Title	Sympathetic denervation exacerbates zymosan-induced temporomandibular joint hypernociception in rats
Authors	Livia Eduarda Cabrera Corrêa Tales Ferruccio Vicentini Rosin Débora Simões de Almeida Colombari Daniel Breseghello Zoccal Eduardo Colombari Pedro Lourenço Katayama
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Session	20 – Dor e inflamação

Abstract, Ethics Committee Number*, and Keywords	<p>Several lines of evidence have demonstrated that the sympathetic nervous system (SNS) modulates pain and inflammation. In this study, we investigated the role of the SNS in modulating pain in the zymosan-induced temporomandibular joint (TMJ) hypernociception. Experiments were performed on adult male Holtzman rats (~300 g). Experimental procedures were approved by the Ethical Committee in Animal Experimentation of the Araraquara School of Dentistry, São Paulo State University (protocols 17/2019 and 06/2023). In the first set of experiments, we employed a retrograde neuroanatomical tracing method and confirmed previous studies showing that the sympathetic innervation to the TMJ originates from the ipsilateral superior cervical ganglion (SCG). Next, we sought to identify if this sympathetic innervation would play a role in zymosan-induced TMJ hypernociception. Zymosan (1 mg in 40ul sterile saline/rat) or sterile saline (40 ul/rat) was injected into the left TMJ of Sham rats and of rats previously (7 days before) subjected to left SCG surgical excision (SCG-X). The mechanical nociceptive threshold of the left TMJ region was assessed 7 days after zymosan or saline injections using an electronic Von Frey equipment. Sham+saline rats (n=12) displayed a TMJ mechanical nociceptive threshold of 150.1 ± 29.5 g whereas Sham+zymosan rats (n=12) showed a significantly lower threshold (89.7 ± 18.6 g, $p < 0.0001$). Surprisingly, SCG-X+zymosan rats (n=9) presented significantly lower values (62.9 ± 18.3 g) compared both to Sham+saline ($p < 0.0001$) and Sham+zymosan ($p < 0.05$). SCG-X+saline group (n=6) displayed a similar threshold (124.9 ± 17.7 g) compared to Sham+saline animals. Our results suggest that the SNS can negatively modulate pain in rats with zymosan-induced TMJ hypernociception. Ongoing research aims to explore different timepoints and the involved mechanisms.</p> <p>Keywords: Pain, Inflammation, Sympathetic Nervous System, Nociception.</p> <p>Financial support: This work was financed in part by the São Paulo Research Foundation (FAPESP; grant #2023/07741-8) and by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) Financial code 88887.890650/2023-00).</p>
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Title	Análise da resposta inflamatória das células dTHP-1 tratadas com hidróperóxido de urato
Authors	<u>Antônio P. S. Pratti</u> Beatriz P. da Silva Flávia C. Meotti
Affiliations	Institute of Chemistry, Biochemical department – University of São Paulo, São Paulo, Brazil.
Session	20 – Pain and Inflammation

Abstract, Ethics Committee Number*, and Keywords	<p>The present study aimed to evaluate the signaling response of differentiated Human Monocytic Leukemia Cells (dTHP-1) to treatment with urate hydroperoxide (HOOU), an oxidant synthesized in the inflammatory oxidative burst by the enzyme myeloperoxidase in the presence of uric acid. It is known that macrophages respond to oxidative stimuli by activating two main inflammatory pathways: the NF-κB and Nrf2 pathways. However, little is known about the effects of HOOU on these pathways. Furthermore, hyperuricemia is related to cardiovascular disease, such as atherosclerosis, then the influence of HOOU on the main inflammatory pathways can be related. Treatments were performed with 1 μg/mL LPS, 5 ng/mL TNF-α, 20 μM tBHQ, 50μM HOOU, or mobile phase (FM). The cells content were separated into two enriched cytosolic and nuclear fractions, to evaluate the translocation of the transcription factor by Western-Blot. After that, the effectiveness of the methodology was evaluated. The results show effectiveness in enriching the nuclear fraction, with almost total absence of Lamin B1 (nuclear protein) in the cytosolic fraction. However, the presence of β-actin (cytosolic protein) in the nuclear fraction was observed, indicating the need to add more washes to the protocol. Finally, the translocation of NF-κB and Nrf2 was evaluated and the results showed that, in cytosolic fraction, there was more Nrf2 in the HOOU group than the control group. Furthermore, for the nuclear fraction, a band for NF-κB was observed only in the positive control and HOOU groups. These results suggest that HOOU plays a role in the inflammatory pathways by modulating the translocation of NF-κB and NRF-2, but more research is needed to improve the hypothesis. Thus, future steps for the project include: PCR analysis of the genes that are regulated by those transcription factors and ELISA assay for cytokines regulated by NF-κB.</p> <p>Keywords: Inflammation, Uric acid, atherosclerosis, NF-κB, urate hydroperoxide</p>
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Title	Influence of lipid mediators derived of omega-3 fatty acids on degeneration and regeneration of skeletal muscle tissue
Authors	Gabriela Grassmann Roschel ¹ Marcela Bego Nering de Sousa ¹ Douglas Souza Oliveira ² Eduardo Purgatto ³ Vanessa Moreira ¹
Affiliations	1 – Pharmacology Department, Federal University of São Paulo, São Paulo, Brazil. 2 – Center of excellence in new target discovey (CENTD), Butantan Institute, São Paulo, Brazil. 3 – Pharmaceutical Sciences Faculty, University of São Paulo, São Paulo, Brazil.
Session	Dor e inflamação

Abstract, Ethics Committee Number*, and Keywords	<p>Lipid mediators derived from omega-3 fatty acids (LM3) as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) exhibit anti-inflammatory properties. LM3 are synthesized by enzymatic pathways that can undergo acetylation by acetylsalicylic acid (ASA). However, the potential proregenerative effect of LM3 on skeletal muscle are unknown. Male Swiss mice (n=4) (6 weeks/CEUA5123050421) received intraperitoneal (i.p.) injection of fish oil nanoemulsion (FON) (6µg/g DHA; 10µg/g EPA/500µL/animal), or 0.9% saline (Sal) for 7 days (d). Then, they received injection of <i>Bothrops asper</i> snake venom (Bav) (2.5mg/kg/50µL) in left gastrocnemius muscle, or Sal into contralateral muscle. A distinct group received acetylsalicylic acid (ASA) via i.p. (117mg/kg/500µL) every 3d. Gastrocnemius were collected at 24h, 3, 7, and 28d post-Bav injection for quantification of interleukin (IL)-1β, IL-6, IL-15, irisin (IRI), leukemia inhibitory factor (LIF), and osteonectin (OST) using commercial kits. Expression of myogenic transcription factors Myf5, MyoD, and Myf6 were assessed by western blot, and plasma creatine kinase activity (CK) was measured by a commercial kit. Data showed a reduction in IL-1β post-Bav injection at 24h, 3 and 7d in the FON-treated groups by 23%, 46%, and 78%, respectively (p<0.05). IL-6 was reduced at 24h post-Bav injection in both FON (47%) and FON+ASA (48%) groups (p<0.05), and at 3d post-injection by 47% and 74%, respectively (p<0.05), compared to Bav alone. LIF and IRI were reduced by 32% and 68% at 24h post-Bav in both FON and FON+ASA groups, respectively, compared to Bav group (p<0.05). In contrast, high levels of OST, MyoD, and Myf5 (30%, 43% and 42%, respectively) were obtained in FON and FON+ASA groups at 24h and 3d, when compared to Bav-alone (p<0.05). CK level was decreased at 3d post-Bav in both FON and FON+ASA (40%) compared</p>
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to Bav alone ($p < 0.05$). The findings indicate that LM3 derived from EPA and DHA have the potential to enhance muscle regeneration.

Key words: Tissue regeneration; omega-3 fatty acids; myotoxicity.



Title	Virtual screening of bioactivity and molecular docking simulations of carvacrol analogs on cholecystokinin receptors aiming at modulation of neuropathic pain
Authors	Wesley Lyeverton Correia Ribeiro Michelli de Medeiros Bueno Matheus Nunes da Rocha Emmanuel Silva Marinho
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Session	Pain and Inflammation

Abstract, Ethics Committee Number*, and Keywords	<p>The aim was to perform a virtual screening of bioactivity and molecular docking simulations of carvacrol analogs on cholecystokinin (CCK) receptors aiming at modulating neuropathic pain. Thus, a virtual screening of carvacrol (CV) bioactivity and its synthetic derivatives, CV aldehyde, Carvacrol Schiff Base (BSCV), and Cu(II)-Schiff Base Complex (BSCu²⁺), which exhibit known biological activity against <i>Mus musculus</i> and <i>Rattus norvegicus</i>, was conducted. Subsequently, an absorption, distribution, metabolism, and excretion (ADME) study; analyses of molecular lipophilicity potential; MPO analyses and lipophilicity-metabolism efficiency (LipMetE); and prediction of metabolism site and acute toxicity for <i>M. musculus</i> and <i>R. norvegicus</i> were carried out. Finally, molecular docking simulations were performed targeting CCK receptors. Virtual assessment of the structures revealed that synthetic CV analogs have promising potential for treating neural disorders due to their structural similarity to other compounds affecting CCK1 and CCK2 receptors in the central nervous system (CNS). Furthermore, ADME analysis, focusing on MPO, indicated a comprehensive distribution of these substances throughout the CNS, especially BSCu²⁺, which showed a more favorable correlation between pharmacokinetic properties and safety of activity in the CNS. BSCV exhibited high passive permeability, extensive brain distribution volume, and a more positive pharmacodynamic interaction with the CCK2 receptor, making it promising for chronic pain treatment. Thus, it is proposed that synthetic carvacrol analogs, BSCu²⁺ and BSCV, potential CCK2 receptor antagonists, may compete with CCK, enhancing the opioid effect in pain management. These results open new perspectives for understanding the potential mechanisms of action of carvacrol analogs in modulating neuropathic pain, providing insights for validation studies in animal models.</p> <p>Keywords: Virtual screening. Pain management. Cholecystokinin receptors</p>
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Title	Effect of the tea prepared from <i>X. americana</i> barks on the hypernociception and degeneration of articular cartilage
Authors	Bianca Feitosa Holanda ¹ Francisco Glerison da Silva Nascimento ¹ Jessica Kelly Alves Ferreira ¹ Carlos Jorge Maciel Uchoa Gadelha ¹ Mário Rogério Lima Mota ² Maria Gonçalves Pereira ¹ Rondinelle Ribeiro Castro ¹ Ana Maria Sampaio Assereuy ¹
Affiliations	1- State University of Ceará, ISCB, Fortaleza- CE, Brazil. 2- Federal University of Ceara
Session	Pain and inflammation, banner.

Abstract, Ethics Committee Number*, and Keywords	<p>Osteoarthritis is degenerative and disabling, its treatment is ineffective and has a variety of adverse effects. <i>Ximenia americana</i> bark tea is used to treat pain. Experimentally, it has been demonstrated that such barks have analgesic and anti-inflammatory effects and have reduced toxicity. The present study aimed to evaluate the effects of <i>X. americana</i> bark tea (DC.Xa) on hypernociception and degeneration of articular cartilage in osteoarthritis induced by monoiodoacetate (MIA) in rats. animals were treated with DC.Xa (100 mg/kg, p.o.) 24h and daily after the induction of osteoarthritis by MIA (1 mg/ 25 µl, intra-articular) to evaluate hypernociception and histopathology of articular cartilage. To evaluate the mechanism of action, treatment was carried out 1h and daily for 24h/24h until the 6th day after the induction of hypernociception by TNF-α (5 nM) and 1h and 24h after the induction of hypernociception by capsaicin (1 µg). Statistical analysis was performed by ANOVA, followed by Bonferroni. The protocols were approved by the Ethics Committee for the Use of Animals of the State University of Ceará (n° 08146900/2022). DC.Xa reduced joint damage scores (71%, 5 ± 1.7 vs. MIA: 17.5 ± 2.5 OARSI scores). Elevated MIA-induced mechanical threshold from 1st to 6th (24%, 208.1 ± 6 vs. MIA: 159.2 ± 7 AUC), from 7th to 13th (36%, 358.4 ± 14 vs. MIA: 230.7 ± 12 ASC), from the 14th to the 20th (39%, 412.7 ± 33 vs. MIA: 253.3 ± 21 ASC) and from the 21st to the 28th (68%, 534.0 ± 35.43 vs. MIA : 313.6 ± 34.43) days after induction, the mechanical threshold induced by TNF-α from the 1st to the 4th day (39%, 72 ± 3 vs. TNF-α: 44 ± 5 AUC) and the mechanical threshold induced by capsaicin from the 2nd to 24th hours (49%, 153.6 ± 4 vs. capsaicin: 103.3 ± 7 AUC). It is concluded that DC.Xa has an antinociceptive effect and protects articular cartilage in MIA-induced osteoarthritis in rats.</p> <p>Keywords: Sertão plum. Osteoarthritis. Histopathology.</p>
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Title	<i>Ximenia americana</i> barks decoction and polysaccharide fraction reduces inflammatory parameters and histological scores in rats Arthritis Model induced by Zymosan
Authors	Gabriela Fernandes Oliveira Marques ¹ Francisco Glerison da Silva Nascimento ² Paulo Goberlânio de Barros Silva ¹ Maria Gonçalves Pereira ⁴ Rondinelle Ribeiro Castro ³ Ana Maria Sampaio Assreuy ²
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Session	Dor e Inflamação

Abstract, Ethics Committee Number*, and Keywords	<p>The therapeutical approach of the Rheumatoid arthritis (RA), severe arthropathy that affects 0.5-1% of world population, includes anti-inflammatory/analgesic drugs and implies high costs. <i>Ximenia americana</i> or “ameixa-do-sertão”, used popularly in Northeast Brazil to treat inflammatory and painful disorders, contains a diversity of bioactive compounds (including polysaccharides). Previous studies showed that barks decoction and other extracts inhibits inflammatory parameters in an classic and clinical inflammation models. This study investigated the anti-inflammatory effect of decoction and polysaccharides from the barks of <i>X.americana</i> in arthritis model induced by zymosan. The decoction (30, 100 e 300 mg/kg;v.o.) or the polysaccharide fraction-FIId (1 mg/kg; i.v) were administered to Wistar rats 3 h after or 1 h before arthritis induction (Zymosan: 500µg; i.a.), respectively. Inflammatory parameters were evaluated at 6 hours post induction (CEUA/UECE No. 4153018/2018). Zymosan reduced the rats paw withdrawal (zy: 11.4 ± 0.5 g), increased by decoction at 30 mg/kg (1.8 x) and 100 mg/kg (1.7 x). Zymosan increased joint edema (3.0 ± 0.1 mm), reduced by decoction at 100 mg /kg (1.6 ± 0.1 mm). Leukocyte influx caused by Zymosan (54525 ± 8673 cells) was reduced by 82% at 30 mg/kg (9691 ± 3224 cells), 83% at 100 mg/kg (9032 ± 1442 cells) and 82% at 300 mg/kg (9865 ± 1333 cells). FIId (1 mg/kg) also increased the paw withdramal (31.6 ± 3.1 g vs. zy:20.0 ± 0.7 g); reduced joint edema (1.6 ± 0.2mm vs. zy: 2.8 ± 0.1 mm) and reduced neutrophil migration (15008 ± 5794 cells vs. 57,300 ± 14,659 cells). Polymorphonuclear infiltrate, edema signs, tissue necrosis and joint structures disorganization they were reduced in the groups treated with decoction (100 and 300 mg/kg) and FIId (1 mg/kg). Therefore, it can be concluded that decocotion and the polysaccharide fraction inhibit inflammatory parameters of the zymosan-induced arthritis model, confirmed by histopathological analysis.</p> <p>Keywords: Experimental arthritis. Medicinal plants. <i>Ximenia americana</i>.</p>
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Title	Impact of grape pomace extract on healing and inflammation in Danio rerio
Authors	Veiga, Natan Luz, Karine Narloch, Sally Douglas Marcon , Evelyn Cristina Moreira , Victória Bettio Centa , Ariana Locatelli , Claudriana
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Session	Pain and Inflammation

Abstract, Ethics Committee Number*, and Keywords	<p>The utilization of agricultural by-products rich in bioactive compounds for pharmaceutical applications represents a pivotal approach to sustainable development. Grape pomace, a residue from wine production, is notably rich in phenolic compounds, known for their antioxidant and anti-inflammatory properties. This study investigated the potential pharmacological applications of hydroalcoholic extract from Cabernet Franc grape pomace in inflammatory and healing processes using Danio rerio as an animal model due to their genetic resemblance to humans and effective absorption of compounds in aquatic environments. Fish were divided into groups receiving treatments with lipopolysaccharide (LPS), LPS combined with indomethacin (0.08 or 0.16 ppm), or LPS and grape pomace extract (0.08 or 0.16 ppm). Healing efficacy was assessed with a biodegradable film containing the extract applied at a concentration of 1mg/ml. The results indicated a reduction in IL-1β and IL-6 levels by approximately 45% and 50%, respectively, showcasing the extract's anti-inflammatory properties. Histopathological analysis revealed a 40% decrease in peritoneal polymorphonuclear infiltrates, and phenolic content analysis highlighted high levels of tannins and flavonoids. Moreover, lesion sizes in treated fish were significantly reduced by 40%, emphasizing the extract's potential to enhance healing. Given these promising outcomes, further studies are needed to explore the underlying mechanisms and possible applications in human health. Ethics Committee Approval Number: 005.2022.</p> <p>Acknowledgments: FAPESC n° 54/2022, TO n° 2023TR000885, CNPq Doctoral Scholarship n° 69/2022 and the Research and Internationalization sector UNIARP.</p> <p>Keywords: Grape pomace, anti-inflammatory, healing, Danio rerio, phenolic compounds.</p>
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Title	The polysaccharide-extract of <i>Cissus sicyoides</i> leaves presents anti- inflammatory activity in mice mediated by nitric oxide
Authors	Cléo Vanessa Gomes de Queiroz ¹ Gabriela Fernandes Oliveira Marques ³ Rafael Aires Lessa (Quixada) ² Janaina Serra Azul Monteiro Evangelista ¹ Maria Gonçalves Pereira ² Ana Maria Sampaio Assreuy ¹ (fonte Verdana, tamanho 9)
Affiliations	1 – Instituto Superior de Ciências Biomédicas, Universidade Estadual do Ceará, Fortaleza-CE, Brasil. 2 – Faculdade de de Educação, Ciências e Letras do Sertão Central, Universidade Estadual do Ceará (FECLESC-UECE), Quixadá-CE, Brasil. 3 - Centro Universitário Christus-UNICHRISTUS, Fortaleza-CE, Brasil.
Session	Dor e inflamação

Abstract, Ethics Committee Number*, and Keywords	<p><i>Cissus sicyoides</i> leaves are used in the Brazilian folk medicine to treat inflammatory processes and the antinociceptive activity of its aqueous extract has been demonstrated. This study aimed to obtain the polysaccharide-rich extract (PE-Cs) leaves and to investigate its anti-inflammatory effect. Mice received PE-Cs before inflammatory stimuli. The experimental protocols were performed following the guidelines of the Brazilian College of Animal Experimentation (COBEA) and Animal Care and Use Committee of the State University of Ceará (n° 5748564/2015), Fortaleza, CE, Brazil. Paw edema were induced by carrageenan or L-arginine and analyzed by plethysmometry/histopathology. Peritonitis and naive isolated macrophages incubated with PE-Cs were stimulated with carrageenan and fluids evaluated for inflammatory markers. PE-Cs (0.86% yield; 25.4% total carbohydrates, including 9.8% uronic acid) at 0.01 mg/kg inhibited the carrageenan-induced edema by (58%) and L-arginine (73%). Histological analysis showed reduction of leukocyte infiltration and hemorrhage. In the peritoneal fluid, PE-Cs reduced neutrophil migration (92%) and NO₂-/NO₃- (20%). In vitro, PE-Cs reduced NO₂-/NO₃ (30%) released by activated macrophages. PE-Cs exhibit anti-inflammatory activity mediated by nitric oxide in mice models of acute inflammation.</p> <p>Keywords: Acute inflammation; Oxidative stress; “Cipó pucá”, Plant polysaccharide; Carrageenan; Macrophage.</p>
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Title	Effect of Δ^3-carene on the leukocyte recruitment in acute inflammatory response
Authors	Paloma Kênia de Moraes Berenguel Lossavaro João Victor Ferreira Mila Marluce Lima Fernandes Joyce dos Santos Lencina Iluska Senna Bonfá Dalila dos Santos Lencina Mônica Cristina Toffoli-Kadri Saulo Euclides Silva-Filho
Affiliations	Laboratory of Pharmacology and Inflammation, Federal University of Mato Grosso do Sul, Campo Grande, MS, Brazil
Session	Session 20 – Inflammation and pain

Abstract, Ethics Committee Number*, and Keywords	<p>Essential oils, have been used in the treatment of inflammatory conditions, and the constituents of these oils are related to the biological activities of these natural products. The Δ^3-careno (CAR) is a monoterpene present in the pine essential oil. The aim of this study was to evaluate the effect of CAR on the leukocyte recruitment in zymosan-induced peritonitis model in mice. Male Swiss mice (n=5 animals/group) were orally pretreated with CAR (25, 50, or 100 mg/kg), indomethacin (15 mg/kg, reference drug) or vehicle. One hour later, all of the animals received an intraperitoneal zymosan injection (1 mg/cavity) or saline. Six hours after the animals were euthanatized, the cells present in the peritoneal cavity were harvested by introducing 1 mL of phosphate-buffered saline. Counts were then performed in total and differential cells. The zymosan injection promoted an increase of leukocytes count in the peritoneal cavity ($571.5 \pm 127.8 \times 10^6$ cells/cavity) compared with the naive group (saline) ($151,2 \pm 151,2 \times 10^6$ cells/cavity). The CAR treatment at doses of 25, 50 and 100 mg/kg significantly reduced the leukocyte recruitment (56, 68 and 71%, respectively), compared to control group. The decrease in the number of leukocytes was mainly attributable to a reduction of the number of polymorphonuclear. CAR demonstrated anti-inflammatory activity during acute inflammatory response. The experimental protocols were approved by the Ethical Committee on Animal Experimentation of the Federal University of Mato Grosso do Sul (protocol number 1.288/2023).</p> <p>Keywords: acute inflammation; terpenes, leukocyte migration; natural products.</p>
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Title	Anti-inflammatory and antinociceptive activities of trispine inhibitor isolated from <i>Inga laurina</i> (sw.) Willd seeds
Authors	¹ Bonfá, I. S.; ² Muller, J. A.I.; ¹ Lossavaro, P. K. M.B.; ¹ Lencina, D. S.; ¹ Lencina, J. S. ¹ Fernandes, M. M. L.; ¹ Toffoli-Kadri, M. C.
Affiliations	1 Faculdade de Ciências Farmacêuticas, Alimentos e Nutrição, Federal University of Mato Grosso do Sul. 2 University of Queensland
Session	Poster/Banner

Abstract, Ethics Committee Number*, and Keywords	<p>Introduction: Serine proteases (SP) develop a defense mechanism for neutrophil, induce recruitment and activation of immune cell, cytokine production and degradation of the extracellular matrix. However, the deregulated activity of SP or their inhibitors can lead to the development of inflammatory diseases. Natural inhibitors of serine proteases (iSP) modulate inflammation. In the Fabaceae family we found iSP in their composition. <i>Inga laurina</i> species has an iSP (ILiT) in its seeds.</p> <p>Objective: Evaluate the effect of ILiT on the inflammatory and nociceptive response. Method: Leukocyte influx, writhing abdominal and formalin assay were used. Male Swiss mice were distributed in the groups: Water (10 mL/kg); Dexamethasone (dexa; 0.5 mg/kg); ILiT (0.3; 3 or 30 mg/kg); and Morphine (5 mg/kg), in the formalin test. The leukocyte influx was evaluated through the total and differential cell counts. The number of abdominal writhing was counted after acetic acid 0,6% injection and the time of paw licking was recorded in two phases, neurogenic and inflammatory. CEUA (1225/2022). Mean±E.P.M, ANOVA and Bonferroni tests (p<0.05). Results: The influx of polymorphonuclear leukocytes induced by carrageenan (1517.0±182.0 cell/mm³) was increased when compared to saline (110.3±25.7 cell/mm³). The ILiT (0.3 mg/kg) treatment reduced this response (74%). In the writhing test, acetic acid induced 132.0±1.6 abdominal writhing. ILiT (53%) and dexa (52%) were effective in the writhing reduction. In the formalin assay, the animals treated with water presented a paw licking time of 249.8 ± 29.3 s. In the second phase, the treatment with morphine reduced this effect (64%). Dexa and ILiT were not effective in the paw licking reduction time. Conclusion: The ILiT demonstrated anti-inflammatory activity and decrease of inflammatory pain. These response might be related to the inhibition of protease of polymorphonuclear cells that degrade components of endothelial cell junctions, favoring the recruitment of leukocytes.</p> <p>Keywords: serine proteases; inflammation; trypsin inhibitor</p>
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Title	Anti-inflammatory and anti-hyperalgesic properties of <i>Handroanthus heptaphyllus</i> (Vell.) Mattos bark hydroethanolic extract in the acute inflammatory response in mice
Authors	Lencina, D. S.; Lencina, J. S.; Lossavaro, P. K. M. B.; Toffoli-Kadri, M. C.; Silva-Filho, S. E.
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Session	Session 20 – Inflammation and pain.

Abstract, Ethics Committee Number*, and Keywords	<p><i>Handroanthus heptaphyllus</i> (Vell.) Matos is a Brazilian native plant, known as ipê-roxo, and it has been used in folk medicine for pain and inflammation. However, literature has not enough evidence about that use. The aim of this study was to evaluate the effects of <i>Handroanthus heptaphyllus</i> (Vell.) Mattos bark hydroethanolic extract (HEHh) on the mechanical hyperalgesia and edema formation in acute inflammation induced by carrageenan (Cg). The experimental protocol was approved by the Ethics Committee in the Use of Animals of Federal University of Mato Grosso do Sul, under registration No 1.218/2022. Male Swiss mice were pretreated with HEHh (80, 160 or 320 mg/kg, orally), indomethacin (5 mg/kg, reference drug), or vehicle (control group) (n = 5–7 animals/group), 1 hour before intraplantar injection of Cg (300 µg/paw) in the right hind paw of all animals, the contralateral paw received saline solution 0.9% injection. The edema formation was determined by the volume difference between the right and left paw, using a digital pletismometer, in the times of 0.5, 1, 2 and 4 hours after Cg injection. For the evaluation of mechanical hyperalgesia, a digital analgesimeter (Von Frey) was used as a pressure transducer, which records the applied force until the moment of paw withdrawal, 3 and 4 hours after Cg injection. The HEHh treatment reduced edema in all doses at times of 30 minutes, and 4 hours. Also, the effect was observed at times of 1 hour, (at doses of 80 and 160 mg/kg) and 2 hours (at a dose of 80 mg/kg) after Cg injection. The HEHh treatment reduced hyperalgesia at a time of 2 hours (at doses of 80, and 160 mg/kg), and 4 hours (at doses of 80, and 320 mg/kg) after Cg injection. In conclusion, HEHh extract showed anti-inflammatory and anti-hyperalgesic activities during the acute inflammatory response.</p> <p>Keywords: <i>Handroanthus heptaphyllus</i>; acute inflammation; edema formation; hyperalgesia.</p>
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Title	Nox contributes to <i>S. sanguinis</i> resistance to complement immunity and invasion of cardiovascular endothelial cells
Authors	E.M. Franco ¹ L.A. Alves ² D.C. Bastos ³ T.L.S. Araujo ⁴ R.O. Mattos-Graner ¹
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Session	Immunology

Abstract, Ethics Committee Number*, and Keywords	<p><i>Streptococcus sanguinis</i> is a commensal species of the oral cavity commonly involved in opportunistic cardiovascular infections, through unknown mechanisms. This species expresses nox, which encodes a conserved moonlighting NADH oxidase found on the cell surface in <i>S. pneumoniae</i>. The aim of this study was to investigate the role of Nox in the <i>S. sanguinis</i> capacities to evade blood immunity and to invade endothelial cells. An isogenic nox mutant designated SKnox was obtained in the <i>S. sanguinis</i> strain SK36 by double cross-over recombination with a null allele. Deposition of complement C3b on these strains was quantified by flow cytometry after probing C3b on the surface of serum or PBS-treated strains with FITC-conjugated anti-human C3 IgG antibody. Opsonophagocytosis by polymorphonuclear neutrophils (PMN) isolated from peripheral blood were assessed after challenging these cells with FITC-labeled strains under the presence or not of human serum. Antibiotic-protection assays were used to assess strain invasiveness into primary human coronary artery endothelial cells (HCAEC) after 2h of incubation of HCAEC with 1×10^7 cfu (MOI:1:100) of each strain, which were pre-treated with either human serum, heat-inactivated serum or PBS. The SKnox strain showed 2.8-fold increase in C3b binding ($p < 0.05$) and 37% increase in the frequency of opsonophagocytosis by PMN ($p < 0.05$), as well as impaired invasiveness into HCAEC invasion after treatment with serum ($p < 0.05$), when compared to SK36. These findings indicate that Nox contribute to the <i>S. sanguinis</i> capacities to evade complement-mediated immunity and opsonophagocytosis by PMN, as well as to invasiveness into endothelial cells. Thus, Nox might be a therapeutic target for controlling cardiovascular infections by <i>S. sanguinis</i>.</p> <p>Supported by FAPESP (proc. 2021/13074-9) and CAPES (PhD fellowship)</p> <p>Keywords: complement immunity opsonophagocytosis cardiovascular infections Streptococcus sanguinis</p> <p>Committee number 58699622.4.0000.5418</p>
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Title	Microglia derived extracellular mitochondria modulate neuronal survival and function
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Session	<i>SESSÃO ORAL</i>

Abstract, Ethics Committee Number*, and Keywords	<p>Neuroinflammatory disorders are frequently linked to impaired mitochondrial function in neurons. We aimed to determine if mitochondria transfer from microglia to neurons could impact neuronal survival/function. We employed PhamLyz mice expressing the fluorescent protein Dendra2 in microglial mitochondria (CEUA 6301-1/2023). Timelapse imaging unveiled microglia releasing mitochondria into the extracellular space, either independently or encapsulated in vesicles, through in part by GTPase RhoA/ROCK signalling. Hypothalamic neurons effectively internalize microglia-derived mitochondria, with high uptake under injurious conditions, such as rotenone treatment. Furthermore, co-culture between neurons with microglia on inserts with 0.2µm pores, which prevents mitochondrial transfer, increased neuronal death, showing a crucial role of microglia-derived mitochondria in neuronal survival and indicate mitochondrial uptake likely occurs through tunnelling nanotube formation and endocytosis. To discern if specific microglial activation profiles induce neuroprotective mitochondrial release, microglia were stimulated with LPS (inflammatory stimulus) or Mdivi-1 (Drp1 inhibitor) + M1 (fusion promoter) before co-culture. Mitosox labeling revealed increased superoxide production in rotenone-treated neurons, which was intensified by culture with LPS- activated microglia, while Mdivi-1-treated microglia reduced superoxide levels in neurons. Microglia-derived mitochondria enhanced neuronal oxidative phosphorylation. Also, neurons exposed to isolated mitochondria from microglia expressed elevated levels of AgRP and TNFα. In vivo, neurons internalized microglia-released mitochondria, particularly in mice fed a high-fat diet. These findings suggest that microglia influence neuronal survival and function through the transfer of extracellular mitochondria, presenting a potential therapeutic avenue for neurodegenerative and metabolic disorders.</p> <p>Keywords: Microglia, neuron, neuroinflammation, mitochondria</p>
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Title	Could oleic fatty acid improve the aspects related to psoriasis on an experimental mouse model?
Authors	Baccarin, I. B ¹ , Burger, B ¹ , Sagiorato, N.R ¹ , Rodrigues, H.G ¹
Affiliations	1- Faculdade de Ciências Aplicadas, Universidade Estadual de Campinas, Limeira, São Paulo, Brasil
Session	21- Imunologia

Abstract and Keywords	<p>The skin is one of the largest organs, it is a physical barrier and has an active immune system, which could be exacerbated leading to chronic inflammatory skin diseases, such as psoriasis. The pathology of psoriasis involves hyperproliferation of keratinocytes with expressive differentiation, epithelial hyperplasia and parakeratosis. A critical factor is the interaction between immune cells and cytokines, especially the TNF-α/IL-23/IL-17 axis. Psoriasis affects thousands of people worldwide and has no cure yet. Fatty acids have been a successful strategy for the treatment of inflammatory diseases. However, there are few studies that have investigated the effects of oleic acid (OA) on psoriasis. To psoriasis induction, 60 mg of Ixium® (5% of IMQ) was applied on shaved back skin of C57BL/6 mice from day 1 to day 5 (CEUA: 5562-1/2020). At the same time, we performed oral administration of pure OA. The back skin was collected for histological analysis with samples stained with H&E and, to evaluate changes in gene expression by RT-qPCR. Data were analysed by one-way ANOVA or two-way ANOVA followed by post-hoc Bonferroni with $p < 0.05$.</p> <p>The supplementation with OA reduced the percentage of skin thickness ($119\% \pm 6$ (IMQ); $103\% \pm 6$ (IMQ+OA) $n=5$/group). The histological quantitative analyses revealed that OA supplementation led to a significant reduction in the number of layers and epidermis area (in relative unit) comparing IMQ and IMQ+OA. By RT-qPCR we observed that IMQ increased IL-23, IL1-β, IL-23p19, IL-12p40 and IL-22 in comparison to control mice, and administration of OA increased IL-12p40 in comparison to IMQ group ($2,82 \pm 0,42$ (IMQ); $3,92 \pm 0,65$ (IMQ+OA) $n=5$/group). No other alterations were observed.</p> <p>So far, our results suggest that oral supplementation with oleic acid reduces skin thickness, acting mainly on keratinocytes in IMQ mouse model of psoriasis. PSORIASIS; FATTY ACIDS; INFLAMMATION. Funding: FAPESP, CNPq, CAPES finance code 001.</p>
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Title	Participation of the cholinergic anti-inflammatory pathway in wound healing in type I diabetic animals supplemented with linoleic acid
Authors	Sophia Franchi Bruzetti ¹ Roberta Nicolli Sagiorato ¹ Hosana Gomes Rodrigues ¹
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Session	21- Immunology

Abstract, Ethics Committee Number*, and Keywords	<p>Diabetic wounds present greater infiltration of pro-inflammatory cells stimulating chronic inflammation. Cholinergic anti-inflammatory pathway is a central nervous mechanism that regulates peryphery inflammation. Our previous results showed that oral administration of linoleic acid (LA) accelerates tissue repair in diabetic mice compared to unsupplemented mice. So, we decided to investigate if the beneficial effects of LA were dependent of the cholinergic anti-inflammatory pathway. For this, we used homozygous mice for deletion of the alpha7 subunit of the nicotinic acetylcholine receptor (Chrna7^{-/-}) and their wild-type (WT) controls. Diabetes was induced by intraperitoneal administration of streptozotocin 45 mg/kg (CEUA 6058-1/2022). Ten days after the last injection, pure LA 50µl/day was orally administered to Chrna7^{-/-}DLA group during 5 days. WT and Chrna7^{-/-} groups received water. Then, an area of 1cm² was removed from back skin and the wound closure were analyzed until the 21st day. We did histological analyses (Sirius Red and H&E) and RTqPCR. Data were presented with mean ± standard error of the mean and analysed by ANOVA and Bonferroni tests, p<0.05 was considered significant. We observed no differences on wound closure between unsupplemented groups. From the 3rd to 7th day post-wound, there's a delay in wound healing in Chrna7^{-/-}DLA compared to Chrna7^{-/-}. Wounds from Chrna7^{-/-} were more inflamed than the WT group, as expected. Chrna7^{-/-}DLA reduced the inflammation when compared with Chrna7^{-/-}. Sirius Red analysis revealed that LA supplementation improved the fibers organization compared to control groups. Fibronectin expression was lower in the Chrna7^{-/-}DLA compared to Chrna7^{-/-}, on 7th day. Our results suggest that the cholinergic anti-inflammatory pathway isn't essential for skin wound healing in the diabetes context and the beneficial effects of LA supplementation aren't related to this pathway.</p> <p>Keywords: DIABETES, LINOLEIC ACID, WOUND HEALING Funding: FAPESP, CNPq, CAPES finance code 001</p>
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Title	Butyrate demonstrates immunomodulatory effects by attenuating psoriatic inflammation in murine models
Authors	Roberta Nicolli Sagiorato ¹ Beatriz Burger ¹ Vitória Christina Gaspar ¹ Isabela Buquio Baccarin ¹ Hosana Gomes Rodrigues ¹
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Session	21 - Imunologia

Abstract, Ethics Committee Number*, and Keywords	<p>Psoriasis presents as a persistent condition marked by T cell activation, inflammatory mediator release, and excessive keratinocyte proliferation. Butyrate is under investigation for its potential therapeutic role, particularly in immune-related disorders. However, its impact on psoriasis remains unclear. This study aimed to explore the effects of butyrate on experimental psoriasis.</p> <p>Male C57BL/6 mice were induced with chronic psoriasis using imiquimod cream for 6 consecutive days, with reapplication from day 21 to 26. Mice received either 150 mM butyrate in their drinking water or 3 g/kg tributyrin via gavage from day 7 to 28. In another model, psoriasis was induced for 5 days with concurrent butyrate treatment. Measures included skin thickness, body weight, food and water intake, spleen size and weight, and gene expression of tissue resident T cell markers. CEUA: 6059-1/2022. Statistical analysis was performed using one-way or two-way ANOVA with Bonferroni's post-test ($p < 0.05$).</p> <p>Imiquimod application replicated psoriatic inflammation, evidenced by increased skin thickness, redness, and peeling on the dorsal skin, alongside spleen area and weight increase, and decreased body weight compared to controls. Butyrate treatment reduced skin thickness (n: 10; C: 0,27 mm; IMQ: 0,40 mm; IMQ+but: 0,36 mm) in both chronic and acute psoriasis models. By the 28th day of the protocol, CD103 gene expression decreased in butyrate-treated animals (n: 10; C: 1,01; IMQ: 1,32; IMQ+but: 0,66). Tributyrin also displayed some efficacy in mitigating psoriatic inflammation, albeit less prominently.</p> <p>In conclusion, butyrate and tributyrin attenuated psoriatic inflammation, suggesting butyrate's immunomodulatory potential in psoriasis.</p> <p>Keywords: psoriasis, inflammation, immunology, butyrate, tributyrin</p> <p>Funding: FAPESP, CNPq and CAPES (financial code 001)</p>
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Title	Effects of oral administration of inulin-rich diet during experimental chronic psoriasis in mice
Authors	Vitória Christina Gaspar Roberta Nicolli Sagiorato Hosana Gomes Rodrigues
Affiliations	Universidade Estadual de Campinas, Faculdade de Ciências Aplicadas, Limeira, São Paulo, Brazil
Session	21- Immunology

Abstract, Ethics Committee Number*, and Keywords	<p>Psoriasis is an autoimmune disease without cure. The pathology is characterized by hyperproliferation of keratinocytes, hyperplasia and parakeratosis. The disease has an immunological axis that involves the cytokines TNF-α/IL-23/IL-17. Psoriasis affects millions of people around the world, and unconventional treatments are being addressed in the literature, among of them fibers and fatty acids. The aim of the present study was to investigate the effects of high-fiber diet (10% of inulin) on psoriasis manifestation. To induce psoriasis, 60 mg of Ixium® (5% of IMQ) was applied to the back of C57BL/6 mice (CEUA: 6259-1/2023) in two cycles to mimic chronic psoriasis. Animals were treated topically with imiquimod for 6 days. Oral treatment with the diet occurred between the 7th and 26th day. The application of imiquimod was returned on the 21st day and continued until the 26th day. Skin thickness, water and feed consumption, and body weight of the animals were evaluated. Data were analysed by one-way or two-way ANOVA and Bonferroni tests, $p < 0.05$ was considered significant. Skin thickness of the IMQ and IMQ + inulin groups remained significantly increased from the 3rd to 6th and 20th to 28th days, respectively, in relation to group C. In addition IMQ + Inulin group presented reduced thickness compared to IMQ group on days 7,8,9,10,14,16 and 17. Water consumption did not differ among the groups. The IMQ + inulin group reduced feed intake compared to groups C and IMQ. On the other hand, the IMQ group increased feed consumption compared to group C. Group C showed increased weight compared to IMQ and IMQ + Inulin from the 2nd to the 6th day, and also on the 7th day when compared to IMQ. The ingestion of inulin-rich diet did not present relevant effects on psoriasis model.</p> <p>Keywords: PSORIASIS. FATTY ACID. INULIN. INFLAMMATION.</p> <p>Funding: FAPESP, CNPq, CAPES (Finance code:001).</p>
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Title	Dimethyl Fumarate prevents periodontitis progression in an Nrf2 activation-dependent manner. A study in rats
Authors	Andréa Cardinali Romanelli Rosa Vitória Bonan Costa Mariely Araújo de Godoi Angelo Constantino Camilli Bruna Carolina da Silva Isabela Rinaldi Gomes Nogueira Morgana Rodrigues Guimarães Stabili
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Session	Imunologia

Abstract, Ethics Committee Number*, and Keywords	<p>DMF (Dimethyl Fumarate) is a compound used to treat inflammatory diseases, however, its effects on periodontitis have not yet been evaluated. Its biological activities appear to be mediated largely by activation of Nrf2. The aim of this study is to evaluate the effect of DMF on the progression of periodontitis in a model of periodontitis induced in rats, and to investigate the participation of Nrf2 in these effects. Periodontitis was induced by ligature placement around the mandibular first molars. Concurrently, animals (n=10 per group) received DMF (50, 100, 150mg/kg), Nrf2 inhibitor (15 mg/kg); Nrf2 inhibitor + DMF (50mg/kg); or distilled water (positive control group) via intragastric gavage for ten days. A negative control group received only distilled water, without ligature placement. Following euthanasia, the mandibles were evaluated using microcomputed tomography (μCT) for bone loss, stereometric analysis for inflammatory infiltrate and extracellular matrix. Gingival tissue was assessed for biological mediators by RT-qPCR (Superoxide dismutase (SOD) and Catalase), ELISA (TNF) and Malonaldehyde (MDA), by TBARS method). 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, reduced cellular infiltrate, inhibited MDA and TNF levels in gingival tissues, and increased SOD and Catalase expression. Although all doses of DMF showed an anti-osteolytic effect, the lowest dose of the compound (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. DMF has a promising role in preventing periodontitis and is dependent on Nrf2 activation. Keywords: Periodontitis; Dimethyl Fumarate; Nrf2. Ethics Committee number: 12/2021.</p>
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Title	Assessing Eryptosis and intracellular oxidative stress on erythrocytes from peritoneal dialysis patients
Authors	V. A. B. dos Santos, Y. Daitchman, E. S. Dias, J. B. Monte-Alegre, B. A. K. V. Spitzenbergen, G. Bohnen, A. N. Moreno-Amaral
Affiliations	PUCPR
Session	Biology and Kidney Diseases Imunology

Abstract, Ethics Committee Number*, and Keywords	<p>Renal anemia is common in patients with end-stage kidney disease (ESKD). During peritoneal dialysis (PD), red blood cells (RBC) from ESKD patients undergo physiological stress, which may result in premature death or eryptosis. This physiological stress is mainly caused by reactive oxygen species (ROS) since PD may filter essential antioxidants like glutathione (GSH). We aimed to evaluate the intracellular oxidative stress and eryptosis in RBC obtained from PD patients. Blood samples were collected from healthy controls (HC, n=14) or PD patients (n=22), and after RBC isolation, cells were labeled with Annexin-V for Phosphatidylserine (PS), Fluo-4AM for intracellular calcium (icCa²⁺), DCFH-DA for ROS, and Thiol tracker for GSH assessments. PS exposure was higher on RBC from PD patients (10.4±4.5) than on the HC (3.6±2.4), while icCa²⁺ didn't show relevant changes between the two groups. The oxidative stress was evaluated by ROS and GSH levels in RBC from the groups. In PD, ROS was increased (88.3±7.5) compared to the HC (10.3±3.1), while GSH had inverse results, with higher GSH levels in HC (37.2±7.3) compared to PD (12.3±5.1). Therefore, PD patients exhibit elevated eryptosis, partially attributed to heightened intracellular ROS levels, which can be related to renal anemia.</p> <p>Ethics Committee Number: 5.587.701</p> <p>Keywords: peritoneal dialysis, oxidative stress, eryptosis, erythrocytes, chronic kidney disease.</p>
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Title	Exploring thymic involution with aging and metabolic disease at a single cell resolution
Authors	Niedson Correia de Lima Junior ¹ , Leticia de Souza Figueiredo ¹ , Raissa Guimarães Ludwig ¹ , Gabriel Palermo Ruiz ¹ , Deisi Braga Shimo ¹ , Leticia Grillo Guimarães Pereira ¹ , Henry David Mogollon Garcia ¹ , Tereza Cristina Minto Fontes Cal ¹ , Alessandro dos Santos Farias ¹ , Marcelo Alves da Silva Mori ¹
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Session	Session 21- Immunology

Abstract, Ethics Committee Number*, and Keywords	<p>Introduction: Thymic involution is a natural aging process characterized by decreased thymic epithelial cells (TECs) number and increased adipocyte accumulation within thymus. Metabolic dysfunction such as during obesity can accelerate it. Beyond the energy status, microRNAs (miRNA) can also drive this process. Thus, we aimed to investigate the effects of high-fat diet (HFD)-induced obesity, aging, and partial lipodystrophy induced by fat-specific deletion of the miRNA processing enzyme Dicer (AdicerKO) on thymic involution in mice at the single cell level.</p> <p>Methods: To explore the interplay between age, lipodystrophy, and obesity on thymus cellularity, we studied AdicerKO and wild type (WT) mice at different ages (1 month, 6 months, and 18 months). For the 6- and 18-month age groups, we also had mice on a HFD since 2 months of age. Histological analyzes and single-nucleus RNA sequencing (snRNAseq) were performed in the thymus of these animals.</p> <p>Results: At 6 months of age, chow-fed ADicerKO (lipodystrophy model) and HFD-fed WT animals presented a slight anticipation of thymic involution, while HFD-fed ADicerKO mice (severe lipodystrophy model) exhibited marked thymic involution, with the thymus mass being almost entirely replaced by adipocytes. Through snRNAseq, we observed an increase in adipocytes and a decrease in TECs in the thymus of these animals. Additionally, we noted a marked increase with aging of cells we termed "club- like cells", which exhibited high expression of secretoglobulins. Club cells, primarily characterized in the lung, had not been described in the thymus yet, and we aim to validate them.</p> <p>Conclusion: At young adulthood, obesity and lipodystrophy accelerate thymic involution, leading to a more severe phenotype when combined. Under these conditions, thymic involution is characterized by adipocytes replacing much of the thymic area. This was confirmed by snRNAseq data, which also revealed the emergence of "club-like cells" in the thymus with aging.</p> <p>Key words: Thymus; aging; immunometabolism; single-cell</p>
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Title	Identification of genetic contributors to early-onset leprosy: the <i>LRRK2</i> and <i>NOD2</i> perspective
Authors	<ol style="list-style-type: none"> 1. Hallana Nicoli Ribeiro 2. Caroline Riske 3. Isabel Carvalho Oliveira 4. Letícia Fracaro 5. Marcelo Távora Mira
Affiliations	Pontifícia Universidade Católica do Paraná, Curitiba, Brazil
Session	Immunology

Abstract, Ethics Committee Number*, and Keywords	<p>Leprosy is a chronic infectious disease caused by <i>Mycobacterium leprae</i> (<i>M. leprae</i>) exhibiting marked tropism for macrophages of the skin. We employed WGS to explore a family residing in Piauí/Brazil, characterized by a father and grandmother afflicted with leprosy, a mother unaffected and a pair of monozygotic twins who contracted leprosy before the age of 2 (exceptionally rare). Two variants situated within the <i>LRRK2</i> gene (N551K and R1398H), along with one variant located in the <i>NOD2</i> gene (R702W), were found and pinpointed as potential contributors to these extremely early-onset leprosy phenotypes. Studies have reported that the expression of <i>LRRK2</i> is high in peripheral immune cells, particularly in macrophages. Furthermore, mutant <i>LRRK2</i> and the <i>NOD2</i> gene regulate the immune response type and induce autophagy in leprosy patients. To conduct functional assays with these mutations in monocyte-derived macrophages (MDMs) exposed to <i>M. leprae</i> or BCG, we aim in this study to identify individuals in southern Brazil harboring the same genotype as the studied family. The Local Ethical Committee approved the study cohort (5.441.522), which comprised 309 individuals residing in Paraná/Brazil. All peripheral blood samples underwent extraction, quantification, standardization, and genotyping of genomic DNA. 7 and 2 volunteers exhibited an identical haplotype of the twins' grandmother and father, respectively. And 49 displaying the identical haplotypes of the twins' mother. For the European population, the probability of such twin's haplotype occurrence is estimated at 0.0003% and our initiative led to the identification of 1 individual carrying this haplotype. Through the construction of this database, a group of suitable candidates has been successfully identified. These samples will provide valuable insights into the role of variants in the <i>LRRK2</i> and <i>NOD2</i> genes in macrophages suspected to control host susceptibility to leprosy phenotypes.</p> <p>Keywords: Leprosy, Macrophages, Genetic database, Genetic variants, Susceptibility.</p>
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Title	Occupational exposure to pesticides deregulates inflammatory response pathways in breast cancer patients
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Affiliations	1 -Department of Pathological Sciences, Universidade Estadual de Londrina (UEL), Londrina, Brazil, 2- Laboratory of Tumor Biology, Universidade Estadual do Oeste do Paraná, UNIOESTE, Campus de Francisco Beltrão-Paraná, Brazil. 3- Postgraduate Program in Applied Health Sciences, Universidade Estadual do Oeste do Paraná, UNIOESTE, Campus de Francisco Beltrão-Paraná, Brazil. 4- Francisco Beltrão Cancer Hospital, CEONC, Francisco Beltrão-Paraná, Brazil.
Session	21 - Immunology

Abstract, Ethics Committee Number*, and Keywords	<p>Introduction: Breast cancer is a multifactorial disease, which can be related to both genetic and environmental factors. There is a large body of evidence to suggest that exposure to pesticides may contribute to an increased risk of developing cancer. In Brazil, the state of Paraná is among the top five states in pesticide sales nationwide. Its agricultural sector contributes significantly to the Gross Domestic Product (GDP) of the Southwest region, which covers 27 municipalities and is characterized by the extensive use of pesticides. With this in mind, the aim of this study was to evaluate the profile of cytokines in women with breast cancer who had or had not been exposed to pesticides.</p> <p>Methodology: Peripheral blood samples were collected from these 167 women with breast cancer, whether or not they had been exposed to pesticides, in order to quantify the levels of the cytokines IL-1, IL-12, IL-4, IL-17 (5 mL heparinized blood, which was centrifuged at 4,000 rpm for 5 minutes. Plasma aliquots were then frozen at -20 °C until analysis). This study was approved by the Ethics Committee (CAAE 35524814.4.0000.0107). The patients in the study were diagnosed with breast cancer and were treated at the 8th Health Region of the Francisco Beltrão Cancer Hospital, Paraná, Brazil. These women came from 27 municipalities. The tests were carried out at a statistical significance level of 5%.</p> <p>Results: We observed a significant reduction in the levels of all cytokines in women exposed to pesticides compared to those who were not exposed. The Th1 pattern showed a decrease in IL-1 (-28.26±7.48 (p=0.0003)) and IL:12 (-37.90±6.99 (p=0.0001)) levels, while the Th2 pattern showed a depletion of IL-4 (-20.05 ±3.23, p=0.0001), and the Th17 pattern showed a reduction in IL-17 (-90.70±17.78, p=0.0001). Conclusion: This study confirms that exposure to</p>
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pesticides causes significant immunological changes in breast cancer patients. Thus, we highlight that the combination of pesticide exposure and breast cancer results in a systemic reduction of this cytokine in women affected by the pesticide.

Keywords: Breast cancer, cytokines, immune response, pesticides



Title	Study of the metabolic modulation of microglia by leptin in obesity
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Session	[Immunology]

Abstract, Ethics Committee Number*, and Keywords	<p>This work aims to determine how hyperleptinemia modulates microglia in obesity. We used tamoxifen-induced CX3CR1^{CreER/+}:R26^{DsRed/+} animals to control <i>Obr</i>^{fl/fl} CX3CR1^{CreER/+}:R26^{DsRed/+} (CEUA 6086-1/2022) with deletion of <i>Obr</i> only in microglia. We analyzed the effect of HFD intake on hypothalamic inflammation after three days of diet. Control animals had higher food intake without weight or glucose tolerance changes. No expression of pro-inflammatory cytokines in the hypothalamus, liver, and epididymal adipose tissues of animals on HFD. However, in animals that had <i>ObR</i> deletion in microglia, we observed an increase in the expression of the cytokine IL-6 in the hypothalamus, suggesting that <i>ObR</i> participates in the control of hypothalamic inflammation in obesity. To understand the metabolic mechanism of microglia in the process of obesity, we cultivated the animal's primary microglia up to three days after birth. We activated them with leptin (100ng/ml), followed by treatment with palmitate (400μM) for 6 hours. Our findings showed that palmitate directly modulates mitochondrial morphology, reducing their branching. To understand the effect of this morphological change on microglial metabolism, we evaluated OCR and ECAR. Leptin+palmitate treatment was able to increase maximum glycolysis without altering mitochondrial respiration. We also observed that this treatment increased IL-6 secretion, suggesting that leptin makes microglia reactive to a second stimulus. An interesting finding was that deletion of <i>ObR</i> in primary microglia resulted in the loss of leptin treatment-induced effects on IL-6 secretion. We were suggesting that the inflammatory process is <i>ObR</i>-dependent. More research is needed to understand these dynamics. Our partial conclusions are that <i>in vivo</i> the <i>ObR</i> participates in the process of hypothalamic inflammation and <i>in vitro</i> it plays a fundamental role in mitochondrial dynamics and inflammatory processes in obesity</p> <p>Keywords: Lepr; immunometabolism, macrophages;</p>
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Title	SARS-CoV-2 infection alters the expression of purinergic receptors and induces caspase activation in the brain of K18-ACE2 transgenic mice
Authors	Elaine Paiva-Pereira ¹ Letícia Diniz Crepaldi ¹ Milla Souza Pessoa da Silva ¹ Nayara Carvalho Barbosa ¹ Fabiana Cristina Rodrigues ¹ Jairo R. Temezoro ² Christina Maeda Takiya ¹ Dumith Chequer Bou-habib ² Claudio de Azevedo Canetti ¹ Claudia Farias Benjamin ¹ Robson Coutinho-Silva ¹ Luiz Eduardo Baggio Savio ¹
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Session	21 - Imunologia

Abstract, Ethics Committee Number*, and Keywords	<p>The coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The COVID-19 pandemic had catastrophic effects, resulting in approximately 7 million deaths worldwide. In addition to respiratory symptoms, COVID-19 patients may also experience acute neurological complications, which can lead to neuropsychiatric syndromes and cognitive dysfunctions. The mechanisms underlying these neurological symptoms remain poorly understood. Structural components of SARS-CoV-2 can cross the blood-brain barrier and reach the brain, activating pattern recognition receptors (PRRs) in glial cells. The activation of these receptors can induce the secretion ATP that activates purinergic receptors and mediate inflammatory responses. Therefore, we evaluated the effects of SARS-CoV-2 infection on neuroinflammatory parameters and purinergic signaling components in the brain of K18-hACE2 mice. The K18-hACE2 transgenic mice expressing human ACE2 (Ethics Committee: 088/2) were divided into four groups: MOCK; MOCK treated with 50 mg/kg Brilliant Blue G (a specific P2X7 antagonist); SARS; and SARS+BBG. According to the group, the mice received intranasal administration of 50 μL of 2×10^4 PFU of SARS-CoV-2 or Mock solution. Mice were euthanized and tissue samples were collected for further analysis 7 days post-infection. We found an increase in transcript levels for P2Y₂ and P2Y₆ receptors, while the expression of P2Y₁₂ was decreased in the hippocampus and cerebral cortex of infected mice. Furthermore, transcripts for genes related to neuroinflammation were positively regulated, such as IL-1β, IL-6, TNF-α, IBA-1, GFAP, and interferon-related genes in both brain structures. Moreover, the infection increased caspase-1 and caspase-3 activation, indicating activation of cell death. Our initial findings suggest new pathways for understanding mechanisms related to purinergic signaling in the pathophysiology of SARS-CoV-2-induced brain alterations.</p> <p>Keywords: SARS-CoV-2, purinergic signaling, neuroinflammation.</p>
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Title	Decifrando os perfis imunológico e metabólico de macrófagos em proximidade com subtipos distintos de adipócitos
Authors	Matheus Barbosa, Pedro Manoel Mendes de Moraes Vieira, Monara Kaélle Sérvulo Cruz Angelim
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Session	Sessão oral

Abstract, Ethics Committee Number*, and Keywords	<p>Macrófagos, principais células imunológicas presentes no tecido adiposo durante a obesidade, contribui significativamente para a inflamação crônica e resistência à insulina observada em pacientes obesos. Estudos têm evidenciado diferentes fenótipos de macrófagos no tecido adiposo durante a obesidade. Diante disso, nosso objetivo é identificar qual o perfil metabólico e inflamatório de macrófagos quando cultivados com diferentes adipócitos. Inicialmente, temos identificado o perfil de macrófagos da medula óssea de camundongos C57Bl6/J quando cultivados com células 3T3 F442 A diferenciadas em adipócitos, por 24h (CEUA 6301-1). O fenótipo de macrófagos foi analisado por citometria de fluxo, a secreção de citocinas por RT-qPCR e a dinâmica mitocondrial por microscopia. Os dados foram analisados por test-t student, nível de significância $p > 0.05$. Nossos dados preliminares indicam que os macrófagos cultivados com adipócitos exibem um perfil anti-inflamatório: CD301+, Cd11c-, Ly6c+ e Cd11b+, aumento da expressão de arginase e redução da expressão de NOS2 e IL-6, porém com mitocôndrias fragmentadas (redução do volume, da superfície, aumento de esfericidade e número de mitocôndrias pro célula), quando comparados com macrófagos cultivados com a mesma linhagem celular, porém não diferenciada. Os resultados apontam os adipócitos como células capazes de modular o perfil inflamatório e metabólico de macrófagos in vitro. Sabendo disso, seguiremos o projeto verificando o fenótipo dos macrófagos quando em co-cultura com diferentes tipos de adipócitos. Nossos dados contribuem para entendermos como a interação adipocito-macrofago pode modular o perfil inflamatório destas células imunes.</p> <p>Obesidade, tecido adiposo, macrófagos, imunometabolismo</p>
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Title	Impact of p53 on Colorectal Cancer Response to Oxaliplatin and CDK 4/6 Inhibitors
Authors	Letícia Silva Ferraz ¹ , Alana Silva Oliveira Souza ¹ , Julia Souto da Conceição ¹ , Helena Lobo Borges ¹
Affiliations	¹ Federal University of Rio de Janeiro, Institute of Biomedical Sciences
Session	Session 22: Cancer Signaling and Therapy

Abstract, Ethics Committee Number*, and Keywords	<p>Cancer is one of the leading causes of death worldwide and has become a major public health problem due to its increasing incidence in the population and the development of resistance to available therapeutic options. Colorectal cancer (CRC) is the third most common cancer and has a low probability of cure at advanced stages. The TP53 gene, which encodes the p53 transcription factor, acts to control the cell cycle, repair DNA damage and induce apoptosis. In this sense, alterations in the p53 tumor suppressor favor tumorigenesis and are the most frequently found in human tumors. The aim of this study was to evaluate the efficacy of CDK 4/6 inhibitors (abemaciclib and palbociclib) as monotherapy and in combination with the chemotherapy drug oxaliplatin in HCT116 CRC cells with and without p53 silencing. Cell viability was assessed using the Cytation5 multiparametric platform with the Live and Dead kit and the expression of phospho-RB (ser 807/811) and p53 by western blotting. Cell viability curves were performed for both strains and IC10, IC30 and IC50 values were calculated. The p53-silenced strain showed higher values for all the ICs (0.3; 1.5; 3.9 μM respectively) compared to the normal strain (0.07; 0.30; 0.70 μM respectively) for the chemotherapeutic oxaliplatin, and the same was found for the inhibitors. When combining oxaliplatin and the inhibitors, we found combinations with synergistic effects. The expression of phospho-RB (ser 807/811), a cell cycle regulatory protein and the main target of CDK4/6, was decreased when treated with the inhibitor abemaciclib 300 nM (IC50) and palbociclib at 400 nM in the normal lineage, but did not change significantly in the lineage silenced for p53. These data demonstrate the relevance of the presence of genetic alterations in the tumor, showing the decreased sensitivity of these cells to treatment with traditional chemotherapy and in combination with inhibitors. Keywords: Colorectal Cancer, p53 Tumor Suppressor, CDK 4/6 Inhibitors, Oxaliplatin.</p> <p>Keywords: Colorectal Cancer, p53 Tumor Suppressor, CDK 4/6 Inhibitors, Oxaliplatin.</p>
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Title	Evaluating cdk4/6 inhibitor and chemotherapy combinations in colorectal cancer treatment
Authors	Alana Silva Oliveira Souza ¹ Julia Souto da Conceição ¹ Letícia Silva Ferraz ¹ Helena Lobo Borges ¹
Affiliations	¹ Federal University of Rio de Janeiro, Institute of Biomedical Sciences
Session	Session 22: Cancer Signaling and Therapy

Abstract, Ethics Committee Number*, and Keywords	<p>In Brazil, colorectal cancer (CRC) is the second most common and stage IV patients have a low survival rate. Emerging treatments targeting cell cycle enzymes, like CDK4/6 inhibitors, show promise. This study evaluates their efficacy with first-line chemotherapy in CRC. The CDK4/6 inhibitors Palbociclib and Abemaciclib, used to treat advanced breast cancer expressing the Retinoblastoma protein (pRB), the main target of CDK4/6, which is also altered in CRC were used. This study aims to evaluate the efficacy of these inhibitors as monotherapy and in combination with first-line chemotherapy in HCT 116 CRC cells with and without pRB silencing. Cell viability, cell death and autophagy were assessed on a multiparameter platform using the live/dead kit, TUNEL and CYTO-ID, respectively. The cell cycle was assessed by flow cytometry. We found that combinations with different concentrations of chemotherapeutic agents and inhibitors were synergistic. Some combinations were more effective in reducing the number of live CRC cells compared to monotherapy, such as: oxaliplatin 0.6 μM with abemaciclib 35 and 300 nM or palbociclib 400 nM. The combination of oxaliplatin and 35 nM abemaciclib increased cytotoxicity in the cell line with RB silencing, as opposed to the combination with 300 nM. The combination of oxaliplatin and palbociclib increased cytotoxicity only in the cell line not silenced for RB. The combined treatment of oxaliplatin with CDK4/6 inhibitors was not associated with a significant increase in cell death by apoptosis, but was associated with an increase in G1 arrest. Furthermore, the combination of oxaliplatin with CDK4/6 inhibitors inhibited autophagic flux in CRC cell lines. Finally, the combination of oxaliplatin with CDK4/6 inhibitors in a non-tumorous intestinal epithelium cell line did not significantly increase cytotoxicity. These data suggest that the combination therapy of oxaliplatin with CDK4/6 inhibitors is a promising strategy for the treatment of CRC.</p> <p>Keywords: Cyclin-Dependent Kinases (CDK) Inhibitors, Colorectal Cancer, Chemotherapy.</p>
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Title	Synergistic anticancer potential of piplartine-furoxan hybrids: selective cytotoxicity through NO-induced oxidative stress in PC3 cancer cells
Authors	Bruno Zavan ¹ Carolyne B. Braga ² Julio C. Milan ² Matheus A. Meirelles ² Ester Siqueira Caixeta ¹ Ronaldo A. Pilli ² Marisa Ionta ¹
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Session	22 Sinalização e Terapêutica do Câncer

Abstract and Keywords	<p>Combining nitric oxide (NO) donors with anticancer agents represents a promising strategy in cancer treatment, augmenting the effectiveness and specificity of cytotoxic natural products. We synthesized novel hybrids (compounds 10-12) by conjugating piplartine (PPT), a potent cytotoxic compound, with a diphenyl sulfonyl-substituted furoxan moiety, a recognized NO donor. These hybrids underwent rigorous cytotoxic evaluation on diverse human cancer cell lines (MCF-7, PC3, and OVCAR-3), along with non-cancer human cells (MCF10A and PNT2). Compared to their precursors and PPT, hybrids 10-12 exhibited remarkable cytotoxicity. Particularly noteworthy was the heightened sensitivity of PC3 cells to compounds 10 and 12, with IC₅₀ values of 240 nM and 50 nM, respectively, while displaying reduced activity in normal prostate cells (IC₅₀ = 17.8 μM and 14.1 μM, respectively), resulting in impressive selectivity indices of approximately 75 and 280, respectively. Confirmation of NO generation capacity in the hybrids on PC3 cells correlated positively with their cytotoxic effects. Furthermore, the NO scavenger carboxy-PTIO mitigated the growth inhibitory effect of compound 12. Notably, intracellular ROS generation induced by compounds 10 and 12 was observed, with co-administration of the antioxidant NAC protecting against ROS induction by PPT. Mechanistic studies unveiled that compounds 10 and 12 triggered apoptosis in PC3 cells, partially attributed to NO release and increased ROS production. Compound 12 demonstrated superior apoptotic induction, potentially due to elevated NO levels. Additionally, compounds 10 and 12 modulated the expression profiles of key cell cycle regulators, including CDKN1A (p21), c-MYC, and CCND1 (cyclin D1). Overall, our findings underscore the significant therapeutic potential of furoxan NO donors</p>
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tethered to piplartine, particularly compound 12, in cancer therapy, leveraging synergistic NO and PPT effects.

Keywords: piplartine, furoxan, NO, cancer.



Title	Effect of açai consumption (<i>Euterpe oleracea Martius</i>), on inflammatory response in 5-fluorouracil-induced mucositis animal model
Authors	Richard Lyncon da Silva Assis Talita Alves Faria Martins Magalhães Júlia Albuquerque Lélis Melina Oliveira de Souza Karina Barbosa de Queiroz Joana Ferreira do Amaral
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Session	22. Sinalização e Terapêutica do Câncer

Abstract, Ethics Committee Number*, and Keywords	<p>Antineoplastic treatment includes drugs such as 5-fluorouracil (5-FU), which produces intestinal mucositis (MI) as a side effect. MI is characterized by an inflammatory process of the mucosa, with local and systemic clinical repercussions capable of interrupting or prolonging treatment. Consequently, several studies have demonstrated the anti-inflammatory effect of açai (<i>Euterpe oleracea Martius</i>), a fruit typically found in Brazil. This study aimed to investigate the effect of açai pulp on the inflammatory response in an animal model of 5-FU-induced MI. BALB/c mice received (CTL+Açai and MUC+Açai) or did not receive (MUC) pre-treatment with açai pulp at 200g/kg for fourteen days, orally. On the fifteenth day of the experiment, the MUC+Açai and MUC groups received a single intraperitoneal injection of 5-FU (200mg/kg) and were euthanized three (D3) or seven (D7) days after drug administration. At euthanasia, the following were collected: jejunum, ileum, duodenum, and mesenteric lymph nodes for analysis of pro-inflammatory mediators (TNF and IL-1β) and regulators (IL-10). Cytokine measurements were performed using the ELISA immunoassay. The ingestion of açai by MI groups did not alter the production of IL-1β and TNF-α in any evaluated tissue, after data treatment by multiple comparisons using one-way ANOVA and Tukey's post-test. Regarding IL-10, we verified that cytokine concentrations in the jejunum and ileum of the animals were below the sensitivity level of the test. Thus, the results obtained indicate that the inflammation caused by 5-FU in the intestine presents a mechanism different from that caused by increased production of IL-1β and TNF or regulated by IL-10.</p> <p>CEUA/UFOP Approval Protocol: 2016/17</p> <p>Keywords: Mucositis, 5-fluorouracil, Açai, Inflammation.</p> <p>Financial support: UFOP, FAPEMIG (APQ-03385-17)</p>
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Title	Effect of açai consumption (<i>Euterpe oleracea Martius</i>), on antioxidant response in 5-fluorouracil-induced mucositis animal model
Authors	Talita Alves Faria Martins Magalhães Marina Lopes Alexandrino Karine Marlleny Neves Correa Melina Oliveira de Souza Karina Barbosa de Queiroz Joana Ferreira do Amaral
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Session	22. Sinalização e Terapêutica do Câncer

Abstract, Ethics Committee Number*, and Keywords	<p>Intestinal mucositis (MI) caused by antineoplastic chemotherapy is a highly recurrent mucosa inflammatory and oxidant process in patients treated with 5-fluorouracil (5-FU). At present, there is no approved therapy for MI. Although, several studies have demonstrated antioxidant effect of açai (<i>Euterpe oleracea Martius</i>), a typically Brazil fruit. Our group has studied the effects of açai consumption on aspects of mucositis. In this study we aimed to investigate açai pulp effect on antioxidant response in 5-FU-induced MI animal model. BALB/c mice received (CTL+Açai and MUC+Açai) or did not receive (MUC) pre-treatment with açai pulp at 200g/kg for fourteen days, orally. On the fifteenth day of experiment, the MUC+Açai and MUC groups received a single intraperitoneal injection of 5-FU (200mg/kg) and were euthanized three (D3) or seven (D7) days after drug administration. At euthanasia, the following tissues were collected: jejunum for histological analysis, SOD and CAT enzyme activity, total SH group concentration, and total polyphenols; intestinal fluid for sIgA evaluation and MPO enzyme activity. Açai consumption by MI groups promoted resistance and recovery of villous height, restored the villus/crypt ratio, and almost completely regenerated tissue histological appearance. We also observed a reduction in SOD activity, an increase in MPO activity, and sIgA release on D3. MPO enzyme activity was higher and CAT enzyme activity was reduced in animals with mucositis on D3 and D7, respectively. There was no change in total polyphenol content and tissue levels of total SH groups. Our results demonstrated a protective effect of açai pulp components on intestinal injury caused by 5-FU, improvement in antioxidant response, and accelerated tissue repair.</p> <p>CEUA/UFOP Approval Protocol: 2016/17</p> <p>Keywords: Mucositis, 5-fluorouracil, Açai, Oxidative stress, Inflammation.</p> <p>Financial support: UFOP, FAPEMIG (APQ-03385-17)</p>
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Title	Beyond bloodsuckers: exploring the hidden power of <i>ornithodoros brasiliensis</i> tick saliva against tumours
Authors	1- Fernanda Rodrigues Kuhnen 2- Ronaldo Figueira de Oliveira 3- Sheron Campos Cogo 4- Simone Michaela Simons 5- Selene Elifio Esposito
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Session	22- Sinalização e terapêutica do câncer

Abstract and Keywords	<p>The tick <i>Ornithodoros brasiliensis</i>, a member of the Argasidae family, is an ectoparasite that produces toxins by specialized salivary glands, which are effective molecules in modulating coagulation processes, tissue regeneration, itching, and host immune system activation, exhibiting cytotoxic actions. Therefore, the aim of the study is to evaluate the antitumor action of the salivary gland extract (SGE) of <i>O. brasiliensis</i> on Neuroblastoma (NB) cells. The production of the SGE was carried out using ticks fed on domestic rabbit blood. Upon completing blood ingestion, the ticks detached from the rabbits and were carefully dissected in chilled saline solution. SGE was prepared according to the laboratory protocol and then stored in a -80°C freezer. The protein concentration of the SGE was measured using the BCA Protein Assay Kit. In the assays, NB cells from the SH-SY5Y lineage and human fibroblast cells from the HFF-1 lineage, as non tumoral control cells, were cultured. Assays including cytotoxicity, migration, colony formation, apoptosis detection, mitochondrial depolarization, cell cycle, differentiation profile, substrate adhesion, and cell morphology were performed. The SGE exhibited cytotoxic activity starting from a concentration of 3 µg protein/ml, reducing cell viability by 17%, and increased cell death by 8% in apoptosis tests. At higher tested concentrations (50-100 µg protein/ml), a 50% reduction in the percentage of viable cells compared to the control group was observed. The SGE inhibited NB migration and decreased its colony-forming capacity, however, no significant difference was observed in cellular depolarization assays compared to the control group. Based on the findings in this study, it is concluded that the SGE possesses antitumor potential on NB cells, warranting further studies for its better characterization and description.</p> <p>Keywords: Neuroblastoma; Tick; saliva.</p>
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Title	Using nanobody, bioincorporation of non-canonical aminoacids and click chemistry for specific drug delivery to PSMA+ prostatic cancer cell lineage
Authors	Rafael Cerioni Tognato ¹ Carolina Meloni Vicente ² Fábio Dupart Nascimento ³ Jair Ribeiro Chagas ¹ Vitor M. S. B. B. de Oliveira ¹
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Session	22

Abstract, Ethics Committee Number*, and Keywords	<p>PSMA is a membrane metalloenzyme, expressed in prostatic epithelial cell lineages, whose expression increases in cells from castration-resistant prostate cancer. The present study aims the expression and characterization of an anti-PSMA nanobody (NB7), modified by amber codon suppression and labelled with fluorescent and antineoplastic groups, to study the PSMA specific recognition by the nanobody, and to use this nanobody as a specific antitumor drug carrier for prostate cancer cells. Nanobodies are antibody fragments (~15 kDa) made up only of the heavy variable domain derived from heavy-chain-only immunoglobulins expressed by Camellidae and sharks. NB7 was selected from PDB data bank and modified with the addition of the non-canonical amino acid (ncAA) 4-azide-L-phenylalanine during its biosynthesis in <i>E. coli</i> by amber codon suppression, which is based on the presence of an orthogonal aminoacyl-tRNA synthetase/tRNA suppressor pair (pEVOL-Az-Phe) into the expression host cell (BL21(DE3)). The specific bioincorporation of this azide containing residue (Az-Phe) permitted the modification of the NB7 by click-chemistry with DBCO-Cy5 and with different constructions containing the antineoplastic agent MMAE: DBCO-Val-Cit-MMAE, DBCO-MMAE. The click-chemistry reactions were done at physiologic conditions (PBS, pH = 7.4). LNCap (PSMA+) and PC3 (PSMA-) cells were treated with NB7-DBCO-Cy5 at 100, 20, and 5 ug, showing specific ligation only in LNCap membranes. Positive signals of NB7-DBCO-Cy5 were present within lysosomes (lyso-tracker) after 2 hours of incubation only in LNCap cells. NB7-DBCO-Val-Cit-MMAE (MMAE placed after a cat-B cleavable linker) decreased LNCap cell viability after 24 hours of treatment in the concentration of 0,5 uM, while NB7-DBCO-MMAE also showed a measurable effect in cell viability, however this compound required at least 10-fold higher concentration to present toxicity on LNCap cells.</p> <p>Keywords: PSMA, nanobody, click chemistry, non-canonical aminoacids.</p>
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Title	Testing an epigenetic drug to treat patient-derived wilms tumor cells
Authors	Ellen A da Silva, Larissa Akemi Kido, Milena R Marusco, Juliana S Ruas, Mayara F Euzebio, Izilda A Cardinalli, Camila M Daiggi, Renato D Puga, Patricia Y Jotta, Priscila P Zenatti, Mariana Maschietto
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Session	22

Abstract, Ethics Committee Number*, and Keywords	<p>Wilms tumors are embryonic tumors of the kidney, characterized by both the cell and genetic heterogeneity. Similar to other embryonic tumors, Wilms tumors present few recurrently mutated genes in a low proportion of the cases but 70% have imprinting alterations at 11p15. DNA mutation and methylation profilings reported the existence of subgroups that are being explored regarding tumor clinical and pathological characteristics. Following the genetic characterization of 5 cases that were implanted in a NSG mouse model (patient-derived xenografts, PDX), we established the 3D cell culture of Wilms tumors for drug testing. Tumoroids are capable of replicating and sustaining the genetic diversity observed in tumor tissues, offering predictive insights into individual patient responses to drugs. Fragments from one Wilms tumor extracted from PDX were dissociated and 10,000 cells were plated in a 96-well cell repellent plate. After 5 days, the necrotic nucleus was observed in the tumoroids. The tumoroids were treated with three concentrations of the epigenetic drug Panobinostat, a HDAC inhibitor. After 72-hour incubation, the tumoroids were characterized by viability assays. The cell death was determined by measuring ATP levels by luminescence (CellTiter-Glo® 3D Cell Viability Assay, Promega Biotechnology). At 100 nM and 1uM concentration, we observed evidence of increasing cell death from 24 to 72 hours, when they were all dead at the 1uM concentration. The cell viability was confirmed using the LIVE/DEAD™ Viability/Cytotoxicity Kit (Thermo Fisher Scientific) that enabled the visualization of dead and alive cells in the tumoroid. Our preliminary data indicated the efficacy of HDAC inhibitor, a drug already used to treat multiple myeloma, in inducing cell death in Wilms tumors. This study was approved by both CEP (CAAE 44219021.6.0000.5376) and CEUA (0017-2021). Funding: PRONON 2500211368/2019-41.</p> <p>Keywords: Wilms tumor; Embryonic tumor; Tumoroids; Epigenetic; HDAC inhibitor.</p>
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Title	Podoplanin quantification in patients with acute promyelocytic leukemia
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Session	22 - Sinalização e Terapêutica do Câncer

Abstract, Ethics Committee Number*, and Keywords	<p>Acute promyelocytic leukemia (APL) is a hematological emergency associated with high early mortality rates. Recently, the expression of podoplanin (PDPN), that mediates platelet activation in inflammatory contexts, by leukemic cells is as a relevant mediator of the coagulopathy of APL. Samples were obtained from patients with APL at the time of diagnosis at the Clinics Hospital, University of Campinas. Biobank samples from 35 patients with non-APL AML matched for age and sex were used in comparative analyses. Circulating levels of PDPN were measured in plasma using a commercial Elisa kit. Furthermore, of these 35 patients, 12 patients with APL and 7 patients with non-APL AML had PDPN expression quantified using real-time PCR. Biomarkers of coagulation were measured using multiplex immunoassays. The study was approved by the ethics committee and all participants provided written informed consent (39948520.8.0000.5404). Patients with APL are classified into risk groups, which are low, intermediate and high. Fibrinogen was lower in patients with APL when compared to of AML (115.4 (52.2-376.0) vs 383.2 (147.6-638.2) mg/dL, U=30.00; p<0.0001). The Mann-Whitney test showed that soluble PDPN levels were significantly higher in patients with APL compared to other forms of AML (16.4 (0.5-120.5) vs 2.4 (0.37-238 .9) ng/mL, U= 399.00; p=0.01). The levels of P-selectin (7,194 (2,213-30,650) vs 11,292 (2,792-235,307) pg/mL, U=357.00; p=0.002) were lower in patients with APL when compared to other forms of AML. PDPN expression in leukemic blasts was significantly higher in APL compared with non-APL AML. When considered as a categorical variable, the expression of PDPN by real-time PCR was evidenced only in patients with APL (Chi-square test= 8.601;1; P=0.004). PDPN expression is higher in APL compared to other AML. Additional studies are warranted to confirm these findings and further explore the role of PDPN. Keywords: Acute promyelocytic leukemia (APL), APL coagulopathy, PDPN.</p>
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Title	Fractions of the fixed oil of <i>Syagrus coronata</i> (Mart.) Becc. have antiproliferative potential in tumor cells
Authors	Joyce Bezerra Guedes ^{1,2} Andreza Larissa do Nascimento ^{1,2} Rafael de Felício ¹ Débora Munhoz Rodrigues ¹ Beatriz Pelegrini Bosque ¹ Maria Tereza dos Santos Correia ² Danieli Cristina Gonçalves ¹ Daniela Barretto Barbosa Trivella ¹ Márcia Vanusa da Silva ²
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Session	Poster presentation

Abstract and Keywords	<p><i>Syagrus coronata</i>, popularly known as Licuri, is a plant species from the Arecaceae family, occurring in the Caatinga biome. It has broad potential for the development of new drugs, as it is traditionally used for medicinal purposes by the population. The oil extracted from Licuri is reported in the literature to have therapeutic properties, including cytotoxic effects and a chemical composition rich in free fatty acids. To characterize and isolate anticancer compounds from Licuri, the oil was fractionated and the fractions were subjected to tests using tumor cell lines and a reference cell line. Initially, the oil was partitioned into MeOH/H₂O (8:2, v/v), the supernatant dried and fractionated on a silica gel column, initially eluted with the Hexane/Ethyl acetate (8:2) gradient, with the mobile phase concentration gradually increased. The obtained fractions were monitored by TLC and grouped according to similarity in chemical profiles. They were then subjected to biological evaluation tests with three cell lines, HaCat (reference), HCT-116 (colon cancer) and MDA-MB 231 (breast cancer), using a microscale phenotypic assay to monitor cell survival under exposure to chemical compounds. Paclitaxel and DMSO were used as positive and negative controls, respectively. The results indicated two fractions with selective activity, one against MDA-MB-231 (EC₅₀ = 5.96 µg/ml) and another against HCT-116 (EC₅₀ = 6.22). Both did not induce cell death in normal keratinocyte cells (HaCat), with good selectivity indices (SI). Paclitaxel was active in all cell lines, exhibiting low selectivity for cancer cells (SI of 1.8 for HCT-116 and 0.3 for MDA-MB). The fractions were analyzed by UPLC-MS/MS, revealing the presence of new compounds with potential biological activity, which were selected for subsequent isolation processes. The results point to the discovery of new anticancer agents from Brazilian biodiversity with more effective action than the reference compound currently used.</p> <p>Keywords: Caatinga; <i>Syagrus</i>; antiproliferative; fatty acids; chemical composition.</p>
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Title	Development of Pediatric Synovial Sarcoma PDX (patient-derived xenograft) model: histopathological and cytogenetic validation
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Session	22 - Sinalização e Terapêutica do Câncer

Abstract, Ethics Committee Number*, and Keywords	<p>Synovial sarcoma (SS) is a tumor that primarily affects children and adolescents, with an occurrence rate of 2% in the 0-14 age group and a recurrence rate of 25%. Precision medicine aims for more effective and less toxic therapies, adapted to the individual characteristics of each patient. To achieve this purpose, our study focused on validating patient-derived xenografts (PDX) from SS primary samples to assess whether this model accurately reflects the patient tumor’s heterogeneity. Six samples, representing three SS subtypes, previously grown in immunocompromised mice (NSG), were submitted to histopathological and FISH (Fluorescence in Situ Hybridization) analysis on paired PDX samples, only when the patient tested positive for the SS18 rearrangement. Preliminary results indicated that all PDX were classified as SS in both the first and second generations, with predominant fusiform cells, high hypercellularity, necrotic areas, and the presence of mitotic figures being the most common features. However, the majority of PDX exhibited a more aggressive phenotype of SS when compared to primary samples, likely due to natural selection favoring cell clones more prone to proliferation. These findings were supported by a significant increase in mitotic index in the xenografts compared to the patient sample and between PDX generations, as well as the presence of metastasis. The SS18 rearrangement was confirmed in 4 of 5 PDX samples; however, further qPCR analyses are planned to identify the presence of fusion transcripts. In summary, the preliminary findings showed that pediatric SS was well-established in the PDX model, even favoring undifferentiated grades. While initial data show promise, further research, such as molecular characterization, is needed to confirm and explore the potential of PDX in guiding personalized therapies for SS. FAPESP (2023/14392-0); Ronald McDonald House Charities - Brazil (2022067). CEP (CAAE: 44219021.6.0000.5376) and CEUA (0017-2021).</p> <p>Keywords: Synovial Sarcoma, PDX, Pediatric Cancer, Precision Medicine</p>
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Title	HDACIs and bortezomib induce senescence and affect the stem phenotype in salivary gland mucoepidermoid carcinoma cell lines
Authors	Beatriz Marcelli Correia Henrique Thainá Pacheco Moraes Adrieny Ullian Bastos Emília Maria Gomes Aguiar Fabio Daumas Nunes Leijiane Figueira de Sousa
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Session	Cancer Signaling and Therapeutics

Abstract, Ethics Committee Number*, and Keywords	<p>Mucoepidermoid carcinoma (MEC) is the most prevalent malignant tumor in the salivary glands. Resistant to chemotherapy, its treatment consists of surgery, associated or not with radiotherapy, resulting in many sequelae and deformities. We evaluated the action of two HDACIs panobinostat (PAN) and romidepsin (ROM), alone and combined with cisplatin (CIS) or bortezomib (BTZ), a proteasome inhibitor, in MEC cell lines (UM-HMC1, primary tumor; UM-HMC3A, recurrent tumor). The cells were treated with doses corresponding to the IC₅₀ of the drugs, alone or in combination. Then, clonogenicity (colony assay), self-renewal (sphere assay) and cellular senescence were evaluated (β-galactosidase activity). The IC₅₀ values obtained were: ROM (1.9nM in both cell lines), PAN (UM-HMC1= 6.9nM; UM-HMC3A= 6.4nM), CIS (UM-HMC1= 3.33μM; UM-HMC3A= 2μM) and BTZ (UM-HMC1= 5.64nM; UM-HMC3A= 7.56nM). All treatments significantly ($p \leq 0.05$) reduced the number of colonies formed, compared to the untreated group (control). The treatments with HDACI alone were more effective in reducing spheres than the combinations with CIS (P+C or R+C), which in turn increased ($p \leq 0.05$) the number of spheres compared to the control. However, the combination ROM+BTZ was more effective than each inhibitor alone, in both lineages. Except for BTZ, all the other treatments increased ($p \leq 0.05$) the percentage of senescent cells compared to the control, mainly the groups treated with ROM and R+C, in UM-HMC3A. In UM-HMC1, all treatments also increased ($p \leq 0.05$) the percentage of senescent cells. The HDACIs were effective against important properties associated with the tumor stem phenotype inducing senescence and reducing intrinsic resistance to cisplatin, in the lineages evaluated. Moreover, the combination of HDACI + proteasome inhibitor seems to act synergistically against CSCs, being more effective than isolated treatments, and could represent a new therapeutic tool in studies involving salivary gland MEC.</p> <p>Keywords: MEC/ CSCs/ HDACI/Proteasome inhibitor.</p>
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Title	Antitumor effects of the isoflavonoid Brazilin on cancer stem cells from Oral Squamous Cell Carcinoma
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Session	Cancer Signaling and Therapeutics

Abstract, Ethics Committee Number*, and Keywords	<p>Oral Squamous Cell Carcinoma (OSCC) is the main malignant neoplasm of the oral cavity. Its treatment consists of tumor resection surgery, which can be associated with radio and/or chemotherapy. However, the patient survival rate is very low due to relapses and metastasis caused by the lack of efficiency of conventional therapy, which is related to a small, but significant population of cancer stem cells (CSCs). Therefore, new therapeutic approaches have been studied to find more effective drugs against CSCs. Brazilin is an isoflavonoid used in traditional medicine, and recent studies, with other types of tumors, have shown its antitumor properties <i>in vitro</i>. This study evaluated the antitumor effects of Brazilin in two OSCC cell lines, SCC9 (from primary tumor) and LN1 (a metastatic variant of SCC9). For this purpose, the cells were treated with doses corresponding to the IC₅₀ values of Cisplatin (CIS), Brazilin (BRA) and a combination of the two drugs (BRA+CIS). The clonogenicity (colony formation), self-renewal (tumorsphere formation), cell migration (wound healing assay) and CSC markers (western blotting) were evaluated. SCC9 treated with BRA had a reduction in colony formation compared to the untreated (control) group and this reduction was greater for BRA+CIS. BRA alone was not as effective in LN1. BRA alone and BRA+CIS treatment significantly reduced tumorspheres, compared to the control and CIS groups. Regarding cell migration, BRA+CIS and BRA alone promoted reduction, with the combination being more effective. Finally, BRA and BRA+CIS significantly reduced the protein expression of BMI and Nanog, two important stemness markers, with slight differences between the two cell lines. These preliminary results suggest an additive effect between the two drugs (BRA+CIS), indicating Brazilin's good potential for OSCC treatment.</p> <p>Oral Squamous Cell Carcinoma/ Brazilin/ CSCs.</p>
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Title	Morphofunctional alterations of normal human keratinocytes by melanoma-derived extracellular vesicles
Authors	Sophia Nora Baptista Jordana Maria Azevedo-Martins Carmen Veríssima Ferreira-Halder
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Session	22 – Cancer signaling and therapeutics

Abstract, Ethics Committee Number*, and Keywords	<p>Melanomas are remarkably aggressive skin cancers derived from melanocytes. In the literature, there is a consensus that the tumor microenvironment not only supports the worsening of the disease but is also a critical factor in the low therapeutic efficiency frequently observed in patients. Among the components of the tumor microenvironment, keratinocytes, and small extracellular vesicles (sEVs), are closely related to the progression of melanomas, the latter composing particles smaller than 200nm that actively participating in cell-cell communication events. Recent studies suggest the involvement of sEVs in the metabolic pathway modulation of cells in the tumor microenvironment and the reprogramming of target cells, in a process known as tumor education. Therefore, we hypothesized that sEVs may have a relevant role in the morphofunctional and metabolic reprogramming of keratinocytes, resulting in advantages for melanoma cells, such as better survival and migration rates.</p> <p>To validate our hypothesis, sEVs derived from different melanoma cell lineages (Skmel-103 and Skmel-28) were isolated and characterized through nanoparticle tracking analysis (NTA) and transmission electron microscopy (TEM). Experiments to guarantee the quality control of the sEVs extracted were also performed. The impact of sEVs co-culture on human keratinocytes was analyzed through different approaches: MTT reduction, neutral red uptake (NRU), adhesion and migration assays and spheroid formation.</p> <p>Results displayed an increase in cell viability (~25%) and migratory capacity (~40% for t=24h) of keratinocytes in co-culture with melanoma extracellular vesicles. In relation to adhesion, morphological changes are noted in these cells, which showed a reduced number of cellular projections and cytoskeleton structure, as revealed by phalloidin staining. These findings show evidence that melanoma sEVs can modulate the behavior of keratinocytes.</p> <p>Keywords: Melanoma, extracellular vesicles, keratinocytes, morphofunctional.</p>
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Title	Effect of the vapor phase of patchouli essential oil on the cell viability of H292 and MRC-5 cell lines
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Session	22 - Signaling and Cancer Therapeutics

Abstract, Ethics Committee Number*, and Keywords	<p>Due to their pharmacological properties, essential oils (EOs) have emerged as a potential adjuvant therapy for lung cancer. The major compound of the patchouli (<i>Pogostemon cablin</i> Benth) EO, patchoulol, has already shown antitumor activity. However, there is no information about the antitumor activity of the vapor phase (VP) of this EO. The aim of this study was to evaluate the cytotoxicity of the VP of patchouli EO in lung carcinoma cell line (H292) and control cells (MRC-5). As an experimental strategy, the cells were seeded in the eight central wells of the 24-well culture plates and treated for 72 hours with the VP of the EO at different concentrations (0–1000 µg/mL/well), which were added to the 16 remaining cell-free wells. Cell viability was determined by MTT reduction and sulforhodamine B (SRB) staining assays. The concentration of EO capable of generating enough vapor to reduce cell viability by 50% (ICV50) and the selectivity index (SI) were calculated. The cell migration was also assessed using the scratch assay. The VP of the EO reduced the cell viability of the tumor cells in a dose-dependent manner. In the MTT and SRB assays, the ICV50 values obtained for the H292 cells were 215.36 ± 46.04 µg/mL and 220.27 ± 58.75 µg/mL, respectively. For the MRC-5 cells, the ICV50 values founded were 333.08 ± 76.17 µg/mL (MTT) and 315.64 ± 66.28 µg/mL (SRB). The SI value obtained for the tumor cell line was lower than 2. The scratch assay showed that the EO didn't significantly inhibit cell migration in the H292 cell line. Considering the potential antitumor effect of this EO and the SI value reported in this research, it would be advisable to measure an alternative control cell model to validate the SI value. This is important for further evaluating the mechanisms of action of this EO. Keywords: <i>Pogostemon cablin</i>; Lung carcinoma; Cytotoxicity; Cell migration; Selectivity index.</p>
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Title	Violacein as a potential modulator of AXL tyrosine kinase receptor
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Session	22 - Sinalização e terapêutica do câncer

Abstract, Ethics Committee Number*, and Keywords	<p>The tyrosine kinase receptor AXL has been associated with tumor progression and chemotherapeutic resistance in various types of cancer, including melanoma. Our research group reported that violacein, a secondary metabolite derived from <i>Chromobacterium violaceum</i>, was able to decrease AXL expression in metastatic melanoma cells. Since violacein displays a strong induction of tumor cells death, we hypothesized whether this pigment could also inhibit AXL. In this study, we investigated, through docking simulation, whether the chemical structure of violacein could interact with the tyrosine kinase receptor AXL at its catalytic or active sites. Additionally, we investigated whether violacein could induce cell death in metastatic human melanoma spheroids, since the tyrosine kinase receptor AXL activates several signaling pathways essential for tumor survival. Spheroids were obtained using the Bio-Assembler n3D[®] system and treated with 10μM of violacein for 48 hours (n=6). Images were captured using the LumaScope[®] microscope and analysed using ImageJ[®] and GraphPad Prism[®]. The spheroids were also subjected to flow cytometry assay with 7AAD and Annexin (n=12), and the data were analysed using FlowJo[®]. Molecular docking simulation was performed using the FRED software, using the Chemgauss4 scoring function. As a result, we found that violacein significantly reduced the area of melanoma spheroids (p=0.000046) and promoted more than 50% reduction in cell viability. Regarding docking, we found that violacein is capable of interacting with the active site of AXL. Thus, we hypothesize that one of the ways violacein is able to induce cell death in melanoma may be through decreasing both the active and amount of this enzyme.</p> <p>Keywords: violacein, AXL, melanoma.</p>
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Title	Evaluation of glycosaminoglycans in modulating the inhibitory activity of compounds on cysteine cathepsins
Authors	Maria Luiza Gonçalves Gallego Rosa ¹ Dênis Pires de Lima ² Adelino Vieira de Godoy Netto ³ Wagner Alves de Souza Júdice ¹
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Session	Sesion 22 – Sinalização e Terapêutica do Câncer

Abstract, Ethics Committee Number*, and Keywords	<p>INTRODUCTION: The presence of Glycosaminoglycans (GAGs) and the pH of the environment modulate the activity of lysosomal cysteine proteases, which under specific conditions come into contact with intracellular GAGs, cell surface, or extracellular matrix. This interaction directly correlates with tumoral events, infectious, and parasitic processes in the organism, demonstrating the importance of evaluating the effects of GAGs, along with pH variation, on the effects of cathepsin inhibitors. Therefore, our aim was to evaluate the effects of GAGs (Heparin) on the inhibitory activity of chalcone and flavonoid-derived compounds and palladium compounds on cathepsins B and L. METHODOLOGY: To determine the IC₅₀, cathepsin B was incubated in sodium acetate buffer 100 mM; EDTA 5 mM, NaCl 100 mM; Triton X-100 0.01%; 20% glycerol, 3 mM DTT; and cathepsin L in sodium acetate buffer 100 mM; EDTA 5 mM, NaCl 100 mM; 20% glycerol, pH 5.5, 3 mM DTT. Enzymatic activity was monitored by spectrofluorimetry using Z-FR-MCA as substrate ($\lambda_{Ex}=360nm$, $\lambda_{Em}=480nm$; Cat B \rightarrow 40μM, Cat L \rightarrow 25 μM) at 37°C in the absence and presence of heparin (10 μM). RESULTS: Heparin showed little effect on the inhibitory potential of chalcone and flavonoid-derived compounds on cathepsin L activity. Compounds EM-2B, EM-F12A, and EM-F12B were 16, 37, and 67 times more potent in inhibiting cathepsin B in the absence of heparin. Regarding palladium compounds and pH effect in the presence and absence of heparin, activity modulation was more effective at pH 4.5 and 5.5 for cathepsin L, with little variation in IC₅₀ for cathepsin B at the evaluated pH levels. CONCLUSION: We conclude that heparin is capable of modulating the inhibitory effects of chalcone and flavonoid-derived compounds as well as palladium compounds mainly on cathepsin L in an acidic environment.</p> <p>KEYWORDS: Cathepsins, Glycosaminoglycans, Heparin, Chalcones, Flavonoids, Palladium Compounds; Inhibitory Potential.</p>
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Title	Immunohistochemical expression of P21 and MDM2 proteins in colorectal adenocarcinoma samples
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Session	Sinalização e Terapêutica do Câncer

Abstract, Ethics Committee Number*, and Keywords	<p>Colorectal adenocarcinoma (CRC) is one of the most prevalent cancers around the world. Its prognosis depends, among other factors, on the evaluation of the expression of biomarkers, such as <i>APC</i>, <i>KRAS</i> and <i>TP53</i>, the latter expressing the p53 protein. This protein, associated with the worsening of CRC, regulates p21, a key protein in maintaining the cell cycle, and is negatively regulated by MDM2, a protein that is associated with the progression of tumors such as glioblastomas and breast cancer. The specific roles of these proteins in CRC have been investigated, with special interest in expanding the set of biomarkers that can contribute to better defining the prognosis of patients. Thus, the study aimed to evaluate the correlation between the immunoexpression of p21 and MDM2 proteins in CRC, with classic prognostic factors and patient evolution. Being approved by the PUCPR CEP under registration number 58734022.2.0000.0020, 73 cases of CRC were analyzed, from the UFPR Hospital de Clínicas, collected between 2007-2011. These cases are of men and women, aged >18 years, who underwent elective or emergency surgery without receiving neoadjuvant treatment. The samples analyzed were reviewed for histopathological diagnosis and comprised three tumor regions (superficial, intermediate and deep). They were mounted in TMAs and subjected to immunohistochemistry with antibodies specific for p21 and MDM2. The p21 slides were read using the Allred Score and the results obtained were analyzed using the Fischer exact test. MDM2 was read using color morphometry and the results were analyzed using Student's T and Anova tests. The hazard Ratio was used to evaluate the outcome of the markers. MDM2 immunoexpression in the tumor intermediate region was associated with tumor invasion (p = 0.011). Other associations were not observed. This result suggests potential MDM2 to be explored as a prognostic biomarker in CRC.</p> <p>Keywords: Colorectal Cancer; Biomarkers; p21; MDM2</p>
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Title	Effect of stimulation and blockade of β-adrenergic receptors on cell proliferation in <i>in vitro</i> models of colon and skin cancer
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Session	Cancer Signaling and Therapeutics

Abstract, Ethics Committee Number*, and Keywords	<p>Cancer is one of the major global public health issues. Among the molecular mechanisms contributing to its onset and development, stimulation of β-adrenergic receptors (βARs) has proven to be particularly important, with administration of β-antagonists showing potential clinical efficacy. However, further investigations are needed to precisely determine the effects of β-adrenergic stimulation on critical biological processes for tumor initiation and progression, such as cell proliferation. Therefore, the aim of this study was to evaluate the effect of βAR stimulation and blockade on cell proliferation in <i>in vitro</i> models of colon and skin cancer. To achieve this, the clonogenic assay was performed with colon carcinoma (CT26) and melanoma (B16F10) cells treated with propranolol (PRO; non-selective β-antagonists) or isoproterenol (ISO; non-selective β-agonist) at different doses for seven (CT26) or ten (B16F10) days. Cell proliferation was evaluated by absorbance quantification, results are presented in % of response relative to the control and were analyzed using One-way ANOVA. We observed a 78% reduction in proliferation of CT26 cells after treatment with 20 μM of the β-antagonist PRO ($p < 0.001$), while a dose-dependent relationship was observed for B16F10 cells, with reduced proliferation after treatment with 5 (27%, $p < 0.01$), 10 (60%, $p < 0.0001$), and 20 μM (88%, $p < 0.0001$). The β-agonist ISO displayed no significant difference in the proliferation of both cell lines. Therefore, our results suggest that blocking βARs was more effective in modulating cell proliferation of colon carcinoma and melanoma cells, with the latter being more sensitive to treatment. Furthermore, these findings corroborate a robust body of evidence supporting the anti-tumoral role of β-antagonists, indicating a possible clinical efficacy of PRO, which can be further investigated in subsequent research.</p> <p>Keywords: Cancer, β-adrenergic receptors, β-blockers. Funding: FAPESP 2023/17631-5</p>
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Title	Novel cytotoxic and antimigratory thionaphthoquinone: <i>in vitro</i>, <i>in silico</i> and <i>in vivo</i> studies
Authors	Julio Cesar Thurler Júnior ^{1,2} Hugo Figueira de Paula Pinto ³ Mariana Toledo Martins Pereira ¹ Thiago Sardou Charret ¹ Daniela Gonçalves Galasse Rando ⁴ Caroline da Silva Moreira ⁵ Maraysa de Oliveira Melo ³ Rafael Loureiro Simões ⁸ Raquel Carvalho Castiglione ⁶ David Rodrigues da Rocha ⁵ Vinicius D'ávila Bitencourt Pascoal ¹ Leandro Machado Rocha ^{2,7} Aislan Cristina Rheder Fagundes Pascoal ¹
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Session	22 - Sinalização e Terapêutica do Câncer

Abstract, Ethics Committee Number*, and Keywords	<p>From 1981 to 2019, 64.5% of approved anticancer drugs were derived from natural products. Lawsonia, a dye extracted from <i>Lawsonia inermis</i>, is naphthoquinone with many medical uses. In medicinal chemistry, introducing sulfur heteroatoms into the naphthoquinone core can enhance pharmacological effects, including anticancer activity. This study evaluates the cytotoxic and antimigratory effects of novel lawsonia-derived thionaphthoquinones (TNQs) and explores their molecular mechanisms.</p> <p>The IC₅₀ and selectivity index (SI) of the TNQs were determined via MTT viability assays in HeLa and NIH-3T3 cells. Antimigratory activity was assessed over 48 hours using a wound-healing assay in HeLa cells at a non-lethal concentration. Expression of matrix metalloproteinases (MMP) 2 and 9 was quantified by dye-</p>
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based RT-qPCR. Molecular mechanisms of the TNQs were explored through in silico target fishing and molecular docking. Activation of the ERK/p38 pathway was analyzed using Western blotting. ADME properties were predicted with SwissADME software. Acute toxicity was tested over 96 hours in *Danio rerio* embryos (0046-2024/CEUA-Boldrini). LC₅₀ and IC₅₀ values were calculated using non-linear regression from dose-response curves; ANOVA and Dunnett's test were applied to compare treated groups against controls using GraphPad Prism 8.

Among the five newly synthesized TNQs, TNQ10 was the most potent and selective, exhibiting an IC₅₀ of 25.1 μ M (\pm 0.02) and an SI of 3.49. TNQ10 effectively inhibited HeLa cell migration and reduced MMP-2 and MMP-9 mRNA expression. In silico studies suggest JNK1 as a potential target and highlighted TNQ10's drug-like properties. Western blot analysis confirmed TNQ10's activation of the ERK/p38a/JNK pathway, independent of reactive oxygen species. An LC₅₀ of 3.8 μ M was observed in zebrafish embryos. These findings suggest TNQ10 as a promising candidate for further anticancer drug development.

Ethics Committee number: 0046-2024/CEUA-Boldrini

Keywords: Naphthoquinones, Drug Discovery, Anticancer, Natural Products.



Title	Riboflavin photoproducts induce cell death and loss of adhesion in gastric and esophageal cancer spheroids and Barrett's esophagus organoids
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Session	22. Sinalização e Terapêutica do Câncer

Abstract, Ethics Committee Number*, and Keywords	<p>Esophageal adenocarcinoma (EAC) and gastric cancer (GC) are often diagnosed late and have poor survival rates. Our group previously demonstrated that riboflavin photoproducts (iRF) induced apoptosis in leukemia, prostate, and renal cancers, and now we focused on the antitumoral action of iRF on gastric cancer and esophageal cancer cell lines (Kato-III and FLO-1) and compared to normal gastric cells (GES-1) in both 2D and 3D cell culture model (spheroid). We also aimed to evaluate the impact of iRF on Barrett's Esophagus (BE) (a precursor of EAC) on organoids derived from 2 patients, a more complex 3D cell culture model (Ethical approval: UCSF IRB#21-35223). Cell viability in 2D was tested by MTT (iRF 0.5µM - 50µM) and adhesion (iRF 10µM - 50µM) by crystal violet staining and fluorescent labeling with phalloidin (iRF 50µM). The spheroids were obtained by n3D Greiner Bio-One Kit. For the viability test, after 48h with 50µM, the spheroids were assessed by fluorescence microscopy, using calcein to mark live cells, and ethidium homodimer to mark dead cells. The cell-cell adhesion was tested treating the cells with iRF 50µM before the magnetization and followed for 72h. Adhesion and cell death of the organoids were evaluated by counting and labeling the organoids with E-cadherin and cleaved PARP-1, respectively. The results in 2D demonstrated that iRF reduced cell viability, altered cytoskeleton structure, and impaired cell adhesion in Kato-III and FLO-1 but not in GES-1. In 3D, iRF inhibited the spheroid formation of FLO-1 cells and induced cell death and loss of adhesion in Kato-III, but not in GES-1. After 7 days with iRF 50, 75, and 100uM, the organoids from both patients presented less cell viability and altered morphology, which was sustained by the loss of fluorescent E-cadherin intensity and increase of cleaved PARP-1 on the treated ones. These findings highlight the potential of iRF as an antitumor agent.</p> <p>Keywords: Gastric Cancer. Esophageal Cancer. Barrett's Esophagus. 3D cell culture. Spheroids. Patient-derived organoids. Riboflavin.</p>
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Title	Evaluation of the antitumoral potential of vapor phase of the essential oil of <i>Juniperus communis</i> L. on the NCI-H292 cell line
Authors	Luiz Felipe Biuk; Carla Cristine Kanunfre
Affiliations	Postgraduate Program in Biomedical Sciences - State University of Ponta Grossa
Session	22 - Cancer Signaling and Therapeutics

Abstract, Ethics Committee Number*, and Keywords	<p>Essential oils (EO) are a source of compounds with volatile characteristics that present diverse pharmacological activities, such as antimicrobial, antitumoral and antioxidant. However, these activities are few reported in the literature with the exclusive focus in the volatile phase (vapor phase). The objective of this study was to evaluate the antitumor potential of the vapor phase the <i>Juniperus communis</i> L. (Juniper) EO. NCI-H292 cell line from lung carcinoma and MRC-5 lineage from lung fibroblasts (control) were used in cell viability and scratch assays. Treatments with EO were carried out for a period of 24 hours. The cells were seeded in the eight central wells of the 24-well culture plates and treated with the vapor phase of the EO at different concentrations (25-16000 µg/mL/well), which were added to cell-free wells in only one concentration by plate. Juniper OE was purchased commercially and chemical analysis by CG/MS confirmed α-pinene (69%) as the major compound. Cell viability was determined by sulforhodamine B (SRB) staining assays, from which ICV50 and selectivity index (SI) values were calculated. The concentrations of EO capable of generating enough vapor to reduce cell viability by 50% (ICV50) were 1843 ± 600 µg/mL and 13085 ± 919 µg/mL, respectively, to tumor/H292 cells and control/MRC-5 cells, and the SI value correspondent of 7,1 was obtained. The scratch assay showed that the EO at the concentration 1500 µg/mL/well significantly inhibited (35.7%) cell migration in the H292 cells. These findings suggest that the Juniper essential oil, that can be directly delivered to the lung tissue by inhalation, exhibit antitumor activity. So it would be interesting to investigate the possible mechanisms of action of EO to consider it as an alternative to treatment of lung cancer.</p> <p>KEYWORDS: Juniper; Cell viability; Lung cancer; Selective index; MCR-5 cell line</p>
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Title	Importance of in vitro anti-proliferative activity evaluation of natural extracts to identify potential therapeutic use in cancer treatment
Authors	Robson da Silva Pontes Josélia Cristina de Oliveira Moreira Alex Vinícius de Campos Mary Ann Foglio João Ernesto de Carvalho Ana Lucia Tasca Gois Ruiz
Affiliations	LAFTex – Phytochemistry, Pharmacology and Experimental Toxicology Laboratory; FCF - Faculty of Pharmaceutical Sciences; UNICAMP – University of Campinas; Campinas – Brazil
Session	22 - Cancer Signaling and Therapeutics

Abstract, Ethics Committee Number*, and Keywords	<p>Cell culture is an important tool for high throughput screening, allowing a refined selection of candidates to be tested in animal models. The Phytochemistry, Pharmacology and Experimental Toxicology Laboratory (LAFTex) screens the antiproliferative potential of natural products according to NCI-USA methodology. Herein a retrospective analysis of the results obtained over the last couple of years after the implementation of the new cell culture laboratory. A survey was carried out in the LAFTex database, considering projects coordinated by researchers from the LAFTex group, in 2022 and 2023, using the panel of tumor and non-tumor cells. The most frequently used cell panel was formed by 5 human tumor lines [U251 (glioblastoma), MCF-7 (breast adenocarcinoma), NCI-ADR-RES (multidrug-resistant ovarian adenocarcinoma), NCI-H460 (carcinoma of lung, non-small cell type), HT-29 (colorectal adenocarcinoma)] and 2 immortalized lines [HaCat (human keratinocytes) and 3T3 (murine embryo fibroblast)]. Samples encompass extracts enriched <i>Fragraria ssp</i> fractions, <i>Copaifera langsdorffii</i> <i>Bixa orellana</i>, <i>Pterodon pubescens</i>, <i>Arrabidaea chica</i>, among others were analysed. For antiproliferative activity evaluation, sample-treated cells were compared to two controls of untreated cells representing, respectively, the quantities of cells present at the initial time (T0) and after 48 h of exposure (T1). The sulforhodamine B staining was employed to determine viable cells. The results were expressed as the concentration necessary to completely inhibit cell proliferation (TGI, $\mu\text{g ml}^{-1}$). Among these samples, <i>Copaifera langsdorffii</i> and <i>Arrabidaea chica</i> were selected for in vivo testing. <i>C. langsdorffii</i> exhibited antiproliferative activity, reducing cell viability by approximately 50% in all tested tumor cells. Meanwhile, <i>A. chica</i> demonstrated anti-inflammatory activity, inducing collagen synthesis and fibroblast proliferation, improving wound healing activity by approximately 90%. These findings underscore the importance of the initial in vitro screening conducted by LAFTex in identifying natural compounds with therapeutic potential. This approach allows for a careful selection of promising candidates for testing in animal models, thereby contributing to the development of new therapies.</p> <p>Keywords: cancer, cell lines, natural products.</p>
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Title	Study of the glutaminases isoforms structures using expansion microscopy
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Session	22 - Cancer Signaling and Therapeutics

Abstract, Ethics Committee Number*, and Keywords	<p>Recent advances in expansion microscopy, particularly techniques such as Protein-retention Expansion Microscopy (proExM) and one-step nanoscale expansion (ONE), make it possible to visualize detailed molecular-level protein conformational changes using just a confocal microscope, without relying on complex techniques and delicate microscopes such as super resolution microscopy. To this end, a combination of protocols involves: fixing and treating the sample within a polymeric matrix that goes through a process of mechanical expansion, distancing the molecular entities that make up the sample and increasing the resolution obtained at the same rate as the mechanical expansion. In this project, we used proExM to study the isoforms of the glutaminase enzyme, which plays an important role in tumor metabolism by providing biosynthetic blocks and carbon supply to cells. Furthermore, the ability of glutaminase to form filamentous structures suggests an additional regulatory function, highlighting its importance in metabolic adaptation and as a potential therapeutic target.</p> <p>To be able to visualize the two specific glutaminase isoforms, we established BT-549 cell line overexpressing KGA and PC3 cell line overexpressing GAC. We successfully performed proExM on our samples and found ~4x expansion increasing the resolution of the images obtained. 3D reconstruction showed evident gain in the Z axis resolution allowing the visualization of the complex mitochondrial network. GAC formed filaments inside mitochondria when cultivated in media without glutaminase and we are currently applying SRRF image treatment to enable filament structure analysis. KGA also displayed mitochondrial localization when overexpressed in contrast with the previous described nuclear localization of the endogenous enzyme. With these techniques, we aim to resolve cellular ultrastructure, bringing insights into metabolic and structural enzymatic dynamics.</p> <p>Keywords: Glutaminase, Breast cancer, Expansion microscopy</p>
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Title	Isolated retinitis pigmentosa due to variants in HGSNAT
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Session	22 - Cancer Signaling and Therapeutics

Abstract, Ethics Committee Number*, and Keywords	<p>Cell culture Purpose: heparan-alpha-glucosaminide N-acetyltransferase (HGSNAT) is a lysosomal membrane enzyme historically associated with mucopolysaccharidosis type IIIC or Sanfilippo syndrome. Recently studies have demonstrated patients with non-syndromic retinitis pigmentosa (RP) due to HGSNAT variants. The purpose of this study is to expand the phenotypic and genotypic spectrum of HGSNAT non-syndromic retinopathy.</p> <p>Methods: This is a multicenter retrospective study of eleven patients from Casey Eye Institute at Oregon Health & Science University (OHSU), Federal University of São Paulo (UNIFESP), Instituto de Genética Ocular, and INRET Clínica e Centro de Pesquisa. We reviewed ophthalmologic data extracted from medical records, color fundus photography, fundus autofluorescence (FAF), optical coherence tomography (OCT), Kinetic visual field (KVF), and full-field electroretinogram (ERG). All patients underwent certified commercial next-generation sequencing panels.</p> <p>Results: The age of ophthalmologic symptoms onset varied from 15 to 72 (mean 45; median 38) years. The best-corrected visual acuity ranged from 20/20 to 20/80 (mean 20/30; median 20/20). FAF showed midperipheral hypoautofluorescent changes in all eleven patients. OCT revealed an outer retinal atrophy surrounding fovea area in all eleven patients and cystoid macular edema in four patients. KVF testing performed in eight patients showed a pattern of pericentral RP. Full-field ERG showed abnormal recordings with a pattern of rod-cone dysfunction in ten patients, while only patient 1, the youngest, had isolated rod dysfunction. Genetic testing found three homozygous patients with the p.Ala615Thr variant, the most common one in our cohort.</p> <p>Conclusions: There were differences in age of onset between patients; however, patients who had p.Ala615Thr at least in one allele presented with the onset of ocular symptoms after the fourth decade of life.</p> <p>Keywords: Sanfilippo syndrome; HGSNAT; retinitis pigmentosa; rare disease</p>
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Title	Electrophysiological evaluation of the visual pathways from retina to the primary visual cortex in Covid-19 patients
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Session	23 Visão e Oftalmologia

Abstract, Ethics Committee Number*, and Keywords	<p>Covid-19 is an infection caused by SARS-CoV-2, with varied manifestations. The ophthalmological symptoms are atypical, with retinal microvasculature changes and hyper reflexive lesions in the inner plexiform and retinal ganglion cell layers. Visual pathways' evaluation of Covid-19 patients can give a better understanding of the ophthalmologic and neurologic impacts of the disease. This study evaluated 113 eyes from 69 Covid-19 subjects (age 57.1 ± 11.9 years) and 28 eyes from 18 controls (54 ± 17.5 years) through electrophysiological and ophthalmologic exams (Pattern Electroretinogram – PERG and Pattern Visual Evoked Potential -PVEP). We considered 4 components in the PVEP, 3 latencies (N75, P100, N135) and 1 amplitude (N75-P100); and 5 components in the PERG, 3 latencies (N35, P50, N95) and 2 amplitudes (N35-P50, P50-N95). Covid-19 subjects were classified by respiratory compromise, COV 0 (without compromise), COV 1 (up to 25% compromise), COV 2 (25% to 50% compromise), COV 3 (50% to 75% compromise). PVEP N75 latency was significantly longer in the COV 2 group (88,8 ± 10,4; p= 0,0495) relative to the Control (83,9 ± 11,5) and in the COV 3 group (90,7 ± 12,1; p= 0,0483) relative to COV 0 (84,4 ± 15,2). There was significant difference (p=0.0272) in the Retino Cortical Time (RCT, the difference between the P100 PVEP latency and the P50 PERG latency) between the Covid and Control groups. There was no significant difference in the PERG. 4 in 28 eyes presented macular alterations in the control group against 35 in 69 eyes in the Covid-19 group. There was significant correlation (p=0.0151) between ophthalmologic alterations and the N75 PVEP implicit time for the COV</p>
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3 group. Our results indicate that retinal function measured by PERG is preserved, but cortical function at the primary visual cortex was altered, as shown by the PVEP findings in COVID-19 patients. The nerve signal conduction problem, therefore, would originate after the optic nerve's head.

Grant: This work was supported by Sao Paulo State Research Foundation (FAPESP) doctoral fellowship (2019/18487-0) to LCPB, postdoctoral fellowship (2015/22227-2) to KSFV and Thematic Project 2022/00191-0 to DFV, in addition to a Brazilian National Research Council (CNPq) 1A Productivity Fellowship to DFV (314630/2020-1).

*This research was approved by the CEPH of Prevent Senior under the number 38116720 3 3003, by CEPH of HCFMUSP under the number 38116720 3 0000 8114, and by the CEPH of IPUSP under the number 38116720.3.3003.5561.

Key-words: Sars-Cov 2, Covid-19, Coronavirus, PERG, PVEP, RCT.



Title	Morphological analysis of neurons in the inner retina of diurnal and nocturnal snakes
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Session	23- Visão e Oftalmologia

Abstract, Ethics Committee Number*, and Keywords	<p>The evolutionary history of snakes includes unique adaptations of their visual system. Transitions in daily activity patterns and broad differences in habitats and habits propelled the evolution of their visual structures through new adaptive directions, especially in “advanced” (caenophidian) snakes. Several studies investigated the types of photoreceptors and visual opsins in many species. Nocturnal snakes have rod-dominated retinas and three types of cones. Diurnal species have all-cone retinas with four cone-like photoreceptors, one of which is a modified rod. These discrepancies in the outer retina might suggest differences also in the (as yet, largely unstudied) inner retina and neural pathways. We used immunohistochemistry and Golgi stainings to characterize the cell types in retinal sections of 5 diurnal and 5 nocturnal snakes from the Viperidae, Dipsadidae, and Colubridae families (IPUSP Ethics Committee 9284040521). We identified several markers for inner retinal neurons, some of which labeled distinct cell types in diurnal and nocturnal snakes (PKCa, ChAT), while others revealed specific cells depending on the species (TH, PCP4, SCGN). Specifically, the anti-PKCa antibody labeled rod bipolar cells (BCs) in nocturnal snakes, with terminals stratifying in the innermost sublamina of the inner plexiform layer (IPL), while in diurnal species, it revealed a different population of BCs, with terminals stratifying in two sublaminae in the center of the IPL. In nocturnal species, PKCa BCs contact rods exclusively, while in diurnal species, they selectively contact red cones. With Golgi stainings we identified 4 types of ganglion cells, with different soma sizes and processes stratifying in one or more sublaminae of the IPL. This is the first characterization of neuronal diversity in the inner retina of</p>
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snakes, and will contribute to the investigation of post-receptor pathways for visual processing in diurnal and nocturnal snakes.

Keywords:

Snakes, Inner Retina, Bipolar Cells, Ganglion Cells, Immunohistochemistry, Golgi Staining



Title	Does performing manual adjustments in OCT images actually impact the measurements of central macular thickness in patients with IRDs?
Authors	Caio Henrique Marques Texeira Mariana Matioli da Palma Mariana Vallim Salles Juliana Maria Ferraz Sallum
Affiliations	Federal University of São Paulo, São Paulo, Brazil
Session	BRAVO

Abstract, Ethics Committee Number*, and Keywords	<p>The Optical Coherence Tomography (OCT) is important to evaluate the Inherited Retinal Diseases (IRDs) progression. One of the most used OCT systems is the Spectralis (Heidelberg Engineering, Germany). It automatically calculates the retinal measurements, which may be affected by patient's unstable fixation. In an attempt to avoid mismeasurement, it allows the examiner to manually adjust the grid position to the region of interest and the retinal layers segmentation. The purpose of this work is to understand if macular thickness changes when performing manual adjustments in the OCT images of patients with IRDs. This study was conducted at the Federal University of São Paulo and was approved by the Research Ethics Committee (number 6.267.758). The central macular thicknesses were analyzed from prospective and retrospective OCT images as follows: first, the one calculated automatically by the equipment; second, after a manual adjustment of grid position to center in the fovea; finally, after a manual adjustment of the Internal Limiting Membrane (ILM) and Bruch's Membrane (BM) segmentation. Images of 32 eyes, from 16 patients, were included. Patients were affected by variants in the <i>ABCA4</i> (n=7; 43.75%), followed by <i>RPE65</i> (n=5; 31,25%) and <i>CHM</i> (n=4; 25%). There was statistically significant difference between the measures calculated by the equipment and the ones after adjusting the grid position (p=0.002); and between the measures calculated by the equipment and the ones after adjusting the retina segmentation (p=0.014). Since the retina segmentation was performed after adjusting the grid position, and there was no statistically significant difference between these two groups, based on this sample we suggest that only the grid position adjustment is necessary for accurate central macular thickness measurements. This would avoid incorrect segmentation by the examiner and save much time in the image processing.</p> <p>OCT Tomography; Retinal Dystrophy; Macular Thickness.</p>
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Title	Intraspecific differences in retinal function and opsin gene expression indicate distinct visual adaptations in males and females of the lizard <i>Enyalius perditus</i>.
Authors	Vitor Henrique Corredor ¹ Einat Hauzman ¹ André Maurício Passos Liber ^{1,2} Natália Torello-Viera ³ Cristhian David Sua-Céspedes ⁴ Ana Maria de Lauro Castrucci ⁴ Dora Fix Ventura ¹
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Session	Visão e Oftalmologia

Abstract, Ethics Committee Number*, and Keywords	Lizards have sophisticated color vision, with diurnal species possessing all-cone retinas containing up to five visual opsins. In <i>Enyalius perditus</i> , a diurnal Leiosauridae lizard, ecological intraspecific differences exist between males and females. Green-colored males prefer arboreal habitats, while brownish females are ground-dwelling. Such differences may reflect specific characteristics of their visual system. Here, we investigate genetic, morphological, and physiological aspects of the visual system of males and females of <i>E. perditus</i> (Ethics Committee, IP-USP, 1577270421). Fifteen individuals (10 male; 05 female) were recorded for electroretinography (ERG) under dark and light adapted conditions. Retinal function was assessed through different light intensities (white light flashes) ranging from 0.00095 to 9.49 Cd.s/m ² . After the ERG procedures, the animals were euthanized (thionembutal 100 mg/kg), and the eyes were collected for morphological and genetic analysis. ERG analysis revealed that male individuals had higher amplitudes for dark-adapted a- and b-waves, compared to females, at intensities of 0.95 and 9.49 Cd.s/m ² and for the b-wave under photopic stimulation (3.0 Cd.s/m ²). The five visual opsin genes were amplified by PCR and Sanger sequenced. The spectral sensitivity peaks of the opsins were estimated at 358 (SWS1), 448 (SWS2), 494 (RH1), 497 (RH2), and 560 (LWS) nm. Quantitative PCR (qPCR) revealed higher relative expression of the RH2 opsin gene in females. Retinal morphology showed a visual streak with a central fovea in both males and females. The outer retina contains cone-like photoreceptors with approximately 1,070,000 cells (mean density: 20,953.4 ±
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9,352.1 cells/mm²). In conclusion, significant disparities in *E. perditus* retinal response to light, and levels of RH2 opsin gene expression may be associated with sexual selection and/or adaptations to different light conditions in microhabitats predominantly occupied by males and females.

Keywords: lizards; retina; color vision; photoreceptors; cones; opsins; ERG



Title	Analysis and determination of the allelic frequency of opsins in <i>Sapajus nigritus</i>
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Session	Vision and Ophthalmology

Abstract, Ethics Committee Number*, and Keywords	<p>The mammalian visual system comprises photoreceptors, cones, and rods, crucial for visual information processing. Most mammals possess two color vision genes: LWS and SWS1, expressed in L/M and S cones, respectively. In Platyrrhines, the LWS genes, located on the X chromosome, express photopigments in L/M cones, leading to dichromacy in homozygous males and females when combined with S cones. Heterozygous females have two distinct LWS alleles on the X chromosome, resulting in trichromacy when combined with the S cone gene. The color vision diversity in <i>Sapajus</i> makes them a valuable model for visual plasticity studies. This study aimed to analyze the allelic frequency of opsins genes in <i>Sapajus nigritus</i> from Carlos Botelho State Park (PECB) in São Miguel Arcanjo – SP; estimate and compare the spectral sensitivity peaks of these opsins; and correlate allelic frequency with the animals' habitat. Fecal DNA extraction, PCR, and Sanger sequencing were conducted to identify the main spectral absorption sites of LWS opsin. The study was approved by the ethics committee CEUA/IPUSP no. 5871010420. Nine free-ranging individuals were analyzed. We found a combination of 4 alleles: SYT (560 – 563 nm), SFT (546 – 553 nm), AYT (550 – 556 nm), and AFT (542 – 547 nm), with AFT and SFT being the most and least frequent alleles, respectively. Our results suggest that <i>S. nigritus</i> has a greater ability to discriminate brightness differences between medium wavelengths, optimizing their visual function in this region of the spectrum. This could contribute to a high consumption of green leaves and vegetative parts of palms, corroborating the literature. We conclude that the evaluated population in this study exhibits a diversity of 4 visual opsins alleles, with the highest frequency for the allele with a spectral absorption peak between 542 – 547 nm.</p> <p>Keywords: Neotropical primates. <i>Sapajus</i>. Opsins. Color vision. Molecular biology.</p>
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Title	Electroretinograms to sine-wave modulation in fish
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Session	23 – Visão e Oftalmologia

Abstract, Ethics Committee Number*, and Keywords	<p>Fish models are largely used to investigate pathomechanisms in diseases affecting the visual system and in pharmacological manipulations to study retinal physiology. The electroretinogram (ERG) in fish constitute a valuable functional probing tool for pre-clinical trials for retinal diseases treatment. Therefore, detailed mapping of the ERG responses in fish would contribute to the design of experiments using fish models to study the retina. We sought to record ERGs to sine-wave modulation in zebrafish, goldfish and the Nile tilapia. Animal procedures were in accordance with ethical principles of animal management and experimentation established by CEUA/IPUSP 5925190624. Animals were dark adapted for 12 hours before experiments. They were anesthetized by immersion in Eugenol solution (80 mg/mL), maintaining anesthesia with a 40 mg/mL Eugenol solution administered orally by pumping. They were positioned in a recording chamber (Ganzfeld) for ERG procedures, with gold ring electrodes placed centrally on the corneas to record light response patterns. The animals were positioned face down on a mobile platform inside the recording chamber, with the active and reference electrodes positioned intraorally and ground electrode close to caudal end. Photopic ERGs to sine-wave stimuli (mean luminance = 60 cd/m² and 100% contrast) were used to test post-receptoral cone-driven mechanisms that underlie temporal luminance processing. The signals were analyzed using Fast Fourier Transformation to extract the first harmonic amplitudes and phases as a function of temporal frequencies between 3 and 30 Hz. Amplitudes were high at lower temporal frequencies and decreased as temporal frequency increased. Similarly, phases showed a quasi-linearly change as a function of temporal frequency. This sine-wave ERG response profile is also found in mice, but not primates.</p> <p>Keywords: flicker, electroretinogram, fish retina</p>
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Title	Characterization of the visual system in the <i>Pcdh15^{roda}</i> mutant mouse, an animal model for usher syndrome, using electroretinography and optical coherence tomography
Authors	Moreno-Carmona, F.M. ^{1,2} Liber, A.M.P. ^{2,5} Mori, C.M.C. ³ Massironi, S.M.G. ³ Safatle, A.M.V. ⁴ Costa, M.F. ^{1,2} Ventura, D.F. ^{1,2}
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Session	23 – Visão e Oftalmologia

Abstract, Ethics Committee Number*, and Keywords	<p>The <i>Pcdh15^{roda}</i> (<i>rodador</i>) mouse presents an autosomal recessive disorder characterized by deafness, balance dysfunction. Here we investigate if there is also an alteration of visual function. In humans, the orthologous mutation of the <i>PCDH15</i> gene is responsible for Usher Syndrome 1F (US1F), a ciliopathy characterized by deafness, retinitis pigmentosa (RP), and vestibular dysfunction. Electroretinogram (ERG) was recorded in 24 mice (12 <i>rodador</i>; 12 <i>BALB/c</i> controls) under scotopic and photopic conditions, at different light intensities (-3.7; -2.7; -1.7; -0.7; 0.3 log cd.s.m⁻²). Amplitude reductions were observed in the scotopic b-wave [at intensities from -3.7 to -0.7 log cd.s.m⁻², respectively, 26.6% (p=0.001), 24.53% (p=0.038), 29.43% (p<0.001), and 28.67% (p<0.001)], as well as in the photopic a-wave (0.3 cd.s.m⁻²) by 24.79% (p=0.027). No statistically significant differences were observed in implicit time. Optical coherence tomography (OCT) analysis revealed a decrease in total retinal thickness (TR) (p=0.05). These results demonstrate that <i>Pcdh15^{roda}</i> mice, although not faithfully reflecting the RP found in US1F, exhibit significant alterations in ERG, with a reduction in TR thickness. The decrease in amplitudes, preservation of the b/a wave ratio and architecture of the waves in ERG, agree with the findings in the study of <i>Pcdh15^{av-5J}</i>, <i>Pcdh15^{av-Jfb}</i>, and <i>Pcdh15^{R250X}</i> models. The <i>rodador</i> mouse constitutes a new model for the study of deafness, and our results contribute to the characterization of the model for the study of visual function in US1F. USP RECs approval nos.: IP-1492130323; FMVZ-2236310122. Keywords: Electrophysiology, ERG, OCT, Usher, <i>Pcdh15</i>, <i>Pcdh15^{roda}</i>. Financial Support: CAPES Scholarship (Doctorate</p>
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Title	Vision beyond the ordinary: Advances in the treatment of keratoconus using lenticules - A review of the literature
Authors	Luís Henrique Barroso de Paula Johnny Júnior Fernandes de Castro Izabela Rosa Garcia Paiva Lays Fernanda Nunes Dourado
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Session	23 - Visão e Oftalmologia

Abstract, Ethics Committee Number*, and Keywords	<p>Keratoconus is a progressive eye disease characterized by thinning and irregular deformation of the cornea, resulting in visual distortion and reduced acuity. In the last decade, a great number of alternative approaches were investigated to treat this condition. Among the therapeutic options, the use of corneal lenticules has stood out as a promising alternative. The present work aims to review the advances in the treatment of keratoconus using corneal lenticules. For this purpose, the review methodology was focused on searching for articles in the PubMed and EMBASE databases, using the terms “Keratoconus” and “Lenticule”. As inclusion criteria, articles published in the last 10 years were selected and carried out as systematic reviews on the proposed theme. As an exclusion criterion, articles that did not employ the lenticules for keratoconus treatment were removed. Our research resulted in eight systematic reviews published between 2014 and 2024. Three duplicates were excluded, and two publications were removed based on the exclusion criteria, thus, three reviews were selected. According to the published studies, currently, the keratoconus treatment is mostly performed using a lenticule obtained from a donated cornea. For this, the cornea tissue is prepared by an incision by laser to obtain a small fragment of the cornea, a lenticule. The lenticule insertion has proven to be more interesting than other techniques, such as deep lamellar and penetrating keratoplasty because of a reduced rejection rate, accelerated recovery, and facilitated surgical technique. Moreover, due to the small fragment of cornea used in this technique, is possible to use cornea that was previously discarded in cornea transplant surgeries, reducing the waiting time for transplant. Thus, keratoconus treatment using corneal lenticules represents a significant advance, offering an effective and less invasive alternative for improving the keratoconus treatment.</p> <p>Keywords: Corneal transplant, Corneal disease, Laser, SMILE.</p>
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Title	Color vision of an indigenous community environmentally exposed to pesticides
Authors	Bruna Sofia Pedroso Dezincourt ¹ Laenna Morgana Cunha da Silva ¹ Victor Matheus Silva de Miranda ¹ Juliana Nascimento da Silva ¹ Givago da Silva Souza ² Eliza Maria da Costa Brito Lacerda ¹
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Session	Visão e Oftalmologia

Abstract, Ethics Committee Number 6,658,853, and Keywords	<p>The expansion of monoculture over traditional communities in western Pará has raised concerns due to the exposure to inputs such as pesticides being linked to various health problems, including those involving the nervous system. The aim of this study was to assess color vision in an indigenous community environmentally exposed to pesticides. This study was approved by the Ethics Committee on Human Research of UFOPA (#6,658,853). The research is an observational cross-sectional study consisting of 40 individuals (28 women) aged between 22 and 69 years (mean=38, standard deviation=16). The sample was divided into an exposed group (20 indigenous individuals living in rural areas of Santarém on undemarcated land surrounded by soybean plantations) and a matched control group in terms of age and gender (20 individuals living in the state capital). Volunteers underwent visual acuity testing using the Freiburg Visual Acuity & Contrast Test (FRACT) version 3.7, congenital color vision deficiency was analyzed using the Ishihara Pseudoisochromatic Plates, and acquired color vision deficiency was assessed using the desaturated Lanthony D15 test. The eye with the best visual acuity was chosen for color vision study. Statistical analysis included the D'Agostino Pearson test and t-test (alpha=0.05). There was no difference in visual acuity results (p=0.1023). All results from both groups assessed by the Ishihara Pseudoisochromatic Plates showed no more than 2 errors (p=0.2052). Regarding the analysis of the Lanthony D15d test, the S-index was similar between the groups (p=0.1337). The group of indigenous individuals exposed to pesticides showed a higher C-index (p=0.0046) and tilt angle of errors on the color axis (0.0019) than the control group. We concluded that environmental exposure to pesticides is associated with alterations in color vision.</p> <p>Keywords: Neurointoxication. Visual system. Pesticides</p>
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Title	Color vision and luminance discrimination in adolescence
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Session	22 - Visão e Oftalmologia

Abstract, Ethics Committee Number*, and Keywords	<p>Several studies suggest that the achromatic processing pathway undergoes faster development compared to the color processing pathways, potentially impacting color-luminance interaction mechanisms throughout the lifespan, particularly in adolescents, where the visual pathways are still maturing. The aim of this study is to compare the influence of different masking chromatic mechanisms on luminance threshold contrast in adolescents. Twenty participants (15.7±0.8-year-old) with normal vision and normal results in the Ishihara's test, participated in this research. The current study was approved by the Ethics Committee from the Federal University of Pará (#6.546.671). They performed a luminance contrast discrimination test in a stimulus using mosaics with chromatic noise maskers. Four chromatic noise masking protocols were applied. The noise consisted of 10 chromaticities with a vector size of 0.04 u'v' units projected radially from a reference color (CIE 1976: u'=0.194; v'=0.463) near the protan, deutan and tritan confusion lines (protan, deutan and tritan protocols), and a condition without chromatic noise (no-noise protocol). A subset of circles in the stimulus differing in luminance from the background emerges perceptually forming a target with a Landolt-C shape, displayed in 4 possible positions for 3 seconds on each trial. A staircase method controlled the target luminance. The last 6 reversals were used to estimate the luminance contrast threshold of the participant. For comparisons, we applied a one-way ANOVA, with Tukey's test and $\alpha=0.05$. The luminance contrast in adolescents was: protan: 41.3±8.9; deutan: 40.6±6.9; tritan: 40.8±8.1 and no-noise: 26.4±4.7. The chromatic protocols did not differ significantly, but when compared to the no-noise protocol, there was a significant difference ($p\leq 0.01$). These findings suggest that in the adolescence period the maturation rate is equalized across the color confusion axes, which appear to be in the same pace of development.</p> <p>Keywords: development; chromatic mechanisms; achromatic mechanisms.</p>
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Title	Evaluation of therapeutic potential of the type c lectin from <i>bothrops leucurus</i> snake venom for ocular neovascularization treatment
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Session	23 - Visão e Oftalmologia

Abstract, Ethics Committee Number*, and Keywords	<p>Ocular neovascularization is an associated condition in diseases such as Age-Related Macular Disease and Diabetic Retinopathy. It occurs when there is an increase of blood vessels that are fragile and they can rupture, promoting hemorrhagic spots, which causes a reduction in visual acuity. Currently, there are few treatments available, and these are high cost so, the search for new substances with antiangiogenic activity has gained importance. In this scenario, a lectin protein (B. pradoi) from the venom of the <i>Bothrops leucurus</i> snake has stood out for its ability to reduce blood vessels in previous studies. Based on this, the present study aims to evaluate the antiangiogenic activity of B. pradoi in a suture-induced angiogenesis model. For this, the right cornea of Wistar rats received two nylon sutures. On that same day, was started a topical treatment with B. pradoi or positive and negative controls (Bevacizumab and Saline solution, respectively) for seven days. Before and after the last application, the animals will be evaluated using a slit lamp and optical coherence tomography (OCT) to identify changes in the vessels and corneal thickness. Lastly, the animals were euthanized, the eyes enucleated and prepared for histological evaluation and transmission electron microscopy (MET) analysis to observe changes in the ultrastructure tissue (Protocol n°018-2023). Slit lamp images reveals that the topical use of Lectin and Bevacizumab (both in the concentration 3,5 µg/mL) was capable to promotes an expressive reduce in the corneal vessels. Moreover, the OCT shows a reduces on corneal diameter after these treatments. Histological and MET analyses shows a reduction in the number of vessels blood vessels and a lower presence of inflammatory cells, mainly in the B. Pradoi group. These findings contributed to elucidate the antiangiogenic activity of B. Pradoi, that may represent a promising treatment for ocular diseases that affected millions of people around the world.</p> <p>Keywords: angiogenesis, cornea, Lectin.</p>
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Title	Multimodal characteristic of Stargardt cases by ABCA4
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Affiliations	
Session	22 - Visão e Oftalmologia

Abstract, Ethics Committee Number*, and Keywords	<p>Purpose, To compare multimodal characteristics of Stargardt cases by ABCA4 after 10 years of first achieved data.</p> <p>Materials and Methods, Cases of Stargardt disease by variants in the ABCA4 gene were enrolled. From the patients' medical record were collected data about visual acuity, age of symptom onset and multimodal exams, including retinography, fundus autofluorescence imaging (FAF) and optical coherence tomography (OCT). These multimodal exams were acquired in 2013 and compared with others acquired in 2023.</p> <p>Results, At this initial time, 5 cases were enrolled. Their visual acuity kept the same or decreased in 2 lines after the first appointment. The multimodal data show that in cases of disease onset affecting a small foveal area, the progression did not affect the peripheral retina. Differently, cases that present flecks or pigmentary alterations in peripheral retina progressed with diffuse dots of retinal atrophy. In 2 cases were identified a hyperpigmented lesion in peripheral area with a fibrosis-like aspect attached. This lesion can be seen in FAF as a block of retinal autofluorescence. The OCT determinate those lesion in the subretinal space. One case start with perivascular bone spicule pigmentation at the mid-periphery. At the OCT those bone spicule are in the retina and create a shadow projections.</p> <p>Conclusions. The stargardt cases multimodal data are objective information that can be used to evaluate comparing different stages of the disease. This longitudinal analysis is important for the possibility of future therapies and their indicative for different individuals and their disease progressions findings.</p>
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Title	Effects of the association of photobiomodulation using light-emitting diode therapy (LEDT) with resveratrol on oxidative stress and cell viability in C2C12 muscle fibers
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Session	24. Medicina Regenerativa e Biologia do Desenvolvimento

Abstract and Keywords	<p>LED light is a non-pharmacological intervention to promote inflammation modulation, tissue regeneration, and antioxidant action, showing beneficial effects on the dystrophic muscles. Resveratrol has been showing antioxidant and anti-inflammatory properties in different animal models. This study evaluated the release of oxidizing agents and cell viability after treatment with light-emitting diode therapy (LEDT) associated with Resveratrol (RSV) in myotubes of C2C12 culture cells. C2C12 myotubes were submitted to oxidative stress by hydrogen peroxide for 4 and 24 hours (200µM) and treated with LEDT at 5 J/cm² and RSV (6,25µM) to evaluate cytotoxic effects and oxidative stress marker levels. Cultures were divided into seven experimental groups: 1. CTRL; 2. CTRL-LED; 3. RSV-S-LED (stressed cells by 4h + LEDT + RSV by 24h); 4. S-4H-LED (stressed cells by 4h + LED); 5. S-24h-LED (stressed cells by 24h + LED); 6. S-RSV 24H-LED (Cells simultaneously stressed by H₂O₂+RSV by 24h + LED); and 7. S-RSV 4H-LED (Cells simultaneously stressed by H₂O₂+RSV by 4h + LED). The microplates were exposed once at 630 nm, at 9 cm, across the entire plate, for 3 minutes before RSV treatment. Analyzes were performed 24 hours after irradiation. The MTT and Neutral Red assays showed that LEDT presented no cytotoxic effect compared to the non-irradiated cells. All groups submitted to oxidative stress and LEDT showed a significant decrease (by 70%) in cell viability compared to control. The H₂O₂ levels in the RSV-S-LED, S-4H-LED, S-RSV 4H-LED showed no significant difference from the control. In other groups submitted to oxidative stress, the H₂O₂ levels were significantly higher than control. LEDT presented no cytotoxic effects. LEDT associated with RSV failed to reverse the damage caused by oxidative stress. LEDT associated with RSV has antioxidant action, decreasing hydrogen peroxide release. The combined treatment protocol may even provide a potentially therapeutic strategy for muscular dystrophies.</p> <p>Keywords: LEDT; oxidative stress; resveratrol; muscle cell; muscular dystrophies.</p>
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Title	Evaluation of the antioxidant potential and cytotoxicity of resveratrol in C2C12 muscle fibers
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Session	24. Medicina Regenerativa e Biologia do Desenvolvimento

Abstract and Keywords	<p>Resveratrol (RSV) is a nutraceutical with anti-inflammatory and antioxidant properties in several disease models, presenting a potential candidate for the pharmacological treatment of Duchenne muscular dystrophy (DMD). This study aimed to evaluate the antioxidant potential and cytotoxicity of RSV in myotubes of C2C12 culture cells. C2C12 myotubes were submitted to oxidative stress by hydrogen peroxide (H₂O₂ - 200µM) for 4 and 24 hours and RSV treatment (6,25µM) for 4 or 24 hours to evaluate cytotoxic effects and oxidative stress marker levels. Cultures were divided into eight experimental groups: 1.CTRL; 2.RSV-NS (Cells+RSV by 24h); 3.DMSO-NS (Cells+DMSO by 24h); 4.RSV-S (Stressed cells 4h + RSV by 24h); 5.S-24H (Stressed cells by 24h); and 6.S-RSV (Cells simultaneously stressed by H₂O₂+RSV by 24h) and 7.S-RSV 4H (Cells simultaneously stressed by H₂O₂+RSV by 4h). The MTT and Neutral Red assays showed that RSV treatment presented no cytotoxic effect. All stressed groups showed a significant decrease in cell viability compared to the control in the MTT assay (by 86%). The Neutral Red assay showed a significant decrease in cell viability (by 70%) in all stressed groups vs control, however, the RSV-S group showed a 56% decrease, indicating better viability than the other experimental groups. The H₂O₂ levels in the RSV-S group were not significantly different from the control. In other experimental groups submitted to oxidative stress, the H₂O₂ levels were significantly higher than control. Resveratrol presented no cytotoxic effects. The concentration of H₂O₂ used was cytotoxic, significantly reducing cell viability. Resveratrol was unable to reverse the damage caused by oxidative stress. The data suggest that Resveratrol had antioxidant action, decreasing hydrogen peroxide release, highlighting the potential of this nutraceutical as a therapeutic agent for DMD through its antioxidant action.</p> <p>Keywords: Resveratrol; oxidative stress; muscle cell; Duchenne muscular dystrophy.</p>
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Title	Propolis Faveleira's performance in the repair of second intention skin injuries
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Session	24- Regenerative Medicine and Developmental Biology

Abstract and Keywords	<p>The skin is the largest organ of the body, and its integrity is fundamental for overall health; injuries to this organ can compromise quality of life. Repair following any injury requires synchronous cellular and molecular activation that begins at the moment of skin rupture. There is no gold standard protocol for treating skin lesions; because of this, we are seeking new products, as well as understanding the action of faveleira propolis in the inflammatory phase of tissue repair.</p> <p>To this end, Wistar rats with secondary intention dorsal injury (anesthesia and ethical protocol CEUA IBB-5550250222) were treated for three days divided into 7 groups: Physiological (SHAM), Commercial drug controls: Neomycin sulfate 5 mg/g + bacitracin zincium 250 IU/g; Dexpanthenol 50 mg/g and Collagenase 0.6 IU/g, and Tests on hydroalcoholic solutions of Faveleira propolis at 0.5, 1 and 2%. Samples from the lesion region were subjected to morphological analyzes to quantify cellularity and total collagen, by immunolabeling: blood vessels (CD-31), cell proliferation (Ki67), fibroblasts (Anti- S1) and cell proliferation and differentiation (TGF- β) and by the Elisa method the inflammatory mediators: TNF-alpha, IL-1beta, IL-6 and IL-10. Data were analyzed using a statistical method using GraphPad Prism 5.01.</p> <p>The test groups showed 5% to 20% greater lesion retraction than the other pharmacological groups, where a greater number of cells and a greater area of collagen fibers were observed. In the 2% test group, a greater number of fibroblasts (Anti-S1) and differentiating cells (TGF-β) were observed. It was observed that faveleira propolis has anti-inflammatory activity via IL-10, especially at a concentration of 1%.</p> <p>Our data indicate that faveleira propolis, in secondary intention skin lesions, has a similar action to the drugs used in this study as controls, mainly in controlling inflammation and wound retraction in the initial phase.</p> <p>Keywords: faveleira propolis, repair, skin lesions.</p>
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Title	Mast cells in the dermis in the second intention skin healing process under treatment with Brazilian Red Propolis
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Session	Medicina Regenerativa e Biologia do Desenvolvimento

Abstract and Keywords	<p>The skin is the largest organ in the human body and protects internal tissues, making it vulnerable to injury and clinical complications. Tissue repair is a complex process that is divided into three overlapping phases: inflammation, proliferation and remodeling, all of which mast cells are present in. When stimulated, mast cells generate and release granules containing mediators that can accelerate the healing process by activating cytokines and growth factors for cell differentiation. The closure of lesions and the regulation of processes during healing are dependent on the presence of mast cells, but these are the main inflammatory cells found in hypertrophic scars, showing the need for a balance of these cells for efficient healing. Brazilian Red Propolis (BRP) is composed mainly of flavonoids, phenolic compounds that are therapeutically important because they have antimicrobial and antitumor activity.</p> <p>Our aim is to understand the biological activities of mast cells in tissue repair, with a focus on the skin dermis in second intention injuries. To this end, 3 groups of Wistar rats were used, each with 6 animals, treated for 3, 7 and 14 days with 1% red propolis hydroalcoholic extract (group A+P), 1% red propolis cream (group P+P) (CEUA-IBB: 9793211119 and 3695260923), and then submitted to skin excision in the dorsal region. The histological sections were stained with 1% toluidine blue at pH 2.5 to count the number of mast cells in the dermis. The results obtained were submitted to statistical analysis which showed that there was no statistical difference between the number of mast cells using 1% PVB in the two formulations tested, indicating that the action does not depend on the administration vehicle.</p> <p>Keywords: Mast cells, Skin healing, Brazilian red propolis</p>
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Title	The influence of graphene concentration in PCL/Graphene scaffolds: gene expression and histological investigation for bone regeneration
Authors	Julia Eduarda Schneider Gregório ¹ Camila Lorencetti ² Cintia Sena Bueno ¹ Guilherme Ferreira Caetano ^{1,2}
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Session	24 - Regenerative Medicine and Developmental Biology

Abstract, Ethics Committee Number*, and Keywords	<p>In cases of extensive bone loss, such as trauma and tumor resection, current strategies are not entirely effective. Scaffolds offer a promising alternative for bone regeneration. PCL is a biocompatible material, but there is no bioactivity. The addition of graphene can enhance the biological, physical, and mechanical properties. This study aimed to evaluate the osteogenic and inflammatory response of using PCL/Graphene scaffolds in a critical bone defect in rat model. A total of 24 Wistar rats were divided into 4 groups: PCL, G1, G3, and G5, each one with specific concentration of graphene (0 wt%, 1 wt%, 3 wt%, and 5 wt%, respectively). Animals were operated under anesthesia to create a 24mm² bone defect, and the scaffolds were implanted. The animals were euthanized after 60 days and the bone/scaffold samples were collect. The gene expression related to osteogenesis (<i>Bmp-2</i>, <i>Sost</i>, and <i>Spp1</i>) and inflammation (<i>Il-6</i>, <i>Cd68</i>, and <i>Arg-1</i>) was assessed using real-time quantitative PCR (RT-qPCR). The results were presented using the 2^{-ΔΔCt} method. The formation of mineralized tissue was assessed by histomorphometry (Mallory's Trichrome staining), evaluating the percentage of area. The data was analyzed using the one-way ANOVA test, followed by the Bonferroni post-test or the Kruskal-Wallis test, followed by the Dunn post-test. The G5 group showed higher <i>Bmp-2</i> and <i>Spp1</i> gene expression (10 times and twice, respectively) compared to the PCL group. However, for the <i>Sost</i>, the G1 group showed higher expression (three times). The G5 group presented higher inflammatory gene expression when compared to the other groups. The molecular results obtained were confirmed by histomorphometry, which showed larger area of mineralized tissue formation in G5 group. The use of PCL scaffolds with 5% graphene positively influenced the inflammatory and osteogenic response, enhancing the bone regeneration process.</p> <p>Ethics Committee Number: CEUA (007/2023). Keywords: regenerative medicine, bone regeneration, graphene</p>
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Title	Exploring neuronal differentiation potential of dental tissues stem cells through neurosphere formation
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Session	24 – Regenerative Medicine and Developmental Biology

Abstract, Ethics Committee Number*, and Keywords	<p>Apical papilla (SCAP) and dental pulp (DPSC) stem cells are adult cells with self-renewal capacity and hold potential for enhancing neuron differentiation. Their common origin from the neural crest in the ectoderm suggests they may naturally express neuronal-like markers. One of the techniques used to initiate neuronal differentiation is through 3D cultivation using neurospheres. This study aims to characterize DPSC and SCAP and neurospheres generated during the first steps of neuronal differentiation. This project was approved by the Local Ethics Committee (67807123.8.0000.0020). The cells were isolated from third molars and cultured. The expression of neuronal markers like βIII-tubulin and nestin was evaluated by immunofluorescence. For neurosphere induction, the cells were cultured for five days using DMEM/F12 medium supplemented with EGF, FGF and B27. Neurosphere characterization was made on days two and five with classification sizes as small (<50μm), medium (51-250μm) and large (>251μm), with results in means, standard deviations and percentages. Both DPSC and SCAP exhibited a mesenchymal stem cells (MSC) profile and expressed βIII-tubulin and nestin before neurosphere formation. SCAP showed a significant difference in quantity, while DPSC expressed a significant difference in size between days. During the cultivation period, there were no significant difference in size or quantity between the cell types. Medium-sized classification was predominant for both SCAP and DPSC. On day five, the percentage of medium size neurospheres decreased for SCAP (58.6%;55.6%) due to the increase in large neurospheres (2.38%;5%). For DPSC, it increased for medium (57%;63.5%) and large sizes (1.63%;7%), indicating cellular aggregation. Thus, DPSC and SCAP exhibited MSC characteristics and efficacy in neurosphere formation. Additionally, they showed a greater quantity of medium sized neurospheres, which may present a more favorable condition for neuronal markers expression.</p> <p>Keywords: dental pulp; apical papilla; ectoderm; 3D cultivation; mesenchymal stem cells.</p>
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Title	Histological evaluation of PCL/Graphene scaffolds at different concentrations for bone tissue engineering
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Session	24 - Regenerative Medicine and Developmental Biology

Abstract, Ethics Committee Number*, and Keywords	<p>Bone tissue engineering is looking for new approaches to repair critical bone defects. The use of graphene (G) scaffolds are able to improve the physicochemical and mechanical properties of biomedical polymers, such as polycaprolactone (PCL) and stimulates osteoblast adhesion, proliferation, and bone formation. This study aimed to conduct a histological evaluation of the application of PCL/G scaffolds in rat calvaria. The scaffolds were obtained by fusing PCL pellets and graphene nanosheets. A total of 24 Wistar rats were divided into 4 groups: PCL, G1, G3, and G5, containing 0 wt%, 1 wt%, 3 wt%, and 5 wt% graphene, respectively. The scaffolds were implanted in 24mm² critical bone defect, created by surgical procedure. On the 30th day, the animals were euthanized, and the scaffold/bone defect area was collected for histological analysis. The samples were decalcified and subjected to routine histological procedures. Cross-sections of 4.0µm thickness were made and stained with Mallory's Trichrome. Photomicrographs were captured at 200x magnification, and the images were analyzed using ImageJ software. The following parameters were evaluated: percentage of connective tissue area, osteoid tissue, and mineralized tissue. The data was compared using the one-way ANOVA test, followed by the Bonferroni post-test or Kruskal-Wallis followed by the Dunn post-test. PCL, G3 and G5 groups presented around 60% of connective tissue, while G1 group presented around 40%. There was a similarity between the groups for osteoid tissue. All groups were quantified around 15%. However, regarding the mineralized tissue, the addition of graphene to the scaffolds increased its formation, particularly in group G1, which showed 40%, while, G3 and G5 12%, and PCL only 2%. The use of scaffolds with 1% graphene (G1) showed greater formation of mineralized tissue and therefore greater potential for bone regeneration.</p> <p>Ethics Committee Number: CEUA (007/2023). Keywords: tissue engineering, scaffolds, graphene</p>
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Title	Scaffolds composed of polycaprolactone, hydroxyapatite and carbon- nanotubes combined with electrical stimulation in bone regeneration
Authors	Cintia Sena Bueno ¹ Júlia Eduarda Schneider Gregório ¹ Camila Lorencetti ² Brenda Carolina Pereira ² Mayara dos Santos Deuri ² Gabriel Cyrillo Felisberto Viana ² Guilherme Ferreira Caetano ^{1,2}
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Session	24 - Regenerative Medicine and Developmental Biology

Abstract, Ethics Committee Number*, and Keywords	<p>The tissue engineering brings new alternatives to solve the challenges of critical bone defects. The scaffolds act to provide cell adhesion, growing and differentiation. The ceramic composites are similar to natural bone components, like hydroxyapatite (HA). The carbon-nanotubes (CNT), besides improving the mechanical proprieties, are capable of mimetize the fibrous proteins and provide electrical conductivity. Moreover, the application of electrical stimulation (ES) combined to the use of electrical conductive scaffolds could activating in signaling pathways. This study aims to evaluate whether this combination improves the mineralization potential and stimulates signaling pathways for bone repair. Female Wistar rats were divided in groups: PCL; PCL/CNT and PCL/HA/CNT; and subdivided considering the intensity of the electric estimation: no electric stimulation; 10µA; 50µA and 100µA. The current was applied 3 times a week for 3 minutes. After 14 days of treatment, euthanasia was performed and the samples were collected, followed by histomorphometric and molecular analysis. Our partial results demonstrated the use of PCL/CNT and PCL/CNT+10µA intensity increased mineralized tissue formation. However, the relative expression Cacna1d gene in PCL/CNT+10µA was higher than PCL and PCL/CNT. However, the PCL/HA/CNT scaffold presented increased mineralized tissue in only 50µA intensity. Interesting, this groups also presented higher Cacna1d gene expression compared to the others, and higher Camk2 gene compared to PCL/CT/HA+10µA. The use of ES is able to activate signaling pathways, stimulating calcium influx and interaction with calmodulin, which can explain the increased mineralization process. PCL/CNT+10µA can improve bone formation, and the addition of hydroxyapatite can influence the scaffold/bone tissue</p>
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interaction with the electric current intensity, since it also showed improved bone formation, but applying higher ES intensity.

Ethics Committee: CEUA – 002/2023

Keywords: bone repair; scaffold; calmodulin; hydroxyapatite; carbon nanotubes; electric stimulation.



Title	Therapy with exosomes for bone regeneration in experimental calvaria defects <i>in vivo</i>: systematic review
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Session	24 Regenerative Medicine and Developmental Biology

Abstract, Ethics Committee Number*, and Keywords	<p>Tissue engineering has created possibilities for the advancement of bone regeneration, which may culminate in an ideal therapy for the treatment of bone defects. Thus, the efficiency of exosome (Exo) can be deduced. Hence, this research aimed to analyze, by systematic review, the effectiveness of therapy with Exo for bone regeneration in experimental calvaria defects <i>in vivo</i>. The study was conducted following the recommendations of PRISMA. The electronic literature search was performed until January 2024 using PubMed/MEDLINE, Scopus, and Cochrane Library databases to answer the PICO question: "Would therapy with Exo be efficient for bone regeneration?" Bone regeneration was considered the primary outcome. The administration of Exo, the origin cells of Exo, and the molecular pathways were analyzed, too. The risk of bias was analyzed according to the criteria of SYRCLE's RoB tool. A total of 3718 articles were analyzed, and after applying the eligibility criteria and excluding duplicate articles, 56 articles were selected. Bone regeneration was evidenced after using Exo with a significant increase in the volume of mineralized bone per unit of bone volume (BV/TV) and bone density (BMD) compared to not using Exos. The majority of studies associated Exo with scaffolds for applications in bone defects. And, bone marrow stem cells and adipose stem cells were more used in the extraction of Exo. An increase in the indices of BMP-2, OCN ALP, and Runx2 was observed, indicating the osteogenesis pathway. Angiogenesis pathway was observed, too. The risk of bias analysis identified high scientific evidence in most of the selected studies. It was concluded that therapy with Exo has the potential for bone resorption in experimental defects <i>in vivo</i>, showing promise for this use.</p> <p>Keywords: exosome, extracellular vesicles, bone regeneration</p>
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Title	Neuroprotective Effects of Dental Pulp Stromal Cells in a Parkinson's Disease Model
Authors	Letícia Fracaro ¹ Evellyn Mayla Azevedo ² Agner Henrique Dorigo Hochuli ¹ Jessica Ilkiw ² Mateus de Oliveira Lisboa ¹ Fabiane Barchiki ¹ Ellen Larissa Bail ² Alejandro Correa ³ Alexandra Cristina Senegaglia ¹ Marcelo de Meira Santos Lima ² Paulo Roberto Slud Brofman ¹
Affiliations	1 - Core for Cell Technology, Pontifícia Universidade Católica do Paraná (PUCPR), Curitiba, Brazil 2 - Neurophysiology Lab -Universidade Federal do Paraná (UFPR), Curitiba, Brazil 3 - Laboratory of Basic Biology of Stem Cells, Carlos Chagas Institute, Fiocruz-Paraná, Curitiba, Brazil
Session	24 - Medicina Regenerativa e Biologia do Desenvolvimento

Abstract, Ethics Committee Number*, and Keywords	<p>Parkinson's disease (PD) is a neurodegenerative disorder caused by the death of dopaminergic neurons. Current treatments for PD predominantly focus on relieving motor symptoms, emphasizing the crucial need to explore novel therapeutic approaches targeting neuroprotection and/or neuroregeneration. In this context, dental pulp mesenchymal stromal cells (DPSCs) emerge as a treatment option, as they are easily collected, express neuronal markers, and share the same embryonic origin as neurons. This study aimed to assess the impact of intranigral infusion of DPSCs with and without neuronal differentiation in a PD animal model. This project was approved by the Local Research Ethics Committee (CAAE 28861419.2.3001.0102)/CEUA 1372). DPSCs were isolated, characterized, and evaluated for chromosomal integrity. Part of the cells was induced into neuronal differentiation (DPSCd). PD was induced in rats using 6-hydroxydopamine, followed by transplantation of 100,000 cells after seven days. DPSCs were transduced for cell tracking. Locomotor activity was tested pre-and post-treatment. Seven days post-treatment, the animals were euthanized, and the density of dopaminergic neurons was evaluated using immunohistochemistry for tyrosine hydroxylase. DPSCs exhibited typical mesenchymal stromal cell traits and normal karyotypes without clonal chromosomal abnormalities and showed increased expression of Nestin and βIII-tubulin after differentiation. Both DPSCs and DPSCd were detected at the treatment site. Behavioral analysis revealed a significant locomotor decline post-neurotoxin, which improved after DPSC and DPSCd infusion. Both cell types helped reduce dopaminergic neuron death, supporting their role in motor control. The results demonstrated that DPSCs were</p>
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able to reverse motor impairment related to an increase in the density of dopaminergic neurons. These results suggest that DPSCs have neuroprotective potential and may be a therapeutic strategy for PD.

Keywords: Neuronal differentiation, mesenchymal stem cells, g-banding, Open field test.



Title	Exploring Heterogeneity in Dental Pulp Stem Cell Subpopulations for Regenerative Medicine
Authors	Anna Beatriz Seara Schlickmann Ana Helena Selenko Tatiane Mie Arakaki Moraes da Silva Paulo Roberto Slud Brofman Leticia Fracaro
Affiliations	Core for Cell Technology – Pontifícia Universidade Católica do Paraná, Curitiba, Paraná
Session	Medicina Regenerativa e Biologia do Desenvolvimento

Abstract and Keywords	<p>Dental pulp stem cells (DPSC) arouse great interest in basic and clinical research due to their regenerative potential and the ease of obtaining them through surgical procedures. Morphological variations have been observed in DPSCs cultured <i>in vitro</i>, suggesting the existence of distinct subpopulations of mesenchymal stem cells (MSC) in dental pulp. This study aims to characterize DPSC subpopulations to identify the most suitable sample for research. This project was approved by the Local Research Ethics Committee (5.409.680). Samples from third molars were collected and subpopulations were isolated. The dental pulp was separated into root (DPSC-R), crown (DPSC-C), and whole population (DPSC), which were subjected to enzymatic digestion and expanded <i>in vitro</i>. Samples were characterized by flow cytometry and differentiated into adipogenic and osteogenic lineages according to the criteria of the International Society for Cellular and Gene Therapy (ISCT). Furthermore, differentiation was quantified by spectrophotometry. To assess cell proliferation, an assay was used that detects the incorporation of the nucleoside EdU into DNA. Data were expressed as mean and standard deviation. DPSC and DPSC-R exhibited MSC characteristics, including adherence to plastic, fibroblastic morphology, and surface marker expression following ISCT criteria. However, DPSC-C exhibited a different morphology than expected and showed expression greater than 2% for CD14. DPSC, DPSC-R and DPSC-C exhibited calcium crystals in osteogenic differentiation and lipid vacuoles in adipogenic differentiation. When evaluating proliferation, there was no statistical difference between subpopulations. The results suggest differences between DPSCs and their subpopulations that could affect their regenerative potential. Therefore, it is important to consider these specific characteristics in regenerative medicine in basic, preclinical, and clinical research.</p> <p>Key words: characterization, proliferation, differentiation, quantification, adipogenic, osteogenic.</p>
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Title	Therapy with extracellular vesicles for the treatment of bone resorption due to osteoporosis: systematic review
Authors	Francisco Mônico Moreira Virginia Amarin Frões de Moraes Graziela Garrido Mori
Affiliations	Medicine Course of Presidente Prudente, University of Western São Paulo (UNOESTE) FAPESP Process N° 2023/11276-9
Session	24 Regenerative Medicine and Developmental Biology

Abstract, Ethics Committee Number*, and Key-words	<p>Therapies for osteoporosis show side effects, and the search for new approaches to solve these problems is essential. Extracellular vesicles (EVs), used in regenerative medicine, may represent an alternative for the treatment of osteoporosis. Thus, the objective of this research was to analyze, by systematic review, the effectiveness of EVs for the treatment of bone resorption resulting from osteoporosis <i>in vivo</i>. The study was conducted following the recommendations of the PRISMA. The electronic search was made until December 2023 using the PubMed/MEDLINE, Scopus, and Cochrane Library databases to answer the PICO question: “Would extracellular vesicle therapy be efficient for the treatment of bone resorption?” Control of bone resorption and bone regeneration were considered the primary outcomes. The molecular pathways and origin cells of EVs were also analyzed. The risk of bias was analyzed according to the criteria of SYRCLE’s RoB tool. A total of 680 articles were analyzed, and after applying the eligibility criteria and excluding duplicate articles, 14 articles were selected. The control of bone resorption and bone regeneration were evidenced after using EVs in comparison to not using them. Various studies showed an increase in bone density close to or similar to conditions without osteoporosis. A decrease in RANKL and TRAP and an increase in the levels of OPG, ALP, and Runx2 were observed, indicating the clastic inhibition and the osteogenesis activation pathways, respectively. Intravenous or intraperitoneal injections and stem cells from bone marrow or adipose tissue were the most used. The risk of bias analysis identified high scientific evidence in most of the selected studies. It was concluded that therapy with EVs has the potential to control bone resorption as a result of osteoporosis <i>in vivo</i>, showing promise for the treatment of this disease.</p> <p>Keywords: exosome, extracellular vesicles, bone resorption</p>
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Title	Exploring the therapeutic potential: Urine-derived stem cells
Authors	Maria Luiza de Castro ¹ Mateus de Oliveira Lisboa ² Leticia Fracaro ² Marcelo Távora Mira ¹
Affiliations	1 – Core for Advanced Molecular Investigation, Graduate Program in Health Sciences, School of Medicine and Health Sciences. Pontifícia Universidade Católica do Paraná (PUCPR), Curitiba, Brazil. 2 – Core for Cell Technology, School of Medicine and Health Sciences. Pontifícia Universidade Católica do Paraná (PUCPR), Curitiba, Brazil.
Session	Medicina Regenerativa e Biologia do Desenvolvimento

Abstract and Keywords	<p>The therapeutic potential of urine-derived stem cells (USCs) has been increasingly understood, having similar mechanisms to mesenchymal stem cells (MSCs), with a high potential for differentiation and immunomodulation. The use of USCs in research has been increasingly frequent. Thus, the characterization of USCs and evaluating the genetic stability is necessary. The assessment of the genetic stability of these cells is essential to show the reliability of the results in research and minimize the risk of tumorigenesis in clinical application. This study aimed to characterize urinary stem cells as mesenchymal cells, show the expression of neural markers and demonstrate their genetic stability. The study was approved by the Local Ethics Committee (5.128.887). Cells were obtained through the urine of healthy subjects with their consent. For USCs isolation, the samples were washed with PBS and antibiotics, centrifuged, and the cells were cultivated in a 6-well plate precoated with 0,1% gelatin in a specific culture medium (DMEM/F-12 + REBM) supplemented with REGM. Subsequently, the cells were characterized by flow cytometry using antibodies to MSCs, USCs and Neural markers. For the assessment of genetic stability, the classical cytogenetics technique was applied (G-banding). After immunophenotypic characterization, the USCs showed a positive expression for CD13, CD29, CD73, CD105, CD 271, Vimentin, Fibronectin, and β-catenin and a reduced expression for CD14, CD19, CD45, CD56, CD146, CD324 and HLA-DR. Furthermore, the cytogenetics analysis USCs showed normal karyotypes with no clonal chromosomal abnormalities. The cells showed MSCs, USCs and Neural markers and normal karyotypes. Through this study, it was possible to observe the similarity of USC with mesenchymal stem cells, which could be an alternative source for cell therapy, as these cells are easy to obtain, and collection is minimally invasive without risk to the donor and showed genetic stability during cultivation.</p> <p>Keywords: alternative MSC source, immunophenotyping, G-banding</p>
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Title	Nerve integrity is enhanced, preventing muscle atrophy and fibrosis, after experimental nerve repair associated with heterologous fibrin biopolymer
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Affiliations	1- Graduate program in Surgery and Translational Medicine, Medical School/UNESP, Botucatu. 2- Department of Structural and Functional Biology, Institute of Biosciences/UNESP, Botucatu.
Session	24 Medicina Regenerativa e Biologia do Desenvolvimento

Abstract, Ethics Committee Number*, and Keywords	<p>Nerve injuries represent a challenge, given their frequent occurrence and substantial impact on the quality of life. Although neurorrhaphy is a well-established technique for treatment, achieving significant morphofunctional recovery remains difficult. The Heterologous Fibrin Biopolymer (HFB), a Brazilian sealant devoid of human blood components, emerges as a potential adjuvant when associated with neurorrhaphy, as it helps mitigate nerve, muscle, and neuromuscular junction degeneration post-injury. In this study, we investigated the effects of nerve reconstruction via neurorrhaphy associated with HFB 120 days after repair of the right sciatic nerve. Twenty adult male Wistar rats (CEUA-FMB 1402/2021) were divided into four groups (n=5/group): Control (C), sciatic nerve was visualized; Denervated (D), neurotmesis was performed; Neurorrhaphy (N) and Neurorrhaphy+HFB (NB), after neurotmesis, the stumps were reconnected by neurorrhaphy (2 points) and in the NB group HFB was added. The tibial cranialis muscle and peroneal nerves were collected for histological analysis. Muscle morphology analysis revealed that groups NB and C exhibited healthy and proximal myofiber characteristics, while group D displayed degenerated fibers and N showed intermediate myofiber preservation. Morphometrically, the NB group was similar to C group, both differing from N, showing higher values of area, perimeter, maximum and minimum diameters, maximum and minimum Feret, and lower values of intramuscular collagen and central nuclei count. Further, NB showed an increase in the number of intact axons, notably indicating an increase in the frequency of axons with a healthy G ratio/axon diameter ratio. These results endorse that the substantial increase in regenerated axons in NB provided further muscle reinnervation and prevented muscle degeneration. These findings highlight the beneficial effects of HFB in nerve repair, surpassing those achieved by neurorrhaphy alone.</p> <p>Keywords: neurorrhaphy; degeneration; regenerative medicine.</p> <p>Fapesp (2022/07551-1)</p>
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Title	In vivo evaluation of osteogenic gene expression of using PCL/graphene scaffolds and electrical stimulation for bone regeneration
Authors	Maisa Campagnollo ¹ Letícia Fernanda Prado ¹ Luigi Enryco Nieri Contrera ¹ Julia Venturini Helaehil ^{1,2} Guilherme Ferreira Caetano ^{1,2}
Affiliations	1 – University Centre of Herminio Ometto Foundation, Araras 13607-339, SP, Brazil 2 – Division of Dermatology, Department of Internal Medicine, Ribeirão Preto Medical School, São Paulo University (USP), Ribeirão Preto 14049-900, SP, Brazil
Session	Regenerative Medicine and Developmental Biology

Abstract and Keywords	<p>The regeneration of critical bone defects requires the use of transplantation to auxiliate the bone repair process. However, they are methods invasive and have clinical limitations. In relation to these failures, tissue engineering proposed a new therapeutic method, less invasive and non-immunogenic, approach through the use of biomaterials in the form of a three-dimensional, denominated scaffolds, which depending on their composition, are biocompatible and promote angiogenesis. Synthetic polymers like a polycaprolactone (PCL) have biocompatibility, bioresorption and structural and mechanical stability, but they are destitute of bioactivity. However, PCL combined with graphene nanopolymer at 0.75% was showed to be an alternative, enhancing the bone regeneration, and also providing electroconductive properties. Furthermore, electrical stimulation (ES) can potentialize the repair process once cels depending on bioelectricity. The objective of this research was to investigate the application of PCL scaffolds with graphene in higher concentrations and the application of ES in experimental osteogenesis. Male Wistar rats were subjected to a critical defect in the calvaria and divided considering treatments: PCL/Graphene with different percentage of graphene (1%, 3% and 5%) combined to ES applications at 10, 30, 50 and 100uA. ES was applied twice a week for 3 minutes. After 30 days, samples were collected to evaluate of genes expression (RT-qPCR) involved in osteogenesis. In relation of <i>Runx-2</i>, <i>Col1a1</i> and <i>Osterix</i> genes, the expression the group of 5% at 10uA was superior to the of PCL/graphene without ES and other groups. The profile changes only in <i>Bmp-2</i> gene, because 3% at 30uA group showed higher expression, however him still in highlighted. Therefore, the use of PCL scaffolds composed by 5% of graphene associated with 10uA promotes a better modulation of genes</p>
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related to osteogenesis.

Keywords: bone regeneration, graphene, electrical stimulation, scaffolds

CEUA- FHO 010/2023



XXXVIII REUNIÃO ANUAL DA FESBE
 XXII REUNIÃO ANUAL DA BRAVO
 XVIII CONGRESSO DA SBCAL
 III CONGRESSO DOHAD BRASIL
 II CONGRESSO DA SBBA
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FRONTEIRAS DAS CIÊNCIAS BIOMÉDICAS E SEUS DESAFIOS

Title	Histological evaluation of the use of different concentrations of graphene scaffolds and application of electrical stimulation in bone repair
Authors	Letícia Fernanda Prado ¹ Maisa Campagnollo ¹ Luigi Enryco Nieri Contrera ¹ Julia Venturini Helaehil ^{1,2} Guilherme Ferreira Caetano ^{1,2}
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Session	Regenerative Medicine and Developmental Biology

Abstract and Keywords	<p>Due to numerous cases involving bone defects, tissue engineering is known to be promising proposal, seeking to reduce graft failures, through the use of three-dimensional structures, called scaffolds. The synthetic polymer polycaprolactone (PCL) was showed to support the bone regeneration, due to its biocompatibility, bioresorption and structural and mechanical stability, however its hydrophobic properties and lack of bioactivity restrict its effectiveness. Together with graphene nanopolymer, it was showed to be a promising composite, resulting from adequate cell viability, cell proliferation and differentiation. The addition of graphene also provides electrical conductivity. The use of electrical stimulation (ES) can potentialize the bone repair process, because of needing of tissue bioelectricity. This study aims to evaluate the therapeutic potential of bone tissue regeneration using PCL scaffolds produced with different concentrations of graphene, 1%, 3% and 5%, and electrical stimulation at different intensities, 10, 30, 50 and 100uA. The surgical procedure was performed to insert the scaffolds in 72 male Wistar rats, which were subjected to treatment with electrical stimulation for 3 minutes, twice a week for 30 days. After euthanasia, the collected samples went for histological processing and then for histomorphometric analysis, considering the connective, osteoid and mineralized tissues. The 3% PCL/graphene scaffold group combined with at 50uA ES treatment presented greater mineralized tissue when compared to the other groups. Regarding osteoid tissue, it was noted that the 1% at 100 uA group presented lower proportion, while the 5% at 50 uA group presented greater area of osteoid tissue compared to the other groups. It can be considered the use of graphene 3% at 10, 30 and 50 uA, and graphene 5% at 10 uA presented greater therapeutic potential for the bone repair process.</p> <p>Keywords: bone regeneration, scaffolds, electrical stimulation. CEUA- FHO 010/2023</p>
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Title	Assessment of osteogenic pathways using 1%, 3% and 5% graphene scaffolds with electrical stimulation in critical bone defects
Authors	Julia Venturini Halaehi ¹² Luigi Enryco Nieri Contrera ¹ Maisa Campagnollo ¹ Leticia Fernanda Prado ¹ Guilherme Ferreira Caetano ¹²
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Session	24 - Regenerative Medicine and Developmental Biology

Abstract, Ethics Committee Number*, and Keywords	<p>Tissue engineering emerges as an alternative to the clinical limitations presented by grafts through the production of scaffolds. Electroconductive materials such as graphene can assist in the repair process, especially when associated with electrical stimulation (ES). Furthermore, this combination can modulate the response of signaling pathways such as Wnt/B-catenin and Ca²⁺/CaM, directly influencing the repair process of this tissue. The objective of this work was to evaluate the gene expression of the Ca²⁺/CaM and Wnt/B-catenin pathways in different concentrations of graphene scaffolds associated with different intensities of electrical stimulation in critical bone defects. The study was approved by CEUA-FHO (10/2023). Male Wistar rats were subjected to a critical bone defect of 25mm² in the calvaria and divided into groups considering the concentration of graphene in the scaffold (1%, 3% and 5%) and ES applications (10, 30, 50 and 100uA). ES was applied twice a week for 3 minutes. After 30 days, samples were collected to evaluate gene expression (RT-qPCR). In 30 days, the 5% 10uA group showed higher expression of Wnt1 and B-catenin compared to the other groups. The 5% 30uA group, although it showed a similar expression of Vgcc to the other groups of the same concentration, the same group showed higher expression of CamkII together with the 5% 100uA group. The 5% 100uA group showed lower expression of Wnt1, Wnt5a and B-catenin compared to the other groups. These results may be related to the modulation of the osteogenesis process, since Wnt5a is related to osteoclastogenesis, while the other pathways are directly related to osteoblastogenesis. The use of scaffolds composed of different concentrations of graphene associated with ES was able to modulate the expression of genes related to the Wnt and Ca²⁺/CaM pathways, especially at a concentration of 5%.</p> <p>Keywords: Tissue engineering, critical bone defects, repair process, <i>scaffolds</i>.</p>
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Title	Effects of chondrocyte secretome on mesenchymal stem cell differentiation
Authors	Paula Fernanda Petruf Ribeiro ¹ Bianca Polak Furman ¹ Ana Paula Camargo Martins ² Debóra Regina Daga ¹ Daniela Boscaro Marsaro ¹ Paulo Roberto Slud Brofman ¹ Carmen Lúcia Kuniyoshi Rebelatto ¹
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Session	Poster; Regenerative Medicine and Developmental Biology

Abstract, Ethics Committee Number*, and Keywords	<p>Regenerative medicine (RM) is an emerging interdisciplinary field, focusing on repairing and regenerating cells, tissues, and organs injured by accidents, chronic illnesses, or congenital defects. Mesenchymal stem cells represent an interesting tool for RM, due to their capacity to differentiate in mesenchymal lineages and promote tissue regeneration. The differentiation potential is of interest for the development of advanced therapies for chondral injuries, a high prevalence condition with poor healing capacity. This study aims to evaluate the chondrogenic differentiation of human mesenchymal stem cells (hMSC) treated with conditioned medium (CM) from ovine chondrocytes. This study was approved by the local ethics committee, under approval numbers CAEE Fundação Oswaldo Cruz 39054120.3.3001.5248; PUCPR 39054120.3.0000.0020; CEUA 01990/IV. Cartilage samples were processed by explant and enzymatic digestion, expanded in standard conditions, and exposed to 48 hours without fetal bovine serum for the CM collection. The CM was centrifuged to remove cellular debris and stored at -80°C until use. Umbilical cord hMSC were cultured in micromass and treated with CM and conventional differentiation medium to induce chondrogenic differentiation. Controls were performed for both groups. The differentiation protocol was carried out for 21 days, after which cell pellets were fixed with formalin for histochemistry and immunohistochemistry. It was possible to observe tissue structure with HE staining consistent with cartilage structures, while blue toluidine staining revealed the presence of glycosaminoglycans. Immunohistochemistry demonstrated that hMSC treated with CM showed collagen II labeling, as expected after chondrogenic differentiation. These histological findings allow us to claim that the CM was effective in inducing</p>
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chondrogenic differentiation of hMSC, providing valuable data for future research

Keywords: Regenerative Medicine; Conditioned Medium; Chondrogenic



Title	Characterization and in vivo biocompatibility assessment of a decellularized porcine uterine ECM-based graft aiming tissue repair applications
Authors	Luan Stefani Lima ¹ , Gustavo Henrique Doná Rodrigues Almeida ¹ ; Rafael Oliveira Bergamo ¹ ; Raquel Souza da Silva ¹ ; Beatriz Lopomo ¹ ; Giovanna Vitória Consani Santos ¹ ; Jaqueline de Carvalho Rinaldi ² ; Mariana Sversut Gibin ³ ; Herique Dos Santos ³ ; Francielle Sato ³ ; Mauro Luciano Baesso ³ ; Luzmarina Hernandez ² ; Ana Claudia Oliveira Carreira ^{1,4}
Affiliations	1 – Laboratory of Tissue Engineering and Cell Therapy, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil 2 – Department of Morphological Sciences, State University of Maringá, Paraná, Brazil 3 – Department of Physics, State University of Maringá, Paraná, Brazil 4 – Center of Human and Natural Sciences, Federal University of ABC, São Paulo, Brazil
Session	24 - Medicina Regenerativa e Biologia do Desenvolvimento

Abstract, Ethics Committee Number*, and Keywords	<p>Biomaterials derived from biological matrices for tissue repair has been widely investigated due to their great therapeutic potential in regenerative medicine, once they are able to induce cell proliferation, tissue remodeling and angiogenesis in situ. This study aimed to characterize a biomaterial derived from decellularized porcine uterine horns in order to produce biological grafts for tissue repair applications. This research was conducted in agreement with the institutional ethics committee regulations of the University of São Paulo (CEUAX no. 5764121222) and State University of Maringá (CEUA no. 1210/ 2023). For decellularization, porcine uterine horns segments (n=10) were washed with distilled water, immersed and agitated on SDS and Triton X-100 solutions, followed by ultrasonication. To evaluate the decellularization efficiency, we performed DAPI staining and DNA quantification. In addition, we performed spectroscopic analysis by FTIR and Raman to evaluate physico-chemical alterations on the scaffolds. Scanning electronic microscopy was used to ultrastructural characterization. For biomechanical characterization, for both native and decellularized samples, maximum pulling force, maximum elongation and stiffness were measured. Additionally, in vitro performance and in vivo biocompatibility was also evaluated to attest their ability to be applied as a biological graft for tissue repair. Results showed the protocol was efficient to promote cell removal and the scaffold general structure and ECM composition remained preserved. The scaffolds were cytocompatible, allowing cell growth and survival. In terms of biocompatibility, the matrices did not induce any signs of immune rejection in vivo, demonstrating indications of integration by the host after 30 days of im-plantation. In summary, these findings attested that dUT</p>
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scaffolds could be explored as a bio-material for regenerative purposes, not only restricted to the reproductive field.

Keywords: uterus, extracellular matrix, scaffold, xenogeneic graft



Title	Use of Propolis in skin lesions of second intention. Revisional study
Authors	Monique Livia da Silva ¹ Andréia Cristina Miranda Alves ¹ Cláudia Helena Pellizzon ²
Affiliations	¹ Postgraduate Program in Biotechnology and ² Department of Structural and Functional Biology, São Paulo State University (UNESP), Institute of Biosciences, Botucatu, Brazil.
Session	24- Regenerative Medicine and Developmental Biology

Abstract, Ethics Committee Number*, and Keywords	<p>The skin, a vital organ, is a robust physical and chemical barrier, safeguarding the entire human body from harmful microbial agents and dehydration. In the event of an injury, the process is intricate, unfolding in three distinct stages: hemostasis/inflammation, proliferation, and remodeling. Skin lesions pose a significant health concern, leading to substantial morbidity and treatment expenses. Numerous innovative products have emerged, effectively reducing the repair time and facilitating skin reconstruction.</p> <p>We reviewed the literature on the use of propolis in the healing processes of the skin of second intention in the last 10 years. The inclusion criteria were in vivo experimentation, use of topical propolis, and texts in English, and the exclusion criteria were review articles, case reports, clinical trials, and use of isolated substances.</p> <p>The objective was identifying studies on skin retraction using propolis in experimental models. We meticulously approached this review process, presenting the main models used and the current state of these studies.</p> <p>As a result, we found 54 articles, of which the inclusion criteria approved 21. Studies have involved the antibacterial activity of propolis, as well as antioxidant activity in fibroblasts and keratinocytes. In the <i>in vivo</i> excision model, we had several circular, square, and multiple lesions in the dorsal region and different experimental animals with 1 strain of SK1 mi ce (hairless), 1 Swiss mouse, 4 Balb/C mice, 2 Sprague Dawley rats, 11 Rattus novergicus rats, 1 rabbit, and 1 dog. The studies present a great diversity of studies with different methods of analysis, which allows us to affirm the need to create protocols to define the elements of studies for new treatments.</p> <p>We propose unifying the analyses to enable comparative testing and suggest a possible study protocol for the use of propolis in skin repair.</p> <p>Keywords: "propolis," "wound healing," "Skin"</p>
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Title	Development and characterization of acellular placental membranes as a novel biomaterial for tissue engineering applications
Authors	Luan Stefani Lima ¹ , Gustavo Henrique Doná Rodrigues Almeida ¹ ; Rafael Oliveira Bergamo ¹ ; Raquel Souza da Silva ¹ ; Beatriz Lopomo ¹ ; Giovanna Vitória Consani Santos ¹ ; Jaqueline de Carvalho Rinaldi ² ; Mariana Sversut Gibin ³ ; Herique Dos Santos ³ ; Francielle Sato ³ ; Mauro Luciano Baesso ³ ; Luzmarina Hernandez ² ; Ana Claudia Oliveira Carreira ^{1,4}
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Session	24 - Medicina Regenerativa e Biologia do Desenvolvimento

Abstract, Ethics Committee Number*, and Keywords	<p>The development of scaffolds derived from decellularized tissues has emerged as a promising approach for tissue reconstruction, biomimetic microenvironments and tissue repair therapies. Among the several available sources to obtain acellular and non-immunogenic matrices to modulate as bioactive biomaterials, fetal membranes have been explored. Therefore, this study aimed to establish an optimized protocol to generate acellular porcine placental scaffolds for tissue engineering applications. For that, term porcine placentas (n=5) were decellularized using a combination of detergents (0.1% SDS and 0.5% Triton X-100) and ultrasonication. To evaluate the decellularization efficiency, DNA quantification and DAPI staining were performed. To evaluate the scaffolds structure, histological and ultrastructural analysis were carried out. Then, samples were collected for FTIR and Raman spectroscopy for physico-chemical evaluation. Moreover, samples were sterilized and cultured with L929 and HaCat cell lines for 10 days to attest cytocompatibility in vitro. Cell viability and the interaction between cells and scaffolds were assessed. DNA quantification revealed a decrease of 90% in the content and DAPI staining showed nuclei absence. Histological analysis did not showed significant alterations in collagen, GAGs and elastic fibers distribution. Scanning electronic microscopy (SEM) did not indicate any signs of fiber degradation or signs of cellular debris. Spectroscopy data showed no alterations in all bands related to collagen, elastin, proteoglycans and GAGs content in the scaffolds compared to the native tissue. Regarding the cellular assays, SEM images evidenced both cells attached and anchored on the scaffolds. Cell viability rates were also above 90%, which attested biomaterial's cytocompatibility. In conclusion, the generated acellular</p>
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placental membranes had a preserved microstructure, composition, allowed cell attachment, and survival.

Keywords: placenta, biomaterial, extracellular matrix, scaffolds, tissue engineering



Title	Cellular understanding of the effects of physical activity in diabetes: analysis of didactic strategy applied to high school students
Authors	Felipe Ramos Melo André Williams Bazzo Fernandes Fernanda Yoshiko Souza Nishi Vinícius de Lima Montello Jardim Luciane Candeloro Ludimila Canuto Faccioni
Affiliations	Federal University of Mato Grosso do Sul, Campo Grande -MS, Brazil
Session	25 - Educação, História e Filosofia da Ciência, Comunicação Científica

Abstract, Ethics Committee Number*, and Keywords	<p>Education plays a fundamental role in raising awareness and adopting strategies aimed at making young people's lives healthier, preventing them from modern diseases such as diabetes. Glucose is the primary energy molecule in the human body, internalized by adipocytes and myocytes through GLUT4 carriers expressed in the plasma membrane through insulin action. In diabetes, insulin absence or dysfunctionality leads to hyperglycemia. This clinical state has been on the rise worldwide among the youth. Physical activities are considered an ally in controlling diabetes, due to it stimulating insulin-independent GLUT4 translocation to the cell membrane. This knowledge is essential for the practice of physical activity to be conscious among young people. This paper proposes an educational activity to allow comprehension of the effects of exercise in adjusting the glucose levels in the blood. This action-research methodology was approved by the Institution's Ethics Committee (56119522.0.0000.002). Questionnaires were applied, pre and post dynamics, to 16 students of a public High School institution in Campo Grande, MS, Brazil. The dynamic was composed by a ludic sequence of models about the exercise-mediated glucose intake, using a box that mimicked a cell, with movable portals (GLUT4) and balls representing glucose. The more exercise, the more portals became available, allowing the balls to efficiently enter the box. Before the activity, the students had little to no knowledge about glicemic homeostase and the importance of physical activity for it, though recognized the importance of insulin for glucose absorption. After the dynamic, however, 81,25% of the students understood that GLUT4 acts heightening intracellular glucose and 68,8% understood that physical exercise favors GLUT4 translocation to the plasmatic membrane. The activity seems to promote the subjects' knowledge about glucose regulation and allows conscientization of the physical activity in diabetes treatment.</p> <p>Keywords: insulin resistance; material education; health; prevention</p>
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Title	Biological science teacher training: an inclusive teaching proposal for fostering scientific literacy based on the theory of critical meaningful learning
Authors	Ivana Elena Camejo Aviles Eduardo Galembeck
Affiliations	Unicamp, Campinas, Brazil.
Session	[25: Educação, História e Filosofia da Ciência, Comunicação Científica]

Abstract, Ethics Committee Number*, and Keywords	<p>We are conducting translational research focused on intervention, application, and didactic mediation in the classroom. Our proposal is to develop a dialogical, participatory, and transformative methodology aimed at sensitizing biology teachers during their initial and ongoing training based on Remote Laboratories. Our methodology consists of five phases: review of the state of the art and various approaches to the problem, planning, implementation, validation, and evaluation of the educational innovation strategies developed in the project. Our goal is to contribute to overcoming the challenge of training science teachers capable of responding to the constant demands and changes of today's society. The results of the first phase have been systematized in various directions of interest, including the state of initial and ongoing training of science teachers in the current social context, constructivist approaches and focuses in science teaching, as well as alternative, active, and democratic strategies of didactic experimentation, including remote laboratories. Based on these results, our research will follow the following steps to achieve various investigative directions related to the initial and ongoing training of biology teachers in investigative approaches, through Inquiry and problem-solving, as well as the democratization and availability of Science education through the implementation of Remote Experimentation in public schools in Campinas and neighboring regions. The ethics number is in process.</p> <p>Keywords: science teacher training; critical meaningful learning; scientific literacy; constructivist teaching approaches; remote laboratories.</p>
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Title	Remote experimentation laboratory: an inquiry activity for critical meaningful learning of photosynthesis
Authors	Ivana Elena Camejo Aviles Eduardo Galembeck
Affiliations	Unicamp, Campinas, Brazil.
Session	[25: Educação, História e Filosofia da Ciência, Comunicação Científica]

Abstract, Ethics Committee Number*, and Keywords	<p>The Remote Experimentation Laboratory has the potential to teach about the epistemological, problematic, and social process of constructing scientific knowledge, which is fundamental for consolidating scientific literacy, as well as for the development of metacognition. The aim was to construct a remote photosynthesis experiment proposing an investigative didactic-pedagogical approach. The results of this interdisciplinary research for the construction of the remote photosynthesis experiment resulted in: determination of the physical and biological components of the remote experiment; organization and usability of Arduino and Raspberry Pi based on their development on the Educational Technology Laboratory Server; insertion into the laboratory's remote experiment management system; proposal for teaching photosynthesis as an investigative activity with remote laboratories available at https://www.lte.ib.unicamp.br/portal/experiments.php?idExperiment=139. In this photosynthesis experiment, it is possible to observe and measure variables such as: light intensity; humidity percentage; variations in the mass of biological material; air temperature, among others. The next challenges aim for this and other remote experiments developed by the Educational Technology Laboratory to be used to increase the possibilities of teaching sciences and biology in public schools in Campinas and regions, increasing their levels of inclusion and free access to didactic experimentation.</p> <p>Keywords: remote experimentation; investigative focus; metacognition, meaningful learning, inclusive science education.</p>
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Title	A new proposal for inquiry activity using a low-cost remote acid-base titration
Authors	Author Ricardo Cenamo Cachichi Author Eduardo Galembeck Author Ivana Elena Camejo Aviles
Affiliations	State University of Campinas (Unicamp), Campinas, Brazil
Session	25

Abstract, Ethics Committee Number*, and Keywords	<p>Students' reports on remote experiments indicate that their engagement can be enhanced by incorporating synchronous aspects. Synchronous elements for remote experiments can be achieved using videos, with control being carried out by the students themselves. For this experiment, a low-cost system was built consisting of an Arduino with an Ethernet Shield connected to a pH sensor and a relay. Two peristaltic pumps, a stirrer, and an LED were connected to this relay. Additionally, a system composed of a Raspberry Pi B and a webcam is used for real-time transmission of experiment images. The experiment is available on the website https://www.lte.ib.unicamp.br/portal/experiments.php?idExperiment=136. Upon accessing the page, the user receives instructions and proceeds to add acid or base to sweep the pH range from 0 to 14, observing the colour of the solution via video. At the end of the additions, users can consult data in books and on the internet to identify the indicator based on the colour of the turning points. Seven indicator options are provided for them to choose from. After this research stage, the user fills out a form with their choice(s) and answers some evaluation questions about the experiment. So far, 114 internet users have responded to the form. The next stage of the project involves implementing the experiment in an investigative manner for high school students and evaluating the results. In a future application, the experiment will be conducted with high school senior students, with the help of a facilitating teacher who will be in the classroom guiding the students on how to operate the experiment page, without compromising the investigative nature of the activity. Keywords: Distance Learning / Self-Instruction, Internet / Web-Based Learning, Problem Solving / Decision Making, Acids / Bases.</p>
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Title	Are neuromyths in education relevant to teaching and learning? Development of the educational neuromyths diagnostic tool
Authors	Pedraga, Bruna Carolina Lopes Lellis-Santos, Camilo
Affiliations	Mestranda – Universidade Federal de São Paulo, Instituto de Ciências Ambientais, Químicas e Farmacêuticas, Diadema, Brasil Orientador - Universidade Federal de São Paulo, Instituto de Ciências Ambientais, Químicas e Farmacêuticas, Diadema, Brasil
Session	25 - Educação, História e Filosofia da Ciência, Comunicação Científica

Abstract, Ethics Committee Number*, and Keywords	<p>Introduction: Neuroscience applied to education is a growing field in contemporary times, yet its convergence with teacher education presents many challenges. Deficient teacher training and the dissemination of neuroscience information without scientific evidence contribute to the emergence of neuromyths: misconceptions about the function and role of the brain in the learning process. Objectives: To conduct a systematic literature review on questionnaires used to investigate neuromyths and develop and validate a diagnostic tool to assess belief in educational neuromyths among Brazilian teachers. Methodology: A referred article-based literature review was conducted through the Research Rabbit tool, using Professor Jelle Jones' research group's study on neuromyths in education. Questionnaires containing items that reveal beliefs and knowledge about neuroscience that may impact education were selected. A diagnostic tool containing true (neurofacts) and false (neuromyths) information about neuroscience was developed and submitted for validation by experts in educational neurosciences. Results: Forty-one items were selected from 44 articles, representing 58.5% of items addressing neuromyths. Most items related to neuromyths were associated with neuroeducation (66.6%); however, the majority of neurofacts corresponded to neurobiology (58.8%). Among the set of items, only 4 were considered of little relevance or irrelevant to education. The item "we only use 10% of the brain" was the most cited (83%) in the articles and indicated by 89% of experts as highly relevant. Conclusion: This careful selection of educational neuromyths and validation by experts is essential to develop a reliable diagnostic tool to orient teacher training and discourage the adoption of simplistic didactic strategies based on neuromyths.</p> <p>Keywords: neuroscience, education, neuromyths, diagnostic tool, teachers.</p> <p>CAAE nº. 71318823.5.0000.5505.</p>
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Title	Effect of the educational game 'Ecological Relationships' on high school students' learning and perception
Authors	Amandha Christine da Silva Souza ¹ Pedro Henrique Franco Bento ¹ Pietra Soares de Sousa ¹ Dianne Almeida da Silva Muniz ² Fernanda Klein Marcondes ¹
Affiliations	1 – Universidade Estadual de Campinas, Piracicaba, Brasil. 2 – Escola Estadual Prof. José Martins de Toledo, Piracicaba, Brasil.
Session	Pôster

Abstract, Ethics Committee Number*, and Keywords	<p>The teaching and assessment methodologies employed by teachers significantly influence the learning and academic success of their students. Educational games represent a strategy that can effectively address students' lack of concentration and attention while fostering the development of student autonomy. It has been observed that the use of educational games in the classroom leads to enhanced learning outcomes compared to traditional theoretical lectures. This study aims to assess the impact of the educational game "Ecological Relationships" on the learning outcomes of 30 first-year high school students at a public school in Piracicaba-SP. This evaluation was conducted through pre- and post-tests, along with a questionnaire gauging student perceptions. The game consists of 12 images illustrating ecological relationships and 12 corresponding cards with their respective names. Ethical approval for this research was obtained from the Research Ethics Committee of the Piracicaba Dental School/UNICAMP (CAAE 0873219.8.0000.5418). Prior to the intervention, a pre-test was administered, and the educational game activity replaced the traditional lecture. Fifteen days after using the game, students completed a post-test featuring questions similar to those in the pre-test. Additionally, students were asked to rate the helpfulness of the educational game on a like- Likert scale ranging from 0 (not helpful) to 10 (very helpful). The scores from the pre- and post-tests were compared using the Student's paired t-test. The mean score obtained in the post-test (3.10 ± 1.48) was significantly higher than that of the pre-test (6.52 ± 1.78, $p < 0.05$). Furthermore, the average response regarding student perception was 8.18 ± 1.40. These preliminary findings suggest that the game effectively enhanced learning outcomes related to ecological relationships.</p> <p>Keywords: TEACHING. BIOLOGY. GAMEFICATION.</p>
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XXXVIII REUNIÃO ANUAL DA FESBE
XXII REUNIÃO ANUAL DA BRAVO
XVIII CONGRESSO DA SBCAL
III CONGRESSO DOHAD BRASIL
II CONGRESSO DA SBBA
2 A 5 DE JULHO 2024, CAMPINAS/SP
FRONTEIRAS DAS CIÊNCIAS BIOMÉDICAS E SEUS DESAFIOS

Title	Estudo de caso de uma formação continuada de professores na Educação Infantil em Blumenau (SC) - reflexões sobre o trabalho com neurociência e aprendizagem
Authors	Ana Paula Kuczmynda da Silveira
Affiliations	Instituto Federal de Educação, Ciência e Tecnologia de Santa Catarina - Câmpus Gaspar
Session	[31 - Semana Nacional do Cérebro]

Abstract, Ethics Committee Number*, and Keywords	<p>Esta pesquisa está vinculada ao <u>Grupo de Pesquisas em Multiculturalidade, Interseccionalidades e Formação de Professores</u>, dentro da linha de pesquisa “Letramento e numeramento como ferramenta de empoderamento social” e constitui um estudo de caso em desenvolvimento em um Centro de Educação Infantil (CEI) de Blumenau (SC) desde 2022. A pesquisa abarca um projeto de formação continuada de professores, envolvendo todos os profissionais de educação da unidade escolar e vem desenvolvendo-se a partir da realização de encontros e ações de formação continuada em serviço, que envolvem cerca de 48 horas por ano. O projeto tem como foco o processo de ensino e aprendizagem na e para a educação infantil na contemporaneidade, a partir da elaboração e realização de projetos de ensino (Hernández, 1998; Barbosa; Horn, 2008) direcionados ao trabalho com interações e brincadeiras que atuem significativamente na zona de desenvolvimento proximal (Vygotsky, 1989, 1999) dos estudantes, de maneira a colaborar com o processo de construção de conhecimentos e da autonomia de cada um. A formação continuada vem congregando de forma profícua a abordagem de temáticas próprias da educação infantil - aqui pensada como educação integral na perspectiva humanizadora (Brasil, 2010, 2018) - e as reflexões sobre sociocognição e aquisição da linguagem apresentadas por estudos neurocientíficos atuais e por Vygotsky (1999). Dentre os primeiros, realçamos os conceitos de criança, infâncias e culturas infantis; a percepção de escola como território de construção de individualidades que se renovam diariamente; os processos de aprendizagem e ensinagem com base em interações e brincadeiras. Dentre os últimos, elencamos o conceito de sujeito sócio-histórico-cultural, o papel mediador da linguagem na atribuição de sentido ao mundo, a si e ao outro, o desenvolvimento das funções executivas superiores e os conceitos de autonomia, flexibilidade cognitiva e autoeficácia/motivação.</p> <p>Keywords: Educação Infantil; processo de ensino e aprendizagem; sociocognição; educação integral.</p>
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This research is linked to the Research Group on Multiculturalism, Intersectionalities and Teacher Training, within the line of research “Literacy and numeracy as a tool for social empowerment” and constitutes a case study being developed in an Early Childhood Education Center (CEI) in Blumenau (SC) since 2022. The research covers a continuing teacher training project, involving all education professionals at the school unit and has been developing through meetings and continuing in-service training actions, involving around 48 hours a year. The project focuses on the teaching and learning process in and for early childhood education in contemporary times, based on the elaboration and implementation of teaching projects (Hernández, 1998; Barbosa; Horn, 2008) aimed at working with interactions and games that act significantly in the zone of proximal development (Vygotsky, 1989, 1999) of students, in order to collaborate with the process of building knowledge and autonomy for each person. Continuing training has been fruitfully bringing together the approach to themes specific to early childhood education - here thought of as integral education from a humanizing perspective (Brazil, 2010, 2018) - and reflections on sociocognition and language acquisition presented by current neuroscientific studies and by Vygotsky (1999). Among the first, we highlight the concepts of children, childhoods and children’s cultures; the perception of school as a territory for the construction of individualities that are renewed daily; learning and teaching processes based on interactions and games. Among the latter, we list the concept of socio-historical-cultural subject, the mediating role of language in attributing meaning to the world, to oneself and to others, the development of higher executive functions and the concepts of autonomy, cognitive flexibility and self-efficacy/ motivation.

Keywords: Child education; teaching and learning process; sociocognition; integrated education



Title	Perceptions of scientists and artists by public high school students: Normal science, crisis, or revolution?
Authors	Luciano Rogério Destro Giacóia Daniela Carvalho dos Santos Ariane Leite Rozza
Affiliations	Department of Structural and Functional Biology, Institute of Biosciences, São Paulo State University (UNESP), Botucatu, Brazil
Session	Poster

Abstract, Ethics Committee Number*, and Keywords	<p>Conducted during the "Turning the Cell Inside Out" summer course at Unesp Botucatu, this study employed a quali-quantitative methodology to analyze the perceptions of 20 public high school students about scientists and artists before and after the course. Students answered questionnaires and drew scientists and artists at the beginning and end of the course. The results showed significant changes in students' perceptions. For scientists, there was an increase in associations with "Woman" (from 15 to 18), "Man" (from 16 to 19), "Young" (from 13 to 17), "Funny" (from 9 to 15), and "Fun" (from 12 to 15), indicating a more diversified view. Negative characteristics such as "Bad-tempered" (from 2 to 7) and "Angry" (from 1 to 6) also increased, suggesting a more complex perception. For artists, the changes were smaller, with adjustments in "Woman" (from 17 to 16) and "Elderly" (from 12 to 14), and an increase in "With many friends" (from 14 to 16). Negative characteristics such as "Bad-tempered" and "Angry" also increased (from 2 to 7 and from 3 to 7). The analysis of the drawings showed little evolution, indicating that stereotypical visual representations remain. Students' reflections illustrate the complexity of this transformation, highlighting the discovery that scientists can be "funny" and "fun" and that artists can be seen in various contexts. The discrepancy between changes in descriptive perceptions and the stagnation in visual representations can be interpreted through Thomas Kuhn's theory of the evolution of science. The changes suggest a phase of "Normal Science," but the lack of significant change in visual representations indicates that a "Crisis" has not yet been reached. To promote a true "Revolution" in perceptions, it is crucial to challenge and expand stereotypical views, facilitating the transition to more diversified and inclusive paradigms.</p> <p>Keywords: university extension; normal science; crisis; revolution; Thomas Kuhn</p>
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Title	Instigating curiosity about cell biology in basic education through the project “BioCel na escola”
Authors	Cristiane Figueiredo Pinho ¹ , Maria Clara Betete da Silva Fonseca ¹ , Vinícius Luis Rocha da Silva Maria ¹ , Ana Clara Pacheco de Santana ¹ , Bianca Dionísio ¹ , Danielly Luiza de Castro Souza ¹ , Eduardo Branco Sávio ¹ , Julia Braga Carvalho ¹ , Júlia Landeira Gomez Chamelete ¹ , Raquel Mendes Hikosaka ¹ , Sophia Oliveira ¹ , Aline Cristina Zago ¹ , Sergio Pereira ¹ .
Affiliations	¹ School of Sciences, Sao Paulo State University - UNESP
Session	Session: 25 - Educação, História e Filosofia da Ciência, Comunicação Científica

Abstract, Ethics Committee Number*, and Keywords	<p>Teaching Cell Biology during basic education is important for explaining natural phenomena, life cycles of organisms and contextualizing everyday events for young students. However, teaching abstract cellular processes and structures that are difficult to visualize can make learning frustrating in schools. Therefore, it is a current challenge to insert additional tools that encourage more active student participation in the process of building learning, contributing to the improvement of basic education and spreading enthusiasm for modern science and technology. To bring elementary school students closer to academic and scientific reality, undergraduate students of the Sao Paulo State University (Unesp) created an extension project called “BioCel na escola”. This extension project aims to popularize the knowledge in Cell Biology among elementary school students in the region of Bauru (city of Sao Paulo), contributing to improving the quality of basic education, in addition to presenting the public university and the scientific career as possibilities for the future of students. The project began its activities in 2023 and 6 school visits have been carried out to UNESP, totaling around 200 young students and their teachers. On each visit, students participate in dynamics that aim to explore their curiosity, such as bingos about intracellular organelles and cell division; experiments on photosynthesis; and practical activities using the optical microscope to observe prokaryotic and eukaryotic cells. The students demonstrated interest in the topics covered, proactivity in running experiments and excitement about getting to know UNESP. The school teachers confirm that the students' interest in Science increased after the visits. Preliminarily, we conclude that we are successfully contributing to the teaching of Cell Biology in schools, disseminating scientific knowledge generated at public universities and offering the prospect of entering university for young students.</p> <p>Keywords: Cell Biology, Education, experiments, microscope.</p>
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Title	Unveiling the experimental competencies of students during smartphone-assisted experimentation: an eye-tracking study
Authors	Giovanna Tonzar-Santos, Marcos Ligori, Sandra Mara Medeiros, Maria Eduarda dos Santos Verginio, Camilo Lellis-Santos
Affiliations	Programa de Pós-Graduação em Ensino de Ciências e Matemática, Universidade Federal de São Paulo, Instituto de Ciências Ambientais, Químicas e Farmacêuticas – Campus Diadema
Session	25 - Educação, História e Filosofia da Ciência, Comunicação Científica

Abstract, Ethics Committee Number*, and Keywords	<p>Developing experimental competencies is a cornerstone for high-quality biology education. Smartphone-assisted experimentation (SAE) is a technological innovation that can facilitate experimental biology classes. This study aimed to identify if fixation and gaze patterns are related to the experimental competencies of undergraduate science students when performing tasks during SAE. Undergraduate students (N=12) having different expertise in performing experiments through SAE were exposed to hands-on activity to study heart rate. Expert students (EXP) are undergraduate teaching assistants with previous experience in SAE, and novice students (NOV) have no previous experience in SAE in studying the human body. The lesson protocol encompassed collecting, analyzing, and constructing a graph of heart rate values at baseline and after performing two tasks, one involving mental effort and the other physical effort. Fixation and gaze points were captured using a Tobii Pro-Glasses 3 device, and data were analyzed using the IV-Attention filter of Tobii Pro Lab. The Unifesp ethics committee approved the study (CAAE: 69850223.3.0000.5505). As a result, novice students differed from expert students in their eye-tracking patterns and attentional focus. Graph construction required more time and attention for both groups. Heart rate calculations required significantly more cognitive effort from NOV students compared to EXP students (EXP 938.3 ± 228.3 vs NOV 1532.0 ± 249.4; $P < 0.001$). The table and graph were the elements of the lesson protocol that demanded the most attention. However, the cognitive effort in manipulating the information collected during the mental effort task was higher for NOV students than for EXP students. Therefore, the level of expertise in SAE of students is related to their patterns of attentional focus and eye trajectory. The mathematical elements require higher levels of attention from students during learning activities in experimental biology.</p> <p>Keywords: Experimentation, smartphone, biology education, active learning, heart rate</p>
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Title	Genomic stability after cell culture of bone marrow-derived menseschymal stem cells using chromosome analysis
Authors	Isabeli do Nascimento Masson Isadora May Vaz Valderez Ravaglio Jamur Thalita Bastida Vieira Carmen Lúcia Kuniyoshi Rebelatto Paulo Roberto Slud Brofman
Affiliations	Pontifícia Universidade Católica do Paraná, Escola de Medicina, Núcleo de Tecnologia Celular, Curitiba, PR, Brazil.
Session	Poster presentation

Abstract and Keywords	<p>Mesenchymal stem cells (MSCs) are adult multipotent cells with a fibroblastoid-like conformation. For its immunomodulatory properties, low immunogenicity, and high proliferative rate, these cells are being widely explored as advanced therapy products (ATPs). Stem cells are originate from many sources, mainly bone marrow due to their promising treatment results. However, during cell culture some genomic instabilities may arise, highlighting the impotence of cytogenetic tests as a method of quality control for ATPs. The project was approved by the Research Ethics Committee (n° 6.599.501). To evaluate the stability of bone marrow-derived MSCs, five samples were cultivated for G-banding conventional test and cytokinesis-block micronucleus assay. G-banding allows the visualization of numeric and structural alterations in chromosomes by karyogram analysis. Micronucleus assay numerically identifies the alterations by calculating the nuclear division index (NDI). These alterations are caused by nuclear division, which can be nucleoplasmic bridges, buds, and micronucleus. The conventional test and micronucleus assay demonstraded chromossomal stability in four samples, with normal karyotypes (46,XY) and NDIs greater than 1, indicating nuclear division. The fifth sample showed some instability during both analysis. While G-banding resulted in a karyogram that showed a recurrent loss of chromosome 19 (45,XX,-19[7]/46,XX[13]), the micronucleus assay had a normal NDI but with a higher number of nucleoplasmic bridges than the other samples' results. Bone marrow-derived MSCs showed a significant viability for its use as ATPs but the cells are still susceptible to genetic instabilities. Therefore, it's possible to determinate the necessity of cytogenetic tests for quality control to the development of safe and efficient therapies.</p> <p>Keywords: Stem cells, bone marrow, G-banding, micronucleus assay, cytotenetics.</p>
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Title	Evaluation of the efficacy of hyaluronic acid, niacinamide, and PDRN formulations of epithelial tissue: an animal model study
Authors	Carina Dupas ¹ , Íris Maria Carvalho Rogatto ¹ , Livia Maria Rodrigues Claro ¹ , Durval Coladetti Junior ² , Fernanda Flores Navarro ¹ , Fernando Russo Costa do Bomfim ¹
Affiliations	¹ Centro Universitário da Fundação Hermínio Ometto ² Tradexx USA Inc.
Session	26 - Biologia do Desenvolvimento e Medicina Regenerativa

Abstract, Ethics Committee Number*, and Keywords	<p>Tissue bioremodelers are agents that contribute to maintaining skin health, anti-aging and more elastic appearance. This study aimed to evaluate the thickness and modeling of epithelial tissue in Wistar rats after the application of tissue bioremodelers. After approval by CEUA-FHO (13/2023), fifty-four Wistar rats were assigned into three groups: Control (C, saline solution, n=18), Remodeler 1 (R1, 3.2% hyaluronic acid, n=18), Remodeler 2 (R2, 2.0% hyaluronic acid+0.1% niacinamide+0.5% PDRN, n=18), and Remodeler 3 (R3, 2.2% hyaluronic acid+0.2% niacinamide+0.75% PDRN, n=18). Application of 0.1mL on the dorsum of the animals of R1, R2, and R3 with the bioremodelers occurred at days 0, 7, and 14 after anesthetic protocol with Ketamine (0.3mg/kg) and Xylazine (0.1mg/kg). Group C received applications of sterile saline solution with the same protocols. Euthanasia was performed at 7, 14, and 21 days after the applications by deep anesthesia with Ketamine (0.9mg/kg) and Xylazine (0.3mg/kg) and cardiac puncture. Samples from the dorsum of the animals containing thick skin were removed, processed histologically, and stained with hematoxylin and eosin. Measurements of epithelial tissue areas (μm^2) were made with Image J and statistical analysis, ANOVA test, was performed with median values\pmstandard deviation and Tukey's post-test ($p < 0.05$). The area of epithelial tissue analyzed at 7 days showed significant differences between C (943245 ± 291257) and R3 (482051 ± 82397) and between R2 ($1008 \times 103 \pm 151491$) and R3, $p = 0.0048$. The analyses at 14 days showed significant differences between C (968968 ± 293037) and R2 (757149 ± 195377) and between C and R3 (745458 ± 110093), $p = 0.0317$. The analyses at 21 days did not show significant differences between the groups, $p = 0.7545$. The bioremodelers are effective at 14 days after application, reducing the total area of epithelial tissue, which suggests less tissue decompression formation, with R2 showing better results in the parameters analyzed.</p> <p>Key-words: skin, hyaluronic acid, bioremodelers</p>
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Title	Effects of different tissue bioremodelers on collagen production in Wistar rats
Authors	Livia Maria Rodrigues Claro ¹ , Íris Maria Carvalho Rogatto ¹ , Carina Dupas ¹ , Durval Coladetti Junior ² , Fernanda Flores Navarro ¹ , Fernando Russo Costa do Bomfim ¹
Affiliations	¹ Centro Universitário da Fundação Hermínio Ometto ² Tradexx USA Inc.
Session	26 - Biologia do Desenvolvimento e Medicina Regenerativa

<p>Abstract, Ethics Committee Number*, and Keywords</p>	<p>Connective tissue is important in the integrity and function of the skin, and dermo cosmetic products are widely used to improve skin structure, but their specific effects on connective tissue are not fully understood. This study aimed to evaluate the effects of tissue bioremodelers on collagen production in Wistar rats. The study was approved by CEUA-FHO (13/2023). Wistar rats (n=72) were assigned into 4 groups (n=18/group): control (C, saline solution), Bioremodeler 1 (R1, 3.2% hyaluronic acid), Bioremodeler 2 (R2, 2.0% hyaluronic acid+0.1% niacinamide+0.5% PDRN), and Bioremodeler 3 (R3, 2.2% hyaluronic acid+0.2% niacinamide+0.75% PDRN). Animals received 0.1mL of each bioremodeler intradermally in the dorsal region at days 0, 7, and 14, after anesthetic induction with Ketamine (0.3mg/kg) and Xylazine (0.1mg/kg), while animals in group C received sterile saline solution. Euthanasia was performed at 7, 14, and 21 days after bioremodeler application (n=6/group/time) following deep anesthesia with Ketamine (0.9mg/kg) and Xylazine (0.3mg/kg) and cardiac puncture. Samples from the dorsal region of thick skin were collected, subjected to histological processing and stained with hematoxylin and eosin for connective tissue measurements. Measurements were made using Image J software, and statistical analysis (ANOVA) was performed between experimental groups, with median±standard error and Tukey's post-test (p<0.05). Connective tissue length (µm) at 7 days showed no significant differences between groups C (1010x103± 83846), R1 (829999±74149), R2 (867383±59110), and R3 (898711±84295), p=0.5688. At 14 days, significant differences were found between groups R3 (914408±76690) and control (729793±29307), p=0.0345. At 21 days, significant differences were again found between groups R3 (1002x103±34427) and control (746109±48092), p=0.0152. Tissue bioremodelers did not differ from each other, with an increase in connective tissue length observed only in R3 with two applications.</p> <p>Key-words: Skin booster, collagen, skin remodeling</p>
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Title	Laser therapy and açai gel improve the macroscopic and histopathological aspects of tendon injuries in rats
Authors	Luana de Souza Bastos; Áurea Gabriela Rodrigues Mendes; Cauã da Silva Magalhães; Lucas da Silva Trindade; Marcela Monteiro Ribeiro Lemos; José Lopes de Mendonça Neto; Luana Ketlen Reis Leão da Penha; Karen Renata Herculano Matos Oliveira; Evander de Jesus Oliveira Batista; Anderson Manoel Herculano Oliveira da Silva; Suellen Alessandra Soares de Moraes.
Affiliations	Federal University of Pará
Session	Developmental Biology and Regenerative Medicine

Abstract, Ethics Committee Number*, and Keywords	<p>Açaí extract has anti-inflammatory, antioxidant and analgesic properties, thereby qualifying it as a potential pharmacological agent. Additionally, studies report that low-intensity laser (LBT) can stimulate the proliferation of fibroblasts and collagen synthesis, favoring tissue repair. Consequently, the synergistic application of açai with LBT may be a therapeutic alternative in the management of tendon repair. This study aimed to evaluate the effects of isolated or combined treatment açai gel and LBT on the macroscopic and histopathological aspects of Achilles tendon injury in the proliferative phase of repair. For this, 40 Wistar rats (CEUA nº 9091250620) were divided into control group (CTRL), injury (INJURY), 10% açai gel (INJURY+GA), 2J infrared laser (INJURY+LAS) and combined treatment (INJURY+LAS+GA). After 14 days, the macroscopic characteristics of the tendon were evaluated by a semi-quantitative scale. Posteriorly, the tendons were collected and the tissue organization pattern was analyzed by H&E and staining with DAPI to quantify the number of cells. We demonstrated improvement in the macroscopic aspects of the tendon in the treatment groups (INJURY+GA: 10.2 ± 0.8; INJURY+LAS: 9.6 ± 1.1; INJURY+LAS+GA: 10.2 ± 0.8, $p < 0.004$) compared to INJURY (INJURY: 12.8 ± 1.3). Concerning tissue organization, all treatment groups had an improvement in the cell orientation pattern, with fibers more aligned and in parallel if compared to INJURY, however, this improvement was more evident in the INJURY+LAS+GA group. There was an increase in the number of cells in all injured groups (INJURY: 1266.8 ± 269.9; INJURY+GA: 1525.8 ± 370.1; INJURY+LAS 2133.6 ± 488; INJURY+LAS+GA 1414.9 ± 364, $p < 0.007$) if compared to CTRL (355.3 ± 65.4), being more evident in the laser group ($p < 0.001$). Combined or isolated treatment with açai gel and LBT improves the macro and microscopic aspects of the Achilles tendon and Laser therapy alone promotes an increase of 6 times in the number of cells.</p> <p>Keywords: tendon injury; tissue repair; açai; laser therapy.</p>
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Title	Analysis of exposure to malathion on cardiac vascular remodeling during juvenile and peripubertal periods in rats
Authors	1-Camille Angélica Pereira da Silva 1-Manuela de Jesus Silva 1-Carina Aparecida Teixeira Santos 2- Luciana Sanae Ota 3- Glaura Scantamburlo Alves Fernandes 3- Rafaela Pires Erthal 1-Francis Lopes Pacagnelli
Affiliations	1 – Universidade do Oeste Paulista, Presidente Prudente, Brazil. 2- UNIFADRA, Dracena, Brazil 3 – Universidade Estadual de Londrina, Londrina, Brazil
Session	DOHAD e desreguladoresendócrinos

Abstract and Keywords	<p>Introduction: Malathion consists of an organophosphate insecticide, cholinesterase inhibitor, used worldwide for domestic and agricultural police protection. The exposure of individuals to toxic agents during the cardiac growth period can negatively influence cardiovascular adaptations. Thus, this study aimed to analyze the effects of malathion on the thickness of the heart arterioles in the exposed during the juvenile and peripubertal periods. Methods: 18 female Wistar rats were used, with an initial age of 21 days (PND21), from the Central Animal Facility from the Universidade Estadual de Londrina. The study was approved by the Ethics Committee (protocol 21053.2019.71). The animals were randomly distributed into three experimental groups (n=6 animals/group). Two groups of animals were treated with malathion at doses of 10 mg/kg (Mal 10) and 50 mg/kg (Mal 50) per body weight, via gavage. The control group (CG) received only the vehicle (soybean oil) in an equal volume. At PND 60, the rats were anesthetized and euthanized. Heart (left ventricles) were collected for the evaluation cardiac vascular remodeling. The thickness of the coronary arterioles was demonstrated through transverse histological sections in the left ventricle (LV) using Verhoeff Van Gienson (VVG) staining, by measuring the diameter at 4 points in the arteriolar tunica media, on each arterioles lamina that occupy its well-defined layers were selected. Kruskal Wallis test followed by Dunn was used to compare the results between the experimental groups. Differences were considered significant when $p < 0.05$. Results: There was no statistically significant difference between the three groups (CG: 12.94 μm (11.46-17.33); Mal10: 14.35 μm (12.01-15.66; Mal50: 14.65 μm (13.81-16.51), $p=0.772$. Conclusions: Rats exposed during juvenile and peripubertal periods to low doses of malathion do not cause changes in cardiac arteriolar thickness. Keywords: Malathion, arteriolar thickness, heart, postnatal development.</p>
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Title	Fetal effects of the treatment of <i>Caryocar brasiliense</i> fruit oil during the rats pregnancy
Authors	Camilly Fernandes ¹ Paulo Roberto da Silva Filho ¹ Thais Campos Ribeiro ¹ Débora Cristina Damasceno ² João Vitor Alves Damacena ¹ Betânia Branco Tiago de Queiroz ³ Rafaianne Queiroz Moraes-Souza ¹ Paula Becker Pertuzatti Konda ³ Larissa Lopes da Cruz ¹ Gustavo Tadeu Volpato ¹
Affiliations	¹ Laboratory of System Physiology and Reproductive Toxicology, Institute of Biological and Health Sciences, UFMT, Brazil ² Postgraduate Course on Tocogynecology, Laboratory of Experimental Research on Gynecology and Obstetrics, Botucatu Medical School, UNESP, Brazil. ³ Department of Food Engineering, Institute of Exact and Earth Sciences, UFMT, Brazil
Session	Poster

Abstract and Keywords	The <i>Caryocar brasiliense</i> pulp oil is widely used in Brazilian food and traditional medicine, without knowledge of effect during pregnancy, especially in fetal development. The objective of this study was to evaluate the fetal repercussions of the treatment of <i>C. brasiliense</i> pulp oil during pregnancy in rats. This study was approved by the Ethics in the Use of Animals Committee – Araguaia (23108.001988/13-1). Wistar rats, 90 days old, were mated, and the pregnant rats were randomized into two experimental groups (n=12 rats/group): treated with water (Control) and treated with <i>C. brasiliense</i> oil (Treated) at dose of 1000 mg/kg. The treatment was performed daily orally during pregnancy (days 0 to 21). On day 21 of pregnancy, laparotomy was performed and the fetuses and placentas were weighed. The fetuses were analyzed for congenital anomalies. The mean values were analyzed by the ANOVA followed by Tukey's test. To compare proportions, Fisher's exact test was used. Differences were considered significant when $p < 0.05$. The maternal treatment with <i>C. brasiliense</i> oil promote increase fetal weight (Control: 5.3 ± 0.3 vs Treated: 5.5 ± 0.5 g) and fetuses classified as large to gestational age (Control: 4.1% vs Treated: 12.1). There was no change in placental weight, placental efficiency and fetal anomalies. In conclusion, the consumption of <i>C. brasiliense</i> oil during pregnancy causes
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	<p>macrosomia and impairs fetal development, indicating warning in the use of oil of this plant during pregnancy.</p> <p>Keywords: Medicinal plants; Food toxicology; Pequi; Pregnancy; Fetuses.</p>
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Title	Investigation of the neuroadaptive effects of maternal physical exercise on the offspring of rats subjected to an obesogenic diet: focus on mitochondrial gene expression
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Affiliations	1 – Programa de Pós-Graduação em Ciências Biológicas - Bioquímica, Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil
Session	03/07

Abstract, Ethics Committee Number*, and Keywords	<p>Diets rich in energy, coupled with sedentary lifestyles, contribute significantly to the prevalence of obesity. Conversely, engaging in regular physical exercise serves as a protective factor against chronic non-communicable diseases. The concept of Developmental Origins of Health and Disease (DOHaD) paradigm, posits that intrauterine conditions exert a lasting influence on the health and disease susceptibility of adults. The objective of our study was to assess the impact of gestational exercise on the mRNA expression of genes associated with mitochondrial dynamics in the cerebellum of offspring exposed to obesity-inducing diets. Wistar rats underwent five weekly sessions of 30 minutes of swimming, starting one week previous and followed throughout pregnancy. Following weaning, the offspring were fed either a standard or an obesogenic diet (high in fat and sucrose). Upon reaching postnatal day (PND) 90, the cerebellum was collected. All experimental protocols were approved by CEUA/UFRGS, under the number 39441. The expression levels of mitochondrial genes including PGC1α, TFAM, and MFN1 were assessed using RT-qPCR, with TUBA1B and GAPDH serving as reference genes. Our preliminar findings revealed no significant alterations in mitochondrial biogenesis as evidenced by the expression of PGC1α and TFAM. However, mitochondrial dynamics were notably influenced by an upregulation of MFN1 expression in response to maternal physical exercise combined with an obesogenic diet, particularly in female offspring. Furthermore, the expression of the GAPDH gene, initially included as a normalization control, exhibited increased levels in response to maternal exercise, an obesogenic diet in the offspring, and both factors combined, in male offspring. These results suggest that maternal exercise induces neuroadaptive changes in mitochondrial gene expression in offspring exposed to obesity-inducing diets in adulthood, in a sex-dependent way.</p> <p>Keywords: DOHaD; Maternal Exercise; Obesogenic Diet; Mitochondria; Cerebellum; Offspring.</p>
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Title	Metabolic imprinting induced by neonatal overfeeding modulates butyrate production in an experimental insulin resistance model
Authors	Nicolly Neves Freitas ^{1,2} Miliane Martins de Andrade Fagundes ^{1,2} Gustavo Silveira Breguez ³ Érika Cristina da Silva Oliveira Siqueira ⁴ Aline Ribeiro Rezende Abreu ^{1,2} Karina Barbosa de Queiroz ^{1,2}
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Session	Abordagens pré-clínicas em DOHAD

Abstract and Keywords	Metabolic imprinting is used to describe postnatal experiences that affect long-term health risks, such as insulin resistance (IR). Lactation may help prevent disturbances in the organism by increasing butyrate levels by gut microbiota, a short-chain fatty acid which is related with improvement in insulin sensitivity. Previous studies from our group has shown that neonatal overfeeding (OF) model mitigates the IR caused by a high sugar diet (HSD) after 8 weeks. Approved by Federal University of Ouro Preto CEUA (n°2245040518). Wistar rats (P0, n=38) were randomly distributed into normal feed litters, with 8 pups/dam (NF, n=19) and overfeeding litters, with 4 pups/dam (OF, n=19). At 21st day, 9 recently weaned animals were euthanized (NF=5; OF=4), and the remaining were divided into 4 groups: NF fed a standard diet (STD-NF, n=7) or NF fed a HSD, 79% carbohydrates (HSD-NF, n=7); and OF fed a STD (STD-OF, n=7) or OF fed a HSD (HSD-OF, n=7) during 8 weeks. After euthanasia, the cecum fecal content was collected and frozen at -80°C. HPLC evaluated fecal butyrate production (µmol/g feces). Shapiro-Wilk assessed data normality, Student's T test litter size effects and groups comparison, TWO-WAY ANOVA followed by Bonferroni post-hoc test. OF increased butyrate (5.34±2.12) in post- weaning rats compared to NF (1.93±1.05), P=0.0122. After 8 weeks of HSD, OF effects also increased butyrate (P<0.0001) as observed in STD-OF group (21.77±3.55) compared to STD-NF (6.20±1.20); and at
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HSD-OF group (24.50 ± 7.63) compared to HSD-NF (10.06 ± 2.61). Thus, it was observed that increases in butyrate levels was due to OF effects, which has persisted even after 8 weeks of diet. Since butyrate is associated with IR improvement, it is important to further explore the mechanisms that explain the relationships between these variables in order to better understand our results. Acknowledgments: UFOP, CCA, FINEP, CAPES code 001, FAPEMIG APQ-02511-22, APQ-04983-22 and BDP-00094-22.

Keywords: metabolic imprinting; insulin resistance; neonatal overfeeding; butyrate.



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 XXII REUNIÃO ANUAL DA BRAVO
 XVIII CONGRESSO DA SBCAL
 III CONGRESSO DOHAD BRASIL
 II CONGRESSO DA SBBA
 2 A 5 DE JULHO 2024, CAMPINAS/SP
FRONTEIRAS DAS CIÊNCIAS BIOMÉDICAS E SEUS DESAFIOS

Title	Neonatal overfeeding activates adaptive thermogenesis of brown adipose tissue and ameliorates insulin resistance induced by high-sugar diet in young rats
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Session	Prêmio DOHAD Brasil (28-Abordagens Pré-clínicas em DOHAD)

Abstract, Ethics Committee Number*, and Keywords	Our model of metabolic imprinting induced by neonatal overfeeding (OF) resulted in the activation of adaptive thermogenesis in brown adipose tissue (BAT), with attenuation of insulin resistance (IR) induced by a high-sugar diet (HSD) intake in adulthood. We aimed to evaluate the long-term effects of neonatal OF in the development of IR and adaptive thermogenesis in the BAT of young rats fed with HSD. After approval by the Ethics Committee on Animal Use of the Federal University of Ouro Preto (UFOP), Protocol 2245040518, metabolic imprinting was investigated by manipulating litter size. After birth (P0), male Wistar rats (n=48) were randomly assigned to either normal feeding (8 pups/dam;NF) or OF (4 pups/dam). Post-weaning (21 days), the animals were further divided into 4 groups: NF litter (i) fed a standard diet (STD, Nuvilab® chow diet, n=11) or (ii) a HSD [condensed milk-based "palatable" diet high in carbohydrates (79%), n=13]; OF litter (iii) fed a STD or (iv) a HSD (n=12), for 8 weeks. At the end of the diet protocol, the animals were euthanized, BAT was removed the degree of phosphorylation at serine 473 of AKT [pAKT(Ser473)], and protein expression of UCP1 was assessed. The effects
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of litter size and post-weaning diet were determined by TWO-WAY ANOVA, followed by Bonferroni post-hoc test. Small litter resulted in neonatal OF, with an increase in neonatal weight [OF, 59.05 ± 8.32 versus NF, 53.65 ± 3.03 grams ($P < 0.01$)]. Moreover, neonatal OF attenuated IR HSD-induced by up-regulating pAKT (Ser473) [HSD-OF, 1.26 ± 0.17 versus HSD-NF, 0.61 ± 0.18 ($P < 0.001$)], and also activates adaptive thermogenesis by up-regulating UCP1 [HSD-OF, 3.33 ± 0.43 versus HSD-NF, 2.55 ± 0.35 ($P < 0.001$)]. These results suggest that metabolic imprinting induced by neonatal OF can activate adaptive thermogenesis, mitigating the IR induced by HSD after 8 weeks. This study was financed by the CAPES code 001, FAPEMIG APO-04983-22, CCA-UFOP, and UFOP.

Keywords: metabolic imprinting; neonatal overfeeding; insulin resistance; adaptive thermogenesis; UCP1; pSer473AKT



Title	Postnatal overfeeding mitigates hepatocytes histomorphological changes caused by a high sugar diet
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Session	Abordagens Pré-clínicas em DOHAD

Abstract, Ethics Committee Number*, and Keywords	<p>The postnatal period is able to modulate the onset of chronic diseases in adulthood, such as insulin resistance (IR). A high sugar diet (HSD) is linked to the IR and histological alterations in the liver, such as inflammation and ballooning of hepatocytes, whereas litter size may result in neonatal overfeeding (OF), influencing these outcomes. We aimed to assess the histomorphological effects of OF on the liver of HSD-fed rats. (CEUA/UFOP nº2245040518) Wistar rats (n=49) were randomly allocated into a normal feed litter (NF, 8 pups/dam, n=24) or OF (4 pups/dam, n=25) after birth (P0). After weaning, animals were fed a commercial (STD) or HSD (condensed milk-based formulation, 79% carbohydrates): STD-NF, n=11, or HSD-NF, n=13; STD-OF, n=12 or HSD-OF, n=13. After 8 weeks, the animals were euthanized and the liver was fixed with methanol-DMSO and analyzed according to the NAFLD Activity Score (NAS) method (ANOVA 2-way, Bonferroni post-hoc). Litter size (P=0.0228), post-weaning diet (P<0.001) and interaction (P=0.0228) influenced NAS, with diet HSD increasing NAS, while OF decreased it (1.231±0.9268 and 0.7692±0.8321, respectively). Analyzing the indices separately, litter size (P=0.0358), post-weaning diet (P=0.0072) and interaction (P=0.0358) influenced ballooning, with HSD increasing it (0.6923±0.8549). Inflammation was influenced by the effect of post-weaning diet (P=0.0102), whereas OF attenuates the inflammation caused by HSD (0.1538±0.3755 and 0.3846±0.5064, respectively). Steatosis was not influenced by the variables. Thus, HSD alters liver histomorphology, increasing NAS, inflammation and hepatocyte ballooning, while neonatal OF mitigates these changes, suggesting its protective effect in the model. This work was carried out with funding from FAPEMIG - APQ-04983-22, CAPES Financing Code 001, CCA and UFOP, to FINEP and FAPEMIG project APQ-02511-22, which have allowed the adaptation of the CCA.</p> <p>Keywords: metabolic imprinting, neonatal overfeeding, insulin resistance, high-sugar diet, inflammation, ballooning</p>
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Title	Characterization of the exposure-gene-disease triad: environmentally relevant endocrine disruptor mixture predisposes to prostate carcinogenesis
Authors	Ana Beatriz Ratto Gorzoni ¹ Odin Serodio Mettifogo ² Edson Assunção Mareco ³ Thaina Cavalleri Sousa ¹ Maria Luiza Silva Ricardo ¹ Karianne Delalibera Hinokuma ¹ Andreia Yuri Yoshigae ¹ Leticia Pereira de Souza ¹ Ariana Musa de Aquino ⁴ Wellerson Rodrigo Scarano ⁵ Leonardo de Oliveira Mendes ^{1,6}
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Session	27 - Abordagens Pré-clínicas em DOHaD

Abstract, Ethics Committee Number*, and Keywords	<p>Many studies examining the effects of endocrine disruptors on various organs often fail to accurately replicate the full extent of human exposure. Therefore, our objective was to assess the impact of exposure to an endocrine disruptor (ED) mixture, spanning from intrauterine development to adulthood, on global gene expression in the prostate and its tissue consequences. Pregnant Sprague-Dawley rats were allocated into two groups: Ctrl (received corn oil, administered orally) and ED Mix (received a mixture comprising 12 ED compounds including phthalates, pesticides, UV filters, BPA, and butylparaben, diluted in corn oil and administered orally at 32.11 mg/kg/day). Pregnant or lactating rats received the ED Mix from gestational day 7 to postnatal day 21 (PND21), and male offspring continued receiving the mixture until PND220, when they were euthanized. The ventral prostate was processed for next-generation sequencing (HiSeq-2500 Illumina) and histological analysis (CEUA/UNOESTE:6034). Transcriptome analysis identified 52 differentially expressed genes, with 32 exhibiting a fold-change greater than 2 compared to the control group. These genes are associated with increased cell survival (e.g., Cacybp - 4.54-fold, Bclaf1 - 4.14-fold) or tumor progression and metastasis (e.g., Need4 - 3.56-fold, Prpf4b - 3.55-fold). Notably, the differentially expressed ARCN1 gene, which plays a crucial role in intracellular membrane trafficking, was linked to an increased risk of prostate cancer mortality in humans. Genetic alterations were mirrored in the tissue microenvironment, with 80% of animals in the ED Mix group exhibiting pre-neoplastic lesions. In conclusion, exposure to an ED mixture alters the expression of genes involved in the carcinogenesis process. This indicates a potential relationship between gene expression, environmental exposure, and the development of prostate cancer. (Funding: FAPESP – 2018/24044-0).</p>
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Title	Undernutrition at suckling phase malprograms long-term dysfunction in brown adipose tissue's thermogenic marker in rat-offspring
Authors	Ginislene Dias Souza ¹ , Aline Milena Dantas Rodrigues ¹ , Ana Caroline Schoenberger Kipper ¹ , Antonio José Rocha Ribeiro ¹ , Rodrigo Mello Gomes ² , Júlio Cezar de Oliveira ¹
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Session	Abordagens Pré-clínicas em DOHAD

Abstract, Ethics Committee Number*, and Keywords	<p>Nutritional insults early in life have been associated with metabolic diseases in adulthood. We aimed to evaluate the effects of maternal food restriction during the suckling period on metabolism and interscapular brown adipose tissue (iBAT) thermogenically involved proteins in adult rat offspring. The study protocol was approved by the Ethics Committee (number 23108.709618-2015-21). Wistar lactating rats were subjected to a food restriction (50%) during the initial 2/3 of lactation (FR50 group), while control mothers were fed <i>ad libitum</i> throughout lactation (CONT group). At birth, the litter size was adjusted to eight pups, and weaning performed at 22 days old. Body weight, food and water intake were assessed every two days. High- (HCD, 4,589 cal) and normal-caloric diet (NCD, 3,860 cal) preferences, as well as food intake during the dark part of the cycle, were assessed. At 100 days old, the rats were euthanized, and blood and tissues were removed for further analyses. Adult FR50 rats, although hyperphagic ($P < 0.001$), were leaner ($P < 0.001$) than the CONT group. The FR50 rats, were normoglycemic ($P = 0.962$) and had hypertriglyceridemia ($P < 0.01$). In addition, the FR50 rats were dyslipidemic ($P < 0.01$), presenting with a high atherogenic risk by the Castelli indexes ($P < 0.01$), had a higher iBAT mass ($P < 0.01$), fewer $\beta 3$ adrenergic receptors ($\beta 3$-R, $P < 0.05$) and higher iBAT expression of uncoupled protein 1 (UCP1, $P < 0.05$) and peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α, $P < 0.001$) than the CONT rats. In conclusion, maternal food restriction during early breastfeeding programs rat offspring to have a lean phenotype, despite hyperphagia, and increased iBAT UCP1 and PGC-1α protein expression.</p> <p>Keywords: Food restriction; Lactation; Thermogenesis; Uncoupled protein 1; Metabolic programming.</p>
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Title	Effects of gestational protein restriction on nephrogenesis of male mice offspring
Authors	Marina Silva Folguieri ¹ Bruno Calsa ¹ Patricia Aline Boer ¹ José Antonio Rocha Gontijo ¹
Affiliations	1 – Fetal Programming and Hydroelectrolyte Metabolism Laboratory, Nucleus of Medicine and Experimental Surgery, Department of Internal Medicine, FCM, Unicamp, Campinas, SP, Brazil.
Session	28 - Abordagens Translacionais e Clínicas em DOHAD

Abstract, Ethics Committee Number*, and Keywords	<p>We have demonstrated that protein restriction leads to 28% reduced nephrogenic cells at 17th gestational days of rats and here our objective was to investigate key growth factors and developmental mechanisms in a previous period that can be related to the genesis of this reduction. C57BL/6J and C57BL/6-Tg (CAG-RFP/EGFP/Map1c3b) 1Hill/J mice were bred at 8-10 weeks of age (ethic committee #5481-1/2020). The female mice were randomly divided into two groups that received diet containing normal protein t (NP-17%) or low protein (LP-6%) content. At 14th gestation day, the male pups were weighed and kidneys were processed for PCR-array of 84 growth factor genes, immunohistochemistry and autophagy dynamic analyses. The results surprisingly showed that the LP pups had 4% greater body mass (0.1035 ± 0.03) than the NP (0.09977 ± 0.02, $p=0.005$). Additionally, the number of cells per metanephric cap (CAP) area was 10% higher in LP (0.02 ± 0.003) compared to NP (0.018 ± 0.005, $p=0.04$). Also we found three downregulated (<i>Csf2</i>, <i>Il1b</i>, <i>Il2</i>) and 7 upregulated (<i>Bmp2</i>, <i>Csf3</i>, <i>Fgf8</i>, <i>Gdnf</i>, <i>Bmp7</i>, <i>Fgf3</i>, <i>Ntf3</i>) genes. The autophagy process and its regulators (AMPKα and mTOR) were unaltered. There was a reduction in activated caspase 3 in the UB of the LP (30105 ± 18947 vs NP: 61112 ± 39327, $p=0.02$) group. The hypoxia-inducible factor-1α expression was also reduced in the BU (12.8 ± 12.59 vs NP: 21.96 ± 9.511, $p=0.007$) and CAP of LP (16.57 ± 10.64 vs NP: 26.75 ± 11.25, $p=0.01$) animals. The vascular endothelial growth factor expression was not altered. Conversely to observed in postnatal progeny of older rat fetuses, for the first time, we demonstrated that gestational protein restriction causes an increase in the key growth factors, in body mass and in CAP cells number at 14th GD in male mice.</p> <p>Keywords: Gestational protein restriction, Fetal programming, autophagy, renal growth factors, kidney nephrogenesis.</p>
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Title	High-calorie diet ingestion by breastfeeding mothers induces hyperphagia and obese-phenotype earliest in male than female rat offspring
Authors	Aline Milena Dantas Rodrigues, Ana Caroline Schoenberger Kipper, Mateus Silva Campos, Antonio José Rocha Ribeiro, Ginislene Dias Miranda, Júlio Cezar de Oliveira
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Session	Abordagens Pré-clínicas em DOHAD

Abstract, Ethics Committee Number*, and Keywords	<p>Introduction: Obesity, considered one of the main dysfunctions of energy metabolism, when present at critical life stages, can program metabolic dysfunctions in offspring. Objectives: To evaluate the weight gain and milk intake of newborn males and female rats, whose mothers ate a high-calorie diet during lactation, and to analyze the caloric content of breastmilk. Methodology: Lactating Wistar rats were fed a high-calorie diet and 10% sucrose solution from delivery until the end of lactation (Ob group), while control mothers (Cont) ingested a standard diet. Pups were weighed every two days, and milk intake measured on the 6th, 11th, and 16th days of life. On the 12th day of lactation, milk samples were collected for biochemical analysis. Approval protocol by the ethics committee (23108.017073-2019-56). Results: Ob-mothers display adiposity index 83.14% higher than Cont ones (P<0.01). Regarding the pups, on the 6th day of life, Ob-male consumed 37.4% more milk than Cont-male, and Ob-female consumed 63.1% more than Cont-feale (P<0.001). On the 11th day, Ob-male consumed 40.8% more, and Ob-female consumed 58.16% more than their counterparts (P<0.05). On the 16th day, Ob-male consumed 49.09% more (P<0.001), and Ob-female consumed 45.37% more than their counterparts (P<0.01). In relation to the counterparts, the pup's body weight was increased by 21.7% in Ob-male and by 10% in Ob-female (P<0.05). By its turn, Ob-male were 18.65% heavier than Ob-female (P<0.001), while there was no significant difference between Cont-male and Cont-female. Breastmilk calorie was 50.9% higher in Ob-mother than Cont-mother (P<0.05). Conclusion: High-calorie diet consumption by lactating mothers increases calorie-milk composition, which can influences male rat-offspring to develop hyperphagia and body weight gain earliest than female rat-offspring.</p> <p>Keywords: Maternal obesity, Lactation, Hyperphagia</p>
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FRONTEIRAS DAS CIÊNCIAS BIOMÉDICAS E SEUS DESAFIOS

Title	Impact of maternal high-fat diet and CCR2+ monocytes on adult offspring metabolism
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Session	Poster

Abstract, Ethics Committee Number*, and Keywords	<p>Monocytes expressing CCR2, the receptor for monocyte chemoattractant protein-1 (MCP-1), migrate to the hypothalamus of high-fat diet (HFD)-fed rodents. Using CCR2-deficient mice (CCR2^{RFP/RFP}) and heterozygous mice (CCR2^{RFP/WT}), we explore the lasting impact of maternal HFD on offspring hypothalamic CCR2 recruitment, chemokine profile, and some metabolic parameters. The experiments were approved by the Committee on Ethics in Animal Use (CEUA 6312-1/2023) of UNICAMP. Offspring were weaned at P21. From P21 to 9 weeks, mice were fed standard (SD) or HFD, then subjected to a glucose tolerance test and divided into SD or HFD groups for 24 h. Brains were harvested for confocal microscopy analysis; white and brown adipose tissues and liver were dissected for histological analysis; and the hypothalamus was harvested for qPCR analysis. Male CCR2^{RFP/RFP} offspring from HFD-fed dams had higher body mass at P21, normalized over time. At 9 weeks, female CCR2^{RFP/RFP} progeny from HFD-fed dams exhibited glucose intolerance, without body mass differences. Similar trends were observed in CCR2^{RFP/WT} offspring, but both sexes were heavier at P21 and glucose intolerant at 9 weeks. Hypothalamic CCR2+ cells infiltrate the hypothalamus of CCR2^{RFP/RFP} offspring from HFD-fed dams, suggesting that other chemokines beyond CCL2 participate in this process. Likewise, we found increased expression of various chemokines in the hypothalamus of progeny from HFD-fed dams. Metabolic dysfunctions were reinforced by morphological analysis and were exacerbated after a 24-hour HFD challenge. Increased caloric intake was also observed in male and female CCR2^{RFP/WT} progeny from HFD-fed dams post-challenge. Maternal HFD triggers lasting metabolic changes in offspring. CCR2-deficient mice do not show protection from these metabolic alterations and CCR2 chemotaxis to the hypothalamus, suggesting the involvement of other chemokine receptors and chemokines in HFD-induced CCR2+ monocyte recruitment.</p> <p>Keywords: monocyte; metabolic programming; hypothalamus.</p>
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Title	Effects of gestational protein restriction on cardiac development of male mice offspring
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Session	Abordagens Translacionais e Clínicas em DOHAD

Abstract, Ethics Committee Number*, and Keywords	<p>Studies demonstrate that protein restriction during pregnancy leads to a reduction in heart weight and the number of cardiomyocytes, here our objective was to investigate whether the process of autophagy and apoptosis could be participating in these cardiac changes. The C57bl/6 and CAG-RFP/EGFP/Map1lc3b mice were used for breeding (Ethics Committee #5481-1/2020). The pregnant mice were randomly divided according to their diet, in which they received normal protein (NP-17%) or low protein (LP-6%), and the fetuses were collected at 14 and 18 gestational days (GD).</p> <p>Surprisingly we observed that the male offspring of the 14GD LP had greater body mass, and the cardiac area was 89% greater in the LP group. However, with 18GD the LP group had lower body mass, and no difference was found in the cardiac area. At both ages, there was no significant difference in the progression of the autophagy process. With 14GD, we observed a 150% increase in mTOR, and there was no difference in AMPKα, as no change in these proteins was found in the hearts with 18GD. Although there was a 63% increase in hypoxia-inducible factor-1α (HIF-1α) in the hearts of the LP group with 14GD, no difference was observed in vascular endothelial growth factor. At 18GD the HIF-1α was 22% reduced in LP. With 14DG, we observed 270 % increased expression of cleaved caspase 3 in the heart of the LP group, and no difference was observed in cellular proliferation (PCNA), and these two proteins in animals with 18DG.</p> <p>We demonstrate, for the first time, that gestational protein restriction causes an increase in body mass and heart area. There is a strict relationship between increased mTOR pathway activity and HIF-1α stimuli that could be inducing cellular differentiation. Additionally, we suppose that is occurring an induction of caspase 3-dependent differentiation. This exacerbated differentiation to the detriment of the mitose process can be involved in the reduced cardiomyocyte number after birth.</p> <p>Keywords: Gestational protein restriction, autophagy, heart development, cell differentiation</p>
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Title	Prostatic Tissue Remodeling vs. Environmental Exposure: Assessing an Endocrine Disruptor Mixture's Impact on Aging through the Lens of DOHaD
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Session	27 - Abordagens Pré-clínicas em DOHaD

Abstract, Ethics Committee Number*, and Keywords	<p>In recent years, there has been a surge in studies focusing on Endocrine Disruptors (EDs), environmental compounds known to alter hormone production and linked to chronic diseases. However, these studies often overlook the diversity of human exposure, typically concentrating on isolated or small-group assessments of EDs. Consequently, there is a pressing need for research that evaluates the effects of perinatal exposure on aging. The aim of this study was to investigate the histopathological aspects of the ventral prostate in elderly rats exposed to a mixture of EDs during gestation and lactation, mirroring human exposure scenarios. Pregnant Sprague-Dawley rats were randomly assigned to two experimental groups (n=10/group): the Ctrl Group (receiving corn oil as vehicle) and the ED Mix Group, which received a mixture of 12 compounds (including phthalates, pesticides, UV filters, bisphenol A, and butylparaben) diluted in corn oil (2ml/kg) at a dosage of 32.11mg/kg/day via gavage. Treatment was administered from gestational day 7 to postnatal day 21 (PND21). Male pups continued to receive water and feed ad libitum until PND440, when they were euthanized in accordance with the CEUA Protocol 6034. There was an increase in the epithelial (p<0.0001) and stromal (p=0.0012) compartments, accompanied by a decrease in the luminal compartment (p<0.0001). Additionally, the ED Mix group exhibited increased epithelial height (p=0.0002) and nuclear parameters, including perimeter (p<0.0001) and area (p<0.0001), with a more rounded nuclear phenotype (p=0.0128). Furthermore, there was a notable increase in the deposition of type I (p<0.0001) and type III (p<0.0001) collagen fibers. The exposed group also showed an elevated number of total (p=0.0034) and intact mast cells (p=0.0002) in the prostatic stroma. In conclusion, exposure to the mixture of EDs during gestation and lactation negatively impacts prostatic tissue remodeling during aging. (Funding: FAPESP – 2018/24044-0).</p> <p>Keywords: endocrine disruptors, prostate, environmental exposure.</p>
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Title	DOHAD in Cerebral Palsy model: sex differences of perinatal treatment with quercetin in muscular strength
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Session	28: Preclinical interventions in DOHAD

Abstract, Ethics Committee Number*, and Keywords	<p>Perinatal quercetin supplementation has been associated with neuroprotective effects in experimental hypoxia-ischemia (HI) models, including experimental Cerebral Palsy (CP). However, the effects of this treatment on different sexes remain underexplored. Therefore, we aim to evaluate the effects of perinatal administration of quercetin on the muscle strength of male and female rats with CP. After ethical approval (n.005/2022), 40 male and 40 female Wistar rats were divided into groups according to the injury and treatment: Control + saline (CS, n=20), CP + saline (CPS, n=20), control + Quercetin (CQ, n=20), and CP + quercetin (QCP, n=20). The CP model includes maternal LPS (200mcg/,10µl/g I.P.) from day P17 until P21, associated with perinatal anoxia (12 min) on day P1 after the birth of the pups. Quercetin treatment (50mg/kg, 10µl/g I.P.) was administered during gestation, concomitant with LPS injection, and also 0h, 24h, and 48h after anoxia, always in the progenitor/lactating rat. The body weight was measured from P1 until P25. The muscular force was evaluated with the GripStrength test, in P25. For statistics, we performed ANOVA 2-way + Bonferroni´s post-test. The body weight and strength of PCS were lower than CS CQ and CPQ (p<0,05). Furthermore, females of the CQ group performed better than all the other groups (p<0,05), suggesting an increase of strength in healthy females but not in males. Thus, we concluded that quercetin improves the muscular force in CP rats. Moreover, quercetin effects are more expressive in females than males. Further research is warranted to investigate the mechanisms associated with this sex difference observed.</p> <p>Keywords: muscular strength; DOHAD; quercetin; cerebral palsy.</p>
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Title	Sex-specific impact of maternal high-fat diet on hypothalamic CCR2 recruitment and chemokine profile in offspring
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Session	Oral

Abstract, Ethics Committee Number*, and Keywords	<p>Upon a high-fat diet (HFD), peripheral monocytes expressing CCR2, the chemokine receptor of CCL2, infiltrate the hypothalamus. Their transcriptome profile shows significant sexual dimorphism, prompting inquiry into the influence of fetal programming on early-life differences. Here we assessed the impact of maternal diet on offspring hypothalamic CCR2 recruitment, chemokine profile, and metabolic parameters using CCR2-deficient mice (CCR2^{RFP/RFP}) and heterozygous mice (CCR2^{RFP/WT}). Ethical approval was obtained from the Committee on Ethics in Animal Use (CEUA 6312-1/2023) of UNICAMP. Offspring were weaned at post-natal day 21 (P21), gender-separated, and weighed. Brain slices underwent confocal microscopy, while white and brown adipose tissues (WAT and BAT, respectively) and the liver were histologically examined. qPCR analysis was performed on the hypothalamus, and blood was collected for biochemical analysis. Offspring from HFD-fed dams exhibited higher body and fat mass, elevated total cholesterol, triglycerides, and fasting glycemia compared to standard diet (SD) littermates, regardless of sex or CCR2 expression. Brain slices from CCR2^{RFP/RFP} showed significant CCR2 monocyte presence in the hypothalamic parenchyma of HFD offspring compared to SD, implying the involvement of other chemokines in this chemotaxis process. Gene expression analysis of the hypothalamus revealed a pattern of higher transcripts of chemokines and their receptors, and pro-inflammatory cytokines in HFD offspring, with sex-based distinctions. Metabolic dysfunction was evident in these mice, as indicated by pronounced fat accumulation in BAT, WAT, and liver on H&E staining. Maternal HFD caused metabolic dysfunction in offspring, independent of sex or CCR2. Sex differences in hypothalamic chemokine expression from HFD dams suggest alternative chemokines and receptors mediate CCR2+ monocyte recruitment, modulating the inflammatory response and the subsequent metabolic dysfunction.</p> <p>Keywords: chemokines; metabolic programming; hypothalamus; monocytes.</p>
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Title	Maternal low-dose caffeine intake during lactation increases the risk of metabolic dysfunction in response to fructose overload in adult female Wistar rat offspring
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Session	28 - Abordagens Pré-clínicas em DOHAD

Abstract, Ethics Committee Number*, and Keywords	<p>High caffeine intake during pregnancy increases the offspring risk to metabolic dysfunction. However, the impact of perinatal low-dose of caffeine intake on metabolic risk of offspring in response to a second insult is not reported in the literature. Here, we tested the response of offspring exposed early to low doses of caffeine to a second insult, i.e., a fructose overload in adulthood. For this, pregnant Wistar rats received intragastric caffeine (25mg/kg/day) or vehicle during gestation or lactation: CON, CAF GEST and CAF LACT groups. Litters were adjusted to 8 pups by dam. At postnatal day 170 (PND170), we substituted the water bottle for a fructose solution (10%) for 10 days before the euthanasia of male and female offspring. Data were analyzed by one way ANOVA followed by post-test Tukey (without fructose) and by t-test Student (with fructose). Protocol approved: 26/2019. Perinatal caffeine did not change the body weight, adiposity, plasma cholesterol and triglyceride at PND180, regardless window of caffeine exposure or offspring sex. However, CAF GEST and CAF LACT female exhibited lower plasma insulin (-34.7%). During the fructose overload test, the volume of fructose drunk was not different among experimental groups. In CON female, fructose only increased plasma triglyceride (+31%) compared to CON without fructose. In CAF LACT female, fructose increased triglyceride (+21.6%), body weight (+12%), retroperitoneal (+33.8%), gonadal (+34.7%) and mesenteric (+46%) adiposity, and plasma insulin (+91%) compared to CAF LACT without fructose. In CAF GEST female, fructose increased plasma insulin (+19.5%) compared to CAF GEST without fructose. Concerning the males, only in CAF GEST group, the fructose increased mesenteric adiposity (+46%). Exposure to caffeine during lactation turned the adult female offspring more susceptible to the effects of fructose. Therefore, even at low doses, maternal caffeine intake increases the risk of metabolic dysfunction due to a second insult.</p> <p>Keywords: maternal caffeine, fructose overload, second insult, lactation, gestation, DOHAD</p>
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Title	Biometric and biochemical parameters in Wistar rat dams and pups in the model of maternal exposure to polystyrene microparticles during gestation and lactation
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Session	27 - Abordagens Pré-clínicas em DOHAD

Abstract, Ethics Committee Number*, and Keywords	<p>Worldwide, more than 350 million tons of plastic/year are produced, a scenario that was intensified with the Covid pandemic. Plastic components can act as endocrine disruptor chemicals and their residues of various sizes dispersed in the air, soil, water and food have already been found in the placenta and breast milk, being associated with impacts on the endocrine and cognitive system of offspring. Here, we studied if exposure to microplastics (MP) during gestation and lactation can cause dysfunction in the dam and pups of both sexes at weaning. The experimental design was approved by the Ethics Committee (031/2022; 032/2022). Nulliparous Wistar rats were mated for 24 h (2 females: 1 male). From the 7th gestational day until weaning, dams received vehicle (Filtered water–control group; n=9) or 10 µm polystyrene microparticles (25 µg/kg body weight–MP group; n=8). Biometric and biochemical parameters of dams, male (M) and female (F) pups were evaluated at weaning (21 days), as well as the biochemical evaluation of milk. Data were analyzed by Student's t test, considering $p < 0.05$ as significant. MP dams had lower body mass during pregnancy (-16%, $p = 0.0134$) and lower plasma cholesterol at weaning (-34%, $p = 0.0381$). Food intake, visceral fat mass, blood glucose, plasma triglycerides, milk cholesterol and triglycerides, plasma leptin and insulin were not changed in MP dams. At birth, MP males and females showed no change in body weight and nasoanal length. From birth to weaning, MP males and females showed lower weight gain (M=-10%, $p = 0.0426$; F=- 13%, $p = 0.0193$). These pups had higher plasma triglycerides at weaning (M=+93%, $p = 0.0457$; F=103%, $p = 0.0490$). MP females had higher plasma cholesterol at weaning (+40%, $p = 0.0107$). Food intake, visceral fat mass, blood glucose, plasma leptin and insulin in the offspring were not significantly altered. Our preliminary findings suggest that perinatal exposure to microplastics affects body mass and lipid metabolism of dams and pups.</p> <p>Keywords: Microplastic, Polystyrene, Lactation, Pregnancy</p>
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Title	Nanoplastic and its effects as an endocrine disruptor chemical in a metabolic programming model
Authors	Nelyana Oliveira Serpa ^{1,2} , Nathalia Medeiros Nehme ¹ , Iala Milene Bertasso ¹ ; Luiz Cláudio Oliveira do Espírito Santo ¹ ; Vitor Hugo Santos Duarte Pinheiro ¹ ; Alex Christian Manhães ³ ; Beatriz Souza da Silva ¹ ; Egberto Gaspar de Moura ¹ ; Luana Lopes de Souza ¹ ; Rosiane Aparecida Miranda ¹ ; Andrea Claudia de Freitas Ferreira ² ; Patrícia Cristina Lisboa ¹ .
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Session	28 – Abordagens pré-clínicas em DOHaD

Abstract, Ethics Committee Number*, and Keywords	<p>Nanoplastics potentially act as endocrine disruptor chemicals, but the impact of exposure to this pollutant on metabolic programming remains poorly understood. Here we studied if maternal exposure to nanoplastics during pregnancy and lactation can lead to long-term dysfunction in the metabolic profile of both sexes offspring. Ethics Committee: 031/2022; 032/2022. Wistar rats were paired for 24h (2 females: 1 male). From the 7th day of pregnancy until the end of lactation, dams received vehicle (filtered water – control group; n=9) or 100 nm polystyrene nanoparticles (25 µg/kg of body weight; n=8). Biometric, biochemical, and behavioral parameters of male and female offspring were assessed at puberty (45 days) and adulthood (120 days). Data were analyzed by Student's T-test. At 45 days, females exhibited a reduction in fat mass (-17%, p<0.05) and an increase in lean mass (+2%, p=0.045). Both males and females showed a decrease in triglyceride levels (-39% and -38%, p<0.05). At 120 days, females showed an increase in food intake (+3%, p<0.010). In the food preference test, males showed reduced preference for the standard diet (-50%, p<0.05) and preference for the high-fat diet (+12%, p<0.05). Between female offspring, only the preference for the standard diet was reduced (-80%, p<0.01). No differences in leptin and insulin levels between sexes and ages were observed. In the elevated plus maze (EPM) and the open field (OF), only males, irrespective of age, exhibited increased anxiety-like behavior (p<0.05). No changes were observed in locomotor activity in either the EPM or OF. Females showed no change in EPM or OF. These findings suggest that nanoplastics can influence body mass in a sex-dependent manner during puberty, a critical phase when adipose tissue plays a crucial role in sexual maturation. Besides, adult male progeny exhibited an increased preference for a high-fat diet, possibly linked to the anxious profile detected in the test.</p> <p>Keywords: Nanoplastic; Programming; Endocrine disruptors; Puberty; Adulthood.</p>
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Title	Long-term effects of maternal exposure to polystyrene microparticles during gestation and lactation on biometric, biochemical and behavioral parameters of both sexes rat progeny
Authors	<u>Nathália Medeiros Nehme</u> ¹ Nelyana Oliveira Serpa ¹ Luiz Cláudio Oliveira do Espírito Santo ¹ Beatriz Souza da Silva ¹ Luana Lopes de Souza ¹ Egberto Gaspar de Moura ¹ Rosiane Aparecida Miranda ¹ Alex Christian Manhães ² Vitor Hugo Santos Duarte Pinheiro ² Iala Milene Bertasso ¹ Patrícia Cristina Lisboa ¹
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Session	27 - Abordagens Pré-clínicas em DOHAD

Abstract, Ethics Committee Number*, and Keywords	<p>Plastic production in the world exceeds 350 million tons/year and the Covid pandemic was one of those responsible for worsening this situation. Plastic particles of different sizes are dispersed in the air, soil, water and food, in addition to being detected in the placenta and breast milk. Plastic components act as endocrine-disrupting chemicals and are associated with impacts on the endocrine and cognitive systems of offspring. We assessed whether maternal exposure to microplastics (MP) during pregnancy and lactation can cause long-term dysfunction in offspring of both sexes. The experimental model was approved by the Ethics Committee (031/2022; 032/2022). Wistar rats were mated for 24 h (2 females:1 male). From the 7th gestational day to weaning, dams received vehicle (Filtered water–control group; n=9) or 10 µm polystyrene microparticles (25 µg/kg of body weight–MP group; n=8). We evaluated biometric, biochemical and behavioral parameters (food preference, elevated plus maze and open field) of offspring of both sexes at puberty (45 days) and adulthood (120 days). Data were analyzed using Student's t test, considering $p < 0.05$ as significant. At 45 days-old, MP males showed a reduction in plasma cholesterol (-27%, $p = 0.0167$) and triglycerides (-39%, $p = 0.0004$). At 120 days-old, MP males showed a reduction in food intake (-3%, $p = 0.0071$) and plasma leptin (-43%, $p = 0.0419$). In the food preference test, MP males showed lower preference for high-fat diet (-10%, $p = 0.0134$). In the open field test, regardless of age, MP males showed an increase in anxiety-like behavior ($p < 0.05$). MP females, regardless of age, did not show significant changes in the biometric, biochemical and behavioral parameters studied. Our preliminary data suggest that maternal exposure to microplastics during pregnancy and lactation leads to sex-dependent changes, with males being more affected by MP.</p> <p>Keywords: Microplastic, Polystyrene, Puberty, Adult, Endocrine system</p>
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Title	Female progeny whose dams were tributyltin-exposed during pregnancy and lactation shows endocrine and reproductive disorders throughout life
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Session	Abordagens Pré-clínicas em DOHaD

Abstract, Ethics Committee Number*, and Keywords	<p>Tributyltin (TBT), a biocide organotin used as an antifouling in boat paints, can contaminate marine foods affecting organisms and human health. TBT acts as endocrine disruptor chemical (EDC) causing a phenomenon called imposex, that is an emergence of male sexual characters in female gastropods. As everyone can be exposed to TBT, including women of reproductive ages, we studied if the maternal exposure to low and high TBT doses during pregnancy and lactation can cause long-term dysfunctions in female offspring. Ethics committee approved this model (CEUA/010/2019). Wistar rats were mated and after pregnancy detection, dams were randomly separated into 3 groups: dams received ethanol (0.01%; Control); TBT100 (100 ng/kg body weight, bw) or TBT1000 (1000 ng/kg bw), from the 7th gestational day until the end of lactation by gavage (n=7/group). We analyzed in female offspring from postnatal day (PND) 21, 45 and 180: biometric parameters, plasma insulin, leptin, gonadotrophins, sex hormone and pancreatic damage by Transmission Electron Microscopy (TEM). Statistical analysis: One Way Anova with Dunnett's post-test; p<0.05. TBT100 induced low birth weight in female offspring, while TBT1000 promoted low bw in PND180. TBT caused low lean mass from PND60 until adulthood. Both TBT doses reduced plasma leptin at PND21 (-45%, -70%, respectively), but insulin is reduced only in TBT100 (-43%). At PND45, TBT groups presented increases in plasma testosterone (+2-fold). TBT100 presented reduced FSH (-50%). At adulthood, TBT1000 did not show alterations in plasma hormones, but had altered estrous cycle as well as TBT100 (prolonged diestrus). Regardless of the dose or age, TBT caused damage pancreatic structural damage. Taken together our data indicate that perinatal exposure to TBT causes endocrine dysfunctions throughout life of the female progeny, which can lead to diabetes and infertility.</p> <p>Keywords: Tributyltin; hormonal dysfunction; female progeny.</p>
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Title	Gestational caloric restriction affects offspring's brain mitochondrial dynamics
Authors	João Victor Marques ¹ , Vinícius Stone ² , Vanessa-Fernanda Da Silva ² , Bernardo Gindri dos Santos ² , Pauline Maciel August ² , Vitor Gayger-Dias ² , Carlos Alberto Saraiva Gonçalves ^{1,2} , Cristiane Matté ^{1,2}
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Session	27

Abstract, Ethics Committee Number*, and Keywords	<p>Caloric restriction (CR) is well-documented for promoting health and preventing metabolic disorders and chronic non-communicable diseases (CNCDs), but its effects during development, including its role as a non-pharmacological strategy to protect against CNCDs, remain poorly understood. Previous data from our lab showed improvement in offspring's brain mitochondrial health and positive effects in antioxidant enzyme activation, when moderate CR was applied to dams. Here, we used a 20% CR in dams during pregnancy, and the offspring's prefrontal cortex was evaluated for antioxidant and mitochondrial markers. The project was approved by local Ethics Commission (n 38809). Our data showed a reduction on the immunocontent of SIRT 3 [t(8)=2.816, p=0.0226] and TFAM [t(10)=2.253, p=0.0479] in the prefrontal cortex. We observed no alteration on SIRT1 [nucleus: t(5)=0.9030, p=0.4079; cytoplasm: t(10)=0.3328, p=0.7462], AMPK [t(8)=1021, p=0.3371], Akt [t(10)=0.7886, p=0.4486], PGC1α [nucleus: t(5)=1.161, p=0.2982; cytoplasm: t(7)=1.210, p=0.2657], mitofusin [t(10)=0.7850, p=0.9390], DRP1 [t(9)=0.4189, p=0.6851], and NRF2 [nucleus: t(6)=0.7128, p=0.5027, cytoplasm: t(7)=0.7342, p=0.4867] expression. In previous works, we demonstrated a robust activation of antioxidant defenses, as well as mitochondrial function. On the other hand, we are reporting a reduction in SIRT3 and TFAM in this study, which could affect the mitochondrial metabolism and dynamics. Despite these seemingly contradictory findings, they are still preliminary results, and further studies are needed to elucidate the mechanisms related to the effect of gestational caloric restriction on offspring neurodevelopment.</p> <p>Keywords: DOHaD; Caloric Restriction; Mitochondria; Cortex; Offspring.</p>
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Title	Effects of maternal curcumin supplementation on the offspring in an experimental model of protein restriction during pregnancy
Authors	Da Silva, G.G.C ¹ ; Rezende, J.P.O ¹ ; De Souza, A.B.F ^{1,2} , Machado-Junior, P.A. ¹ ; Valença, S.S ² ; Bezerra, F.S. ¹
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Session	27 - Abordagens Pré-clínicas em DOHAD

Abstract, Ethics Committee Number*, and Keywords	<p>This study evaluated the influence of curcumin intake in females subjected to restricted protein intake on offspring at 24 hours of age. This project was approved by CEUA-UFOP under No. 6251150822. Male and female C57BL/6 mice aged between 8 and 10 weeks were used. After confirmation of pregnancy, females were divided into 5 groups: normoprotein (NP), low protein (LP), and LP groups that received curcumin by gavage once a day at doses of 50 mg/kg (LPC50), 100 mg/kg (LPC100), and 200 mg/kg (LPC200). During pregnancy, the females were observed and the pups were euthanized at 24 h of age. Food consumption in grams and kcal was similar between groups during the first week of gestation ($p > 0.05$), but during the last week of gestation food consumption was higher in the LP group (29.86 ± 4.93 g) compared to LPC50 (18.46 ± 0.85 g) and LPC200 (20.91 ± 4.38 g). At the beginning of pregnancy, there was no difference in the body mass of the females, but at the end of pregnancy, the LPC50 [27 (25.50-27)] and LPC200 [26.50 (25.25-29.25)] groups had lower body mass compared to NP [34.50 (33-36)]. In terms of offspring, the LP [1.00 (0.97-1.12)] had lower mass compared to NP [1.16 (1.07-1.20)]. LP [2.20 (2.10-2.30)] was shorter compared to NP [2.40 (2.40-2.50)] and LPC50 [.50 (2.40-2.65)] was longer compared to LP. In the blood, the leukocyte count was higher in LP (5.11 ± 0.81) compared to NP (3.67 ± 0.68), and the administration of curcumin at three doses (50 mg/kg: 2.05 ± 0.23; 100 mg/kg: 2.53 ± 0.47; 200 mg/kg: 2.62 ± 0.30) resulted in a decrease in the cell count compared to LP. Similarly, the lymphocyte count was higher in LP (4.331 ± 0.7392) compared to NP (3.200 ± 0.5991), and LPC50 (1.71 ± 0.22), LPC100 (2.04 ± 0.42), and LPC200 (2.25 ± 0.30) showed lower counts compared to LP. Preliminary results suggest that the low protein diet promotes growth restriction and inflammation in offspring, and curcumin appears to improve offspring growth.</p> <p>Keywords: <i>Curcuma longa</i>, Curcumin, protein restriction, intrauterine growth restriction</p> <p>Funding: UFOP, FAPERJ</p>
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Title	Impact of maternal caloric restriction on signaling pathways related to mitochondrial dynamics and oxidative stress in the offspring's hippocampus
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Session	27

Abstract, Ethics Committee Number*, and Keywords	<p>Calorie restriction (CR) is well documented as an intervention capable of promoting health and increasing longevity in adult and human animal models. However, the effects of CR during fetal development are still not well understood. In this sense, we explored markers of mitochondrial dynamics and signaling pathways (DRP1, mitofusin, NRF2, TFAM, sirtuins, PGC1-a, PI3K/Akt and AMPK) that affect the hippocampus in adult female Wistar rats (approved by the local Ethics Committee on the Use of Animals, under number 38,809). The Wistar rat dams in the CR group received food reduced by 20% in relation to the control. One pup per litter was used in each Western Blot analysis. Low weight gain was identified in CR mothers compared to the control group on the first day of gestation. Maternal CR promoted a decrease in the expression of TFAM [t(9)=3.291), p=0.0094] and DRP1 [t(10)=2.399, p=0.0374] in the hippocampus. No change in SIRT1 [nucleus: t(6)=0.001271),p=0.9990; cytoplasm: t(7)=1.183,p=0.2753], SIRT 3 [t(10)=1.040e- 010, p>0.9999], AMPK [t(9)=1402, p=0.8916], Akt [t(10)=0.4041, p=0.6946], PGC1a [nucleus: t(6)=0.06823, p=0.9478; cytoplasm: t(7)=0.7805; p=0.4607], mitofusin [t(10)=0.1971, p=0.0.8477] and NRF2 [nucleus: t(5)=1.454, p=0.2058,cytoplasm:t(7) =1.195, p=0.2711] expression was verified. This intervention was able to prevent oxidative damage to hippocampal proteins. With this, we then have benefits related to the redox state and mitochondrial health of offspring in which the matrix was subjected to CR. However, more studies are necessary to better understand the effect of CR on offsprings' brain development.</p> <p>Keywords: Calorie restriction, Oxidative stress, Pregnancy, Cell Signaling, Hippocampus, Mitochondria, DOHaD.</p>
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Title	Maternal organophosphorus pesticides exposure and the association with sex-dependent behavioral changes in the adult offspring
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Session	Abordagens Pré-clínicas em DOHAD
Abstract, Ethics Committee Number*, and Keywords	<p>Organophosphorus pesticides (OPPs) are the most used worldwide and have been associated with changes in the central nervous system, inducing cognitive dysfunction. We hypothesize that maternal exposure during pregnancy and lactation to glyphosate and acephate, pesticides most used in Brazil, may alter offspring behavior in adulthood. On the 7th day of gestation, pregnant rats were exposed by gavage to vehicle (filtered water; Control group; CON); glyphosate (5mg/kg of body weight, bw-GLY5 or 0.5mg/kg bw-GLY0.5); or acephate (4.5mg/kg bw-ACE4.5 or 0.45mg/kg bw-ACE0.45), n=8/group. At adulthood (120-days-old), we evaluated the male and female offspring's body weight gain, food intake, food preference test offering a standard (SD) and a high-fat (HF) diets for 30 min and 12h, the anxiety-like behavior using the elevated plus maze (EPM) test, and locomotor activity by open field (OF) test. Protocol approved: CEUA 004/2020. Statistical analysis: One Way Anova with Dunnett's post-test (considering p <0.05). All groups of both sexes, except ACE0.45 group, had low birth weight compared with CON. In adulthood, there is no difference in body weight of offspring perinatally exposed to both pesticides. However, except ACE0.45 females, all animals showed reduced food intake during the study. Regarding feeding study, as expected CON group ate more HFD; ACE4.5 females only showed high preference for after 30 min (SD+2.9x vs CON). In the EPM, ACE4.5 males showed an increase in total entries (+59%; higher locomotor activity). GLY5 females showed reduction in total entries (-29%; lower locomotor activity) and ACE4.5 females showed a reduction of % of open arms (-30%; anxious-like behavior). Maternal OPPs exposure has adverse effects on biometric and behavior parameters of the adult progeny, depending on the dose, pesticide and sex of the offspring. We suggest that maternal OPPs can increase risk for cognitive dysfunctions leading to health consequences in the long term.</p> <p>Keywords: Organophosphorus pesticides, maternal exposure, offspring behavior.</p>



Title	Perigestational high-sucrose intake leads to early NAFLD onset in the offspring of Wistar rats
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Session	Preclinical approaches in DOHaD

Abstract, Ethics Committee Number*, and Keywords	<p>Metabolically unbalanced intrauterine environment elevates the risk of late-in-life chronic non-communicable diseases. Maternal high-sugar intake has been shown to negatively affect the offspring glycolipid metabolism. Thus, this study aimed to investigate whether the onset of non-alcoholic fatty liver disease (NAFLD) occurs early in rats exposed to high-sucrose diet (HSD) during gestation and lactation. Female rats were fed an isocaloric HSD (25% sucrose, n=4) or regular chow (10% sucrose, CTR, n = 4) from preconception through lactation. Male and female offspring were switched to a control diet at weaning, and the groups were euthanized at 30 or 90 days old (n=5-8/group/sex) (CEUA/UFMA 23115.002915/2023-62). HSD dams exhibited higher body weight from preconception (+9.7%, p<0.01) through gestation (+6.5%, p<0.05), and lactation (+4.1%, p<0.05). Maternal liver weight decreased by 10.7% in the HSD group (p<0.05) with no significant histological changes observed. In comparison to CTR, HSD mothers displayed elevated serum triglycerides (+95.5%, p<0.05), impaired glucose tolerance (+29.7%, p<0.01), and a higher post-lactation TyG index (+9.72%, p<0.05). HSD offspring showed higher body weight gain (+12.1%, p<0.001) during lactation, but only male offspring maintained it over 90 days. At 30 days old, no sex-based metabolic differences were observed between CTR and HSD offspring. However, at 90 days old, HSD males exhibited lower glucose tolerance (+41,5%, p<0.001), and elevated serum triglycerides (+111.3%, p<0.05), while HSD females showed reduced serum cholesterol (-27.1%, p<0.05), as compared to CTR offspring. Hepatic steatosis was only evident in HSD offspring from both sexes at 30 days old but was worsened by 179.9% (p<0.01) in males at 90 days old. In conclusion, our findings underscore the importance of precociously evaluating metabolic markers in the offspring of metabolically dysfunctional mothers, particularly for early tracking of NAFLD onset and development.</p> <p>Keywords: Added sugars; intergenerational outcomes; NAFLD.</p>
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Title	Maternal caffeine intake in a low dose during gestation or lactation promotes insulin resistance in female Wistar rat offspring at adulthood
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Session	28 – Abordagens Pré-clínicas em DOHaD

Abstract, Ethics Committee Number*, and Keywords	<p>Pregnancy and lactation periods are susceptible to maternal diet promoting different outcomes on offspring metabolism. 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, lactation or both periods on glucose metabolism of offspring is unknown. 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. Fasting glycemia were evaluated of offspring from both sexes at birth, weaning, puberty and adulthood. The oral glucose tolerance test (OGTT) and the insulin signalling in skeletal muscle were performed at adulthood. Data were analysed by one way ANOVA followed by Dunnett's post-test. Protocol approved: 26/2019. CAF groups showed unchanged glycemia at birth, weaning and puberty. Regarding adult females, CAF GEST showed fasting hyperglycaemia (+15.5%), while CAF GEST and LACT showed glucose intolerance during the OGTT. CAF female GEST showed higher glycemia at all points of test (15': +45%, 30': +43%, 60': +28%, 120': +18%), CAF LACT showed higher glycemia at 15 (+38%), 30 (+39%) and 60 (+29%) minutes. In the soleus muscle, we observed lower pAKT (-63%) and GLUT4 expression (-73%) only in the female CAF LACT, with no change in pIRS1. Female CAF GEST showed a nonsignificant lower GLUT4 content (-54%). We did not observe changes in OGTT nor in the insulin signalling proteins in the male CAF offspring. Therefore, even in a low dose, maternal caffeine intake isolated to gestation or lactation promotes oral glucose intolerance and insulin resistance only in female offspring. Interestingly, the maternal caffeine intake during continuous period (CAF GL) did not change the glucose homeostasis of offspring.</p> <p>Keywords: maternal caffeine, gestation, lactation, insulin resistance, DOHaD</p>
	FeSBE2024-611



Title	Spermidine decreases lipid profile and meliorates glucose intolerance in diabetic female mice
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Session	Abordagens Pré-clínicas em DOHAD

Abstract, Ethics Committee Number*, and Keywords	<p>Type 2 diabetes mellitus (T2DM) is one of the main causes of death globally. Spermidine (SPD), as autophagy inducer, can improve energy metabolism, but their impact on type T2DM remains unclear. Our aim was to assess the effect of SPD on T2DM induced by high-sucrose diet (HSD). Post-weaning female Swiss mice were divided into CTR, HSD, and SPD groups. HSD and SPD groups received a standard chow and 30% sucrose solution <i>ad libitum</i> for 20 weeks to induce diabetes. SPD group also receiving oral spermidine (20 mg/kg, by gavage) since the 12th week of induction. Morphometric and biochemical analyses were performed in blood, liver and white adipose tissue (WAT). The number of animal ethics committee was 23115.008036/2023-44. At the end, HSD group presented increases in the glycemic levels (150±6.54 vs 122±3.85 mg/dl), weight gain (45.7±2.77 vs 38.4±1.35 g), body mass (Lee Index: 339.3±39.9 vs 320.1±3.8), and energy consumption (13.4±0.98 vs 2.9±0.1 Kcal/10g BW) when compared to CTR. These data were corroborated with accumulation of retroperitoneal (0.15±0.02 vs 0.08±0.01 g/10g BW), periovarian (0.86±0.08 vs 0.47±0.08 g/10g BW), and mesenteric WAT (0.29± 0.02 vs 0.16±0.02 g/10g BW) in HSD vs CTR. SPD treatment do not ameliorate these alterations. However, compared to CTR, SPD group showed reduction in serum triglycerides (TG) level (105.9±8.4 vs 139.9±2.4 mg/dl) and insulin resistance by TyG index (8.9±0.09 vs 9.3±0.03). Moreover, SPD treatment attenuates hepatic TG (10.8±1.81 vs 13.4±1.73 mg/g), hepatic total fat (357.6±38,33 vs 391.4±48.48 mg/g), glucose intolerance (AUC: 36593±909.5 vs 40380 ± 3400) and a tendency to improve insulin sensitivity in WAT measured by <i>ex vivo</i> lipolysis (4.5±0.37 vs 5.2±0.37 glycerol µg/mg/h) compared to HSD. Therefore, these findings showed that SPD decreases lipid alterations and improves glucose homeostasis in T2DM female mice, suggesting your therapeutical potential for management of T2DM and its comorbidities.</p> <p>Keywords: Spermidine; Type 2 Diabetes Mellitus; High-sucrose diet.</p>
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Title	Peripubertal metformin administration attenuates metabolic dysfunction in adult female offspring of rats perigestationally fed a high-sucrose diet
Authors	Bruno Eduardo Lopes de Macêdo ¹ , Jéssica Furtado Soares ¹ , Odara Champoudry da Silva ¹ , Jaqueline Pessoa Pontes ^{1,2} , Camila de Fátima Carvalho Brito ¹ , Thamys Marinho Melo ^{1,2} , Lucas Martins França ^{1,2} , Antonio Marcus de Andrade Paes ^{1,2}
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Session	Abordagens pré-clínicas em DOHAD

Abstract, Ethics Committee Number*, and Keywords	<p>According to the DOHAD concept, an adverse perigestational environment can lead to poor health outcomes at adulthood. However, due to its high plasticity, puberty has emerged as a unique metabolic window for pharmacological and non-pharmacological interventions aiming to prevent such outcomes. Thus, this study aimed to investigate whether metformin administration during peripuberty could improve the metabolic status of adult female rats whose mothers were perigestationally fed a high sucrose diet (HSD, 25% sucrose). Female Wistar rats (30 days old) were divided into two groups and fed with HSD (n=6) or regular chow (CTR, n=6) for 8 weeks before mating, continuing throughout gestation and lactation. From the postnatal day (pnd) 30 to 51, corresponding to the peripubertal period, female offspring of HSD rats was orally treated with metformin (MET, 300 mg/kg/day, n=8) or vehicle (HSD, 1 ml/kg/day, n=8). Female offspring of CTR rats received only vehicle (CTR, n=10). All the offspring groups were fed regular chow from weaning through euthanasia at pnd 105 (CEUA/UFMA 23115038755/2018-22). HSD dams showed increased body mass, hypercholesterolemia, and steatohepatitis compared to the CTR ones. The HSD offspring showed increased body weight at birth (+10%, p<0.01) and weaning (+11%, p<0.0001), as compared to CTR, which was not kept upon entering puberty. At euthanasia, HSD offspring showed increased serum triglycerides (+82%, p< 0.0001), impaired glucose tolerance (+75% AUC GTT, p<0.05), and increased TyG Index (+7%, p< 0.0001) in comparison to CTR. The MET group did not present any of these outcomes. On the other hand, metformin treatment did not prevent liver steatosis, which was verified in both HSD and MET groups. Therefore, peripubertal administration of metformin attenuated metabolic disorders in female adult rats born to HSD-fed rats, but did not prevent the onset and development of non-alcoholic fatty liver disease.</p> <p>Keywords: added-sugars, metformin, peripuberty, NAFLD.</p>
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Title	Maternal low-dose caffeine intake during the perinatal period affects fetal growth, thyroid function, and behavior of Wistar rat offspring in a sex dependent manner, regardless corticosterone levels
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Session	28 - Abordagens Pré-clínicas em DOHAD

Abstract, Ethics Committee Number*, and Keywords	<p>Perinatal caffeine crosses the placental barrier, affecting the fetal growth from heavy caffeine users, through mechanisms such as fetal corticosterone overexposure. Here, we tested the impact of low maternal caffeine intake on the risk of intrauterine growth restriction (IUGR). Female pregnant Wistar rats received intragastric caffeine (CAF) (25mg/kg/day) or vehicle during gestation and lactation periods (CAF group vs CON group). At birth, litters were adjusted to 8 pups per dam, and the body weight, length and plasma corticosterone were evaluated. In dams, the body weight gain, accumulated food intake and plasma hormones were measured at the end of lactation period. At adulthood, behavior tests, as elevated plus maze (EPM) and open field (OF) were performed. Test t-student was used. Protocol approved: 26/2019. CAF dams did not exhibit food intake changes (-10%; $p < 0.07$) but showed reduced body weight gain (-4.2%) and adiposity (-31%). At birth, CAF female offspring showed lower body weight (-6%), without changes in the nasoanal length. At end of lactation, CAF dams showed hypercholesterolemia (+36.6%), without alter the plasma cholesterol of offspring at birth and weaning. Unexpectedly, CAF dams did not change serum corticosterone, as well as pups at birth and weaning. CAF dams showed reduced plasma T3 (-56%), without changes in serum T4 and TSH. At weaning, CAF male offspring showed lower plasma T3 (-71.5%), without changes in serum free T4. Adult CAF female had reduced levels of anxiety-like behavior in the EPM test, while CAF males had higher locomotor activity in OF test. Then, even a low maternal caffeine intake affects the fetal growth and development, regardless changes in the plasma corticosterone. In addition to IUGR in CAF female offspring, the CAF male offspring should show impairment of development, due the maternal and early hypothyroidism. These early changes can affect the behavior in a sex dependent manner.</p> <p>Keywords: maternal caffeine, IUGR, hormones, perinatal period, DOHAD</p>
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Title	Placental fatty acid profile of animals that consumed a high-fat diet before and during pregnancy
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Affiliations	DOHAD
Session	Abordagens Pré-clínicas em DOHAD

Abstract, Ethics Committee Number*, and Keywords	<p>Introduction: During pregnancy, there is an increase in metabolic, physiological and nutrient demands. Studies have already demonstrated changes in the maternal plasma lipid profile in the presence of obesity, associated comorbidities, insufficient gestational hormones and placental function with impaired fetal growth. Furthermore, the contribution of maternal metabolism derived from the excessive accumulation of lipids in the placental tissue and its effect on fetal development is uncertain. Therefore, the objective of this study was to evaluate the fatty acid (FA) profile of the placenta of animals submitted to high-fat diet. Methodology: Female Swiss mice (CEUA-5608-1/2020) were fed a high-fat diet (45%) for 4 weeks before pregnancy and categorized as prone (HF-P) or resistant to obesity (HF-R) according to the weight gain profile. Afterwards, they were subjected to mating and on day E19.5 the pregnancy was interrupted for sample collection. The lipid profile in the placenta was evaluated by Chromatography Coupled to Mass Spectrometry and the data analyzed using one-way ANOVA test, followed by Tukey's post hoc test for $p < 0.05$. Results: No differences were observed between groups in total and saturated FA. However, polyunsaturated FA (PUFAs) was decreased in HF-P compared to the control (CT) and HF-R. In a more detailed analysis, it was found that this decrease was mainly due to the reduction in $\omega 6$ in the prone group. In general, the samples presented more $\omega 6$ than $\omega 3$, however, there was no difference in $\omega 6/\omega 3$ ratio. Among other PUFAs there was a trend to reduction in C18:2 and a decrease in C20:4 in the HF-P. Finally, C18:1 monounsaturated FA were increased in the HF-P group and there was a tendency for C20:1 to increase in the same group. The results of the simple regression showed a negative relationship between gestational weight and C18:2-n6. Conclusion: Thus, our results show that the impairment in fetal development observed previously in the HF-P may be related to changes in the profile of placental fatty acids, especially PUFAs.</p> <p>Keywords: placenta, fatty acids, obesity</p>
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Title	Aerobic training avoids vascular damage in the offspring, independent of sex, of Wistar rats submitted to gestational variable stress
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Session	27- Abordagens pré-clínicas em DOHaD

Abstract, Ethics Committee Number*, and Keywords	<p>Exposure to stress during pregnancy can impact fetal development and vascular function in adult offspring and it is known that aerobic exercise is capable of mitigate the harm induced by stress. The aim of the study is to evaluate the effects of aerobic training on vascular function in male and female offspring of Wistar rats subjected to variable stress. Study approved by the ethics committee (9518170621) with 4 offspring groups (n=8/group/sex) of <i>Wistar</i> rats: Control(C), Control+training(CT), Stress(S), and Stress+Training(ST). The stress protocol was carried out from the 14th to the 21st day of pregnancy, daily, through different stimuli. At 60 days, offspring underwent treadmill training for 2 months at moderate intensity. At 120 days, thoracic aorta was collected for vascular reactivity and protein quantification by Western Blot (eNOS, GPx). Results described as mean±SEM. One-way/two-way ANOVA with post-test, significant at $p \leq 0.05$. The training protocol demonstrated its effectiveness, since the trained animals reached greater speed in the final test (Male $p=0.003$; Female $p < 0.001$). Vasoconstrictor reactivity to phenylephrine showed no differences (Male C: 6.65 ± 0.30; S: 7.31 ± 0.30; CT: 7.36 ± 0.20; ST: 6.96 ± 0.24; $p=0.15$; Female C: 6.65 ± 0.30; S: 7.31 ± 0.30; CT: 7.36 ± 0.20; ST: 6.95 ± 0.24; $p > 0.05$). Stress had impaired vasodilation to acetylcholine, which was improved</p>
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by training, in male (C:99.90±2.08; S:86.36±2.88; CT:103.00±2.13; ST:99.88±2.67; p<0.0001) and female (C:106.40±3.59; S:78.50±7.43; CT:99.26±0.74; ST:104.70±2.54; p<0.0001). In males, stress decreased GPx expression in aorta (C:1.11±0.10; CT:1.06±0.16; S:0.60±0.05; ST:0.91±0.13; p=0.02). No differences were observed in eNOS expression (Male C:1.10±0.23; CT:0.88±0.24; S:0.81±0.20; ST:1.26±0.30 p=0.18; Female C:1.09±0.06; CT:0.88±0.27; S:0.78±0.15; ST:1.06±0.21; p=0.18). Gestational stress impaired vasodilation, by different pathways in males and female offspring and aerobic training prevented the damage.

Key words: gestacional variable stress, aerobic training, vascular reactivity.



Title	Effect of high sucrose diet in adult rats, malnourished in adolescence, on adiposity and glycemc homeostasis
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Session	Abordagens Pré-Clinicas em DOHaD.

Abstract, Ethics Committee Number*, and Keywords	<p> Introduction: Nutritional insults in critical periods of life development programs long-term metabolic diseases. The intake of hypercaloric foods is related to the development of obesity and associated comorbidities. Objective: To evaluate the effect of high sucrose diet on body composition and glycemc homeostasis in adult rats subjected to food restriction during adolescence. Methods: Male and female Wistar rats were subjected to 50% food restriction during adolescence (from 30 to 60 days-old) and then divided (RADS and RA) to receive or not water containing 30% sucrose until 120 days-old. At the end of the experiment, body weight, adiposity index and glycemc homeostasis were evaluated. The experimental protocol was approved by the CEUA (#23108.085904/2023-07) of UFMT. Data are expressed as mean and standard deviation and were analyzed using GraphPad Prism. Results: There was no statistical difference in calorie intake, but the high sucrose diet increased body weight, visceral fat and adiposity index, regardless of gender. The adiposity index showed no difference between the RA male and female groups, while the RADS females showed greater fat accumulation. There was glucose intolerance in the RADS groups for both sexes when compared to the RA groups, and there was no influence of sex on either diet. There was no difference among the groups in the insulin tolerance test. Conclusion: The intake of a high sucrose diet in adolescent food-restricted animals is associated with increased weight and fat, as well as glucose intolerance. The data suggests that the metabolic programming caused by food </p>
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	<p>restriction in adolescence and the subsequent consumption of a high sucrose diet leads to higher accumulation of fat, which can lead to the progression of chronic diseases. keywords: Metabolic programming; malnutrition; metabolic syndrome.</p>
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Title	Late hepatic and neurological impairments resulting from overt diabetes in pregnancy
Authors	Thamys Marinho Melo ¹ Nayra Oliveira Sousa ^{1,3} Jefferson Oliveira De Almeida Santos ¹ Luiza De Katulica Rodrigues Oliveira ¹ Maysa Souza de Alencar ¹ Jonas Rodrigues Sanches ¹ Bruno Eduardo Lopes De Macedo ¹ Nathalia Késsia Corrêa Rodrigues ^{1,3} Rômulo Brênno Lopes Fróes ^{1,2} Jaqueline Pessoa Pontes ^{1,2} Karla Frida Torres Flister ¹ Lucas Martins França ^{1,2} Antônio Marcus de Andrade Paes ^{1,2} Bruno Araújo Serra Pinto ^{1,3}
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Session	Abordagens Pré-clínicas em DOHAD

Abstract, Ethics Committee Number*, and Keywords	Overt diabetes in pregnancy (OD) significantly impact the future development of metabolic alterations and neurological impairments. OD creates an adverse intrauterine environment due to elevated blood glucose levels (≥ 126 mg/dL) that predisposes the fetus to future diseases such as non-alcoholic fatty liver disease (NAFLD) and neurological disorders. In our study, we explored metabolic, hepatic, and neurological issues in adult rats exposed to OD. Wistar rats (n=5/group; 120 days) were divided into three groups: control (CTR), not subjected to interventions; type 2 diabetes mellitus (T2DM), receiving 40% sucrose solution for 12 weeks; and OD, comprising offspring of pregnant rats given streptozotocin (STZ; 40 mg/Kg; 13th day of pregnancy). We assessed body morphometry, glycemic and lipid profiles, insulin resistance (IR), liver histopathology, and behavior/cognition using open field, water maze, and object recognition tests. All experiments were ethically approved (CEUA/UFMA n° 23115000747202290). Mothers administered STZ developed OD, showing severe hyperglycemia (475 ± 17.6 mg/dL), weight loss, and IR. OD offspring exhibited reduced litter size and neonatal hyperglycemia (114 ± 4.5 mg/dL) compared to CTR offspring. In adulthood, OD animals displayed a diabetogenic profile marked by hyperglycemia, dyslipidemia, and insulin resistance without obesity. Liver evaluation revealed impaired function and NAFLD characterized by microvesicular steatosis with lobular inflammation and ballooning. Additionally,
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they exhibited anxiety behavior and deficits in hippocampus-dependent and independent learning and memory. Conversely, the T2DM group exhibited a more severe diabetogenic profile, visceral obesity, behavioral changes resembling OD, and a less severe NAFLD with microvesicular steatosis only. Thus, our findings underscore the critical role of adverse conditions during gestation and neonatal stages, such as OD, in shaping future metabolic and neurological outcomes.

Keywords: Overt diabetes in pregnancy; Type 2 diabetes mellitus; non-alcoholic fatty liver disease; neurological disorders



Title	High protein dietary intervention or high sucrose withdrawal equally revert metabolic and cognitive impairments induced by high sucrose intake
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Session	Abordagens Pré-clínicas em DOHaD



Abstract, Ethics Committee Number*, and Keywords	<p>High sucrose intake led to metabolic syndrome (MetS) and cognitive decline. Low-sugar diets like high-protein (HP) diets and reducing sugar intake have shown promising results in addressing these issues. This study compares the effects of HP diet and sugar withdrawal on metabolism and cognition of rats with sucrose-induced MetS. Male Wistar rats (30 days old) were divided into two groups: CTR (standard chow, n=5) and HSD (30% sucrose-rich solution, n=15), monitored for 30 weeks. At week 18, the HSD group was divided into HSD/HSD (continuing sucrose, n=5), HSD/CTR (sucrose withdrawal, n=5), and HSD/HP, (HP diet 45%, n= 5). MetS progression, cognition (Water Maze and Novel Object Recognition), hippocampus atrophy, and gene expression of BDNF (neuroplasticity), Ire1α, Perk and ATF6 (endoplasmic reticulum stress) and CHOP (apoptosis) were evaluated. All procedures were approved by CEUA-UFMA (23115017437201828). We show that HSD/HSD group had an increase in weight gain (320%; p<0.0001), central obesity (6.14%; p<0.01), hypertriglyceridemia (378%; p<0.0001), fasting and fed disglycemia (15,62% and 18,55%, respectively; p<0.01) glucose intolerance (46,7%; p<0,01), hyperinsulinemia (79%; p<0,01), and in insulin resistance (23,18%; p<0,0001) compared to the CTR. HSD/HSD also shows reduction in spatial (37%; p<0,01) and episodic memory consolidation (23,8%; p< 0,005). Along with gene overexpression of Ire1α (526%; p<0,05), Perk (900%; p< 0,0001), ATF6 (340%; p<0,05) and CHOP (310%; p<0,05), which leads to hippocampal atrophy (reduction of 27%; p<0,01). Both HSD/CTR and HSD/HP equally reversed all the metabolic disturbances, improved cognition, increased neuroplasticity (264% and 246% of BDNF expression, respectively; p<0.05), while alleviating hippocampal ER stress and neurodegeneration. We suggest that a HP diet and reducing sucrose intake equally show promise as interventions to prevent early cognitive decline and neurodegenerative diseases induced by excessive sucrose.</p> <p>Keywords: High-protein diet, Sucrose, Metabolic syndrome, ER stress, Cognitive improvement.</p>
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Title	Altered pathways of liver branching morphogenesis of 14- day-old male rat fetuses programmed by low protein diet
Authors	Lopes MG, Calsa, B, Folguieri, MS, Boer PA and Gontijo JAR.
Affiliations	Fetal Programming and Hydroelectrolyte Metabolism Laboratory, Nucleus of Medicine and Experimental Surgery, Department of Internal Medicine, FCM, Campinas, São Paulo, Brazil.
Session	Poster

Abstract, Ethics Committee Number*, and Keywords	<p>The DOHaD experimental research has shown that gestational protein restriction is related to the rise prevalence of metabolic dysfunction in adult life. The liver is the largest internal organ providing essential metabolic, exocrine, and endocrine functions, and changes in the hepatogenic process can be related to the genesis of observed metabolic impairment. Between e10–15, the liver bud originating from the endoderm undergoes accelerated growth as it is vascularized and colonized by hematopoietic cells to become the significant fetal hematopoietic organ. Thus, in the present study, we investigate gene expression and proteins of key developmental signaling pathways in the liver of male fetuses from mothers submitted or not to protein restriction. The C57BL/6 mice (8–10 weeks) were used for breeding (ethics committee 6029-1/2022). After pregnancy confirmation, female mice were randomly divided into a normal-protein (NP 17% casein) or low-protein (LP 6% casein) diet group. On the 14th gestational day (14GD) the liver from male fetuses were processed for immunofluorescence. Immunofluorescence shows a significant increase in mTOR ($p=0.0001$) expression, coupled with a notable reduction in AMPK ($p=0.0192$) in LP offspring. mTOR, a key regulator of various cellular processes, including cell growth, proliferation, motility, survival, protein synthesis, autophagy, and transcription, also plays a crucial role in sensing nutrient, oxygen, and cellular energy levels. The reciprocal relationship between mTOR and AMPK pathways, where the ATP-sensitive AMPK inhibits the mTOR pathway and downregulates ATP-consuming protein synthesis, has been well- established. Our findings underscore the importance of mTOR and AMPK pathways in cellular homeostasis and their potential implications in various physiological and pathological conditions.</p> <p>Keywords: Gestational protein restriction, Fetal Programming, Liver development, immunofluorescence.</p>
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FRONTEIRAS DAS CIÊNCIAS BIOMÉDICAS E SEUS DESAFIOS

Title	Sex-dependent endothelial response at different stages of life in offspring from mothers subjected to gestational stress
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Session	27- Preclinical approaches in DOHaD



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Abstract, Ethics Committee Number*, and Keywords	<p>Under stress, occur physiological responses that can increase the risk of cardiovascular disease. These responses can be passed between generations and are sex-specific with different impacts throughout life. The aim of this study is to evaluate the sex-dependent endothelial response at different stages of life in offspring subjected to pregestational stress. Ethics Committee(9518170621). The stress protocol for the mothers was from the 14^o to 21^o day of pregnancy. After birth, offspring were divided in 4 groups (n=8) according to stress and sex: Control 60 days; Control 90 days; Stress 60 days; Stress 90 days. The blood was collected for corticosterone analysis. Aorta was collected for vascular reactivity (concentration-response curve of acetylcholine and phenylephrine). Results are described as mean±SEM. Comparison between groups was performed using One-way ANOVA. Significant when $p < 0.05$. Regarding mothers, it was observed higher corticosterone levels, confirming the effectiveness of the stress protocol, in the S group (C: 1.51 ± 0.35; S: 3.38 ± 1.18; $p = 0.04$). For offspring, no significant differences between C and S, in both sexes, for body mass (60 days-Male $p = 0.07$; Female $p = 0.94$; 90days-Male: $p = 0.31$; Female $p = 0.69$). For vascular function, there were no differences in vasoconstrictor response to phenylephrine, in both sexes for 60 days (Male C: 7.83 ± 0.65; S: 7.80 ± 0.63; Female C: 7.46 ± 0.72; S: 8.86 ± 1.83; $p = 0.05$) and 90 days (Male C: 7.29 ± 0.24; S: 7.84 ± 0.93; $p = 0.09$; Female C: 6.99 ± 0.46; S: 7.56 ± 0.45; $p = 0.02$). Regarding vasodilator responses, group S showed lower reactivity independent of sex for 60 days (Male C: 107.4 ± 17.24; S: 86.92 ± 17.71; $p = 0.002$; Female C: 94.44 ± 6.51; S: 85.21 ± 10.55; $p = 0.07$) and 90 days (Male C: 100.7 ± 6.84; S: 92.08 ± 9.02; $p = 0.04$; Female C: 101.2 ± 9.85; S: 81.23 ± 16.03; $p = 0.004$). Gestational stress impaired endothelium-dependent vasodilation in offspring of rats submitted to stress, regardless of sex or stage of life, without damage to vasoconstriction.</p> <p>Keywords: Stress, metabolic programming, sexual dimorphism, cardiovascular diseases, vascular function.</p>
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Title	Perinatal bisphenol A exposure disrupts hormonal receptor expression in mammary gland
Authors	COELHO, Rafael Henrique Borges ¹ ; AGUIAR, Leonardo Araújo Caires de ¹ ; ASSIS, Vinícius Savazo ¹ ; SILVA, Stella Bicalho ¹ ; GRIGIO, Vitor ¹ ; BEDOLO, Carolina Marques ¹ ; VILAMAIOR, Patrícia Simone Leite ¹ ; RUIZ, Thalles Fernando Rocha ^{1,2} ; TABOGA, Sebastião Roberto ^{1,2}
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Session	Poster session

Abstract, Ethics Committee Number*, and Keywords	<p>The mammary gland (MG) is an organ susceptible to hormonal oscillations during reproductive cycles. Estrogens and progesterone are sexual hormones that regulate epithelial and stromal changes. It is important to consider that females are exposed to bisphenol A (BPA), an environmental pollutant (a polycarbonate plastic monomer) and a compound that mimics estrogenic effects, leading to endocrine disruption. Thus, this study aimed to analyse the hormonal receptors in MG after BPA perinatal disruption and pro-carcinogenic induction. To assess carcinogenic development under an endocrine-disrupting microenvironment, female gerbil (6mo) was exposed perinatally to BPA and divided into 3 experimental groups: a BPA group (only BPA exposure – 50 µg/kg/daily), an induced to carcinogenesis submitted to ethyl-N-nitrosourea (ENU) and a vehicle group treated with Oil, (CEUA/IBILCE nº 239/2022). Values of histopathological analysis (% of positive cells) was expressed as mean ± standard deviation. The expression of estrogen receptor alpha (ERα) in the MG was increased in ENU-induced females (25.60 ± 8.22) in comparison to BPA (11.05 ± 4.54). The estrogen receptor beta (ERβ) was similarly expressed in both groups. There was a decrease of expression on progesterone receptor (PR) after pro-carcinogenic induction with ENU (27.60 ± 8.07), compared to BPA (50.12 ± 3.76), which had an increase in PR expression. Prolactin receptor (PRLR) did not show significant differences in both groups. In summary, the results showed a potential to developed breast cancer Era positive is enhanced by the BPA disruption, one of the most aggressive cancers reported to the MG.</p> <p>Key-words: Histopathology, endocrine disruption, DOHAD.</p>
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Title	Increased neoplastic potential in the gerbil male prostate related with androgenic modulation after perinatal exposure to the endocrine disruptor bisphenol A
Authors	AGUIAR, Leonardo Araújo Caires de ¹ COELHO, Rafael Henrique Borges ¹ ASSIS, Vinícius Savazo ¹ SILVA, Stella Bicalho ¹ GRIGIO, Vitor ¹ VILAMAIOR, Patricia Simone Leite ¹ RUIZ, Thalles Fernando Rocha ² TABOGA, Sebastião Roberto ¹
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Session	Poster session

Abstract, Ethics Committee Number*, and Keywords	<p>Androgenic level is a remarkable issue for the complexity of prostate cancer. Moreover, in a disturbed scenario due to endocrine disruptors exposure, such as bisphenol A (BPA), it is especially relevant. Therefore, we aimed to evaluate the prostatic repercussions under androgenic modulation after adult male gerbils perinatally exposed to BPA. The animals (n=50) (Ethics Committee Protocol: CEUA/IBILCE 240/2022) were randomly divided into 5 groups: a control group and four exposed to BPA perinatally (50 µg/kg/daily). BPA-exposed males were submitted to treatments with androgen supplementation, androgen deprivation (surgical castration), or androgen blockage (enzalutamide). At 12-month age, the animals were euthanized, and the ventral prostate was collected and subjected to histopathological analyses (expressed in mean ± standard deviation). Androgen supplementation enhanced hyperplasia (35.1 ± 9.16) and prostatic epithelial neoplasia (19.17 ± 5.88) compared to the control (11.71 ± 9.74 and 4.03 ± 1.74, respectively); whereas the blockage with enzalutamide reduced hyperplastic foci (17.89 ± 2.98) compared to supplementation. Under BPA exposure, androgen receptor (AR) (39.34% ± 6.99) was decreased in the stroma, as well as estrogen receptor beta (ERβ) (55.24% ± 22.76), compared to the control (62.31 ± 7.25 and 82.61 ± 12.36, respectively). With androgenic deprivation, AR (38.05±19.28) drastically decreased in the epithelium compared to the other groups (83.45 ± 8.81, 78.08 ± 6.23, 64.58 ± 19.61 and 79.13 ± 6.621). Thus, response to differential androgenic scenarios was observed under endocrine disruption by BPA. The prostatic lesions development was altered in the prostate after BPA exposure, and testosterone supplementation aggravates this scenario. Therefore, the neoplastic potential was decreased due to AR expression reduction after testosterone deprivation, unlike the blockage group, in which receptors were not modulated, but pre-neoplastic lesions were reduced.</p> <p>Keywords: Prostate Cancer; Histopathology; EDCs</p>
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Title	DOHAD and endocrine therapies: repercussions on the mammary gland after perinatal endocrine disruption in a rodent model
Authors	Thalles Fernando Rocha Ruiz ¹ Stella Bicalho Silva ² Vitor Grigio ² Rafael Henrique Borges Coelho ² Paula Rahal ² Marília de Freitas Calmon ² Patricia Simone Leite Vilamaior ² Ellen Cristina Rivas Leonel ³ Sebastião Roberto Taboga ^{1,2}
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Session	Abordagens Pré-clínicas em DOHAD

Abstract, Ethics Committee Number*, and Keywords	Perinatally exposure to bisphenol A (BPA) leads to consequences for female reproductive organs still revealed. Also, women are supplemented with hormones for therapeutic purposes. Thus, we aimed to evaluate the mammary gland (MG) under hormonal treatment after BPA perinatal disruption and pro-carcinogenic induction. Female gerbils (N=50) were exposed to BPA (gestation/lactation) and induced to carcinogenesis with N-ethyl-N-nitrosurea (ENU) with subsequently treated for 5 weeks with different hormones: 17- β -estradiol (E2); 17-alpha-Ethinylestradiol (EE2); Testosterone cypionate (TE); or Dehydroepiandrosterone sulfate (DHEA-S). Pro-neoplastic control (ENU) and only BPA groups were also performed. MG samples were collected (CEUA/IBILCE 239/2022) and destined for histopathological analysis and Western Blotting (mean \pm SD). ENU-treated samples enhanced microinvasive carcinoma (Mc) (21.1% \pm 9.5) and ductal carcinoma in situ (DCIS) (52.3% \pm 8.9). Also, estrogen receptor α (ER α) positive neoplastic lesions in MG were induced (25.6% \pm 8.3) compared to the expression of other receptors (progesterone – PR; HER2/Neu; androgen – AR). E2 also enhanced pre-neoplastic lesions (Mc: 32.4% \pm 13.2; DCIS: 21.5% \pm 6.9), but only EE2 presented this feature (Mc: 39.7% \pm 11.9; DCIS: 27.1% \pm 7.3) related with high expression of ER α (17.6% \pm 7.7) and PR (81.5% \pm 12.2). Females treated with TE showed enhanced hyperplastic foci (53.7% \pm 11.2) and high expression of AR (73.2% \pm 8.7) and ER α (24.1% \pm 9.6). DHEA-S presented only
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an increase of HER2/Neu in comparison to other groups ($69.1\% \pm 11.7$). Related to epithelial lesions, DHEA-S promoted a slight increase in hyperplasias ($47.5\% \pm 9.4$) compared to other groups, but not in comparison with TE. Thus, our results demonstrated that ER-positive epithelial lesions were developed after ENU induction, and estrogens (E2 and EE2) aggravated this scenario, whereas androgenic compounds (TE and DHEA-S) influenced the receptors' expression with hyperplastic increase.

Keywords: Estrogens; androgens; cancer; receptors.



Title	Estrogen and androgen receptors in female prostate under endocrine disruption and carcinogenic conditions
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Affiliations	1 – Universidade Estadual Paulista – Unesp, São José do Rio Preto 2 – Instituto de Biologia – Unicamp, Campinas
Session	28 – Abordagens Pré-clínicas em DOHAD

Abstract, Ethics Committee Number*, and Keywords	<p>Estrogen and androgen are endogenous hormones that regulate estrous and reproductive cycles in females. The project evaluated the hormonal receptor profile in the female prostate of Mongolian gerbils (<i>Meriones unguiculatus</i>) under endocrine disruption and induced to pro-carcinogenic process. Females were exposed during the perinatal period to 50 µg/kg daily and induced by N-Ethyl nitrosourea (ENU) to carcinogenesis, as previously stated for several glands. A control group was performed, only with BPA exposure, and other 2 groups were performed with ENU exposition in adulthood: exposure during 2 months to 17-β-estradiol (E2) and 17-α-ethinylestradiol (EE2), used as hormonal supplementation. Analyzed and statistical data analysis was provided as mean ± standard deviation. The estrogen receptor alpha (ERα) was highly expressed in the epithelium in the E2-induced group (39.01% ± 3.87) compared to the BPA and BPA + ENU groups (24.58% ± 6.11 and 24.65% ± 8.12, respectively). In stromal compartment, the E2 group (32.67% ± 10.31) was reduced compared to the BPA + ENU group (56.48% ± 15.38). Exposure by the estrogen receptor beta (ERβ) reported a reduction in epithelial cells in the EE2 group (32.31% ± 13.0) compared to the BPA and E2 groups (56.48% ± 10.79 and 54.04% ± 6.40, respectively). There were no relevant statistical differences for ERβ expression in the stroma. The androgen receptor (AR) presented a decrease in the epithelium of the BPA plus ENU-induced groups (28.34% ± 6.26, 33.68% ± 6.10, 25.88% ± 4.70) concerning the group exposed to only BPA exposure. In the stroma, all groups (50.28% ± 15.67, 46.80% ± 7.65, 54.21% ± 4.98) were statistically different from the group exposed only to ENU (33.02% ± 10.09). Thus, E2 increased the expression of both ERα and ERβ, which was also increased by BPA, and reduced the expression of EE2 in the epithelium. However, in the analysis of AR, there was a drastic reduction in its expression, which may show androgen-independent lesions.</p> <p>Keywords: Epithelium; Stroma; Hormones; Glands.</p>
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Title	Can the late effects of exposure to glycotoxins early in life be attenuated by N-acetylcysteine supplementation?
Authors	Marcos Divino Ferreira-Junior Keilah Valéria Naves Cavalcante Ariel Penha Carvalho da Mota Rodrigo Mello Gomes
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Session	27 - Abordagens Pré-clínicas em DOHaD

Abstract, Ethics Committee Number*, and Keywords	<p>Previous data relates early exposure to glycotoxins and the predisposition to increased oxidative stress in several organs in adulthood. Glyoxalase system detoxifies methylglyoxal, a glycotxin precursor, being coupled to the glutathione cycle. Despite the effects of glycotoxins exposure during lactation were mainly observed at adulthood, here we sought to evaluate this effects during early life, when intervention is still possible. The present study aimed to evaluate the effects of the supplementation with N-Acetylcysteine (NAC), in a model of early life methylglyoxal exposure. Experimental protocols were approved by the institutional Ethics Committee for the Use of Animals (007/21). Pregnant Wistar rats were separated into two groups: CO and MG – respectively orally injected with PBS and methylglyoxal (60 mg/kg). Injections were performed from postnatal day (PND) 3 to PND14. Male and female pups (8/litter) were weaned at PND21, and at PND30 MG offspring were separated into two groups and daily injected i.p. until PND45 with PBS (Veh) or NAC 200 mg/kg (NAC). At PND45 the animals were underwent to glucose tolerance test (GTT), and then they were euthanised for sample collection. MG exposure led to lower body weight at weaning, in both male and female. In addition, only male offspring present higher fasting glycemia compared to CO counterparts. At PND45, despite the sex-specific alterations in the fasting glycemia at weaning, during the GTT, no relevant differences were noted between the groups. At PND45 the body weight of both MG and NAC groups were similar to CO group. However, there are sex-specific differences in the white and brown adipose tissues. Noteworthy, there is a decrease in BAT in male offspring in the MG exposed offsprings. Taken together, our results indicates that MG exposure can affect adipose tissue development. However, more experiments are ongoing to show the molecular effects of NAC supplementation on BAT and WAT metabolism.</p>
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Title	Maternal protein restriction combined with postnatal sugar consumption alters liver proteomic profile and metabolic pathways in adult male offspring rats
Authors	Isabelle Tenori Ribeiro, Luis Antonio Justulin Jr
Affiliations	UNESP- Sao Paulo State University, Department of Structural and Functional Biology, Institute of Biosciences, Botucatu, SP, Brazil
Session	27 - Abordagens Pré-clínicas em DOHAD

Abstract, Ethics Committee Number*, and Keywords	<p>Aims: This study aimed to investigate the effects of maternal protein restriction (MPR) and early postnatal refined sugar (SUG) consumption on liver morphology, serum biochemistry, and global hepatic protein expression in adult male offspring rats. Main methods: Male offspring of mothers fed a normal protein diet (NPD) or a low protein diet (LPD) during gestation and lactation were divided into four experimental groups: Control (CTR), Control+Sugar (CTR+SUG), Gestational and Lactational LPD (GLLP), and Gestational and Lactational LPD+Sugar (GLLP+SUG). The animals started consuming sugar water on postnatal day 21 (PND 21), and on postnatal day 90 (PND 90), the male offspring were euthanized for various analyses. Key findings: Sugar consumption reduced food intake and increased water consumption in CTR+SUG and GLLP+SUG groups. The GLLP and GLLP+SUG groups exhibited reduced body weight, and total and retroperitoneal fat compared to the CTR and CTR+SUG groups. The CTR+SUG group displayed elevated blood glucose levels while the CTR+SUG and GLLP+SUG groups displayed hepatocyte vacuolization associated with increased hepatic glycogen content compared to the CTR group. Hepatic catalase (CAT) activity increased in the GLLP group compared to the CTR group. Proteomic analysis identified 223 differentially expressed proteins (DEPs) among experimental groups. While in the GLLP group, the DEPs enriched molecular pathways related to cellular stress, glycogen metabolic pathways were enriched in the CTR+SUG. The association of sugar consumption (second hit) amplifies the effects of MPR, deregulating molecular mechanisms related to metabolism and the antioxidant system. Significance: These results reinforce the importance of maternal diet for offspring health and highlight the post-natal nutritional environment as a risk factor for hepatic disorders throughout the lifespan.</p> <p>Keywords: maternal protein restriction, sugar consumption, liver, metabolism, proteomic</p>
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Title	Desnutrição na adolescência aumenta a suscetibilidade de quebra da homeostase glicêmica associada à ingestão hipercalórica
Authors	Marcos Thiara Chaves dos Santos ^{1,2} , Beatriz Portela Castro ^{1,2} , Antonio José Rocha Ribeiro ^{1,2} , Carlos Eduardo Albanez ^{1,2} , Gabriel de Oliveira Bianchi ^{1,2} , Mateus Silva Campos ^{1,2} , Ana Caroline Schoenberger Kipper ^{1,2} , Aline Milena Dantas Rodrigues ^{1,2} , Isabelle Zanata Fabiane ² , Joskame Saint Paul ^{1,2} , Júlio Cezar de Oliveira ^{1,2}
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Session	27

Abstract, Ethics Committee Number*, and Keywords	<p>Introdução: A adolescência, por ser um estágio de maturação neuroendócrina, é tida como uma janela de programação para disfunções metabólicas, onde insultos como a desnutrição atuam como fatores de risco para o surgimento dessas disfunções. Objetivos: Avaliar o efeito sexo dependente de uma dieta hipercalórica sob o metabolismo de ratos previamente desnutridos na adolescência. Metodologia: Dos 30 aos 60 dias de vida, ratos Wistar machos e fêmeas foram submetidos a restrição alimentar de 50% (grupos RM e RF), enquanto os controles (CM e CF) ingeriram ração <i>ad libitum</i>. Dos 60 aos 90 dias parte desses grupos receberam ração hipercalórica e solução de sacarose à 10% (RMOB, RFOB, CMOB e CFOB) e os 90 aos 120 dias dieta padrão. O peso corporal foi aferido a cada dois dias, e aos 120 dias foram eutanasiados para coleta de sangue e tecidos (Aprovação do comitê de ética nº 23108.021234-2024-73).</p> <p>Resultados: Aos 60 dias, os ratos RM foram 32,81% menor que os CM, enquanto os RF foram 22,15% menor que os CF (P<0,001). Aos 90 dias, em relação aos ratos CM, o grupo RM foi 15,54% menor e o CMOB 7,88% maior (P<0,01). Quando comparado ao RM, o grupo RMOB foi 7,57% maior (P<0,01). Comparando-se às fêmeas CF, o grupo RF foi 8,34% menor (P<0,05), enquanto o CFOB embora não diferente foi 5,45% maior. Embora o grupo RFOB tenha sido 2,46% maior, não diferiu do RF. Aos 120 dias, tanto o peso quanto a adiposidade não diferiram entre os machos; contudo, as fêmeas RF foram mais leves do que as CF (-7,47%, P<0,05), embora as RFOB tenham acumulado 48,53% mais gorduras do que as RF (P<0,01). Em relação aos controles, a glicemia de jejum, aos 60 dias, foi reduzida em 11,22% nos ratos RM (P<0,05), enquanto nas fêmeas não diferiu. Aos 120 dias, a glicemia aumentou em 15,94% nos ratos RMOB <i>versus</i> RM (P<0,05) e em 15,60% nos RFOB <i>versus</i> RF (P<0,01).</p> <p>Conclusão: A dieta hipercalórica em ratos previamente desnutridos na adolescência induziu maior fragilidade na homeostase glicêmica em ambos os sexos.</p> <p>Palavras-chave: Adolescência, Desnutrição, Obesidade</p>
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Title	Malnutrition during adolescence increases the susceptibility to disruptions in glucose homeostasis associated with hypercaloric intake
Authors	
Affiliations	
Session	

Abstract, Ethics Committee Number*, and Keywords	<p>Introduction: Adolescence, as a stage of neuroendocrine maturation, is considered a critical window for programming metabolic dysfunctions, where insults like malnutrition act as risk factors for the development of these dysfunctions. Objectives: To evaluate the sex-dependent effect of a hypercaloric diet on the metabolism of rats previously malnourished during adolescence. Methodology: From 30 to 60 days of age, male and female Wistar rats were subjected to a 50% food restriction (groups RM and RF), while the controls (CM and CF) consumed food ad libitum. From 60 to 90 days, some of these groups received a hypercaloric diet and a 10% sucrose solution (RMOB, RFOB, CMOB, and CFOB), followed by a standard diet from 90 to 120 days. Body weight was measured every two days, and at 120 days, the rats were euthanized for blood and tissue collection (Ethics committee approval nº 23108.021234- 2024-73). Results: At 60 days, RM rats were 32.81% smaller than CM rats, while RF rats were 22.15% smaller than CF rats ($P < 0.001$). At 90 days, compared to CM rats, the RM group was 15.54% smaller and the CMOB group was 7.88% larger ($P < 0.01$). Compared to the RM group, the RMOB group was 7.57% larger ($P < 0.01$). Compared to CF females, the RF group was 8.34% smaller ($P < 0.05$), while the CFOB group, although not significantly different, was 5.45% larger. Although the RFOB group was 2.46% larger, it did not significantly differ from the RF group. At 120 days, both weight and adiposity did not differ among males; however, RF females were lighter than CF females (-7.47%, $P < 0.05$), although RFOB females accumulated 48.53% more fat than RF females ($P < 0.01$). Compared to the controls, fasting glucose at 60 days was reduced by 11.22% in RM rats ($P < 0.05$), while it did not differ in females. At 120 days, glucose increased by 15.94% in RMOB rats compared to RM rats ($P < 0.05$) and by 15.60% in RFOB rats compared to RF rats ($P < 0.01$). Conclusion: A hypercaloric diet in rats previously malnourished during adolescence induced greater fragility in glucose homeostasis in both sexes.</p>
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Title	Maternal diet rich in sucrose or fat alters murinometric parameters and energy expenditure in young offspring
Authors	¹ Luciana Caroline Paulino do Nascimento ¹ Mayara da Nóbrega Baqueiro ¹ Maiara de Jesus da Silva ² Mina Desai ² Michael Ross ¹ Adriana Souza Torsoni
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Session	Abordagens Pré-clínicas em DOHAD

Abstract, Ethics Committee Number*, and Keywords	<p>Nutritionally unbalanced maternal diets may increase the risk of cardiometabolic diseases in offspring. We aimed to evaluate the effects of maternal diet rich in sucrose or fat on murinometric parameters and energy expenditure in offspring. This study was approved by the Ethics Committee on the Use of Animals at UNICAMP, nº5639-1/2020. Female C57/BL6 mice from the 5th week of life began adapting to the experimental diets for 8 weeks, being distributed into three groups according to the diet received: Control–CT; High sucrose-HS; and High fat-HF, remaining on their respective diets during gestation and lactation. At 21 days of life, the offspring were weaned and started receiving CT diet on the days of calorimetry until euthanasia on d28. HS and HF dams have greater weight compared to CT (CT=22.30 vs HS=24.43 vs HF=27.01); however, regarding the adiposity index, it was higher in relation to HS group (HS=1.226 vs HF=1.888). The ovarian adipose tissue content (CT=0.225 vs HF=0.477 vs HS=0.187) and retroperitoneal (CT=0.047 vs HF=0.117 vs HS=0.041) HF dams increased compared to CT and HS. Additionally, fasting blood glucose values remained higher in relation to HS group in the pre-gestational period (HS=92 vs HF=111) and after weaning (HS=91 vs HF=111). In offspring, HF females had greater weight (CT=17.6 vs HF=24.3), fat mass (CT=0.84 vs HF=1.38) and lean (CT=6.226 vs HF=9.404) compared to CT. HF males similarly presented greater weight (CT=18.34 vs HF=24.50), fat mass (CT=1.07 vs HF=1.47) and lean (CT=7.97 vs HF=11.02). Indirect calorimetry demonstrated a reduction in CO₂ production in HF females (CT=7367 vs HF=4632), in respiratory rate (CT=1.098 vs HF=0.9783) and heat production (CT=0.2886 vs HF=0.2588). In HS and HF males, there was a reduction in respiratory rate (CT=1.033 vs HF=0.9692 vs HS=1.017). Both the HS and HF diets favor greater weight gain in mothers, but the effects on body composition and energy expenditure are more pronounced in the offspring of mothers fed the HF diet.</p> <p>Keywords: Western diet; Metabolic programming; Weight gain.</p>
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Title	"We're pregnant... now what?"
Authors	¹ Luciana Simone E. F. Vargas; Cristiane Matte
Affiliations	Federal University of Rio Grande do Sul; Associação DOHAD Brasil; Latin-American DOHAD Regional Society
Session	29 - DOHAD, doenças maternas e intervenções perinatais

Abstract, Ethics Committee Number*, and Keywords	<p>The intrauterine environment profoundly shapes individual health by responding to external influences, particularly impacting metabolic programming during embryonic development, largely through epigenetic and hormonal alterations. This field of study, known as Developmental Origins of Health and Disease (DOHAD), focuses on averting chronic non-communicable diseases like cardiovascular disease, diabetes mellitus, and neurodegenerative disorders. The outreach initiative "We're Pregnant... Now What?" aims to bridge the gap between scientific research on DOHAD and the general population, particularly targeting pregnant women or those considering pregnancy. It seeks to democratize crucial health information typically confined to academic circles. The project entails producing a podcast based on meticulously researched scripts derived from scientific literature, health guidelines, and studies sourced from reputable databases such as PubMed. Additionally, it involves leveraging an Instagram platform to disseminate podcast episodes and informational cards. To date, our Instagram account has amassed a following of over a thousand individuals, with weekly informational cards reaching our audience. Currently, we are in the process of editing podcast episodes, slated for release every fifteen days, starting in May. Our aspiration is that this initiative will broaden understanding of DOHAD principles, empowering expectant mothers and those planning pregnancies to cultivate healthy gestational environments and safeguard against future diseases through simple interventions during this critical period.</p> <p>Keywords: pregnancy; DOHAD; first 1000 days; health; science divulgation.</p>
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Title	Effects of Brazil nut oil (<i>Bertholletia excelsa</i>) supplementation on body composition, biochemical parameters and bone health in female rats programmed by early weaning
Authors	Mariana Sarto Figueiredo ¹ Luisa Maria Tavares da Silva de Campos ² Gleiciane Teixeira Souza ² Beatriz Alem Nascimento de Araujo ² Bianca Freitas de Barros ² Cynthia Gomes Teles ² Bruna Almeida Nascimento ³ Karla de Araujo Coutinho ³ Alana Louzada Millions Monteiro ⁴
Affiliations	¹ Departament of Nutrition and Dietetic, Faculty of Nutrition, Universidade Federal Fluminense, Niteroi, Brazil. ² Pos-graduated in Nutrition Science, Faculty of Nutrition, Universidade Federal Fluminense, Niteroi, Brazil. ³ Graduated in Nutrition, Faculty of Nutrition, Universidade Federal Fluminense, Niteroi, Brazil. ⁴ Pos-graduated in Medical Science, Faculty of Nutrition, Universidade Federal Fluminense, Niteroi, Brazil.
Session	29

Abstract, Ethics Committee Number*, and Keywords	Abstract: Intake of vegetable oils, mainly sources of polyunsaturated fatty acids (PUFAs), may play a key role in bone metabolism and contribute to the prevention of obesity. We aimed to evaluate the effects of supplementation with Brazil nut oil on body composition, biochemical and bone parameters in female rats programmed by early weaning. Sixty female rats were used (30 offspring from control dams (C); and 30 offspring from early weaning dams (EW). In PN150, offspring from control group were subdivided into: 1) Control/ gavage with saline (CS, n=10), 2) Control gavage with soybean oil (COS, n=10); 3) Control gavage with Brazil nut oil (CCAS, n=10); and offspring from EW were subdivided into: 1) EW with saline solution gavage (EWS, n=10), 2) EW with soybean oil gavage (EWOS, n=10); 3) Early weaning gavage Brazil nut oil (EWCAS, n=10). Animals received gavage for 30 days (dose 0.5ml/100g body mass). In the present study, we demonstrated that supplementation with Brazil nut oil in control offspring reduced food consumption (-20%, p<0.05), fasting glycemia (-16%, p<0.05), total cholesterol (-21%, p<0,05) and LDL-cholesterol (-19%, p<0.05), and increased retroperitoneal adipose tissue compartments (TAR, +59%, p<0.05), peri -ovarian (TAO, +58%, p<0.05), and reduced brown adipose tissue (-20%, p<0.05) and bone mineral density (-5%, p<0.05) in the
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femur. In offspring programmed for early weaning supplemented with Brazil nut oil, we observed a reduction in food consumption (-26%, $p < 0,05$), serum TGO and TGP (-26% and -26%, $p < 0.05$, respectively). EWCAS group presented lower femur bone area (-22%, $p < 0.05$), width (-5%, $p < 0.05$) and elastic modulus (-24%, $p < 0.05$), and higher fasting blood glucose (+24%, $p < 0.05$) and femur length (+2%, $p < 0,05$). Thus, Brazil nut oil can act as a good non-pharmacological nutritional intervention in control offspring. However, some health benefits disappeared in adult offspring programmed by early weaning.

Committee number: 9204110520

Keywords: Brazil nut oil, Body Composition, Biochemical parameters, Bone parameters, Early weaning



Title	Maternal diabetes and glyceimic repercussions in successive generations
Authors	Maria Eduarda Picoles Gomes ¹ Verônyca Gonçalves Paula ¹ Yuri Karen Sinzato ¹ Rafaeliane Queiroz Moraes-Souza ¹ Vinícius Soares Barco ¹ Maysa Rocha Souza ¹ Franciane Quintanilha Gallego ¹ Gustavo Tadeu Volpato ² Débora Cristina Damasceno ¹
Affiliations	¹ Postgraduate Course on Gynecology and Obstetrics, Laboratory of Experimental Research on Gynecology and Obstetrics – UNIPEX, Botucatu Medical School, UNESP, Brazil. ² Laboratory of System Physiology and Reproductive Toxicology, Institute of Biological and Health Sciences, UFMT, Brazil.
Session	Poster

Abstract and Keywords	<p>Introduction: The exposure of embryos and fetuses to intrauterine hyperglycemia causes short and long-term consequences for the health of the offspring. Considering that maternal diabetes impairs pancreatic function and, consequently, glucose metabolism in offspring, whether in humans or in laboratory animals, the present study aims to evaluate glyceimic levels in adult offspring of diabetic mothers.</p> <p>Methods: Blood samples from diabetic, and daughters and granddaughters of non-diabetic (control) and diabetic rats were obtained in adulthood (> 90 days of age) during the Oral Glucose Tolerance Test (OGTT). Next, the Area Under the Curve (AUC) was calculated. All data were obtained from our research laboratory database. Ethics Committee for the Use of Animals at our Institution approved all protocols used in the studies. The data analyzed came from groups of Spague-Dawley adult female rats, which were constituted as follows: 1) Control (C), diabetic (D), female offspring of diabetics (OD) and granddaughter offspring of diabetic rats (GO). P < 0.05 was considered as the significant limit.</p> <p>Results: At the beginning of OGTT (fasting = time 0), groups D, OD and GD showed higher glyceimic levels compared to group C. After administration of glucose overload in the animals, within 30 min of OGTT, the group OD (176 ± 26) and GD (197 ± 17 mg/dL) and, after 60 min, OD (144 ± 14) and GD (149 ± 18) showed higher glyceimic, compatible with group D. At the end of the OGTT (120 min), all groups had glyceimic return with normoglycemia. Regarding AUC, rats D (12,748 ± 3,254), OD (16,716 ± 1,448) and GD (14,499 ± 2,611 mg/dL/min) had a greater amount of circulating glucose in the two hours of the test compared to group C (8,828 ± 2,072). Furthermore, the OD group presented a higher AUC than the D group.</p> <p>Conclusion: In adulthood, the animals presented glyceimic changes due to the presence of intrauterine hyperglycemia since the first generation, confirming the transgenerational effect of diabetes.</p> <p>Keywords: animals, diabetes, generation, fetal development, pregnancy</p>
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Title	Diabetes during pregnancy damages the pancreatic islet development of offspring
Authors	Vinícius Soares Barco ¹ , Maysa Rocha de Souza ¹ , Isabela Lovizutto Lessi ¹ , Verônyca Gonçalves Paula ¹ , Yuri Karen Sinzato ¹ , Franciane Quintanilha Gallego ¹ , Gustavo Tadeu Volpato ² , Débora Cristina Damasceno ¹
Affiliations	¹ Course of Postgraduate on Tocogynecology, Unesp_Univ Estadual Paulista, Botucatu, São Paulo State, Brazil. ² Federal University of Mato Grosso (UFMT), Barra do Garças, Mato Grosso State, Brazil.
Session	Sessão de Pôster 1 (03/07 – 17h00 às 19h00)

Abstract, Ethics Committee Number*, and Keywords	<p>Maternal diabetes can lead to structural and functional changes in the fetal pancreatic islets, which increases the risk of developing metabolic disorders in adulthood. We aimed to evaluate the glycemia at birth and the endocrine pancreas morphology of the rat offspring on days 18 (GD18) and 21 (GD21) of pregnancy, postnatal day 5 (PND 5), and 15 (PND 15) from dams with hyperglycemia. Diabetes was chemically induced in female rats during the neonatal period to reproduce the glycemia of human Type 2 <i>Diabetes mellitus</i>. Adult nondiabetic (control-C) and diabetic (D) rats were mated and killed on days 18 and 21 of pregnancy. The offspring were evaluated on GD18 and 21, and PND 5 and 15 for pancreatic immunohistochemical analysis for insulin, Ki-67 (cell proliferation), and cleaved caspase-3 (cell apoptosis). $P < 0.05$ was considered a significant statistical limit. The local ethical committee approved this study (Permit number: 11/916). On GD18, the endocrine pancreas of fetuses of D dams presented disorganized cell distribution, a higher ratio of insulin-stained cells (0.44 ± 0.13 vs 0.33 ± 0.09), and a decreased ratio of Ki-67-positive cells (0.14 ± 0.18 vs 0.17 ± 0.16) than the control group. On GD21, the newborns of D dams presented hyperglycemia, increased pancreatic cell proliferation (0.47 ± 0.21 vs 0.32 ± 0.10), and a higher ratio of cleaved caspase-3-positive cells (0.30 ± 0.12 vs 0.26 ± 0.12) than the control. The morphological analysis of the pancreatic islets showed a reduced area in the pups of D dams on PND5 (10539.99 ± 5737.40 vs 13853.05 ± 4725.80) and PND15 (10317.92 ± 5315.30 vs 13103.45 ± 6586.40) than the control. In conclusion, maternal hyperglycemia causes an impaired fetal pancreatic islet structure, which results in fetal hyperglycemia at birth, contributing to functional pancreatic changes in the neonatal period.</p> <p>Financial Support: FAPESP (11/18519-7, 11/23642-2 and 2022/15499-0).</p> <p>Keywords: Hyperglycemia, perinatal period, endocrine pancreas, murine, fetal programming.</p>
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Title	Effects of <i>Salvia hispanica</i> L. oil supplementation on body composition, biochemical parameters and redox balance in rats programmed by early weaning
Authors	Julia Teixeira de Oliveira Meirelles ¹ Gleiciane Teixeira Souza ² Gabriel de Alcantara Noblat ³ Luisa Maria Tavares da Silva de Campos ² Bianca Freitas de Barros ² Beatriz Alem Nascimento de Araujo ² Carolina de Oliveira Ramos Petra de Almeida ⁴ Alana Louzada Millions Monteiro ³ Anderson Junger Teodoro ⁵ Mariana Sarto Figueiredo ⁵
Affiliations	¹ Graduated in Nutrition, Faculty of Nutrition, Universidade Federal Fluminense, Niteroi, Brazil. ² Pos-graduated in Nutrition Science, Faculty of Nutrition, Universidade Federal Fluminense, Niteroi, Brazil. ³ Pos-graduated in Medical Science, Faculty of Nutrition, Universidade Federal Fluminense, Niteroi, Brazil. ⁴ Pos-graduated in Food and Nutrition, Faculty of Nutrition, Universidade Federal do Estado do Rio de Janeiro, Rio de Janeiro, Brazil. ⁵ Departament of Nutrition and Dietetic, Faculty of Nutrition, Universidade Federal Fluminense, Niteroi, Brazil.
Session	29 – DOHaD, doenças maternas e intervenções perinatais

Abstract, Ethics Committee Number*, and Keywords	Abstract: Early weaning can lead to endocrine-metabolic disorders, favoring the development of diseases at adulthood. Diets containing vegetable oils, such as <i>Salvia hispanica</i> L. oil, contain significant amounts of bioactive compounds and essential fatty acids, which can contribute to disease prevention due to their antioxidant potential. Sixty male rats were used, 30 offspring from control dams (C); and 30 offspring from early weaning dams (EW). In PN150, offspring from control group were subdivided into: 1) Control/ gavage with saline (CS,n=10), 2) Control gavage soybean oil (COS,n=10); 3) Control gavage chia oil (CCO,n=10); and offspring from EW were subdivided into: 1) EW gavage saline (EWS,n=10), 2) EW gavage soybean oil (EWOS,n=10); 3) EW gavage chia oil (EWCO,n=10), during 30 days (dose 0.5ml/100g body mass). At PN180, were analyzed body composition, biochemical parameters and serum redox balance. At PN180, CCO and EWCO reduced food intake (-33% and -26%, p<0.05, respectively). No changes were observed in body bone parameters among the control and early weaning offspring. EWCO presented higher body fat mass (+24%, p<0.05) and lower lean body mass (-7%, p<0.05). CCO offspring presented lower retro-peritoneal adipose tissue (-31%, p<0.05), visceral fat mass (-27%, p<0.05), total cholesterol (-20%, p<0.05), LDL (-27%, p<0.05), alkaline phosphatase (-28%, p<0.05). EWCO presented higher retroperitoneal, peri-mesenteric adipose tissues and visceral fat mass (+38%, +28% and +30%, p<0.05, respectively) and lower alkaline phosphatase (-24%, p<0.05). In serum, CCO presented higher tiol (1.6x fold increase, p<0.05), DPPH (+9%, p<0,05), FRAC (+7%, p<0.05) and ORAC (+19%, p<0.05), followed by lower
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FOX (-7%, $p < 0.05$). EWCO showed higher catalase and DPPH in serum (+44% and +46%, $p < 0.05$, respectively) and lower FOX and ORAC (-20% and 10%, $p < 0.05$). Thus, chia oil supplementation for 30 days can provide a nutritional strategy to prevent or mitigate adverse events induced by EW.

Committee number: 9204110520.

Keywords: Salvia hispanica, Body composition, Biochemical parameters, Redox Balance.



Title	Transgenerational effects of maternal diabetes
Authors	Franciane Quintanilha Gallego Vinícius Soares Barco Maysa Rocha de Souza Verônyca Gonçalves Paula Débora Cristina Damasceno
Affiliations	Laboratory of Experimental Research on Gynecology and Obstetrics, Course of Postgraduate on Tocogynecology, Botucatu Medical School, Sao Paulo State_Unesp, Botucatu, São Paulo State, Brazil.
Session	29 - Abordagens Translacionais e Clínicas em DOHaD

Abstract, Ethics Committee Number*, and Keywords	<p>The mechanisms of this transgenerational effect have been studied, however, there are few studies evaluating the transgenerational effect in diabetic F2 generation. To evaluate the transgenerational effects of the hyperglycemic intrauterine environment on lipid and glycemic profiles, pancreatic beta (β)-cells, and reproductive performance in granddaughters (F2 generation) at adulthood, and check the blood glucose levels and body weights of great-granddaughters (F3 generation) at birth. Female Sprague-Dawley rats were used, and distributed to nondiabetic rat granddaughters (from the control generation: F2_CG) and granddaughters from the diabetic generation (F2_DG). The F2_CG and F2_DG rats were mated, and at the end of pregnancy, blood and pancreas samples were obtained for biochemical analyses and pancreatic morphology, respectively. Their pups (F3) were assessed for glycemic analysis and body weight classification (small - SGA, adequate- AGA, and large for gestational age- LGA). A minimum confidence limit of 95% ($p < 0.05$) was considered significant. The local institution approved all the methods adopted in this study (Protocol CEUA Number: 21/1375). F2_DG already presented hyperglycemia (confirmed by abnormal oral glucose tolerance test), hypertriglyceridemia (blood measurement by spectrophotometer), insulin resistance (by higher HOMA-IR index), and greater body weight before pregnancy. At the end of pregnancy, the F2_DG showed increased rates of embryonic losses before and after implantation. F3_DG newborns presented hyperglycemia, and a higher percentage of newborns were considered as LGA. The outcomes of diabetes over the generations were as harmful as or more so than the first generation with diabetes. This experimental model confirms that diabetes is a silent disease that causes alarming impairments in a transgenerationally increasing manner. This confirms the need to consume a strict glucose intake to obtain appropriate glycemic control.</p> <p>Financial Support: CAPES (001), FAPESP</p> <p>Keywords: Transgenerational diabetes, pregnancy, macrosomia, fetal programming, rats</p>
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Title	Evaluation of reproductive parameters of childhood of female rats exposed to chloroquine in uterus
Authors	Nicolas Oliveira Viana ¹ Karina Nicole Sobota ¹ Vinicius Antônio Hiroaki Satu ² Glaura Scantamburlo Alves Fernandes ³ Daniela Cristina Ceccatto Gerardin ¹
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Session	Translational and clinical approaches in DOHaD

Abstract, Ethics Committee Number*, and Keywords	<p>According to the Developmental Origins of Health and Disease, maternal exposure to drugs can affect the functional development of organs in childhood and adult life. Chloroquine (CQ) is a 4-aminoquinoline drug used in the treatment of some immune diseases, such as rheumatoid arthritis and systemic lupus erythematosus, due to its anti-inflammatory action; and it is used as an antimalarial, inhibiting the parasite's ability to metabolize the heme group in red blood cells. Understanding this, the effects of CQ during pregnancy and its offspring were analyzed even if CQ crosses the placental barrier and is considered safe for pregnant women. It is necessary to analyze the effects of the drug in childhood, to understand the effects of the drug on pre and postnatal development and avoid consequences such as birth defects, growth problems, and delays in organism development. Considering this, this project was developed with the main to evaluate reproductive parameters of the offspring of female rats in childhood after maternal gestational exposure to CQ. For this, pregnant adult Wistar rats received different doses of CQ by gavage (24, 48 or 72 mg/kg/day) or distilled water as a control (CTR), from gestational day (GD) 15 to 21. From postnatal day (PND) 0 to 21, the offspring's weight and anogenital distance (AGD) were analyzed and, on PND21, the animals were euthanized for weighing of uterus and ovary organs. Results were considered statistically significant if $p \leq 0.05$ and compared by Analysis of ANOVA or Bonferroni (CEUA/Uel 063.2020). Thus, a significant difference was observed in the AGD relative of the offspring (CTR: 2.773 ± 0.09; CQ72: 3.327 ± 0.15), both in</p>
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the PNG21 (Bonferroni, $p=0.0013$). Other parameters were similar between the groups. These results indicate that exposure to CQ during development can alter the reproductive of female offspring. Also, further studies are needed to show if CQ can change other parameters.

Keywords: Chloroquine, gestational exposure, anogenital distance, reproductive parameters



Title	Maternal repercussions of <i>Strongyloides venezuelensis</i> infection during rats pregnancy
Authors	Thais Campos Ribeiro Beatriz Digigov Santana Rondon Ailys Karoline Sergio Gisele Rabelo Alves Camila Iohana Negrão Paulynne Silva Fagundes Gustavo Fernando Milhomens Paes Barreto Luana Carolina Hochberger Madileine Francely Américo Rafaianne Queiroz Moraes-Souza Gustavo Tadeu Volpato
Affiliations	Laboratory of System Physiology and Reproductive Toxicology, Institute of Biological and Health Sciences, UFMT, Brazil
Session	Poster

Abstract, Ethics Committee Number*, and Keywords	<p>Strongyloidiasis is a helminthiasis with a wide global distribution, whose etiological agent is <i>Strongyloides stercoralis</i> in humans. However, the effects of this infection during pregnancy have not yet been well elucidated. Thus, this study aimed to evaluate the maternal repercussions of <i>Strongyloides venezuelensis</i> infection on the pregnancy of rats. The local Animal Ethics Committee approved all procedures (23108.028129/2023-84). Wistar rats were mated and randomly distributed into two experimental groups (n=12 rats/group): Control (C) and Infected (I). After mating, 2000 L3 larvae of <i>S. venezuelensis</i> were inoculated subcutaneously (day 0 of pregnancy). During pregnancy, the number of eggs per gram of feces (OPG), body weight, and water/food consumption were monitored daily. On day 21 of pregnancy, the rats were anesthetized and laparotomy was performed. Maternal organs were weighed and the uterine horns were removed to obtain data on maternal reproductive performance. $P < 0.05$ was considered as the statistical significance limit. The infected group presented a peak in OPG (~9,500 eggs) on day nine of pregnancy. Infection with <i>S. venezuelensis</i> during the pregnancy period showed a decrease in maternal weight gain and water consumption, and an increase in the weight of the heart, kidneys, and spleen. These changes may have contributed to the impairment of maternal reproductive performance, demonstrated by the increase in post-implantation losses (C=9.8% vs I=23.8%). With these findings, it was possible to conclude that strongyloidiasis causes negative changes in the maternal parameters analyzed.</p> <p>Keywords: <i>Strongyloides venezuelensis</i>, pregnancy, abortion, rats.</p>
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Title	Evaluation of aortic reactivity in adult male rats maternally exposed to cyantraniliprole during gestation and lactation
Authors	Carolina Oliveira Gonçalves Kaio Yuri Morimoto Ana Camila Ferreira de Menezes Glaura Scantamburlo Alves Fernandes Graziela Scaliante Ceravolo
Affiliations	State University of Londrina, Londrina, Brazil
Session	28 - Translational and Clinical Approaches in DOHaD

Abstract and Keywords	<p>Several studies confirm the harmful effects of exposure to pesticides on human health. Cyantraniliprole is a highly lipophilic insecticide, which can easily cross biological barriers, allowing offspring of pregnant women exposed to this compound to receive it indirectly. The study investigated the vascular effects of intrauterine and lactational exposure to cyantraniliprole in the male rat offspring. Experiments were approved by the CEUA-UEL (OF. CIRC. CEUA No. 20/2020 and OF. CIRC. CEUA No. 122/2021). Pregnant Wistar rats were treated with either water (CTR group) or cyantraniliprole (CYA - 1 mg/kg/day) from gestational day 5 to the end of lactation. Male offspring at 90 days of age were used for in vitro evaluation of aortic reactivity to phenylephrine (phenyl), acetylcholine (ACh), and sodium nitroprusside (NPS) in the presence (E+) or absence of endothelium (E-). Maximum response (Rmax, grams or % of relaxation) and pD2 were compared between groups using ANOVA or t-Student test ($p < 0.05$), with data presented as mean \pm standard error, n=number of rats/group. The study found that, the Rmax for phenyl (E+ and E-) was similar between the groups (CTRE+: 2.10 ± 0.28 (6) vs CYAE+: 2.13 ± 0.19 (7) and CTRE-: 3.21 ± 0.16 (6) vs CYAE-: 3.46 ± 0.12 (7)), the same occurred for ACh (CTR: 76.61 ± 4.15 (5) vs CYA: 79.74 ± 3.53 (9)), and for NPS (CTR: 95.22 ± 1.97 (6) vs CYA: 96.23 ± 1.23 (6)). There was also no difference in pD2 for the different vasoactive drugs. These results suggest that intrauterine and lactational exposure to cyantraniliprole did not affect vascular reactivity in adult male offspring. However, further studies are warranted to investigate the toxicity of this pesticide in other systems.</p> <p>Keywords: Insecticide; Vascular Function; Maternal Treatment; DOHaD</p>
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Title	Paternal obesity induced by lactation overnutrition induces metabolic changes in their offspring in the prepuberty
Authors	Polyana Keiko Shishido ¹ , Larissa Rugila dos Santos Stopa ¹ , Rhauany Pelisson Guergolette ¹ , Fabiano Takeo Komay Tsutsui ¹ Lucas Leonardo França de Oiveira ¹ , Ana Luiza Machado Wunderlich ¹ Ana Carolina Valverde da Silva ² , Maria Clara Alves Schroeder ² Yhassodara Narimatsu Oliveira ² , Raul Henrique Tonin dos Santos ² Emanuelle Passos Cerqueira ² Lucas Valente ² Cassia Thaís Bussamra Vieira Zaia ^{1, 2} Ernane Torres Uchoa ^{1, 2}
Affiliations	1- Postgraduate Program in Physiological Sciences, State University of Londrina, Londrina, PR, Brazil 2 – State University of Londrina, Department of Physiology, Londrina, PR, Brazi
Session	Poster

Abstract, Ethics Committee Number*, and Keywords	<p>The prenatal period, childhood, and adolescence are critical periods of development characterized by high plasticity. Origins of Paternal Health and Disease (POHaD), is an extension of the DOHaD paradigm. In rodents, litter size reduction is one of the experimental models used to induce early obesity by increasing the amount of breast milk to pups. Thus, this work evaluated, in the prepuberty, the offspring of fathers of normal litter (NLO) and small litter (SLO). Body weight, food consumption, glucose tolerance, nasoanal and tibia lengths, Lee index and the weights of visceral white adipose tissues, ovaries, uterus, adrenals, gastrocnemius and soleus muscles, and testes, as well as plasma concentrations of cholesterol, triglycerides, glucose, high-density lipoprotein (HDL), and Tyg index were analyzed in NLO and SLO of both sexes. Cages consisted of 4 females and 4 males, housed separately according to sex and group. Body weight and food consumption were from PND 25-30. GTT and euthanasia were performed on PND 29 and 30 in females, and on PND 31 and 32 in males, respectively. Parametric analyses Student's t-test or repeated-measures ANOVA and nonparametric Mann-Whitney tests were used. A significance level of $p < 0.05^*$ was considered for all statistical analyses. (CEUA nº 18310.2019.03, OF. CEUA nº 164/2019). Male offspring of small litter fathers showed decreased Lee index [$t(22) = 2.372$, $p = 0.0268$], tibia length [$t(22) = 2.731$, $p = 0.0122$] and HDL plasma levels [$t(26) = 2.590$, $p = 0.0155$], despite the increased food intake [$t(23) = 2.534$, $p = 0.0185$] and weight of</p>
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gastrocnemius muscle [$t(24)=2.622$, $p = 0.0150$]. Female offspring of small litter fathers showed reduced HDL plasma levels [$U=11$, $p = 0.0003$] and higher glucose plasma levels [$t(22)= 2.753$, $p= 0.0116$]. There were no differences in the other parameters. The results of the current study show that, in the prepubertal period, males seem to be more sensitive to paternal obesity by early overfeeding.

Keywords: postnatal overfeeding; PoHaD; metabolism; weaning



XXXVIII REUNIÃO ANUAL DA FESBE
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III CONGRESSO DOHAD BRASIL
II CONGRESSO DA SBBA
2 A 5 DE JULHO 2024, CAMPINAS/SP
FRONTEIRAS DAS CIÊNCIAS BIOMÉDICAS E SEUS DESAFIOS

Title	Vitamin C administration in the peripubertal period attenuates glycemic disbalance of young adult male rats with lactation overnutrition
Authors	Guergolette, R.P. *, Stopa, L.R.S., Aguiar, D.D., Shishido, P.K., Costa, I. R., Bertozzi, M.M., Zaia, C.T.B.V., Leite, C.M., Verri, W.A., Fernandes, G.S.A., Uchôa, E.T.
Affiliations	Departamento de Ciências Fisiológicas, UEL, Londrina/PR.
Session	Poster

Abstract, Ethics Committee Number*, and Keywords	<p>Reduction of litter size is used to induce early overnutrition. Excess ingested nutrients leads to an imbalance between oxidative and antioxidant compounds, which causes oxidative stress. Exogenous antioxidants, such as vitamin C (vitC), have been used for therapeutic purposes to mitigate oxidative stress and metabolic comorbidities of obesity. Thus, the aim of this work was to evaluate the effect of the administration of vitC in the peripubertal period of male Wistar rats with lactation overnutrition. On postnatal day (PND) 0, litter size was adjusted to <i>normal litter</i> (NL–10 pups) or <i>small litter</i> (SL–3 pups). Oral treatment with tap water (<i>vehicle</i>) or vitC (50mg/rat/day) was performed for 30 days (PND 30 until 59), and animals were analyzed on PND 60, forming 4 groups: <i>NLv</i>; <i>NLvitC</i>; <i>SLv</i>; <i>SLvitC</i>. Slv animals showed: glucose intolerance (18422 ± 1068 vs. 16989 ± 1389), hyperglycemia (169 ± 8.4 vs. 130.1 ± 1.9), increased Lee index (0.2856 ± 0.01135 vs. 0.2726 ± 0.0068), weights of retroperitoneal (0.664 ± 0.056 vs. 0.538 ± 0.046) and epididymal (0.977 ± 0.0767 vs. 0.853 ± 0.056) adipose tissues, decreased plasma triglycerides (113.3 ± 8.2 vs. 89.4 ± 6.4) and HDL (40.6 ± 9.9 vs. 55.6 ± 12.7), as well as higher liver lipoperoxidation (LP) (1.6 ± 0.2 vs. 1.3 ± 0.2), increased superoxide anion (NBT) (20.3 ± 1.2 vs. 27.2 ± 3.9) in the gastrocnemius muscle (GM), and reduced GSH (109.4 ± 5.6 vs. 116.8 ± 9.1) in the soleus muscle (SM). In SL animals, vitC reduced glucose intolerance (17107 ± 700.5) and glycemia (147.6 ± 2.8), reduced liver ABTS (406.8 ± 196.1 vs. 558.3 ± 113), increased liver GSH (115 ± 1.7 vs. 124.4 ± 5.95), increased NBT (27.2 ± 11.1 vs. 33 ± 5.7) and reduced FRAP (670.1 ± 76.2 vs. 747.6 ± 113.1) in the GM, as well as decreased GSH (101.1 ± 8.1 vs. 109.4 ± 5.6) and FRAP (485 ± 72 vs. 543 ± 74.4) in the SM. Thereby, the vitC administration in the peripubertal period reversed glycemic disbalances in young adult male rats with lactation overnutrition, without improving oxidative stress in the liver and skeletal muscles.</p> <p>– CEUA/UEL nº 87.2020; 024.2020.</p> <p>Keywords: litter size manipulation; overnutrition; vitamin C; glycemic disbalances.</p>
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Title	Influence of the western diet and exposure to the herbicide glyphosate on mouse prostate
Authors	Pinha, V.C. ^{1*} ; Rocha, V.A. ¹ ; Aquino, A.M. ² ; Magosso, N. ¹ ; Souza, P.V. ¹ ; Barbisan, L.F. ¹ ; Scarano, W.R. ¹ .
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Session	Abordagens Translacionais e Clínicas em DOHaD

Abstract, Ethics Committee Number*, and Keywords	<p>The prostate is highly sensitive to various environmental factors, specially endocrine disruptors. Both nutritional factors, such as the western diet (WD), a hypercaloric dietary pattern, and toxic agents, such as the herbicide glyphosate, are related to changes in the prostatic microenvironment that can influence its morphophysiology and lead to adaptive or carcinogenic responses. Thus, in order to examine the combined effects of the WD and glyphosate on the prostate, male C57BL/6J mice were divided into five groups (n=10). G1 received AIN-93G chow and filtered water; and groups G2-G5 were fed with a hyperlipidic and hypercaloric diet rich in sucrose and sugar, for six months. During the same period, the animals received glyphosate, at doses of (G3) 0.05, (G4) 5 and (G5) 50 mg/kg b.w/day (CEUA: 5897260423). Data of weight and body fat were measured. The animals underwent a glucose tolerance test, and the ventral prostate (VP) was collected for histological and molecular analysis (RT-qPCR) for gene expression related to cell repair (<i>Tp53</i>, <i>Casp3</i>), androgen receptor (<i>Ar</i>) antioxidant defense (<i>Cat</i>, <i>Gsr</i>, <i>Gpx3</i>) and methylation (<i>Dnmt1</i>). Statistical analysis was performed using GraphPad Prism; data are presented by mean \pm SD. The WD increased body and fat weight and induced glucose intolerance. Morphological changes and increase in the number of mast cells were observed in G2. Glyphosate (G4 and G5) reduced the quantity of mast cells altered by WD. Gene expression analysis showed a decrease of <i>Ar</i> and <i>Casp3</i> expression in G2 compared to G1, and increase in G5 compared to G2. Additionally, there was an increase of <i>Tp53</i> expression in G2 and a decrease in the glyphosate exposed groups compared to G2. No statistical difference was found in antioxidant enzymes and methylation markers. Our results showed that WD caused significant changes in VP, resulting in a microenvironment susceptible to pathological alterations, and glyphosate was able to modulate the effects caused by WD.</p> <p>Keywords: prostate, obesity, glyphosate, reproductive toxicology.</p>
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Title	Gestational and lactational exposure to a phthalate mixture modulates prostate proteome in offspring rats based in the human secretome
Authors	Souza, P.V.¹ , Magosso, N. ¹ , Rocha, V.A. ¹ , Moreira, M.F. ¹ , Fioretto, M.N. ¹ , Pinha, V.C. ¹ , Barbosa, F.C. ¹ , Maia, G.A. ¹ , Aquino, A.M. ² , Scarano, W.R. ¹ .
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Session	Abordagens Translacionais e Clínicas em DOHaD

Abstract, Ethics Committee Number*, and Keywords	<p>Phthalates are endocrine disrupting chemicals used as plasticizers for enhancing the flexibility of plastic products and are ubiquitous in human life. Prostate is critical for reproductive success and holds interest since Prostate Cancer (PCa) has a high incidence. DOHaD postulates that exposure to stressors, such as phthalates, during the susceptibility windows can be associated with diseases in adulthood. Here, we aimed to identify the effects on the prostate proteome of offspring rats exposed to phthalates during early life, focusing on secreted proteins in humans related to prostate oncogenesis, to identify potential biomarkers. CEUA: 9748091019. Pregnant SD rats were divided into 3 groups (n=10): C: Control group; T1: 20 µg/kg/day and T2: 200mg/kg/day treatment groups. The mixture was based in the metabolites of phthalates found in pregnant women, composed of 21% DEHP, 35% DEP, 15% DBP, 8% DiBP, 5% BBzP and 15% DiNP. Pregnant rats were treated from GD10 to PND21. At PND22 and 120, male rats (n=4-6/group) were euthanized, and the proteomic profile of ventral prostate was obtained. Differentially proteins in the proteome were compared to data of secreted proteins predicted in humans available in the HPA platform. The unregulated common proteins were cross-referenced with the differential gene expression data with both healthy and neoplastic prostate tissue. The treatment decreases several proteins of endoplasmic reticulum, crucial to the protein folding and secretion machinery, impairing prostate development, such as Hsp90b1 and Pdia3 and 6. Western blot showed an increase in AR (p =0,03) and ER (p =0,041) abundance at PND22 in T2 group, and a decrease in PRDX3 (p = 0,038 and 0,044) in all doses at PND120. Thus, several of those proteins altered in our analysis have their gene expression modified in PCa patients, alerting that phthalate alters the tissue microenvironment, which may increase the susceptibility to oncogenesis.</p> <p>Keywords: Prostate, phthalates, DOHaD, Bioinformatics</p>
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Title	Maternal deprivation affects hippocampal oxidative stress but not recognition memory of female rats; multicomponent training can positively modulate the oxidative balance
Authors	Gabriela Cristiane Mendes Gomes Ben-Hur Souto das Neves Giulia Azevedo Martinez Pâmella Andréa Machado da Silva Laranja Pinto Larissa de Almeida Dias Anna Cecília Perretto Vieira de Souza Ana Carolina de Souza da Rosa Karine Ramires Lima Marcelo Gomes de Gomes Pamela Billig Mello-Carpes
Affiliations	Physiology Research Group, Stress, Memory and Behavior Lab, Federal University of Pampa, Uruguaiana, RS, Brazil
Session	30 DOHAD, doenças maternas e intervenções perinatais

Abstract and Keywords	<p>Maternal deprivation (MD) is an animal model for investigating early life stress (ELS) effects, showing anatomical, neurochemical, and behavioral alterations. Exercise is studied due to its cognitive benefits and oxidative stress improvement. However, most studies of MD and exercise focus on aerobic exercise, and most do not include females as research subjects. We aimed to investigate the effects of multicomponent training (MCT) on cognition and redox balance in female rats submitted to MD. Pregnant female Wistar rats and their offspring were used (IRB 008/2023). Females from different offspring were divided into four groups: I) Control (CT); II) MD; III) MCT; and IV) MD+MCT (n=10-12/group). MD protocol was performed with MD groups for 10 days. The litters were weaned on the 21st day, and MCT groups executed the MCT for six weeks, with alternate practice of aerobic, anaerobic, and cognitive exercises. Recognition memory was evaluated using an object recognition (OR) task based on the ability to discriminate a familiar object from a new one. The percentage of total exploration time for each object was calculated and compared to a theoretical mean of 50% (one sample t-test). All groups explored the new object for more than 50% of the total time of exploration (CT P=0.0002; MD P<0.0001; MCT P<0.0001; MD+MCT P<0.0001), demonstrating that, unlike males, the adult female rats OR memory is not affected by MD. However, we detected significant differences in the oxidative balance. Lipid peroxidation data showed a reduction related to MCT (P=0.0082). ROS presents increased levels in MD compared to MCT (P=0.0102) and lower levels in MCT than CT (P=0.0021). The total antioxidant capacity was reduced in MD compared to CT (P<0.0001) and increased in MD compared to MD+MCT (P=0.0102). These data contribute to comprehension of the role of MCT in the ELS and the sexual dimorphism in MD-related effects.</p> <p>Keywords: Neonatal stress, Cognition, Exercise, Redox balance, Sex, Hippocampus</p>
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Title	Caryocar Brasiliense oil during pregnancy in rat: maternal repercussions
Authors	Paulo Roberto da Silva Filho ¹ Thais Campos Ribeiro ¹ Camilly Fernandes ¹ Débora Cristina Damasceno ² João Vitor Alves Damacena ¹ Alliny Alves de Araujo ¹ Betânia Branco Tiago de Queiroz ³ Rafaienne Queiroz Moraes-Souza ¹ Paula Becker Pertuzatti Konda ³ Larissa Lopes da Cruz ¹ Gustavo Tadeu Volpato ¹
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Session	Poster

Abstract and Keywords	<p><i>Caryocar brasiliense</i>, known as Pequi, is a plant used in folk medicine for various therapeutic purposes. However, details about use of oil fruit effects during pregnancy are limited. Thus, the objective of this study was to evaluate the effects of <i>C. brasiliense</i> oil on maternal parameters and reproductive performance in pregnant rats. This study was approved by the Ethics in the Use of Animals Committee – Araguaia (23108.001988/13-1). Wistar rats, 90 days old, were mated, and the pregnant rats were randomized into two experimental groups (n=12 rats/group): treated with water (Control) and treated with <i>C. brasiliense</i> oil (Treated) at dose of 1000 mg/kg. The treatment was performed daily orally during pregnancy (days 0 to 21). Body weight, food and water intake were measured weekly. On day 21 of pregnancy, the rats were anesthetized and laparotomy was performed. Maternal organs were weighed and the uterine horns were removed to obtain data on maternal reproductive performance. P<0.05 was considered as the statistical significance limit. The treatment with <i>C. brasiliense</i> oil caused changes in the indirect parameters of maternal toxicity, as decreased food intake (Control: 20.7±2.2 g vs Treated: 15.3±1.7 g), body weight (Control:</p>
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321.6±18.4 g vs Treated: 303.9±13.1 g), maternal weight gain (Control: 50.0±53.5 g vs Treated: 25.2±41.9 g), and relative liver weight (Control: 4,12±0,27 vs Treated: 3,87±0,20 g/100g p.v.). In addition, consumption of the plant oil caused increase in percentage of preimplantation loss (Control: 5,8% vs Treated: 19,74%) and decreased in litter weight (Control: 69.6±7.7 g vs Treated: 58.8±11.7 g). The results indicate that consumption of *C. brasiliense* oil during pregnancy induces maternal toxicity and impairs embryonic implantation, indicating that the oil should be avoided during pregnancy.

Keywords: Pequi, maternal toxicity, pregnancy, anti-implantation, rat



Title	Administration of graphene oxide nanoparticles during pregnancy in rats: fetal repercussions
Authors	Gisele Rabelo Alves ¹ Ailys Karoline Sérgio ¹ Beatriz Digigov Santana Rondon ¹ Camila Iohana Negrão ¹ Paulyne Silva Fagundes ¹ Larissa Lopes da Cruz ¹ Rafaienne Queiroz de Moraes Souza ¹ Jackson Antônio Lamounier Camargos Resende ² Débora Cristina Damasceno ³ Gustavo Tadeu Volpato ¹
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Session	Poster

Abstract and Keywords	<p>Nanomedicine is currently an area that receives great attention in scientific studies. Among the nanoparticles (NPs) used in nanomedicine, graphene oxide (GO) has stood out due to its many applications. However, there are few studies that document the effects of GO during pregnancy. Therefore, the objective of this study was to evaluate the fetal repercussions of GO administration during pregnancy in rats. The local Animal Ethics Committee approved all procedures (23108.022228/2019-76). Wistar rats were mated and randomized into three experimental groups (n=12 rats/group): rats received water (C) or GO at a dose of 2.5 (GO 2.5) or 5.0 (GO 5.0) mg/Kg. Administration of GO was performed daily orally during pregnancy (days 0 to 21). On day 21 of pregnancy, laparotomy was performed and the fetuses and their respective placentas were immediately removed and weighed, for subsequent analysis of fetal anomalies. P<0.05 was considered as the statistical significance limit. The results showed that the administration of GO did not have a teratogenic effect. However, the administration of GO at a dose of 5.0 mg/kg caused an increase in fetal (C=5.2±0.5g; GO 2.5=5.3±0.6g; GO 5.0=5.7±0.4g) and placental (C=0.45±0.07g; GO 2.5=0.45±0.06g; GO 5.0=0.49±0.01g) weights. Furthermore, these rats showed an increase in fetuses classified as large (LGA) for gestational age (C=3.8%; GO 2.5=3.7%; GO 5.0=16.8%) and in ossification sites compared to the other groups. Therefore, the results indicate that the administration of GO nanoparticles (at the highest dose) during pregnancy causes fetal macrosomia. These data highlight the importance of research with NPs to verify possible adverse effects of these materials, especially during the gestational period.</p> <p>Keywords: nanoparticles, graphene oxide, pregnancy, fetuses, malformations.</p>
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Title	Diabetes and reproductive consequences in future generations
Authors	Maysa Rocha Souza ¹ , Verônyca Gonçalves Paula ¹ , Franciane Q. Gallego ¹ , Vinicius Soares Barco ¹ , Gustavo Tadeu Volpato ² , Débora C. Damasceno ¹
Affiliations	¹ Laboratory Experimental Research on Gynecology and Obstetrics – UNIPEX, Postgraduate Course on Gynecology and Obstetrics, Botucatu, Medical School, UNESP, Botucatu, São Paulo State, Brazil. ² Laboratory of System Physiology and Reproductive Toxicology, Institute of Biological and Health Sciences, UFMT, Brazil
Session	DOHAD, doenças maternas e intervenções perinatais

Abstract, Ethics Committee Number*, and Keywords	<p>Previous studies show that diabetes causes embryonic and fetal losses due to hyperglycemia and oxidative stress. Daughters of diabetic rats also present gestational outcomes similar to those of their mothers. The main objective was to evaluate the ovarian structures of daughters of diabetic rats to explain the increased embryo-fetal losses resulting from diabetes. In addition, we evaluated embryonic losses before implantation and the pre-embryo morphology of daughters of diabetic rats. Sprague-Dawley daughters of non-diabetic rats (FPC - control) and of those with chemically induced diabetes (FPD) were used. In adulthood, these rats were subjected to the oral glucose tolerance test (OGTT) to calculate the area under the curve (AUC). At day 120 of life, 5 (non-pregnant) rats were euthanized for ovary collection and ovarian follicle counting, and 6 rats were mated with healthy males to obtain pregnancy. Subsequently, these rats were euthanized to remove and count the corpora lutea and obtain pre-embryos for morphological analysis on day 5 of pregnancy. A minimum confidence limit of 95% ($p < 0.05$) was considered significant. The local Animal Research Ethics Committee (Protocol CEUA Number: 1334/2019) approved the protocols performed. The FPD group had higher AUC compared to FPC rats (13.254 ± 416 vs 18.208 ± 1.350), lower number of the primordial follicle (75.8 ± 11.0 vs 34.6 ± 14.6), primary (37.4 ± 12.3 vs 19.8 ± 5.7), secondary (155.8 ± 25.0 vs 88.4 ± 16.4) and antral follicles (63.4 ± 7.5 vs 20.0 ± 7.9). The FPD group had a lower number of normal blastocysts (9.83 ± 2.48 vs 2.33 ± 2.73), a higher number of pre-embryos with developmental delay (0.83 ± 0.72 vs 5.67 ± 6.53), and a higher number of embryonic losses before implantation (0.92 ± 1.00 vs 4.83 ± 4.75) in relation to the FPC group. Therefore, the greatest number of embryo-fetal losses in daughters of diabetic rats is due to intergenerational hyperglycemia, which leads to compromised ovarian follicles.</p> <p>Keywords: Fetal programming; ovarian reserve; pre-implantation; hyperglycemia.</p>
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Title	Evaluation of placental transfer of neomycin, ampicillin and their combination when administered during pregnancy: validation of the gestational dysbiosis model
Authors	Manuela Dainez Aquotti ¹ , Ana Paula Sanches ² , Juliana Mozer Sciani ³ , Georgia Correa Atella ⁴ , Mileane De Souza Busch ⁴ , Gabriel Da Silva Cordeiro ⁵ , Kênia Moreno De Oliveira ⁶ , Marco Aurélio Ramirez Vinolo ⁵ , Everardo Magalhães Carneiro ⁶ , Leticia Ignácio De Souza Zimmermann ² , Liana Verinaud ⁵ , Catarina Raposo Dias Carneiro ¹ , Valquiria Aparecida Matheus ¹ .
Affiliations	1 - Faculty of Pharmaceutical Sciences – Unicamp, Brazil 2 - Faculty of Applied Sciences – Unicamp Limeira, Brazil 3 - São Francisco University, Bragança Paulista, Brazil 4 - Federal University of Rio De Janeiro - Rio De Janeiro – Brazil 5 - Institute of Biology - Unicamp, Brazil 6 - Obesity and Comorbidities Research Center, Unicamp, Brazil
Session	30 - DOHAD, doenças maternas e intervenções perinatais

Abstract, Ethics Committee Number*, and Keywords	<p>Offspring from pregnant with dysbiosis (imbalance in intestinal microbiota) are more susceptible to metabolic disorders. Maternal dysbiosis may alter the availability of short-chain fatty acids (SCFAs) produced by microbiota metabolism, which could be responsible for offspring liability to these disorders. Combined antibiotic administration during gestation serves as an experimental dysbiosis model; yet, its use is controversial as it's unclear whether the drugs could cross the placental barrier and directly harm the offspring. This project aims to determine whether offspring susceptibility is related to reduced SCFA availability or direct action of antibiotics. Pregnant C57BL6J females were divided into 4 groups, treated during gestation in drinking water: control group (CT), no intervention; dysbiosis group (ABX), received water with antibiotic mix (1 mg/mL ampicillin and 0.5 mg/mL neomycin); neomycin group (NEO), received neomycin (0.5 mg/mL); ampicillin group (AMP), received ampicillin (1.0 mg/mL). On the 15th day of gestation, females were euthanized for amniotic fluid and placental collection (CEUA 6278/2023). There was no significant difference in placental efficiency (fetal weight/placental weight) among the 4 experimental groups. Likewise, there was no difference in placental morphology (observed by HE) among the 4 groups, indicating no decrease in the labyrinth zone (vascular network). No concentrations of antibiotics were detected in the amniotic fluid of the treated groups. This analysis was performed by mass spectrometry, with the lowest concentration identification point being 0.5 ng/uL. Thus, the results suggest antibiotics do not directly affect the offspring. Next steps include measuring SCFA concentration in maternal feces and offspring amniotic fluid, and measuring of transforming growth factor beta TGF-β concentration and expression in placental samples by ELISA and PCR, respectively.</p> <p>Keywords: Microbiota, Offspring, Placenta, Antibiotic</p>
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Title	Unveiling complex networks of non-communicable diseases: from the first 1100 days to the end of the 2nd decade of life
Authors	Silas Alves-Costa Bruno Souza Feres Lorena Lúcia Costa Ladeira Erika Bárbara Abreu Fonseca Thomaz Claudia Maria Coelho Alves Rosângela Fernandes Lucena Batista Cecília Claudia Costa Ribeiro
Affiliations	Federal University of Maranhão, São Luís, Brazil.
Session	30 - Abordagens de Saúde Coletiva em DOHAD

Abstract, Ethics Committee Number*, and Keywords	<p>Non-linear complexity is a hallmark present in the human body systems. Thus, we hypothesized that non-communicable diseases (NCDs) form an interconnected network from the first 1100 days of life (100 days of the conceptional period + 270 days of pregnancy + 365 days of the 1st year + 365 days of the 2nd year), which increases in complexity at the end of the 2nd decade of life. We performed complex networks analyses to identify NCDs patterns, the hub (central element of the network), and subsequently investigate the main risk factors underlying these patterns. We analyzed data from the BRISA Cohort (N=731): 25th week of gestation, birth, and 2nd year of life and from the RPS Cohort Consortium, follow-up at 18-19 years (N=2515) in São Luís. From the pre-gestational period, the hub was women being obese before pregnancy (2873 connections); during pregnancy, caries (2636), periodontitis (2388), and anemia (2273) emerged; in the 2nd year, child obesity (2145). Among adolescents, the hub was higher TyG (17231), a marker of insulin resistance, followed by caries (14498). Risk factors were previous cesarean section in the pre-gestational period (1281), ultra-processed foods consumption (4485), and low socioeconomic class (4379) during gestation. In the 2nd year, added sugar consumption was >5% of daily calories (3141), and there was no exclusive breastfeeding (2650). Among adolescents, the risk hub was high sugar consumption (24963), high plaque index (18982), and low socioeconomic class. The complex network of NCDs at 1100 days involved obesity, ultra-processed foods, and high sugar diets in mother-child dyads. In parallel, insulin resistance, caries, hypertension, and high sugar consumption emerged from the network of adolescents. Social disparities were significant for both networks. Public policies promoting healthy food systems, equitable access to healthcare, and economic opportunities can promote overall population health. Ethics Committee #4771/2008-30 and #1302489</p> <p>Keyword: Non-communicable diseases; Complex networks; Early-life determinants</p>
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Title	The relationship between a sensory-cognitive-motor intervention and cognitive, anthropometric, and motor aspects in children with different nutritional states
Authors	Karollainy Gomes da Silva ^{1,4} , Maria Eduarda Rodrigues Alves dos Santos ^{1,4} , Ana Patrícia da Silva Souza ^{1,4} , Ana Beatriz Januário da Silva ^{1,4} , Beatriz Machado Silva ³ , José Maurício Lucas da Silva ⁴ , Mayara Luclécia da Silva ⁴ , Priscyla Evelyn da Silva Albuquerque ⁴ , Robson Feliciano da Silva ⁴ , Letícia Henrique Leite da Silva ¹ , Erica Helena Alves da Silva ⁴ , Antonietta Claudia Barbosa da Fonseca Carneiro ¹ , Williclecia Walkiria Dias Ferreira ⁴ , Sandra Lopes de Souza ¹ , Waleska Maria Almeida Barros ^{2,4}
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Session	Approaches of Collective Health in DOHAD

Abstract, Ethics Committee Number*, and Keywords	<p>The manifestation of malnutrition along with overweight and obesity is determined as a double burden of malnutrition. In recent decades, there has been a gradual decline in malnutrition accompanied by an increase in the prevalence of excess weight. The increased risk of developing childhood overweight and obesity is related to environmental, genetic, and socioeconomic factors. Exergames promote a combination of visual, auditory, motor, and attentional stimuli, providing beneficial impacts on physical, cognitive, and psychosocial variables. Thus, the objective is to investigate the effect of exergames on cognitive, motor, and cardiometabolic findings in children with different nutritional states. This is a prospective experimental longitudinal study, conducted with 48 children with a mean age of 6.95 (± 1.52) years designated according to their nutritional status for intervention or control groups. Intervention participants underwent a 40-minute gaming protocol twice a week for eight weeks. The analyzed outcomes were anthropometric data, lipid and glycemic profile, academic performance, and suggestive changes in cognition and gross motor coordination. The sample consisted of 41.66% eutrophic children, 37.49% overweight and obese, and 20.82% underweight. After stimulation, a reduction in fat percentage was observed for the intervention groups. Furthermore, improvements were observed in academic performance</p>
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and gross motor coordination. Regarding cardiometabolic data, the intervention groups showed a reduction in glycemic profile and blood pressure. Thus, the observations of this study demonstrate that sensory-cognitive-motor stimulation based on the use of exergames seems to have positive effects on anthropometric components, as well as contributing to better academic, cognitive, and motor performance. UFPE Human Ethics Committee: CAE - 60113522.0.0000.5208

Keywords: Nutritional status; childhood obesity; motor skill; cognitive aspects; active games.



Title	Does intrauterine growth restriction impact intelligence throughout life?
Authors	Janielle Ferreira de Brito Lima Rosângela Fernandes Lucena Batista Cecília Claudia Costa Ribeiro de Almeida Vanda Maria Ferreira Simões
Affiliations	Federal University of Maranhão, São Luís, Brazil.
Session	30 - Collective Health Approaches in DOHAD

Abstract, Ethics Committee Number*, and Keywords	<p>Intrauterine growth restriction (IUGR) appears to deprive the fetal brain of necessary nutrients for its proper development. IUGR has been associated with cognitive and intellectual impairments in childhood and adolescence. However, it is still unclear how IUGR may influence intellectual development, the pathways of this association, and whether socioeconomic status (SES) interferes in this association. Thus, the aim is to answer the following questions: Does SES at birth and IUGR have a direct effect on intelligence quotient (IQ) in late adolescence? Is the association between IUGR and adolescent IQ mediated by low birth weight (LBW)?</p> <p>The study was conducted with 313 participants from a birth cohort in São Luís, Maranhão, Brazil, assessed at birth and at 18 to 19 years old. Using structural equation modeling, variables from birth and early life (maternal age, maternal and paternal education, head of household occupation, family income, maternal gestational weight gain, IUGR, LBW, and duration of breastfeeding) and adolescent education were tested as determinants of IQ in late adolescence.</p> <p>The research was approved by the Research Ethics Committee of the University Hospital of the Federal University of Maranhão: Opinion number 1,302,489 of October 29, 2015.</p> <p>It was found that IUGR did not have a direct effect (p-value=0.172) or indirect effect (p-value=0.110) on adolescent IQ, and LBW did not influence this pathway (p-value=0.092). SES showed a positive direct effect of 0.508 standard deviations (p-value<0.001), corresponding to a 5.68-point increase in IQ.</p> <p>Thus, it was evident that better SES at birth had a long-term positive effect on intelligence, while IUGR does not seem to have influenced its development.</p> <p>Keywords: Fetal Growth Restriction; Social Class; Intelligence.</p>
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Title	Suzuki Early Childhood Education (SECE) and its interaction in child development: a perspective in conjunction with DOHaD Theory
Authors	Aline Ananias de Lima ^{1,5} ; Leonilson Oliveira Silva Paz ² ; Karollainy Gomes da Silva ^{1,7} , Maria Eduarda Rodrigues Alves dos Santos ^{1,7} , Ana Patrícia da Silva Souza ^{1,7} , Ana Beatriz Januário da Silva ^{1,7} , José Maurício Lucas da Silva ⁷ , Mayara Luclécia da Silva ⁷ , Priscyla Evelyn da Silva Albuquerque ⁷ , Robson Feliciano da Silva ⁷ , Erica Helena Alves da Silva ⁷ , Antonietta Claudia Barbosa da Fonseca Carneiro ¹ , Waleska Maria Almeida Barros ^{6,7} , Rosana Christine Cavalcanti Ximenes ^{1,3} Sandra Lopes de Souza ^{1,4}
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Session	30 - Education and Scientific Dissemination in DOHaD

Abstract, Ethics Committee Number*, and Keywords	The combination of the Suzuki Early Childhood Education (SECE) Program approach with the Theory of Development Oriented to Environment and Health (DOHaD) in early childhood music education emphasizes the importance of providing enriching and emotionally meaningful experiences from an early age to promote holistic and integrated development in children. An integrative review was carried out with no restrictions on the year of publication or language. The databases used were: ERIC, PubMed, PsycINFO, Web of Science and Scopus, using the descriptors "School Health Services", "Child Development", "Environmental Exposure", with the connective AND (Mesh). The articles selected were subjected to an interpretative analysis aimed at the guiding question. Epidemiological studies (cross-sectional, case-control, cohort, and clinical trial) were included and theses, dissertations, books or book chapters, editorials, newspaper articles, integrative or systematic literature reviews, case studies and experience reports were excluded, as well as works that diverged from the proposed theme. Analysis of the selected studies revealed a final sample of 12 articles. The studies showed significant improvements in verbal and non-verbal communication skills in children who took part in SECE programs, as well as an
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increase in children's self-esteem and confidence after attending music classes based on the Suzuki method. In addition, they indicated a positive correlation between early exposure to music through SECE and the development of cognitive skills such as memory, attention, and logical-mathematical reasoning in preschool children. Thus, the combination of DOHaD theory and the SECE approach in early childhood music education highlights the importance of considering not only musical development, but also children's emotional, social and cognitive development.

Keywords: SECE; child development; DOHaD theory.



Title	Effects of high-fat diet consumption in the perinatal and post-weaning period on bone structure and adipose tissue in adult rats
Authors	Teixeira, R.S. ¹ ; Espírito-Santo, D.A. ¹ ; Cordeiro, G.S. ¹ ; Pereira, P. J. S. ¹ ; Matos, R. J. B. ² ; Deiró, T. C. B. J. ¹ ; Costa, C. A. S. ³ ; Barreto-Medeiros, J. M. ¹ .
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Session	DOHaD and hypernutrition

Abstract, Ethics Committee Number*, and Keywords	<p>The consumption of processed foods rich in saturated fat and refined sugars has led to an increase overweight and obesity cases, which impact the emergence of chronic non-communicable diseases in addition to damage to bone structure. The objective of this work was to evaluate the effects of a HFD in the perinatal and post-weaning period on bone structure and adipose tissue. The study was approved by the EMEVZ/UFBA ethics committee under protocol 04/2019. Male Wistar rats were divided into three groups, CC: formed by rats that received a control diet (4% fat) throughout their life period; HC: formed by rats whose mothers were fed a high-fat diet (HFD-23% fat) only during pregnancy and lactation; and HH: formed by rats that consumed a HFD from the beginning of life until 90 days. The retroperitoneal adipose tissue and femur was collected and weighed on a semi-analytical scale. Subsequently, the structural measurements and biomechanical properties of the femur were analyzed. For statistical analysis, one-way ANOVA was used followed by the Bonferroni test. $P < 0.05$ was considered. The amount of retroperitoneal adipose tissue was lower ($p < 0.0001$) in the CC group (3.89 ± 0.74 g) compared to the HH group (10.58 ± 3.59 g), but with no difference in HC group (4.54 ± 2.01 g). Femur weight was lower ($p < 0.0001$) in the HH group (0.933 ± 0.06 g) compared to the CC group (1.13 ± 0.07 g). The elastic modulus showed no difference ($p = 0.0277$) between the CC group (534620 ± 16700 Mpa), and the others, HC (608508 ± 26156 Mpa) and HH (557879 ± 24909 Mpa). Maximum force was greater ($p = 0.0025$) in the HC group (120.5 ± 2.5 N) compared to the CC group (108.1 ± 2.9 N). Consumption of a HFD during the perinatal and post-weaning period caused an increase in the amount of retroperitoneal adipose tissue and a reduction in the weight of the femur, altering the bone structure, however it did not alter bone quality parameters.</p> <p>Keywords: High-fat Diet, Bone Structure, Perinatal Programming, Adipose Tissue.</p>
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XXII REUNIÃO ANUAL DA BRAVO
XVIII CONGRESSO DA SBCAL
III CONGRESSO DOHAD BRASIL
II CONGRESSO DA SBBA
2 A 5 DE JULHO 2024, CAMPINAS/SP
FRONTEIRAS DAS CIÊNCIAS BIOMÉDICAS E SEUS DESAFIOS

Title	II Semana Nacional do Cérebro no IFSC, campus Gaspar
Authors	Tiburcio, Hagar de Lara Silveira, Ana Paula Kuczmynda da
Affiliations	1 – Departamento de Ensino, Pesquisa e Extensão, Gaspar, Brasil 2 – Direção Geral, Gaspar, Brasil
Session	31 - Semana Nacional do Cérebro

Abstract, Ethics Committee Number*, and Keywords	<p>A Semana Nacional do Cérebro (SNC) é um evento organizado anualmente pela Sociedade Brasileira de Neurociências e Comportamento (SBNeC), sempre no mês de março, com o intuito de difundir estudos sobre o cérebro, cognição, sociocognição, aprendizagem e comportamento. No ano de 2024, o evento foi realizado pelo segundo ano consecutivo, no Instituto Federal de Santa Catarina (IFSC) - Campus Gaspar, tendo como tema "Odisseia Neurocientífica". Este ano, as atividades foram direcionadas aos estudantes dos cursos técnicos de Ensino Médio (EM) e envolveram palestras, oficinas e minicurso preparatório para a Olimpíada Brasileira de Neurociência (OBN), além de videoconferência voltada à formação continuada de professores da educação básica das redes públicas locais. As atividades foram organizadas pela equipe do Núcleo de Acessibilidade Educacional (NAE) do campus e estavam previstas no projeto de ensino "O conhecimento da neurociência para a promoção da aprendizagem, saúde e bem estar: uma ação promovida pelo NAE do campus Gaspar". A exemplo da primeira versão do evento ocorrida em 2023, um de seus objetivos foi envolver o protagonismo discente - inclusive de estudantes que se encaixam no espectro autista - tanto no planejamento e desenvolvimento da programação, quanto ministrando oficinas e apresentando demandas reais a serem abarcadas pelas atividades. Dentre os resultados observados, houve a publicação de posts no Instagram com mais de 570 acessos, a palestra online '<i>Insights da Neurociência na promoção da saúde, bem-estar e aprendizagem escolar do adolescente</i>' com mais de 140 visualizações no Youtube e duas palestras presenciais com aproximadamente 340 participantes. Além disso, cerca de 30 alunos da 4ª fase do curso técnico de química integrado ao ensino médio tiveram práticas com duas psicólogas de como agir em situações de ansiedade. No decorrer de todas as atividades realizadas foi evidente o engajamento e a motivação do público participante.</p> <p>Keywords: Semana Nacional do Cérebro; Neurociências; Campus Gaspar; Núcleo de Acessibilidade Educacional.</p>
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Title	National Brain Week at IFSC, campus Gaspar
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Session	31 - Semana Nacional do Cérebro

	<p>The National Brain Week (SNC) is an event organised annually by the Brazilian Society of Neurosciences and Behavior (SBNeC), always in March, with the aim of disseminating studies on the brain, cognition, sociocognition, learning and behaviour. In 2024, the event was held for the second consecutive year, at the Federal Institute of Santa Catarina (IFSC) - Campus Gaspar, with the theme “Neuroscientific Odyssey”. This year, the activities were aimed at students of High School Technical Courses and involved lectures, workshops and a preparatory mini-course for the Brazilian Neuroscience Olympiad (OBN), in addition to a videoconference aimed at the continued training of basic education teachers from local public networks. The activities were organised by the campus’s Educational Accessibility Center (NAE) team and were part of the teaching project “Neuroscience knowledge to promote learning, health and well-being: an action promoted by NAE campus Gaspar”. Like the first version of the event that took place in 2023, one of its objectives was to involve student leadership - including students who fall on the autistic spectrum - both in planning and developing the program, as well as teaching workshops and presenting real demands to be covered by the activities. Among the results observed, there was the publication of posts on Instagram with more than 570 hits, the online lecture ‘Insights of Neuroscience in promoting adolescent health, well-being and school learning’ with more than 140 views on Youtube and two face-to-face lectures with approximately 340 participants. Furthermore, around 30 students from the 4th phase of the technical chemistry course integrated into high school had practices with two psychologists on how to act in anxiety situations. During all the activities carried out, the engagement and motivation of the participating public was evident.</p> <p>Keywords: National Brain Week; Neurosciences; Gaspar Campus; Educational Accessibility Center.</p>
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Title	Investigating the communication roles of females release calls in anurans
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Session	Oral presentation

Abstract and Keywords	<p>Vocalizations are a conspicuous feature in the life of anuran amphibians. Frog calls can serve different roles depending on the social context. In anurans, vocalizations are almost always associated with males, but females of some species are also known to vocalize. Nevertheless, female frog calls are rare in reproductive and aggressive contexts. The release call is one of the call types commonly observed in the vocal repertoire of male frogs, but females have already been reported to emit release calls. In the latter case, release calls produced by a female assumedly convey information on its reproductive non-receptivity towards an amplexant conspecific male. Here, we report the occurrence of female release calls in two Neotropical tree frog species of the genus <i>Pithecopus</i>. The calls are housed in the Coleção Bioacústica UFMG and were analyzed using Raven Pro 1.6.5. The calls have a relatively simple structure, consisting of a single note with no pulses and a well-defined harmonic series. Differently, the male release calls of the same species differ in lacking pulses and well-defined harmonic structure. Surprisingly, the two occurrences of female release calls in the genus <i>Pithecopus</i> correspond to the two first records for hylids, one of the most species-rich and well-distributed frog families on the planet. Our study raises several questions about the evolution of female calls, especially the release call, and the possible functions mediated by female vocal signaling. Given the rarity of female vocalizations and the scarcity of data regarding female release calls, we intend to test, in future studies, the hypothesis of reproductive non-receptivity in the field using <i>Pithecopus</i> tree frogs as model organisms but also compile additional data on female calls from acoustic repositories in an attempt to characterize the variation patterns of female calls across extant anurans, as well as investigate their multiple functions under different social contexts.</p> <p>Keywords: Anuran bioacoustics, female release calls, hylids</p>
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Title	Maned wolves (<i>Chrysocyon brachyurus</i>) in the Ecological Station of Itirapina-Brazil: temporal and spatial distribution based on long-distance vocalizations recordings
Authors	Angélica Felício da Costa Linilson Padovese
Affiliations	Laboratory of Acoustics and Environment (LACMAM), University of São Paulo (USP), São Paulo, Brazil
Session	32 - Bioacústica

Abstract, Ethics Committee Number*, and Keywords	<p>The maned wolf (<i>Chrysocyon brachyurus</i>; Illiger, 1815) is considered the largest canid in South America. Its vocalizations are classified into several types, including roars-barks, which facilitate long-distance communication and are particularly suitable for passive acoustic monitoring. To study the vocalizations of <i>C. brachyurus</i> at the Itirapina Ecological Station, Brazil, six autonomous recorders, called EcoPods, were deployed on November 9, 2021 and removed on December 24 of the same year. The soundscape of the Itirapina Ecological Station has records of anthropophony, such as party music, and the frequent sound of trains passing by. The geophony identified in the recordings was wind and frequent precipitation, due to the rainy season. The biophony detected was biodiversity, composed of different species of birds, insects, frogs and mammals. The locations within the station with the highest detection rates of maned wolf vocalization, their characteristics, temporal distribution in relation to sunset and moon phases were investigated. The place with the highest concentration of barks coincided with the place where the puppies' vocalizations were recorded, highlighting the importance of vocal communication in parental care.</p> <p>Regarding the temporal analysis of the maned wolf's vocalization, a greater number of vocalizations were detected 8 to 9 hours after sunset, contradicting the initial hypothesis that predicted more vocalizations during the early evening twilight. Nights during the gibbous crescent moon phase, preceding full moon nights, showed increased frequencies of vocal activity. On the other hand, on full moon nights no vocalizations were detected, indicating periods of low energy expenditure, especially for high-energy vocalizations, such as roars and barks. Passive acoustic monitoring has allowed for a less intrusive study of maned wolf behavior for conservation efforts.</p> <p>Keywords: Maned wolf; <i>Chrysocyon brachyurus</i>; vocalizations; bioacoustics; Ecological Station of Itirapina (Estação Ecológica de Itirapina).</p>
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Title	Advertisement calls of the taxocenosis of anuran amphibians from Itinguçu State Park, Peruíbe-SP
Authors	Livia Zanuzzi Barroso ¹ Izabel Gonzalves Velasco ^{1,2} Ivan Sergio Nunes Silva Filho ¹
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Session	34 - Bioacoustics

Abstract, Ethics Committee Number*, and Keywords	<p>Brazil is the country with the world's highest diversity of Anuran amphibians, with 1144 species. For anurans, acoustics are essential for reproduction, territorial defense, and social interactions. The advertisement call is used by males to attract mates and to demarcate the space between them. It is considered a prezygotic reproductive barrier and a trait of taxonomic interest. Because of the taxonomic impediment, many species become extinct before they are described. The main goal is to describe the advertisement call of anurans from Itinguçu State Park (-24°38'37.2"S/-47°01'20.3"W) and compare to those in literature. Recordings were made using recorder Sony ICD-PX240, with monthly fieldworks from April 2022 to February 2023. The data was analyzed in the software Raven Pro 1.5 by the spectrogram, waveform and acoustics parameters of the call. Package seewave in Rstudio was used to create graphic visualizations for every species. The advertisement call of <i>Boana albomarginata</i> consists of a single multi pulsed note, call duration 0.03-0.27s, dominant frequency 1.12-2.67kHz. The call of <i>Boana faber</i> is composed of a stereotyped, pulsed, single note, call duration 0.02-0.11s, dominant frequency 0.34-2.92kHz. The call of <i>Dendrosophus elegans</i> consists of two pulsed notes, call duration 0.06-0.13s, dominant frequency 2.75-3.96kHz, first note is longer while second one has a slightly higher frequency. The call of <i>Phyllomedusa distincta</i> is composed of multiple pulsed notes, usually grouped in pairs, call duration 0.14-0.18s, dominant frequency 1.11-1.46kHz. <i>Rhinella ornata</i> call is composed of multiple pulsed notes, call duration is variable, our results ranged from 1.8s to 4.4s, dominant frequency 1.37-1.55kHz. The call of <i>Scinax cf. ruber</i> consists of a single note with well-defined pulses, call duration 0.15-0.52s, dominant frequency 1.55-3.78kHz. The data from literature presented similar acoustic parameters to those of the species here observed. Sponsor: FAPESP 23/02201-5.</p> <p>Keywords: Acoustics, Atlantic Forest, Taxonomy, Amphibia.</p>
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Title	Vocal sounds of pilot whales (<i>Globicephala</i> sp.) recorded using Passive Acoustic Monitoring during a seismic survey in the Santos Basin, southeast Brazil
Authors	Nara Pavan Lopes Julia Basso Cupertino Aline Mello Mering
Affiliations	TOVERI – Gerenciamento de Projetos Integrados LTDA, Rio de Janeiro, Brazil
Session	Session 34 (Bioacústica)

Abstract, Ethics Committee Number*, and Keywords	<p>The acoustic repertoire of the Delphinidae family is generally summed into three types of sounds: whistles, burst-pulsed sounds and echolocation clicks. Whistles are known to change over geographic regions, and/or ecological and social conditions. Pilot whales (<i>Globicephala melas</i> and <i>Globicephala macrorhynchus</i>) occur in all oceans worldwide and are highly social odontocetes, thus presenting a complex vocal repertoire. However, the acoustic repertoire of Southern hemisphere pilot whales is still poor known. In this study we analysed tonal sounds of pilot whales (<i>Globicephala</i> sp.) collected during a seismic project carried out between February 2023 and April 2024 in the Santos Basin, offshore southeast of Brazil. Data was collected using Passive Acoustic Monitoring (PAM) - SEICHE omnidirectional hydrophones with 4 elements, at 96 kHz samples. A total of 254 minutes of acoustic detections concurrent to sightings of <i>Globicephala</i> sp. were analysed using software Raven Pro 1.6. Whistles were manually analysed using audio-visual inspection of spectrograms (Hamming window of 1024 points of FFT with 60% overlap) and 213 tonal sounds were randomly chosen. The mean values found were: duration, $0,64 \pm 0,38$ s; minimum frequency, 7.4 ± 3.3 kHz; maximum frequency, 12.9 ± 5.3 kHz; peak frequency, 9.3 ± 3.8 kHz; centre frequency 9.4 ± 3.7 kHz; beginning frequency, 9.1 ± 6.4 kHz, and ending frequency, 10.9 ± 5.4 kHz. In Brazil, offshore odontocetes are difficult and expensive to study, thus seismic surveys can be of great value considering the amount of cetaceans' data collected yearly in the country's waters. Although in this study the species identification was not possible, the results are similar to previous acoustic studies of pilot whales in the same region. The compilation of data from different seismic surveys along the Brazilian coast can provide more robust analysis, helping to better understand the vocal repertoire of pilot whales distributed in the southeast of Brazil.</p> <p>Keywords: Delphinidae, <i>Globicephala</i>, whistles, acoustic repertoire, Passive Acoustic Monitoring</p>
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Title	Preliminary characterization of the geographical variation in the advertisement call of <i>Leptodactylus fuscus</i> Schneider, 1799
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Session	34-Bioacoustics

Abstract, Ethics Committee Number*, and Keywords	<p><i>Leptodactylus fuscus</i> Schneider, 1799 is a species of neotropical leptodactylid with a wide distribution, its presence has been recorded across most of South America. The extensive distribution of this species raises various questions regarding possible intraspecific variations within the taxon. In this study, we present preliminary results on the characterization of the advertisement call of eight <i>L. fuscus</i> individuals. We selected one individual from Panama, Guyana, Bolivia, and Uruguay, and four from Brazil, one from Pantanal and three from regions of Atlantic Forest. The recordings were obtained from collections available in the scientific literature and analyzed using the software Raven Pro 1.6 and R. The Bolivian <i>L. fuscus</i> had a call duration of 0,18-0,44 s, while the dominant frequency was 1,11-3,10 kHz. The call from the leptodactylid from Guyana lasted between 0,29-0,44 s, while the dominant frequency was 1,98-2,32 kHz. In the individuals from the Atlantic Forest the variation in call duration was from 0,22 s to 0,40 s, and the dominant frequency varied between 1,29-2,32 kHz. In the Panamanian <i>L. fuscus</i> the advertisement call lasted between 0, 21-0,30 s and its dominant frequency was 1,98-2,41 kHz. From the individual of the Brazilian wetland of Pantanal, the advertisement call had a duration of 0,23-0,43 s, and its dominant frequency showed a variation of 1,03-2,32 kHz. The Uruguayan frog had a advertisement call that lasted between 0,05-0,48 s, with a dominant frequency oscillating from 1,80-2,23 kHz. These findings provide insights into the acoustic characteristics of <i>L. fuscus</i> across different regions, highlighting potential variations in their advertisement calls. Further research on call format and notes/pulses data can delve deeper into the significance of these variations in the context of communication and species differentiation within this widespread anuran species. Sponsor: CAPES; FAPESP 23/02201-5.</p> <p>Keywords: Leptodactylidae. Advertisement call. Anuran.</p>
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Title	Coefficient of variation in call parameters of three species of the <i>Brachycephalus pernix</i> species group (Anura: Brachycephalidae)
Authors	Gabriel S. P. Alves ¹ , Luiz F. Ribeiro ² , Marcos R. Bornschein ¹
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Session	34 – Bioacoustics

Abstract, Ethics Committee Number*, and Keywords	<p>The coefficient of variation (CV) is an index used to classify the variation of parameters in anuran calls, both within individuals (CV_{intra}) and between individuals (CV_{inter}). It takes into account the means (X) and the standard deviation (SD), calculated by the following formula: $CV = (SD/X) * 100$. The resulting value categorizes the parameter as highly variable (dynamic), intermediate, or less variable (static). Reference values are: CV_{intra} < 5% = static and > 12% = dynamic; CV_{inter} < 10% = static and > 20% = dynamic. Dynamic parameters are likely influenced by social and environmental factors, and may play a significant role in mate choice. Static parameters, on the other hand, can be utilized for individual or species recognition. <i>Brachycephalus</i> Fitzinger, 1826 is a genus of small diurnal amphibians from the Atlantic Forest, comprising 41 species not yet evaluated in terms of CV, which is the objective of this study. We analyzed parameters of the advertisement calls of three species from the <i>B. pernix</i> group (<i>B. brunneus</i>, <i>B. curupira</i>, and <i>B. fuscolineatus</i>) deposited at the Museu de História Natural Capão da Imbuia, Curitiba. Recordings were made of each species on the same day. We considered the last six isolated notes before the first note group, or, if absent, the six preceding the last three notes. Five individuals per species were analyzed, evaluating the CV of dominant frequency (kHz), number of pulses per note, and note duration (s). The results indicate that the dominant frequency is static both within and between individuals, while the other parameters show dynamic or intermediate characteristics in all three species. Note duration is correlated with the number of pulses, hence both parameters received the same classification. The dominant frequency can be utilized for individual or species recognition. It remains to be determined whether other species exhibit similar static characteristics relation to this parameter and the range of variation.</p> <p>Keywords: Note centered approach, call variation, intra-specific recognition</p>
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Title	Description of the advertisement and territorial calls of <i>Brachycephalus didactylus</i> (Anura: Brachycephalidae)
Authors	Marina Gonçalves Marques ¹ , Gabriel Silveira Prado Alves ¹ , Luiz Fernando Ribeiro ² , Marcos R. Bornschein ¹
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Session	32 - Bioacústica

Abstract, Ethics Committee Number*, and Keywords	<p><i>Brachycephalus</i> is a genus with a high rate of recent species description, with diagnoses increasingly incorporating acoustic data. <i>Brachycephalus didactylus</i> appears to be a species complex, of which the calls are still unknown. In this study, we describe the advertisement call and a distinct call, tentatively termed territorial, of <i>B. didactylus</i> that we recorded in Teresópolis, Rio de Janeiro, in December 2021. We made recordings with the TASCAM DR-44 recorder and the Sennheiser ME 66 microphone. We used the note-centered approach, considering a set of notes as the call. We conducted analyses in Raven Pro 16.5 software, with FFT 256. We recorded 11 individuals in 10 recordings. The advertisement call (N=10 individuals) consists of one or two notes ($x=1.9$; $SD=0.30$), which have 8-17 pulses ($x=11$; $SD=2.41$). The duration varies from 0.1-0.4 s ($x=0.2$; $SD=0.00$), the lower frequency from 5.94-7.32 kHz ($x=6.70$; $DF=3.59$), the upper from 6.89-8.61 kHz ($x=7.82$; $SD=4.74$), and the dominant from 6.46-8.01 kHz ($x=7.19$; $SD=3.83$). The territorial call (N=3 individuals) presents 3-17 notes ($x=9.40$; $SD=3.29$) containing 1-16 pulses ($x=5.19$; $SD=7.07$). Territorial calls were emitted when at least two individuals were nearby vocalizing synchronously. The advertisement call resembles that of <i>B. sulfuratus</i> and an unidentified species from the southeast coast, both assigned to the same species group as <i>B. didactylus</i> (the <i>B. didactylus</i> group). In turn, the calls of these three species differ greatly from that of <i>B. hermogenesi</i>, which is similar to those of species in the <i>B. pernix</i> group. The discovery of the advertisement call of <i>B. didactylus</i> may assist other researchers in recording other populations and allow comparison based on acoustic parameters. This could help advance taxonomic revisions of the <i>B. didactylus</i> group.</p> <p>Keywords: <i>Brachycephalus didactylus</i>, bioacoustic, advertisement call.</p>
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Title	Using Convolutional Neural Networks in the detection of dolphin clicks through computer vision
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Affiliations	1 – Federal University of Santa Catarina, Florianópolis, Brazil 2 – Federal University of Santa Catarina, Florianópolis, Brazil 3 - BIOSONAR, Florianópolis, Brazil
Session	32 - Bioacústica Poster 03/07

Abstract, Ethics Committee Number*, and Keywords	<p>Passive acoustic monitoring for cetaceans is crucial and demands specialists, consuming hours for audio analysis and cataloging. In Brazil, it's a fundamental tool for IBAMA to oversee seismic companies on the coast, especially in oil and gas explorations. Automating the detection of dolphin acoustic signals is an effective strategy to assist this organization. This project, in its initial phase, employs computer vision and a convolutional neural network for the automatic detection of these clicks, cataloging spectrograms and potentially facilitating the management and analysis of this data. For this study, we used audio spectrograms with a duration of two seconds and a sampling rate of 96000Hz. Data were obtained from recordings provided to IBAMA by seismic research companies. The audios were categorized into sessions with dolphin clicks (positive samples) and without clicks (negative samples). We defined four types of images: single-channel grayscale, single-channel RGB, four-channel grayscale, and four-channel RGB. We used the pre-trained ResNet-18 convolutional network, applying transfer learning due to the dataset size.</p> <p>Images were standardized to 224 pixels and divided into training, validation, and test sets (70/20/10 ratio), maintaining the same number of positive and negative samples in each set. In total, 3444 images were used in each set. The data were processed by the model to extract relevant features. We added a final dense layer with two neurons and a sigmoid activation function for binary classification.</p> <p>Experiments were conducted using the Python language with the PyTorch and Wandb libraries, executed on an NVIDIA GEFORCE RTX 3060 GPU with the Linux operating system.</p>
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Initially, we followed the recommended hyperparameters for binary classification, using the Adam optimizer and cross-entropy loss function, with a batch size of 25 . During training, we monitored loss and accuracy at each epoch. We observed that, in the early epochs, all datasets reached maximum accuracy around the tenth epoch and minimum loss around the twentieth epoch. However, we identified overfitting phenomena in the validation sets, where metrics oscillated, indicating the model's difficulty in generalizing to new data. To mitigate this, we limited training to 100 epochs and implemented early stopping if the loss did not decrease for ten consecutive epochs, in addition to reducing the learning rate from 0.001 to 0.0001. Analyzing the results, we observed a clear distinction among the datasets. Models trained with single-channel images showed more consistent performance, while those with four channels showed inferior and less cohesive results among themselves. In the single-channel datasets, both grayscale and colored images showed similar results. In training, grayscale images achieved a final loss of 0.07748 and 0.07102, respectively, with accuracies of 0.977 and 0.9815. In validation, losses were 0.1559 and 0.1977, with accuracies of 0.9164 and 0.9359, respectively.

On the other hand, in the four-channel datasets, there was a significant difference in performance. Grayscale images achieved a final loss of 0.3191 in training and 0.0691 in validation, with accuracies of 0.8625 and 0.7438, respectively. Colored images recorded final losses of 0.3176 in training and 0.6082 in validation, with accuracies of 0.855 and 0.7331, respectively. Early stopping was activated in both four-channel image configurations. After training, we tested the models on an independent test set, consisting of 562 samples equally divided between positive and negative classes. The results showed that models trained with single-channel images outperformed those with four channels, correctly predicting 515 and 526 samples, respectively, for single-channel models, and 418 and 412 samples, respectively, for four-channel models.

The initial result was positive, highlighting the effectiveness in preventing overfitting by reducing the learning rate. There was a clear performance disparity among datasets, notably between four-channel and single-channel images. The latter performed better, with an accuracy rate of 91-93%, compared to 73-74% for the former. Single-channel images seem to favor discerning crucial characteristics for correct classification, while information overload in four-channel images may divert attention to inadequate features. Further experiments with larger datasets are needed to ensure the model's generalization robustness.

Keywords: Neural Networks, Passive Acoustic Monitoring, Clicks



Title	Did the 2023 annular solar eclipse affect Brazilian soundscapes?
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Session	Bioacoustics (34)

<p>Abstract, Ethics Committee Number*, and Keywords</p>	<p>The sun is the main source of energy for our planet. Many living organisms respond to the sun illumination cycle to adjust their activity budget. On October 14th 2023, the sunlight cycle was disturbed by the alignment of the moon between our star and our planet - an annular solar eclipse - where the moon casts a shadow smaller than the sun on Earth. This study aimed to characterise the possible effect of the annular solar eclipse on the soundscape of different Brazilian habitats across 23 degrees of latitude. Autonomous recording devices were installed in 13 natural sites from the states of Rio Grande do Norte to Santa Catarina. Indices that measure the amount and distribution of sounds in time and in frequency were used to verify if animals responded acoustically to the percent sun coverage during the eclipse. One minute samples taken every 5-10 minutes were used to compare the soundscapes of the days before and after the eclipse and the date of the eclipse, considering one hour before, during and one hour after the eclipse. Acoustic activity and complexity was expected to change proportionally to obscuration at each site during the eclipse. The results show no consistent differences in soundscape indices among days (day before, day of the eclipse, day after) nor correlations with obscuration, but do show differences among areas. The effects of the eclipse on faunal acoustic activity must be investigated at a different level, searching for changes in local species that are contributing to the soundscape instead of overall metrics that characterize it.</p> <p>Keywords: Bioacoustics; Soundscapes; Solar eclipse.</p>
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Title	Geographical variation in the complex song of <i>Pygochelidon cyanoleuca</i> (Aves: Hirundinidae)
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Session	34 - Bioacústica

Abstract and Keywords	<p><i>Pygochelidon cyanoleuca</i> is a common passerine songbird species that occurs in the Neotropics. Despite its abundance, little is known about its vocal repertoire and no studies about variation in song have been made. In this study, we aim to identify variation and to describe potential regional dialects. In total, 210 audio recordings of the species' complex song were gathered from publicly accessible citizen-science databases, namely WikiAves and XenoCanto. To avoid false replicas, recordings containing multiple instances of the analysed vocalization were randomly sampled using R software, with subsequent extraction of the valid segment via Audacity software. The entirety of the recordings will undergo analysis using Raven and R softwares. To mitigate potential cognitive biases, all parameter measurements were conducted by the same researcher. The geographical scope of the study encompasses nine Latin American countries, ranging from Argentina to Venezuela. Notably, Brazil emerged as the most extensively sampled locale, with 167 samples obtained from eight states and the Federal District. Sampling efforts exhibited a pronounced concentration in the south and southeastern regions of Brazil, where <i>Pygochelidon cyanoleuca</i> sightings are more prevalent. The analysed acoustic parameters were maximum frequency, minimum frequency, peak frequency, frequency bandwidth, call duration and pacing.</p> <p>This is ongoing research that has not yet yielded substantial results and conclusions. These will be presented upon completion at the time of the event.</p> <p>Keywords: Passeriformes; comparative analysis; dialects; citizen-science.</p>
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Title	Effects of annular solar eclipse on the vocal activity of Araripe manakin, <i>Chiroxiphia bokermanni</i> (Coelho & Silva, 1998)
Authors	Hipólito Denizard Ferreira Xavier ^{1,2,3} Beatriz Rodrigues de Souza ² Briza Maria de Araújo Mesquita ^{2,4} Cíntia Martins Souza ⁵ Marco Aurélio Crozariol ²
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Session	Poster

Abstract and Keywords	<p>It has been common to observe and document the effects of eclipses on animal behavior, but more studies are important to establish patterns and understand how solar eclipses change and influence these behaviors. This study aims to observe the effects of the solar eclipse on October 14, 2023, on the vocal activity of the diurnal and territorial species, <i>Chiroxiphia bokermanni</i>. The research was conducted between October 12-16, 2023, in Chapada do Araripe, Ceará, Brazil, which featured an annular eclipse between 3:23pm - 5:50pm (85.24% obscuration). Three autonomous recorders were installed near the FLONA Araripe-Apodi and RPPN Oásis Araripe. The devices worked in 4:1 (recording:pause) minute cycles, totaling 45.059 files. A pattern matching (PM) analysis was done in Arbimon's platform with patterns of N=10 and T=0.1. Generalized linear mixed model was used for the count of records/hour (r/h) as the response variable, and the predictor variables were the day of the count, the shift (morning/afternoon), and the solar condition (normal/eclipse/post-eclipse). The model has also considered the correlation between shift and day/solar condition. PM's results in 27.864 matched and confirmed the presence in 1.675 matches, only in RPPN Oásis Araripe. In general, the species vocal activity was between 8am-3pm. Meanwhile, on day 14 the species showed activity after 4pm, 11 records, and an average of 40r/h</p>
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during the eclipse. Apart from this exception, the species was most active in the afternoon (61.75r/h), while activity in the morning (42.25r/h). The highest number of records/day was observed on day 15 with 589 records. From variable predictors, the shift was not significant ($e=13.85/p=0.34310$). However, the solar condition (post-eclipse) could explain the vocalization frequency ($e=33.78/p=0.03981$). From the analysis made with records of the eclipse, it was possible to indicate that there are behavioral changes in the *C. bokermanni*, and in part, are statistically supported.

Eclipses; *Antilophia bokermanni*; Arbimon; AudioMoth; Vocal activity.



Title	Acoustic parameters of whistles from spinner dolphins (<i>Stenella longirostris</i>) in the Atlantic Ocean Equatorial Margin
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Session	[Oral]

Abstract, Ethics Committee Number*, and Keywords	The spinner dolphin (<i>Stenella longirostris</i>) occurs in tropical and subtropical waters. This species is mostly oceanic, but some populations are coastal or island-associated. The tonal sounds emitted by this species have been studied in several areas. But for some regions, such as the Atlantic Ocean Equatorial Margin, these data are not available. Here we present the acoustic parameters of spinner dolphin whistles for the 02°S latitude in areas after the shelf break in Brazil. The data was collected on March 7, 2024 from the hydroceanographic research ship Vital de Oliveira (Brazilian Navy). On this day, two sightings of spinner dolphins were made (at 17:13h and 17:55h). The sounds emitted by
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these animals were also registered using a 4-hydrophone system towed by the ship (AUSSET, 192kHz sampling frequency). The whistles were analyzed in Raven Pro® 1.6 and the following acoustic parameters were extracted: initial, final, minimum, maximum and dominant frequency, bandwidth, duration, number of harmonics, inflection points and steps, and the whistle contour type. In total, we found 210 whistles. The average (\pm SD) minimum and maximum frequencies were 11.58kHz (\pm 2.37kHz) and 18.70kHz (\pm 3.97kHz), respectively. The initial frequency varied from 5.80 to 26.39kHz and the final frequency from 7.39 to 29.80kHz (min, max). The average of dominant frequency and frequency variation was 13.65kHz (\pm 3.31kHz) and 7.12kHz (\pm 4.01kHz), respectively. The whistles lasted 0.67s (\pm 0.40s) on average, with the shortest lasting 0.07s and the longest, 2.12s. The majority (80.4%) of whistles did not present any harmonics, inflection points (59.0%) or steps (74.2%). Regarding the contour type, the most common was the ascending (n=81) followed by the descending (n=38). Given these results are from a single day of recordings, it might not be representative of the entire repertoire but adds information about the acoustic behavior of the species in a region where such information is scarce. Such studies shed light on micro and macroevolution, vocal plasticity and geographic variation in odontocete acoustic communication. Additionally, filling cetacean occurrence information gaps informs better conservation measures.

Keywords: bioacoustics, odontocete, acoustic patterns, occurrence



Title	Evidence of escalated aggressive calling behavior in a Neotropical tree frog
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Session	Poster

Abstract, Ethics Committee Number*, and Keywords	Vocalizations are a conspicuous feature in the life of anuran amphibians. Frog calls can serve different roles depending on the social context. To this date, 13 types of calls are classified under three categories based on social context. One of these is the aggressive call, which generally mediates an interaction between conspecific males and conveys information about the level of aggression. This study describes aggressive calls elicited during physical combat between two males of <i>Pithecopus ayeaye</i> . We also analyzed male aggressive calls emitted out of the context of physical combat and discussed the variation of male aggressive calls in the species related to varying levels of aggression. We conducted the acoustic analysis using Raven Pro 1.6.5; sound files were deposited in the Coleção Bioacústica of the Universidade Federal de Minas Gerais (CBUFMG). The territorial calls emitted in a fighting context (during physical combat) consist of one multi-pulsed note with an increment in sound amplitude throughout the note, its amplitude peak by 96% of note duration. In general, aggressive notes during physical combat and those emitted out of this context have the same temporal envelope and spectral features. However, notes produced during physical combat exhibit a greater number of pulses, a higher pulse repetition rate, and a longer note duration. The differences between
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aggressive calls of *P. ayeaye* produced in distinct contexts could indicate a graded signaling system in the aggressive vocal behavior of males of *P. ayeaye*. Our data match the pattern of escalated aggressive behavior occurring in other vocal anurans by modulating spectral and/or temporal features of their calls.

Acoustic communication, graded signaling, Phyllomedusinae, territoriality.



Title	Exploring the versatility of cellulose in antifungal active release
Authors	Joana Garrossino Magalhães Érika Leão Ajala Caetano Angela Faustino Jozala Denise Grotto
Affiliations	1 – University of Sorocaba, São Paulo, Brazil
Session	Microbiology

Abstract and Keywords	<p>Bacterial cellulose has been widely studied for its versatility, including the controlled release of active compounds. In this context, the combination of natural ingredients with cellulose can generate more efficient and sustainable therapeutic alternatives. This study investigated different forms of the cellulose and their capability to release agents from clove tincture. The Minimum Inhibitory Concentration (MIC) of clove tincture was conducted using the microdilution method in 96-well plates. Each well contained Sabouraud broth, fungi from a food inoculum (10^6 Colony Forming Units/g), and clove tincture (from 0.02 to 20%). Cellulose membrane and nanocrystal, inoculum (10^6 CFU/g) and Sabouraud broth were utilized for the fungi growth test. The test was performed on 24-well plates using 10% clove tincture, the best concentration observed through the MIC results. The results showed the visual fungal growth was observed after 24, 48, and 72h. Fungal growth was observed on the cellulose-loaded membrane with clove tincture after 72 hours. However, the nanocrystal with clove tincture did not exhibit growth after 72h. This study observed that cellulose presentation and the method of active incorporation significantly affect fungal growth. The nanocrystal exhibited delayed fungal growth. The blend of cellulose with clove may serve as a potential antifungal agent for the food industry.</p> <p>Keywords: bacterial cellulose; natural actives; antifungal.</p>
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Title	Antimicrobial evaluation of Jelleine-I against <i>Acinetobacter baumannii</i> and its derivate modified with the chelating agent HYNIC for radiolabeling with technetium-99m
Authors	Adrielle Pieve de Castro ¹ Julio Cesar Moreira Brito ² Simone Odília Antunes ¹ Valbert Nascimento Cardoso ¹
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Session	[33-Microbiology]

Abstract, Ethics Committee Number*, and Keywords	<p>Due to the emergence of current bacterial resistance, interest in new antimicrobial agents has increased rapidly. Jelleine-I is a antimicrobial peptide originally isolated from bee Royal jelly (<i>Apis mellifera</i>) with the short sequence of PFKLSLHL-NH₂. This study aimed to characterize the interaction and antimicrobial activity of Jelleine-I against clinical isolates of multi-resistant <i>Acinetobacter baumannii</i> (MDR-AB) and with the chelating compound HYNIC, radiolabel it with ^{99m}Tc. The peptides were synthesized by the Fmoc strategy and purified by HPLC. Isothermal titration calorimetry and cell lysis assay were employed to investigate peptide-membrane interaction. Microbiological assays were performed against 23 clinical isolates MDR-AB. The HYNIC-βAla-Jelleine-I radiolabeling procedure with ^{99m}Tc was carried out in the presence of co-ligands (tricin and EDDA) and reducing agent (SnCl₂.2H₂O). Radiochemical purity analysis was performed by thin layer chromatography. The affinity study of the ^{99m}Tc-HYNIC-βAla-Jelleine-I complex for bacterial cells was carried out. Jelleine-I showed antimicrobial activity <i>in vitro</i> (MIC: 8-32 μg/mL), demonstrating rapid bactericidal activity (3h) against MDR-AB and was able to lyse the cells of this pathogen through interaction with its cell membrane. Radiochemical purity analyzes of ^{99m}Tc-HYNIC-β-Ala-Jelleine-I showed that the ideal radiolabeling conditions produced a radiochemical purity of 93 ± 1% (n=3). The uptake of the ^{99m}Tc-HYNIC-βAla-Jelleine-I complex by bacterial cells appears to be a concentration-dependent process, since the uptake values increased considerably with increasing bacterial concentration. Jelleine-I is a potential prototype for the development of new antibacterial agents against isolates of MDR-AB and the radiolabeling ^{99m}Tc- HYNIC-βAla-Jelleine-I should be further investigated to optimize the radiolabeled compound in order to use it in the future as specific imaging agent to detect infections caused by <i>A. baumannii</i>.</p> <p>Keywords: Antimicrobial peptides, <i>Acinetobacter</i> infections, Technetium-99m, HYNIC.</p>
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Title	Enhanced biomaterial synthesis employing bacterial cellulose and silk proteins
Authors	Larissa Oliveira Simões Gabriel Pereira Machado Norberto Aranha Denise Grotto Angela Faustino Jozala
Affiliations	University of Sorocaba, Sorocaba, Brazil
Session	Microbiology

Abstract, Ethics Committee Number*, and Keywords	<p>The development of bioproducts with high added value that generate innovative, health-applicable and sustainable products has gained recent attention. Bacterial cellulose (BC), an extracellular polysaccharide produced by bacteria, exhibits biomedical potential due to its biocompatibility. Silkworm cocoon proteins, sericin and fibroin, possess antibacterial and mechanical properties, respectively, enhancing biomaterials. This study aims to optimize BC benefits by associating it with sericin and fibroin. The biomaterials were developed using two different methods. The first, by immersion using 24-well plates, was carried out with the BC membranes already produced, and 1 mL of sericin and fibroin were added distinctively to the membranes. The plates were then placed at 25 °C under agitation and samples were taken at different times between 1 and 24 hours. The second method was carried out simultaneously with the fermentation process. In 24-well plates, sericin and fibroin were inoculated at different times during the fermentation process, between 1 and 3 days. The plates were kept in static culture at 30 °C for 7 days. The results showed that for the immersion methodology, the best period for incorporating fibroin was between 6 and 8 hours, and the amount of protein incorporated was approximately 4.1 mg/mL. For sericin, only 0.15 mg/mL, indicating low incorporation into the BC. For the fermentation process, fibroin showed the best results in terms of thickness and resistance when inserted after 3 days. Sericin, on the other hand, prevents the formation of BC in the fermentation process. The materials obtained were characterized using a scanning electron microscope. Fibroin, when incorporated into bacterial cellulose, proved to have a significant impact as a new biomaterial, with results highlighting a promising additive for improving the properties of BC, making it a more versatile and effective biomaterial for various biomedical applications.</p> <p>Keywords: bacterial cellulose; silk protein; fibroin; sericin; biomaterial.</p>
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Title	Invasive procedures and nosocomial bacterial infection
Authors	Maria Fernanda Leonardi ¹ ; José Ferraz de Oliveira Junior ¹ ; Laura Fernanda Batista ¹ ; Ana Luiza Grizzo Paini ¹ ; Gabriela Maria Zanutto de Oliveira ¹ ; Gabriela Zenatti Gianti ¹ ; Julia Remaih Salata ¹ ; Priscila Aparecida Gatti ¹ ; Walter Manso Figueiredo ¹ ; Camila Linhares Taxini Passos ^{1, 2}
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Session	35 – Microbiologia

Abstract, Ethics Committee Number*, and Keywords	<p>It is known that infections caused by microorganisms are common in hospitals, even though all health protocols are followed. The main causes of nosocomial infections are related to the use of respiratory devices, urinary and intravenous catheters, as well as invasive procedures. The aim of this study was to correlate the use of invasive procedures with nosocomial bacterial infection in a hospital in the interior of the state of São Paulo. This is a cross-sectional analytical study that collected data through <i>in loco</i> analysis of medical records of patients with nosocomial bacterial infections discharged from hospital between 2018 and 2023 (CAAE: 75112423.9.0000.5383). Gender, age and invasive procedures performed during hospitalization were analysed using Microsoft Excel® and GraphPad Prism software. The sample of twelve patients, all of whom were male and all of whom died, with the majority (n=8) over 50 years old. The invasive procedures analyzed were surgery, catheterization, external ventricular shunt (EVS) and ventriculo-peritoneal drain. Two of the patients (17%) underwent some surgery, three (25%) an EVS, one (8%) catheterization, and five (42%) two procedures during the hospital stay. Only two (17%) of the twelve patients in the sample had not undergone any type of invasive procedure. Healthcare-Associated Infections (HAIs) are related to the performance of invasive procedures, knowledge of this relationship will provide targeted attention to patients with the aim of detecting the infection early and carrying out, efficiently and effectively, prevention measures to reduce mortality.</p> <p>Keywords: healthcare-associated infections; resistant bacteria; invasive procedures.</p>
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Title	Environmental challenge: <i>Bacillus subtilis</i> in combating plastic pollution
Authors	Isabelle Teixeira Mello ¹ Patrícia Lius Melo Alves ¹ Joana Garrossino Magalhães ¹ Walter Ruggeri Waldman ² Angela Faustino Jozala ¹
Affiliations	1 – University of Sorocaba, Sorocaba, Brazil 2 – Federal University of São Carlos, Sorocaba, Brazil
Session	Microbiology

Abstract, Ethics Committee Number*, and Keywords	<p>The increase of plastics in the environment is a problem that affects ecosystems and human health. Bioremediation emerges as a sustainable solution to combat it. Hence, the aim of this study was to bioremediate plastic using <i>Bacillus subtilis</i>. Therefore, a preculture was prepared in 135 mL of <i>Tryptone Soy Broth</i> (TSB) containing a suspension of 15 mL of <i>B. subtilis</i>. The preculture was incubated for 24 hours at 35°C with 150 rpm agitation. Treatments were conducted in flasks with concentrations of 50% and 10% TSB, both inoculated with 5 mL of preculture and a 1.5 cm² PET plastic fragment. The flasks were incubated at 35°C with 150 rpm agitation for 4, 7 and 15 days. After incubation, the plastic fragments were collected for surface observation under a conventional optical microscope and Fourier Transform Infrared Spectroscopy (FTIR); and the liquid was filtered through a 0.22 µm filter for biomass quantification. The biomass results for the 10% condition showed a decrease of 3 times from the 4th day to the 7th day, from 42.8 mg/mL to 13.4 mg/mL. On the 15th day, however, it increased 1.2 times, reaching 16.25 mg/mL. For the 50% condition, a progressive decrease in biomass was observed, from 4.7 mg/mL on the 4th day to 9.2 mg/mL on the 7th day, reaching 7.35 mg/mL on the 15th day. Microscopic analysis of the plastic fragments showed a growth pattern in biofilm formation at 7 days, with progressive development of the biofilm at 15 days. The FTIR analysis revealed a decrease in wave frequency from 2969 to 2908, indicating a reduction in C-H symmetrical stretching, after treatments under both conditions. The analyses of the growth process as well as of the plastic treatment suggested the beginning of the degradation process and highlighted the potential of <i>B. subtilis</i> in PET bioremediation.</p> <p>Keywords: Bioremediation; Biodegradation; <i>Bacillus subtilis</i>; plastics.</p>
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Title	Exploring the antioxidant potential of clove and basil extracts for application in natural products
Authors	Ana Carolina Souza Chacon Joana Garrossino Magalhães Érika Leão Ajala Caetano Denise Grotto Angela Faustino Jozala
Affiliations	1 – University of Sorocaba, São Paulo, Brazil.
Session	Microbiology

Abstract, Ethics Committee Number*, and Keywords	<p>The clove (<i>Syzygium aromaticum</i> L. Myrtaceae) is an aromatic plant. This spice is rich in volatile compounds and antioxidants. Basil (<i>Ocimum basilicum</i> L.) is another very common aromatic plant, known for its therapeutic potential and preservation effects. The aim of the study was to evaluate the antioxidant activity of clove and basil extracts to ensure the benefits to natural products. To do so, plate assay methods were used to assess the antioxidant potential, through the DPPH free radical scavenging method for the herbal extracts, and microplate assay of reducing substances by the Folin-Ciocalteu method for phenolic extracts. DPPH analyses confirmed the high antioxidant potential of the extracts even at small concentrations. In 30 minutes, the 5% clove extract had an antioxidant activity (AAO) of 85.56%, and the 1% clove extract had an AAO of 81.30%. In basil analyses, also performed in 30 minutes, the 5% extract had an AAO of 87.70%, and the 1% solution had an AAO of 83%. In the phenolic assay, there was no need to use the 5% extract concentration, as it would not be possible to analyse in the microplate reader. The results of this assay showed that the clove extract had a greater amount of phenolic compounds than to the basil extract (phenolic compounds per µg/mL, in equivalence to gallic acid). It is possible to understand that the dilution of these extractions does not greatly affect their activities, since the decrease in antioxidant property of the diluted 1% actives does not exceed 5% in their AAO. The results emphasise the importance of natural products in combating oxidation and preserving biological and commercial materials. Further research into the specific mechanisms and potential applications of these extracts is warranted to fully harness their conservation and antioxidant properties.</p> <p>Keywords: clove; basil; antioxidant activity; natural products.</p>
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Title	In vitro evaluation of the effectiveness of different porphyrin-mediated photodynamic therapy protocols for <i>Acinetobacter baumannii</i> biofilm inactivation
Authors	Patricio dos Santos Souza Igor Pereira Ribeiro Muniz Gabriel Pinto de Oliveira Santos Vanderlei Salvador Bagnato Francine Cristina Silva Rosa Luciano Pereira Rosa
Affiliations	Federal University of Bahia, Multidisciplinary Health Institute, Vitória da Conquista, Bahia
Session	[Microbiology]

Abstract, Ethics Committee Number*, and Keywords	<p><i>Acinetobacter baumannii</i> is one of the pathogens that causes the most infections in hospitals, with the main problems in combating it being the existence of multi-antibiotic-resistant and even extensively antibiotic-resistant strains and the ability to form biofilms. Given the current difficulty in controlling hospital infections, Photodynamic Therapy (aPDT) has become an interesting strategy as an adjunct to antibiotic therapy. The objective of this study was to evaluate different aPDT protocols for the inactivation of ATCC (19606) and clinical (CCBH24267, CCBH24360, and CCBH24383) biofilms of <i>A. baumannii</i> using porphyrin as a photosensitizer in different concentrations. One hundred and sixty specimens (CP) of acrylic resin were used for the in vitro growth of <i>A. baumannii</i> biofilms, which were divided into groups (N = 10) subjected to treatments with aPDT using porphyrin at concentrations of 5 µg/ml, 20 µg/ml and 40 µg/ml, and red LED (630 nm), irradiance of 12 mW/cm², at times of 3 min, 5 min, and 10 min, generating fluences of 1,08 J/cm², 3,6 J/cm² e 7,2 J/cm², respectively. Control groups were included in which no treatment was performed, treatment only with LED at different times, and treatment only with curcumin at different concentrations. The groups were compared using the Kruskal-Wallis test (95%) with Dunn's post-test. The groups in which only porphyrin, in the three concentrations tested, and all aPDT groups showed a statistically significant difference (p<0.05) in the reduction of log₁₀ CFU/mL compared to the control group. The aPDT protocols tested in this work were efficient in reducing the log₁₀ of CFU/mL of <i>A. baumannii</i> biofilms, with the protocol with porphyrin at a concentration of 5 µg/mL with a fluence of 1.08 J/cm², the which showed better results.</p> <p>Keywords: Photodynamic therapy, porphyrin, <i>Acinetobacter baumannii</i>.</p>
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Title	Curcumin-mediated photodynamic therapy for inactivation of <i>Acinetobacter baumannii</i>: in vitro study
Authors	Luana Moreira Silva Igor Pereira Ribeiro Muniz Gabriel Pinto de Oliveira Santos Vanderlei Salvador Bagnato Francine Cristina Silva Rosa Luciano Pereira Rosa
Affiliations	Federal University of Bahia, Multidisciplinary Health Institute, Vitória da Conquista, Bahia
Session	[Microbiology]

Abstract, Ethics Committee Number*, and Keywords	<p>Hospital infections have been responsible for a large number of morbidity and mortality and are associated with high costs in the public health system. <i>A. baumannii</i> is one of the main pathogens causing infections in the hospital environment and may present reduced susceptibility and/or multiresistant to antibiotic therapy. Photodynamic Therapy (aPDT) has been highlighted as an alternative resource for treating infections. The objective of this study was to evaluate different aPDT protocols for the inactivation of ATCC (19606) and clinical (CCBH24267, CCBH24360, and CCBH24383) biofilms of <i>A. baumannii</i> using curcumin as a photosensitizer in different concentrations. One hundred and sixty specimens (CP) of acrylic resin were used for the in vitro growth of <i>A. baumannii</i> biofilms, which were divided into groups (N = 10) subjected to treatments with aPDT using curcumin at concentrations of 5 µg/ml, 20 µg/ml and 40 µg/ml, and blue LED (460 nm), irradiance of 18 mW/cm², at times of 3 min, 5 min, and 10 min, generating fluences of 3.24 J/cm², 5.4 J/cm² and 10.8 J/cm², respectively. Control groups in which no treatment was performed, treatment only with LED at different times, and treatment only with curcumin at different concentrations were included. The groups were compared using the Kruskal-Wallis test (95%) with Dunn's post-test. The groups in which only light and curcumin were used did not show a statistically significant difference (p>0.05) when compared to the control group. The groups treated with aPDT 5 µg/ml (3 min), 20 µg/ml (5 and 10 min), and 40 µg/ml (3 and 10 min) showed a statistically significant difference (p<0.05) compared to the group control with a decrease in log₁₀ CFU/mL. The aPDT protocols tested were effective in reducing log₁₀ CFU/mL of both the ATCC strain and the clinical strains of <i>A. baumannii</i>, with the protocol with curcumin at 40 µg/mL (10 min) showing the greatest efficiency among the protocols.</p> <p>Keywords: Photodynamic therapy, curcumin, <i>Acinetobacter baumannii</i>.</p>
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XXXVIII REUNIÃO ANUAL DA FESBE
XXII REUNIÃO ANUAL DA BRAVO
XVIII CONGRESSO DA SBCAL
III CONGRESSO DOHAD BRASIL
II CONGRESSO DA SBBA
2 A 5 DE JULHO 2024, CAMPINAS/SP
FRONTEIRAS DAS CIÊNCIAS BIOMÉDICAS E SEUS DESAFIOS

Title	Molecular detection of <i>Fusarium</i> species with mycotoxigenic potential in different conserved forages
Authors	Renato Antonio dos Anjos Karen Sumire Kubo Tiago Miguel Jarek
Affiliations	Pontifícia Universidade Católica do Paraná, Curitiba, Brazil
Session	Microbiology

Abstract, Ethics Committee Number*, and Keywords	<p>In Brazilian livestock farming, forages such as hay and silage are utilized to ensure consistent nutrition for ruminants and mitigate the effects of seasonality. However, these crops can harbor fungal microorganisms, including those of the <i>Fusarium</i> genus. These fungi produce various mycotoxins, such as trichothecenes, fumonisins, and zearalenones, which pose significant health risks to both humans and animals, manifesting in carcinogenic, hemorrhagic, and neurotoxic effects. Given the potential challenges in morphologically differentiating <i>Fusarium</i> species, this study proposes a molecular technique to assist in identifying these species. The focus is on those with mycotoxigenic potential in various conserved forages. This is crucial, as the morphological identification of <i>Fusarium</i> can be restricted in certain cases, with some species considered indistinguishable based solely on morphology. Samples of <i>F. graminearum</i>, <i>F. poae</i>, and <i>F. sporotrichioides</i> were cultured in a growth medium. Simultaneously, these samples were used to establish a wet chamber to verify the viability of DNA extraction and fungal detection by PCR, eliminating the need for prior isolation. DNA was then extracted from isolated <i>Fusarium</i> species as well as from the inoculated hay and silage, followed by PCR using primers proposed by Nicholson <i>et al.</i> (1998), Parry <i>et al.</i> (1996), and Demeke <i>et al.</i> (2005) for <i>F. graminearum</i>, <i>F. poae</i>, and <i>F. sporotrichioides</i>, respectively. Morphological identification and PCR ultimately confirmed the presence of <i>Fusarium</i> species, demonstrating the specificity of the primers. Notably, <i>Fusarium</i> presence was directly confirmed in both hay and silage, enabling quicker and more efficient identification. Furthermore, considering that contaminated forages can be consumed by bovines and subsequently transmitted to humans, posing a significant impact on food safety and health, the development of efficient identification techniques, such as the molecular method, is essential.</p> <p>Keywords: <i>Fusarium</i>. Forage. Morphological identification. PCR.</p>
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Title	Antibiotic susceptibility profile of <i>S. aureus</i> isolated from patients treated at HUAV
Authors	Iris Villela Chagas Jacintho Thiago de Oliveira Cidral Eduardo de Souza Alves Marcondes Petrus Pires Marques
Affiliations	UNIFENAS (Universidade Professor Edson Antônio Velano), Alfenas, Brasil
Session	Poster

Abstract, Ethics Committee Number*, and Keywords	<p>Since the development of the first antibiotics, such as Salvarsan, which was commercialized in 1910 for the treatment of syphilis, there has been considerable progress in the pharmaceutical industry aimed at resolving infections and promoting human well-being. However, the indiscriminate and inadequate use of these drugs has contributed to microbial resistance, resulting in a crisis in controlling these microorganisms, including <i>Staphylococcus aureus</i>.</p> <p>In light of the worldwide crisis of antimicrobial resistance and the emergence of multiresistant bacteria, it is crucial to identify the susceptibility of hospital-dwelling bacteria to ensure effective treatment and explore alternatives if necessary. To this end, this experimental research aimed to investigate the susceptibility profile of <i>S. aureus</i> to vancomycin, cefoxitin, benzylpenicillin, norfloxacin, clindamycin, and erythromycin using the Bauer-Kirby technique. The research involved collecting data on the sensitivity of samples of <i>S. aureus</i> isolated from the sputum of patients hospitalized at the Alzira Velano University Hospital (HUAV) in Alfenas, Minas Gerais, without any age or gender restrictions. Out of the initial 30 samples, those from patients who did not show adequate growth in the amplification process of inoculum preparation, which lasted less than 24 hours, were excluded, resulting in 29 samples. To isolate the <i>S. aureus</i>, the samples of sputum were inoculated on mannitol salt agar, and cultures that grew in less than 24 hours were harvested. The suspensions were then diluted in sterile distilled water to reach a concentration of 0.5 on the McFarland scale and spread evenly on a Petri dish containing Mueller Hinton agar. Discs containing antibiotics at respective concentrations of 30 µg vancomycin, 30 µg cefoxitin, 01 UI benzylpenicillin, 10 µg norfloxacin, 02 µg clindamycin, and 15 µg erythromycin were placed on the surface of the culture medium. The culture</p>
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medium was then incubated at 35°C for 18-24 hours. If the substance was effective against the tested organism, a zone of inhibition formed around the disc. The size of the zone was compared to the antimicrobial sensitivity or resistance criteria of the EUCAST of 2024 (for cefoxitin, benzylpenicillin, ciprofloxacin, levofloxacin, clindamycin, and erythromycin) and the ANVISA of 2005 (for vancomycin) to classify the microorganism. The results of the experiment indicated that there was 79.31% sensitivity and 20.68% resistance to norfloxacin; 13.79% sensitivity and 86.20% resistance to benzylpenicillin; 41.37% sensitivity and 58.62% resistance to clindamycin; 96.55% sensitivity and 3.44% resistance to vancomycin; 37.93% sensitivity and 62.06% resistance to erythromycin; and 82.75% sensitivity and 17.24% resistance to cefoxitin. The data obtained highlighted the significant concern of antimicrobial resistance, with several bacteria demonstrating resistance to multiple commonly used antibiotics. This underscores the importance of identifying the susceptibility of hospital bacteria to ensure effective treatments. While the high sensitivity to vancomycin suggests that there are still therapeutic options for certain bacterial infections, the resistance to other antibiotics, such as benzylpenicillin, clindamycin, and erythromycin, is concerning and indicates the need for additional strategies to address bacterial resistance, such as developing new drugs or implementing more prudent prescribing practices. In conclusion, it is fundamental to explore therapeutic alternatives and continue monitoring antimicrobial resistance to ensure we can efficiently combat bacterial infections and preserve the effectiveness of existing antibiotics.

Keywords: *Staphylococcus aureus*. Methicillin-Resistant *Staphylococcus aureus*. Antibiotic Resistance, Bacterial. Microbial Sensitivity Tests.



Title	Isolation and molecular identification of endophytic fungi from the plants <i>Avicennia schaueriana</i> and <i>Rhizophora mangle</i>
Authors	Laura Almeida Silva Castro ¹ Letícia Fontes Gama ¹ Yoannis Dominguez Rodriguez ¹ Cristiane Angélica Ottoni ¹ Leandro Mantovani de Castro ¹
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Session	33 - Microbiology

Abstract, Ethics Committee Number*, and Keywords	<p>The mangrove is a coastal ecosystem that serves as a transition between terrestrial and marine environments and plays a fundamental role in ecological balance. Brazilian mangroves are mainly composed of three plant species <i>Rhizophora mangle</i>, <i>Laguncularia racemosa</i> and <i>Avicennia schaueriana</i>, which act as habitats for various microorganisms, such as fungi, bacteria and algae. Endophytic fungi are found within these plants and have great potential for biotechnological applications, aiding in the development of a better quality of life. Therefore, different techniques have been used for the identification of these microorganisms. However, due to the similarity of the species, besides to morphological analysis, the use of molecular techniques is necessary. The objective of the present study was to isolate and identify of endophytic fungal communities from the leaves of <i>A. schaueriana</i> and <i>R. mangle</i> plants inhabiting one mangrove in the state of São Paulo, Brazil. The leaves were previously washed thoroughly in running water, superficially sterilized in 70% ethanol, immersed in 4% NaOCl, rinsed in sterile distilled water, cut (1cm²) and placed in potato dextrose agar (PDA) Petri dishes. All plates were maintained at 30 °C and daily examined during three weeks for fungal colonies growing from the leaves fragments. After this period, the fungal colonies were isolated, macroscopically analyzed, and cryopreserved in 20% glycerol. Subsequently, for molecular identification, fungal samples were subjected to the DNA barcode method, using the ITS gene region as a marker. The mangrove plants showed a wide variety of these microorganisms, in which fifteen were defined as lineage, being six from <i>R. mangle</i> and nine from <i>A. schaueriana</i>. Among the obtained lineages, ten are undergoing genetic sequencing. The identification of these endophytic fungal species opens up new perspectives for studies and biotechnological applications, particularly in the agronomy field.</p> <p>Keywords: Mangrove; endophytic fungi; DNA barcode; ITS region.</p>
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Title	The influence of chronotype and physical activity on social jetlag and the sleep quality of medical students at UNICAMP
Authors	Thiago Hideki Shimoura de Oliveira Victor Sanz-Milone Andrea Maculano Esteves
Affiliations	1 – FCM – Faculdade de Ciências Médicas, UNICAMP, Campinas, Brazil 2 – FEF – Faculdade de Educação Física, UNICAMP, Campinas, Brazil 3 – FCA – Faculdade de Ciências Aplicadas, UNICAMP, Limeira, Brazil
Session	35 – Neurociência

Abstract, Ethics Committee Number*, and Keywords	<p>A complex routine, high class workload, night shifts and physical activity or a sedentary lifestyle can significantly impact the quality and quantity of sleep in medical students. Characteristics and phenomena such as chronotype, social jetlag and sleep regularity can positively or negatively influence sleep quality. Thus, the objective of the study was to evaluate the relationships between sleep quality, chronotype, sleep regularity and social jetlag in medical students at UNICAMP. N= 286 students distributed among the 6 years of medicine at UNICAMP, responded to questionnaires (google forms) on sleep quality, chronotype, drowsiness, social jetlag, sleep regularity and physical activity. The results demonstrated a sleep efficiency of 91.71 ± 13.07. However, students had poor sleep quality (7.21 ± 2.74), with the worst results being presented by sixth-year students (9.09 ± 3.7). The students had an indifferent chronotype (50.69), sleep regularity of 79.05 and a social jetlag of 01h39min. Furthermore, 82.32% of students were physically active. The associations demonstrated that morningness was associated with lower sleep latency and good sleep quality, as well as lower values of social jetlag. Having higher sleep regularity was associated with lower values of social jetlag, better sleep quality and less drowsiness. Lower social Jetlag was associated with better sleep quality. Furthermore, a statistically significant difference was found for total sleep time on day 5 of the sleep diary, with the first year showing lower values compared to the sixth year. No associations were found between the practice of physical activity and the variables analyzed. The results suggest that morningness, sleep regularity and lower values of social jetlag were positive indicators for better sleep quality in medical students at UNICAMP.</p> <p>Keywords: Sleep, Chronotype, Social Jetlag, student, physical activity and sleep quality.</p> <p>CAAE: 71026723.0.0000.5404</p>
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Title	Exploring the prevalence of neuromyths among undergraduate students and its relation to previous exposure to neuroscience
Authors	Ana Julia Ribeiro Victoria Marcella Macedo Martins Janine Albuquerque Nogueira Miguel Garcia Rufino Vitória Ferrari Shiroma Fernando Eduardo Padovan-Neto
Affiliations	Laboratory of Neuropsychopharmacology of Neurodegenerative Diseases, Faculty of Philosophy, Sciences and Letters of Ribeirão Preto, University of São Paulo (USP), Ribeirão Preto, Brazil.
Session	Poster

Abstract, Ethics Committee Number*, and Keywords	<p>Neuromyths are misconceptions about the functioning of the Central Nervous System, resulting from misinterpretation of facts established by the scientific community. Neuromyths are widespread in education, among teachers and students. Therefore, dissemination of truthful information and demystify neuromyths must be promoted to develop effective educational practices. This study aims to explore the prevalence of belief in neuromyths and general neuroscience knowledge among undergraduate students. Data was collected via online surveys from 412 undergraduates. Participants completed surveys on sociodemographics, prior exposure to neuroscience, and took general neuroscience knowledge and neuromyths questionnaires. The quantitative data was analysed using Just Another Statistic Program (JASP 0.18.1.0) software, with a 5% alpha significance level. The participants were aged 18-68 years, with 67% identifying as female, 32% as male, and 1 % as other genders. As a result, 85% agreed that understanding neuroscience is essential for their careers and 89% recognized the importance of dialogue between students and neuroscientists. Furthermore, 51 % reported that they had not taken neuroscience courses during their undergraduate studies, 80% did not attend any courses related to the field, and 34% did not receive neuroscientific information from their academic institutions. Those with more comprehensive exposure scored significantly higher on the general neuroscience knowledge questionnaire ($F(3,387) = 9,91, p < .001$). However, exposure to neuroscience had no impact on performance in the neuromyth questionnaire, regardless of the type of exposure ($F(3,387) = 1,91, p = 0,13$). The results suggest that greater exposure to neuroscience is associated with better comprehension of general concepts, but it does not reduce belief in neuromyths, highlighting the importance of spreading appropriate scientific information and improving communication between education and neuroscience.</p> <p>*Research Ethics Committee of the Faculty of Philosophy, Sciences and Letters of Ribeirão Preto, University of São Paulo (FFCLRP/USP; CAAE 74633023.0.0000.5407).</p> <p>keywords: neuromyths; educational neuroscience; undergraduate; students; predictors.</p>
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Title	Finger Tapping Test of two samples with different lifestyles
Authors	Laenna Morgana Cunha da Silva ¹ Victor Matheus Silva de Miranda ¹ Felipe André da Costa Brito ² Givago da Silva Souza ² Eliza Maria da Costa Brito Lacerda ¹
Affiliations	1 – Universidade Federal do Oeste do Pará, Santarém, Brazil 2 – Universidade Federal do Pará, Belém, Brazil
Session	Neurociência

Abstract, Ethics Committee Number 6.036.494, and Keywords	<p>Lifestyle influences people to engage in activities that train different movements, muscles, and skills. This factor could potentially affect the results of more sensitive motor analyses. The aim of the study was to evaluate the results of the Finger Tapping Test application for two samples with different lifestyles. The study was approved by the Human Research Ethics Committee of UFPA (#6.036.494). A cross-sectional observational study was conducted with 2 age and gender-matched groups totaling 84 healthy individuals (52 female). One sample of 42 individuals was collected in Belém, the capital city of the state, in the northeastern region of Pará (population 1,303,403), and another sample of 42 individuals was collected in Santarém, an inland city in the western region of Pará (population 306,480). For motor analysis, the Finger Tapping Test tool from the Momentum Touch platform, protocol #1 (https://acesse.one/momentumtouch), was used. To conduct the test, a mobile phone device was positioned horizontally on a flat platform located in front of the research participant. A square was displayed in the center of the screen, and the participant was instructed to tap the center of the square continuously with the index finger of their dominant hand as quickly as possible for 30 seconds. D'Agostino test, T-test, and Mann-Whitney test (alpha = 0.05) were used for statistical analysis. The results show that both samples did not differ significantly in terms of age (p=0.5000) and minimum interval (p=0.2330). The Belém sample had higher values than Santarém for the variables: ellipse area (p=0.0054) and number of taps (p=0.0371). And the Santarém sample had higher values than Belém for the variables: mean intervals (p=0.0311), standard deviation of intervals (p=0.0024), and maximum interval (p=0.0071). The conclusion of the study indicates that there is a difference in the Finger Tapping Test results for two samples with different lifestyles.</p> <p>Keyword: Movement analysis; Tapping test; Finger movement.</p>
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Title	Long COVID syndrome in skeletal muscle. Case reports.
Authors	Emilly Sigoli; Rosangela A. Antão; Sarah C. G. de Melo; Giovana Bernardes; Wladimir R. Beck; Anabelle S. Cornachione.
Affiliations	Department of Physiological Sciences, Federal University of São Carlos (UFSCar), São Carlos, São Paulo, Brazil.
Session	Neuroscience

Abstract, Ethics Committee Number*, and Keywords	<p>COVID-19 is a multiorgan infection that affects the musculoskeletal system of individuals infected with the SARS-CoV-2 virus, causing fatigue, arthralgia, myalgia, and muscle weakness. The pandemic has negatively affected the lives of millions of people in recent years, causing many individuals who have recovered from the disease to develop new and persistent symptoms; The term to define these symptoms that last weeks to months after infection is Long COVID syndrome. Our objective here was to evaluate the answers provided by an exclusive online questionnaire available in the Brazil, focusing on identifying the main persistent musculoskeletal symptoms in individuals with long-lasting COVID-19. A questionnaire entitled “Research on the effect of COVID-19 in skeletal muscles” was developed and applied on an online platform and shared among the population through social networks, radio and websites. Of the total number of participants (n=500) who completed the form, n=423 tested positive for Covid-19 and are part of this study. Descriptive analysis of the data was carried out and presented as absolute (n) and relative frequency (%). A prevalence of persistent musculoskeletal symptoms was observed, where we highlight the reported difficulty in returning to daily activities and intolerance to physical exercise, as well as the prevalence of fatigue, muscle weakness, and myalgia in individuals who do not practice physical exercise. Long-COVID is a major health problem worldwide, and individuals suffering from persistent symptoms face great difficulty in obtaining adequate evaluation and treatment. This study may contribute to a better understanding of the symptoms and effects of the infection on skeletal muscle after the acute phase. This is a study that was approved by the Ethics Committee of the Federal University of São Carlos (UFSCar) (CAAE: 39913520.3.0000.5504).</p> <p>Keywords: Long COVID syndrome; muscle symptoms; case reports.</p>
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Title	Terapia de exposição através de realidade virtual: conciliando princípios e tratamento da dependência do álcool
Authors	Kaê Leopoldo ^{1,2} João Maurício Castaldelli-Maia ^{1,2,3}
Affiliations	1 – Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo 2 – Instituto HC-Perdizes 3 – Centro Universitário FMABC
Session	BRAVO, Neurociências

Abstract, Ethics Committee Number*, and Keywords	<p>Introdução: Mais de 3 milhões de mortes são atribuíveis ao consumo de álcool por ano. Dentre os agravos, compõem parte considerável os que são 100% atribuíveis ao álcool, como uso abusivo, intoxicação e síndrome de dependência. O impacto destes transtornos relacionados ao uso de álcool se dá não apenas em aumento do número de óbitos, mas também em sua contribuição para anos vividos com incapacidade, ambos deletérios para a saúde pública e bem-estar econômico da população. Dentre os tratamentos para estes transtornos, a terapia de exposição através de realidade virtual (VR-CET) vem se mostrando promissora.</p> <p>Objetivos: Aplicação de VR-CET em pacientes com dependência de álcool (aprovação ética CAAE: 65163822.9.0000.0068), mensurando aceitabilidade, viabilidade, motivação para continuar tratamento, níveis de fissura e ansiedade relativa a estímulos associados ao uso de álcool.</p> <p>Métodos: 15 pacientes diagnosticados com síndrome de dependência de álcool (CID F10.2) passaram por uma sessão de VR-CET. Aplicação de escalas visual-analógicas (EVAs) para mensurar relatos de aceitabilidade, motivação, fissura e ansiedade. Aplicação da escalas SOCRATES para mensurar propensão para mudança de tratamento.</p> <p>Resultados: Pacientes apresentaram níveis de fissura e ansiedade acima da linha basal; demonstraram satisfação com a intervenção e alta motivação para continuar tratamento do tipo. VR-CET é viável para o tratamento de transtornos associados ao uso de álcool. Por fim, salienta-se que uma nova etapa do estudo, analisando efetividade e efeitos de ambientes de socialização, está em andamento no HC-FMUSP.</p>
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Title	Contribution of ALDA-1, activator of enzyme aldehyde dehydrogenase 2, in the progression of Amyotrophic lateral sclerosis in <i>SOD1*G93A</i> mice
Authors	Bárbara N. Krum <u>Luana P. da Silva</u> Luiz R. G. Bechara Isabela V. Boas Lisley S. Ramalho Julio C. B. Ferreira
Affiliations	Institute of Biomedical Sciences - University of São Paulo, São Paulo, Brazil
Session	40 - Neuroscience

Abstract, Ethics Committee Number*, and Keywords	<p>Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease in which reactive aldehydes have a critical role. The enzyme aldehyde dehydrogenase 2 (ALDH2) is associated with eliminating these aldehydes. We investigated the role of ALDH2 on ALS progression by testing a selective ALDH2 activator (AD6626). Wild type and <i>SOD1*G93A</i> mice were treated with either vehicle (VEH - water) or 40 mg/kg of AD6626– n=8-10 per group. Behavioral tests such as Wire hang and Open Field were performed. When comparing <i>SOD1*G93A</i> -VEH X <i>SOD1*G93A</i> -AD6626, ANOVA showed that animals treated with AD6626 stayed longer on Wire hang ($F(33, 252) = 4.371$) and less immobile on Open field ($F(3, 31) = 10.96$). After those tests, the animals were euthanized and its muscles were weighed. Independent of their treatment, there was a decrease in Tibialis anterior (TA) ($F(3, 29) = 55.20$), plantaris ($F(3, 29) = 13.47$; $F(3, 252) = 45.54$) and gastrocnemius ($F(3, 29) = 31.23$) mass in ALS mice when compared to WT mice. We also examined ALDH2 activity and found a decrease in soleus muscle of <i>SOD1*G93A</i> -VEH mice compared to WT-VEH mice. The compound prevented this decrease ($F(3, 28) = 5.474$). Also, we performed the skeletal muscle contractility <i>ex vivo</i>. <i>SOD1*G93A</i> -VEH mice showed a decrease at maximum specific force in soleus ($F(3, 21) = 20.0$) and in EDL muscles ($F(3, 21) = 20.03$). We also carried out histological analysis and evaluated neuromuscular junctions (NMJs), in which an increase of fibrosis in TA muscle in <i>SOD1*G93A</i> -VEH mice when compared to WT mice was noticed; AD6626 was able to prevent that effect in ALS mice ($F(3, 12) = 10.24$), and we found no difference in the section area of TA among the groups. Lastly, a decrease in AChR perimeter of the NMJs in <i>SOD1*G93A</i> -VEH mice when compared to all other groups ($F(3, 189) = 5.016$) was found. Our findings suggest that compound AD6626 seems to contribute to the improvement of some behavioral, biochemical, and histological parameters in ALS.</p> <p>Funding: FAPESP 2021/10692-3 Number of the ethic committee (Comissão de ética do uso de animais do Instituto de Ciências Biomédicas da Unviersidade de São Paulo): 3870190721 Keywords: Therapy; Detoxification; Redox metabolism; Neurodegeneration; Mitochondria;</p>
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Title	Impacts of Social Stress and Cafeteria Diet on Depressive Symptoms and Chronic Pain
Authors	Yasmin Cristina Nunes ¹ Leandro das Neves Bernardi ¹ Marco Oreste Finocchio Pagliusi Jr. ² Cesar Renato Sartori ¹
Affiliations	1 – Department of Functional and Structural Biology, State University of Campinas, Campinas, Brazil 2 – Department of Pharmacology, University of São Paulo, Ribeirão Preto, Brazil
Session	34 - Neurociência

Abstract, Ethics Committee Number*, and Keywords	<p>Depression and chronic pain often co-occur in clinical context, sharing behavioral characteristics and underlying neuroplasticity mechanisms, alongside chronic low-grade inflammation. The interaction between genetic predisposition and environmental factors is crucial in chronic disease development. In this context, stress and dietary habits have emerged as significant environmental contributors, implicated in emotional disorders and chronic pain. However, the impact of an unhealthy diet combined with stress on the severity and chronicity of depressive symptoms and pain remains unclear. In this study, we aimed to explore the effect of the association between social stress and a cafeteria diet on depressive-like behaviors and nociception in mice. For this purpose, male mice were subjected to chronic social defeat stress or to a cafeteria diet, or a combination of both conditions. Following the stress and dietary protocols, depressive-like behaviors were assessed using forced swimming and social interaction tests, while mechanical hyperalgesia was measured via the electronic Von Frey test. Behavioral evaluations were conducted three times over five weeks to assess the persistence of behavioral changes. Our findings indicated that both stress and the cafeteria diet induced persistent mechanical hyperalgesia. Notably, social interest, as measured by the social interaction test, was impacted by stress exposure, whereas behavioral despair, assessed during the forced swimming test, was induced by the cafeteria diet. These results suggest that chronic social stress and the cafeteria diet are potential risk factors for the development of chronic conditions such as depression and pain. Additionally, these environmental stimuli appear to elicit distinct depressive symptoms.</p> <p>Ethics Committee: 5707-1/2021</p> <p>Keywords: Social Defeat Stress, Cafeteria Diet, Depression, Chronic Pain</p>
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Title	Intracellular peptides demonstrate neuroprotective activity in a Parkinson's disease model
Authors	Louise Oliveira Fiametti ¹ Camilla Alves Franco ² Leandro Mantovani de Castro ³ Norival Alves Santos Filho ^{1,2}
Affiliations	1 – School of Pharmaceutical Sciences, UNESP Araraquara, Brazil 2 – Institute of Chemistry, UNESP Araraquara, Brazil 3 – Institute of Biosciences, UNESP São Vicente, Brazil
Session	Neuroscience

Abstract, Ethics Committee Number*, and Keywords	<p>Parkinson's disease (PD) is the second most common neurodegenerative disease in the world, but despite being highlighted in recent research, its cause remains unknown, there is no cure and the treatments available aim only to control the symptoms. Few studies focus on the role of peptides in PD, which have been shown to play a biological role in other neurodegenerative diseases. A previous study of our group (Fiametti <i>et al.</i>, 2021) demonstrated that 9 of 125 zebrafish brain intracellular peptides were altered in a model of 6-hydroxydopamine (6-OHDA). In this context, this study aims to better understand the role of 5 of these 9 peptides. The choice of peptides was based on their precursor proteins: 1) fatty acid protein 7; 2) mitochondrial ribosomal protein S36; 3) MARCKS-related protein 1-B; 4) Excitatory amino acid transporter 2 (EAAT2); and 5) Thymosin beta-4 (Tbeta4). Peptides were chemically synthesized in solid phase using Fmoc chemistry and had the correct synthesis confirmed by mass spectrometry. Zebrafish (<i>Danio rerio</i>) larvae at 4 dpf were treated with 750 µM 6-OHDA, incubated for 24 h, then at 5 dpf were treated with each peptide in different concentrations and incubated for another 24 h. On the 6th dpf, each larva was filmed for 5 minutes to record its swimming behavior, then each video was analyzed by a software (ToxTrac 2024). The parameters observed were mean speed and distance traveled, which were analyzed by one-way ANOVA test. Of the 25 groups studied (5 peptides in 5 concentrations each), 10 showed statistical significance ($p < 0.05$) in comparison with the 6-OHDA-treated larvae in both mean speed and distance traveled. The results suggest that the five peptides have neuroprotective properties and help in the recovery of neuromotor functions in the 6-OHDA Parkinson's disease model.</p> <p>Committee Number: 06/2023 (CEUA of IB/CLP UNESP)</p> <p>Keywords: Parkinson's disease; Intracellular peptides; Zebrafish</p>
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Title	Modulation of energy balance and hypothalamic inflammation by pharmacological activation of GPR139 with TAK-041
Authors	Ester dos Santos Alves ^{1,2} Gabriela Batitucci ^{1,2} Nicolly Porto Marin ^{1,3} Igor Vinicius Sousa Cavaleiro ^{1,4} Alexia Guimarães Batista Augusto ^{1,4} Natália Ferreira Mendes ^{1,3} Licio Augusto Velloso ^{1,2}
Affiliations	¹ Laboratory of Cell Signaling, Obesity and Comorbidities Research Center, University of Campinas, Brazil; ² School of Medical Sciences, Department of Medical Pathophysiology, University of Campinas, Brazil; ³ School of Medical Sciences, Department of Translational Medicine, University of Campinas, Brazil; ⁴ University of São Francisco, Brazil.
Session	Oral

Abstract, Ethics Committee Number*, and Keywords	<p>The hypothalamus regulates energy balance. Excessive intake of saturated fat triggers an inflammatory response in the hypothalamus, affecting eating and energy expenditure. Global obesity rates are on the rise, leading to the search for new treatments. Inhibition of GPR139 in the hypothalamus of rodents has been shown to reduce obesity. The development of the selective GPR139 agonist, TAK-041, offers a promising therapeutic approach for obesity and its associated conditions. This study evaluated the effect of TAK-041 on energy balance in mice. Here we employed a diet-induced obesity model, where 6-week-old male C57BL/6J mice fed a high-fat diet for 6 weeks. Then, these mice were divided into four groups (n=3-5): TAK-041, which was treated orally (3mg/kg/day), semaglutide, treated intraperitoneally (0.5mcg/g/week), combination therapy (TAK+SEMA) for 4 weeks, and the vehicle. Glycemic metabolism was assessed by glucose tolerance test, hypothalamic gene profile by RT-qPCR, and energy expenditure by indirect calorimetry. The Ethics Committee on the Use of Animals of the University of Campinas approved the experimental protocols (6273-1/2023). Our data indicate that the TAK+SEMA group showed reduced body weight and adiposity, alongside lower food intake and higher energy expenditure. This was supported by decreased hypothalamic gene expression of <i>nlrp3</i>, <i>tlr4</i>, and <i>cx3cr1</i>. Conversely, treatment with TAK-041 alone increased glucose tolerance compared to semaglutide or combined therapy, without affecting body mass. Additionally, the TAK-041 group exhibited increased expression of hypothalamic mRNA of <i>Il-6</i>. Taken together, activating of GPR139 by TAK-041 modulates body mass and glucose metabolism, and regulates hypothalamic gene expression of genes involved in high-fat diet-related inflammation and immune activation. Combining TAK-041 with semaglutide enhances these effects, showing promise for treating obesity and its complications.</p> <p>Keywords: GPR139; hypothalamus; obesity.</p>
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Title	The chronic mild stress during gestation can impair sex-specific behavior in elderly offspring
Authors	Amanda Cristina de Souza ¹ , Vinicius Schiavinatto Mariano ¹ , Gabriela Ninin de Carvalho ¹ , Isabele Cristina Gobo Tibiriça ¹ , José Antonio Rocha Gontijo ¹ , Patrícia Aline Boer ¹ .
Affiliations	¹ School of Medical Sciences, State University of Campinas, SP, Brasil.
Session	DOHAD e Fatores Psiconeurais

Abstract, Ethics Committee Number*, and Keywords	<p>Previously our group showed that gestational stress resulted in male and female middle-aged offspring presenting reduced long-term and social memory, anhedonia, and depressive-like behavior. Now, this study aims to evaluate the consequence of chronic mild stress in elderly female and male offspring behavior. (5774-1/2021). The pregnant mice were distributed into control (C) and stress (S) groups from the 6th to 21st gestation day. We applied sucrose preference, open field, elevated plus maze, object recognition, and social interaction test starting at 18 months of life. The S females presented lower sucrose preference [63±7 vs 69±9; p= 0,04]. In the open field test, the female had similar behavior, and the male traveled larger distance (11760±3424 vs 7264±2579; p=0,004) and enhanced activity time (122±20 vs 86±38; p=0,01), outpatient movement (124±27 vs 71±40; p= 0,003), velocity (39±11 vs 24±9; p=0,004), standing time (7,5±5 vs 3±2,7; p= 0,03), jumps (2,5±2 vs 0,9±0,8; p=0,02) and clock movements (6,5±3 vs 3±2; p=0,02), and lower rest time (197±24 vs 224±24; p=0,02). In the elevated plus maze, the S female had similar behavior, and males spent greater time in the open arms (133±35 vs 97±31; p=0,02) than in the close arms. The S female spent more (42±17 vs 14±12; p=0,0003) and the male spent less(37 ±18 vs 63±18; p=0,01) time on the novel object than C, during the long-term memory test. In conclusion we found that exposure to gestational stress modulated the behavioral response in a gender- and age-dependent manner. In females, anhedonia presented in middle age began to express itself as depression-like in old age, and despite the memory loss previously observed, in old age this memory becomes better than that of controls . On the other hand, males who were depressed-like in middle age began to exhibit hyperactive-like and impulsive-like behavior, and the long-term memory loss seen in middle age is maintained into old age.</p> <p><i>Keywords:</i> Gestational stress, Fetal Programming, Behavioral tests, Mother and Offspring behavioral changes</p>
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FRONTEIRAS DAS CIÊNCIAS BIOMÉDICAS E SEUS DESAFIOS

Title	Sex-specific effects of high-fat diet on hypothalamic chemokine expression
Authors	^{1,3} Alexia Guimarães Batista Augusto. ^{1,3} Igor Vinicius Sousa Cavalheiro. ^{2,3} Ester dos Santos Alves. ³ Jonathan Ferreira Campos. ^{3,4} Nicolly Porto Marin. ^{2,3} Licio Augusto Velloso. ^{3,4} Natalia Ferreira Mendes.
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Session	Session 35 (Neuroscience)

Abstract, Ethics Committee Number*, and Keywords	A high-fat diet (HFD) triggers hypothalamic inflammation, releasing proinflammatory cytokines and chemokines. Recent data from our group highlights sex-based differences in chemokine signaling during diet-induced obesity (DIO). While most studies have centered on male mice, the dynamic alterations in hypothalamic chemokine signaling in response to high-fat diet (HFD) exposure in female mice have yet to be investigated. C57BL/6J female mice were divided into SD and HFD groups (short-term: 1 or 3 days; long-term: 7, 14, or 28 days). Weekly body mass recordings and fasting glycemia measurements on the final day were conducted. Hypothalamic gene expression analysis, H&E staining of white and brown adipose tissues and liver, and blood sample analyses were performed. The Committee on Ethics in Animal Use (CEUA) of the University of Campinas approved the experimental protocols (CEUA 6312-1/2023). Long-term HFD groups showed increased body and fat mass, with fasting glycemia rising only in the 3-day HFD group. H&E staining revealed enlarged adipocytes in white adipose tissue (WAT) across HFD-fed groups. Brown adipose tissue (BAT) whitening was also observed across HFD-fed groups, but it was prominent in short-term and 14-day HFD groups. Liver fat accumulation occurred mainly in long-term HFD groups. Blood samples showed elevated plasma total cholesterol (CT) levels in HFD-fed groups (except 7-day HFD). Serum triglycerides initially rose in the 1-day HFD group but normalized later. Hypothalamic gene expression analysis showed dynamic regulation of chemokines and their receptors, with initial increases followed by decreases in long-term HFD groups. Inflammatory gene <i>Tnfa</i> decreased in all
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FRONTEIRAS DAS CIÊNCIAS BIOMÉDICAS E SEUS DESAFIOS

HFD groups, while interleukins II6 and II1b exhibited opposing trends. Our findings suggest that the female hypothalamus responds dynamically to an HFD, with rapid activation of chemokine signaling and immune cell recruitment potentially mitigating diet-induced inflammation.

Keywords: chemokines; sex dimorphism; hypothalamus; inflammation.



Title	The neutrophil-lymphocyte ratio as biomarker of the risk of death in severe cases of COVID-19
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Session	Neurociência

Abstract, Ethics Committee Number*, and Keywords	<p>The identification of clinical characteristics, comorbid conditions and risk factors of SARS-CoV-2 infection are important to predict the progression to severe forms of the disease among hospitalized individuals and to enable timely interventions to prevent poorer outcomes. The neutrophil-lymphocyte ratio (NLR) is one possible biomarker, since blood count is a low-cost exam performed in hospitalized patients with COVID-19, and it has been associated with mortality in patients with cardiovascular and infectious diseases. The aim of present work was to assess the possible role of the neutrophil/lymphocyte ratio (NLR) as a biomarker of the risk of death in patients with comorbidities hospitalized with COVID-19 in a tertiary hospital in southern Brazil. A prospective cohort study with patients with SARS-CoV-2 infection admitted to a hospital in the metropolitan region of Porto Alegre from September 2020 to March 2022 (approved by the Ethics Committee of Research of Grupo Hospitalar Conceição, # 4.280.802). The sample consisted of 185 patients with associated comorbidities, namely hypertension, diabetes mellitus, obesity, cardiovascular, pulmonary, and renal diseases, hospitalized for COVID-19. Of these, 78 died and 107 were discharged alive. The mean age was 66.5 years for the group that died and 60.1 years for the group discharged. Patients that evolved to death showed higher values of troponin, urea, creatinine, potassium and NLR. Surprisingly, there were no differences in values of O₂ partial pressure, serum leukocytes, d-dimer, HCO₃ and pH between the groups. Statistical analysis revealed that a difference greater than or equal to 1.55 in the NLR, from the hospitalization to the 5th day, was associated with a relative risk of death greater than 2. It is concluded that measuring a simple inflammatory marker such as NLR may improve the risk stratification of comorbid patients with COVID-19, and this variable can be a useful severity progression biomarker.</p> <p>Keywords: COVID-19; SARS-CoV-2; comorbidities; biomarkers; neutrophil-lymphocyte ratio; risk of death</p>
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Title	Oleylethanolamide effect on glyceimic metabolism in Alzheimer's disease models
Authors	Caroline Stéfani Plank ¹ Camila Fonseca Balcewicz ¹ Camila Maria Coltri ¹ Maria Júlia Todero ¹ Gabriela Cardoso Posteraro ¹ Eloisa Dai ¹ Fernanda Vitória Leimann ² Rafael Porto Ineu ² Odinei Hess Golçalves ³ Vanessa Ceglarek ⁴ Jean Franciesco Vettorazzi ⁵ Sara Cristina Sagae Schneider ¹
Affiliations	1 – Universidade Estadual do Paraná, Cascavel, Brazil 2 – Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil 3 – Universidade Tecnológica Federal do Paraná, Campo Mourão, Brazil 4 – Universidade Tecnológica Federal do Paraná, Medianeira, Brazil 5 – Universidade Federal da Integração Latino-Americana, Foz do Iguaçu, Brazil
Session	34 - Neuroscience

Abstract, Ethics Committee Number*, and Keywords	<p>Metabolic alterations such as insulin resistance (IR) and type 2 diabetes mellitus (DM2) are risk factors for the development of Alzheimer's disease (AD). In this context, oleylethanolamide (OEA), capable of reversing IR and DM2, is a potential drug for AD treatment and a target in the glycaemic metabolism of streptozotocin (STZ)-induced AD models. This research was approved by the Ethics Committee on Animal Use of the State University of West Paraná (20-21). Male C57BL/6 adult mice aged 60-90 days were used. The animals were separated into groups: CTL (animals that received saline solution, n = 8), ALZ (animals that received 5µL of intracerebroventricular STZ, n = 5), and ALZ-OEA group (animals that received STZ and, two hours later, 20 mg/kg of intraperitoneal OEA). After 17 days, the animals underwent an oral glucose tolerance test (ipGTT), an insulin tolerance test (ipITT) and body weight was assessed during the experimental period. Hepatic GLUT-2 protein expression was evaluated by Western Blot. Data were analysed using ANOVA with Tukey's post hoc ($p < 0.05$) or Kruskal-Wallis with Dunn's post hoc ($p < 0.05$). Blood glucose levels in the ALZ and ALZ-OEA groups were similar and higher than the CTL group, both in fed animals [$F(2, 9) = 22.74$; $p = 0.0003$] and fasted animals [$F(2, 10) = 7.346$; $p = 0.0109$]. There was no difference in ipGTT [$F(2, 10) = 0.6882$; $p = 0.5248$], ipITT [$F(2, 12) = 1.164$; $p = 0.3453$] and body weight (KW</p>
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= 1.014; $p = 0.6213$) among the groups. However, the rate of disappearance of plasma glucose (KITT) in the ALZ and ALZ-OEA groups was similar and higher than the CTL group [$F(2, 12) = 5.430$; $p = 0.0209$]. In conclusion, both DA induction and OEA treatment did not alter GLUT2 expression in hepatic tissue [$K = 0.6667$; $p = 0.7418$]. Therefore, it was observed that AD induction with STZ lead to glycaemic metabolism changes, with OEA treatment proving noneffective in the evaluated parameters.

Keywords: Dementia, oleoylethanolamide, metabolism.



Title	Altered hippocampal doublecortin expression in Parkinson's disease
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Session	Neuroscience

Abstract, Ethics Committee Number*, and Keywords	<p>Parkinson's disease (PD) is a complex neurodegenerative disorder characterized by motor and non-motor symptoms. Motor symptoms include bradykinesia, resting tremors, muscular rigidity, and postural instability, while non-motor symptoms include cognitive impairments, mood disturbances, sleep disturbances, autonomic dysfunction, and sensory abnormalities. Some of these symptoms may be influenced by the proper hippocampus functioning, including adult neurogenesis. Doublecortin (DCX) is a microtubule-associated protein that plays a pivotal role in the development and differentiation of migrating neurons. This study utilized postmortem human brain tissue of PD and age-matched control individuals to investigate DCX expression in the context of adult hippocampal neurogenesis. The use of human tissue throughout this study was in accordance with the Newcastle University Ethics Board (The Joint Ethics Committee of Newcastle and North Tyneside Health Authority, reference: 08/H0906/136). Our findings demonstrate a significant reduction in the number of DCX-expressing cells within the subgranular zone (SGZ), as well as a decrease in the nuclear area of these DCX-positive cells in postmortem brain tissue obtained from PD cases, suggesting an impairment in the adult hippocampal neurogenesis. Additionally, we found that the nuclear area of DCX-positive cells correlates with pH levels. In summary, we provide evidence supporting that the process of hippocampal adult neurogenesis is likely to be compromised in PD patients before cognitive dysfunction, shedding light on potential mechanisms</p>
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	<p>contributing to the neuropsychiatric symptoms observed in affected individuals. Understanding these mechanisms may offer novel insights into the pathophysiology of PD and possible therapeutic avenues.</p> <p>KEYWORDS</p> <p>adult hippocampal neurogenesis, doublecortin, human brain tissue, Parkinson's disease.</p>
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Title	Possible neuroprotective role of adropin in a Parkinson´s disease animal model
Authors	Livia Moreno Lemuchi Ana Flávia F. Ferreira Luiz Roberto G. Britto
Affiliations	Laboratory of Cellular Neurobiology. Department of Physiology and Biophysics. Institute of Biomedical Sciences, University of São Paulo. São Paulo, Brazil.
Session	34-Neurociência

Abstract, Ethics Committee Number*, and Keywords	<p>Parkinson´s disease (PD) is characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNc), followed by motor and non-motor symptoms. This disease has no cure and current treatments are limited, requiring the development of new therapies. A potential therapeutic peptide for PD is adropin, which, among many actions, activates the phosphatidylinositol-3-OH kinase and protein kinase Akt (PI3K/Akt) pathway, promoting anti-apoptosis effects by the inactivation of glycogen synthase kinase-3 (GSK3β). This study aims at evaluating the potential therapeutic effects promoted by the administration of adropin in a PD animal model, through behavioral tests (cylinder and apomorphine tests), and immunohistochemistry/immunoblotting techniques. The PD model was induced in 48 male Wistar rats by the injection of 6-hydroxy-dopamine (6-OHDA) into the right striatum (CPu), the main target of the SNc. We then injected 2,1 ug/kg/day of adropin intraperitoneally for 10 consecutive days. Preliminary analysis of the cilinder test and tirosine hydroxilase (TH) immunoblots of the CPu and SNc confirm that 6-OHDA caused damage to dopaminergic neurons. However, the lack of difference in the results of the cylinder test performed after treatment between the treated and untreated groups provides an inconclusive result regarding the action of adropin, since it is still unclear whether there is a neuroprotective effect masked by compensatory mechanisms or whether this effect is minimal. On the other hand, group averages in the TH expression analyses suggest a clear tendency of reduction in dopaminergic loss in the group treated with adropin. With the next analyses of the expression of the PI3K/Akt/GSK3β proteins, in addition to TH immunohistochemistry and apomorphine tests, we intend to clarify these questions and conclude whether adropin is indeed neuroprotective in this PD model.</p> <p>FAPESP (2023/05573-0) and CNPQ Ethics committee number: 6567270123 (CEUA-ICB/USP)</p> <p>Keywords: Parkinson's disease, 6-OHDA, adropin, neuroprotection</p>
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Title	AD16, a newly developed anti-inflammatory compound, reduces the impact of Parkinson-like neuropathology in mice
Authors	Maria Eduarda Bianchetti Goulart – BIANCHETTI, M. E. Ana Flávia Fernandes Ferreira – FERREIRA, A. F. F. Luiz Roberto Giorgetti de Britto – BRITTO, L. R.
Affiliations	Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil
Session	Neurociência

Abstract, Ethics Committee Number*, and Keywords	<p>Parkinson's Disease (PD) is a neurodegenerative condition characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNc) of the brain, manifesting itself with both motor and non-motor symptoms. A critical element of this pathology is neuroinflammation, which triggers a harmful neurotoxic cycle, exacerbating cell death within the central nervous system. AD16 is a recently identified compound capable of reducing the expression of pro-inflammatory cytokines while increasing the expression of anti-inflammatory cytokines in Alzheimer's Disease models. Consequently, this study sought to comprehend the potential impact of orally administered AD16 in mitigating neurodegeneration and subsequent disease progression in PD. To accomplish this, 6-hydroxy-dopamine (6-OHDA) unilateral striatal injections were employed to induce a PD model in male C57BL/6 mice. Cylinder and apomorphine-induced rotation behavioral tests were conducted to assess motor behavior, and immunohistochemistry techniques were performed to detect tyrosine hydroxylase (TH) and thus label dopaminergic neurons. All animals and procedures were endorsed by the institutional ethics committee in accordance with protocol #6567270123. Results from behavioral tests revealed an enhancement in the motor function of the AD16-treated animals, demonstrated by a higher utilization of the contralateral limb compared to the vehicle-treated group in the cylinder test ($p < 0.0001$; $+43 \pm 0.9\%$), and a reduced number of contralateral apomorphine-induced rotations ($p < 0.0001$; 42 ± 6). Results from immunohistochemistry assays demonstrated an increase in the number of TH-positive cells in the SNc ($p = 0.0006$; $+102 \pm 8\%$) and in the density of TH-positive terminals in the striatum ($p < 0.0001$; $+0,95 \pm 0.5$). Consequently, AD16 emerges as a compound with significant potential for negative modulation of neurodegeneration in the 6-OHDA animal model. of Parkinson's Disease.</p> <p>Keywords: Parkinson's Disease, 6-OHDA, neuroprotection, AD16, neurodegeneration</p> <p>The present study is funded by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) – process #2023/05618-4</p>
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Title	Maternal consumption of the pesticide pyriproxyfen in the gestational period in rats: effects on the motor performance and in the number of dendritic spines in the primary motor cortex of the offspring
Authors	Matheus Henrique Cassias de Lima ¹ Katriane Endiel Pereira ² Rafaela Schelbauer ³ Nicole Jansen Rabello ³ Gabrielle Batista de Aguiar ² Estela Soares Carniel ³ Rafaela Maria Moresco ² Lígia Aline Centenaro ³
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Session	Neurociência

Abstract, Ethics Committee Number*, and Keywords	<p>Pyriproxyfen is a pesticide used in Brazil to control the <i>Aedes aegypti</i> mosquito. However, studies indicate that pyriproxyfen has a molecular structure similar to retinoic acid, a biomolecule derived from vitamin A that regulates neuronal differentiation and hindbrain development during the embryonic period. In this context, this study aimed to evaluate if maternal consumption of pyriproxyfen during pregnancy produce deleterious effects on motor function and in the number of dendritic spines in the primary motor cortex (M1) of the offspring.</p> <p>This study was approved by the Ethics Committee on Animal Use of UNIOESTE (No. N° 15-19 – CEUA, 05/07/2019). Wistar rats were divided into three experimental groups: Negative Control (CT-, n=6) - offspring of rats that drunk potable water during pregnancy; Pyriproxyfen (PIR, n=6) - offspring of rats exposed to Sumilarv® prenatally, a pesticide that has pyriproxyfen as active ingredient and Positive Control (CT+, n=6) - offspring of rats exposed to an excess of vitamin A prenatally. Regarding motor function, the total locomotion time decreased only in the CT+ group (167.3±18.6) compared to the PIR (190.8±20.3) and CT- (231.1±16.0) groups. The frequency of rearing and grooming was similar between the studied groups. In pyramidal neurons of the M1 cortex, the number of dendritic spines in dendrites secondary to the apical was lower in the PIR and CT+ groups (6.5±0.5; 5.4±0.5, respectively) compared to the CT- group (13.9±0.7). There was also a reduction in the number of dendritic spines in the basal dendrites of these neurons in the PIR (3.8±0.2) and CT+ (4.3±0.3) groups compared to the CT- group (7.0±0.4). Thus, pyriproxyfen seems to produce deleterious effects on fetal development that are similar to those caused by the excess of vitamin A, but with less toxicity. The findings of this study point to the need for careful use of this pesticide and the search for alternative methods to control the <i>Aedes aegypti</i> mosquito.</p> <p>Keywords: <i>Aedes aegypti</i>, Teratogenesis, Retinoids, Motor function.</p>
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Title	Behavioral consequences of gestational stress in adult mice: influence of early enriched environment
Authors	Gabriela Ninin de Carvalho ¹ Amanda Cristina de Souza ¹ , Vinicius Schiavinatto Mariano ¹ , Isabele Cristina Gobo Tibiriça ¹ , Patrícia Aline Boer ¹ , José Antonio Rocha Gontijo ¹ .
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Session	DOHAD e Fatores Psiconeurais

Abstract, Ethics Committee Number*, and Keywords	<p>Maternal stress during pregnancy has harmful consequences for the health of the offspring and can alter cognitive, behavioral, and psychomotor development and is important to investigate strategies that can mitigate these effects. The enriched environment (E) has been shown to benefit a variety of neural functions such as improving learning and memory. Thus, our objective was to evaluate the behavior of adult male offspring subjected to gestational stress and exposure to E. The study was approved by the ethics committee (5788-1/2021). Pregnant C57BL/6J mice were divided into two groups: One subjected to the unpredictable stress protocol (S) from the 6th to the 21st day and the other control (C). At 21 postnatal days, male pups were divided into two groups kept for 21 days in an environment: standard (CS and SS) or E (CE and SE). We applied sucrose preference tests, elevated plus maze, and open field tests starting at the 24th week of life. Only animals in the SS group showed a lower preference for sucrose (62 ± 7 vs 69 ± 10, $p=0.03$) but when exposed to the E there was no change concerning the control. Animals in groups SS, CE, and SE spent less time in the open arms compared to those in group C (C: 119 ± 30, SS: 83 ± 38, EC: 86 ± 25, SE: 55 ± 19). These results were corroborated by the open field test as these animals also spent less time in the center. Exposure to the E caused a reduction in the distance (D) and speed (V) only in control animals (D, C: 11801 ± 3640mm vs CE: 8518 ± 4104 mm, $p=0.01$. V, C: 39 ± 12mm/s vs 28 ± 14mm/s, $p=0.01$). Exposure to stress increased standing time (C: 5 ± 3 vs SS: 8 ± 3s, $p=0.04$; CE: 4 ± 3 vs SE: 7 ± 3s, $p=0.02$). The animals subjected to the E rested longer than the control. In conclusion, gestational stress caused depressive-like behavior that was extinguished by exposure to the E. However, both gestational stress and exposure to the E caused anxiety-like behavior in the animals, demonstrating that this is a limiting factor for this intervention.</p> <p><i>Keywords:</i> Gestational stress, Enriched environment, Anxiety and depressive, Behavior.</p>
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Title	Prenatal exposure to piriproxifem pesticide: impacts on gait and dendritic length in neurons of the primary motor cortex of rats
Authors	Rafaela Schelbauer ¹ Nicole Jansen Rabello ¹ Katriane Endiel Pereira ² Gabrielle Batista de Aguiar ² Estela Soares Carniel ¹ Marcelo Alves de Souza ³ Lígia Aline Centenaro ¹
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Session	Neurociência

Abstract, Ethics Committee Number*, and Keywords	<p>In 2014, pyriproxyfen-based pesticides began to be used in an unprecedented way in Brazil to control the <i>Aedes aegypti</i> mosquito. However, pyriproxyfen has a molecular structure like retinoic acid, a substance vitamin A derived, involved in the neurogenesis during the embryonic period. Due to this similarity, it is postulated that pyriproxyfen may have cross-reactivity with retinoid receptors, which may interfere with embryogenesis. In this context, this study evaluated the effects of prenatal exposure to Sumilarv (pesticide whose active ingredient is pyriproxyfen) on gait and dendritic length of the primary motor cortex (M1) neurons. The procedures were approved by the Ethics Committee on Animal Use of UNIOESTE (No. N^o 15-19 – CEUA, 05/07/2019). <i>Wistar</i> rats were divided into three experimental groups: Negative Control (CT-, n=6) - offspring of rats that drunk potable water during pregnancy; Pyriproxyfen (PIR, n=6) - offspring of rats exposed to Sumilarv® prenatally and Positive Control (CT+, n=6) – offspring of rats exposed to an excess of vitamin A prenatally. In relation to gait, no differences were found between the groups in the stride length (CT-: 11.1±0.1; PIR: 10.9±0.2; CT+: 11.6±0.2), support base width (CT-: 4.3±0.0; PIR: 4.3±0.0; CT+: 4.4±0.0) and hindlimb rotation (CT-: 4.3±0.0; PIR: 4.3±0.0; CT+ 4.4±0.0). The basal dendrites length of pyramidal neurons present in layer V of the M1 cortex was also similar among CT- (422.3±197.9), PIR (447.3±209.9) and CT+ (405.7±146.5) groups. These findings indicate that prenatal exposure to pyriproxyfen does not alter motor performance and dendritic length in the M1 cortex. However, previous studies showed that pyriproxyfen causes deleterious effects in the nervous system development based in other brain structure analysis. Thus, more studies are necessary to rule out or not a potential neurotoxic effect resulting from exposure to pyriproxyfen during the prenatal period.</p> <p>Keywords: Motor Function, <i>Aedes Aegypt</i>, Golgi method.</p>
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Title	Implications of prenatal exposure to the larvicide pyriproxyfen on motor function and primary motor cortex in rats
Authors	Rafaela Schelbauer ¹ Gabrielle Batista de Aguiar ² Nicole Jansen Rabello ¹ Katriane Endiel Pereira ² Estela Soares Carniel ¹ Rafaela Maria Moresco ¹ Marcelo Alves de Souza ³ Ligia Aline Centenaro ¹
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Session	Neurociência

Abstract, Ethics Committee Number*, and Keywords	<p>Pyriproxyfen, a pesticide used to control <i>Aedes aegypt</i> mosquitoes in Brazil, has a similar molecular structure to the retinoic acid, a biomolecule that regulates embryonic development. Due to this similarity, it is postulated that pyriproxyfen may have cross-reactivity with retinoid receptors, which may interfere with embryogenesis. In this context, this study evaluated whether the prenatal exposure to Sumilarv® (pesticide whose active ingredient is pyriproxyfen) causes motor deficits or alters cell counts in the primary motor cortex (M1) in rats. The procedures were approved by the Ethics Committee on Animal Use of UNIOESTE (No. N° 15-19 – CEUA, 05/07/2019). <i>Wistar</i> rats were divided into three experimental groups: Negative control (CT-, n=12) – offspring of rats that drink potable water during gestation; Positive control (CT+, n=11) - offspring of rats exposed to retinoic acid prenatally and Pyriproxyfen (PIR, n=15) - offspring of rats exposed to Sumilarv® prenatally. There were no differences between the studied groups in relation to gait quality, using the Basso-Beattie-Bresnahan scale (CT+ 19,92±0,58; CT- 21,00±0,0; PIR 20,57±0,30). However, one animal in the PIR group and another in the CT+ one performed external rotation with one of the hindlimbs during gait initiation, while one animal of the PIR group and four in the CT+ group showed forelimb-hindlimb coordination deficits during walking. A decrease in the number of neurons in the M1 cortex was observed only in the CT+ group (5,17±0,03), with no differences between PIR (5,36±0,03) e CT- (5,34±0,03), while the number of glial cells was similar between the three experimental groups (CT- 1,21±0,02; PIR 1,21±0,01; CT+ 1,19±0,01). This pesticide seems to produce deleterious effects during embrionary period that are</p>
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similar to those caused by retinoic acid, but with less toxicity. The study highlights the necessity for alternative mosquito control methods and advises against using pyriproxyfen in drinking water.

Keywords: *Aedes aegypt*, Retinoic Acid, Teratogenesis.



Title	Physical exercise as a neuroprotective factor in a progressive model of reserpine-induced parkinsonism in rats
Authors	Gabriel Sousa da Rocha Marcelo Nunes da Silva Mylaine Santos Mendonça José Leandro Santos Souza Ana Cleia Alves da Luz Heitor Franco Santos José Carlos Júnio da Silva Lima Marco Aurélio de Moura Freire Katty Anne Amador de Lucena Medeiros Auderlan Mendonça de Gois José Ronaldo dos Santos
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Session	Neurociência

Abstract, Ethics Committee Number*, and Keywords	<p>Introduction: Physical exercise has been shown to promote neuroprotection in models of neurodegeneration. In this sense, the objective of the present study was to evaluate whether physical exercise acts as a neuroprotective factor in a progressive model of reserpine-induced parkinsonism (RES) in rats. Methods: 48 male Wistar rats were used, between 6 and 7 months of age, weighing an average of 350-450 grams, with authorization from the Animal Research Ethics Committee (CEPA) of the UFS (protocol number: 5483070122) . The animals were divided into 6 groups (n = 8 per group): 1- CTR-sedentary (CTR-SED), 2- RES-sedentary (RES-SED), 3- CTR-Exercise (CTR+EXE), 4- RES-Exercise-Previous (RES-EXE-PRE), 5- RES-Synchronous Exercise (RES-EXE-SIN) and RES-Exercise-Late (RES-EXE-TAR). The animals received 10 injections of vehicle (CTR groups) or RES 0.1 mg/kg s.c. (RES groups), one every 48h. On day 20, the rats were anesthetized, perfused, their brains were removed and subjected to immunohistochemistry for Tyrosine Hydroxylase (TH). The mean values for each group were normalized by the mean value of the control group. Results: The RES-SED (0,713 ± 0,218) group showed a lower number of TH-immunoreactive cells in the substantia nigra pars Compacta when compared to the CTR-SED (1,000 ± 0,237) and CTR-EXE (1,047 ± 0,330) groups. The group of animals that underwent physical exercise prior to treatment with RES (RES-EXE-PRE) (0,985 ± 0,175) did not show a statistically significant difference when compared to the CTR-SED and CTR-EXE groups. Synchronous physical exercise (RES-EXE-SIN) (0,699 ± 0,248) and delayed physical exercise (RES-EXE-TAR) (0,695 ±</p>
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0,270) were not able to avoid the reduction in the number of TH-immunoreactive cells caused by RES administration. **Conclusion:** Our study suggests that physical exercise promotes a neuroprotective effect, slowing the progression of RES-induced nigral degeneration in rats.

Keywords: Parkinson's disease, physical exercise, neuroprotection, dopamine



Title	The leading role of students with autism at IFSC-Gaspar in participating in events about autism
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Session	[34 - Neurociência]

Abstract, Ethics Committee Number*, and Keywords	<p>Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by difficulties in social interaction, restricted and repetitive behaviors and interests, according to the Diagnostic and Statistical Manual of Mental Disorders. Therefore, schools that understand the conditions of students with ASD are of fundamental importance for their effective success in learning. Therefore, the Federal Institute of Santa Catarina, Gaspar campus, through the Educational Accessibility Center promoted several opportunities where student protagonism was highlighted with the aim that these high school students understood their characteristics and also their potential as active subjects in society. Among the activities promoted by NAE for the public with ASD was the offer of an origami workshop by a student with ASD from the chemistry course for students on the technical IT course, both from high school, highlighting the importance of the algorithm in folding for later students to understand the steps of computer programming. The student replicated the same workshop during National Brain Week in the years 2023 and 2024. Students with ASD also participated, in April of this year, in the V LAND symposium: interface autism and ADHD held at the University of Extremo Sul Catarinense (UNESC) in the city of Criciúma. At the same event, the student who promoted the origami workshops participated in the art exhibition, exhibiting neurons made in crochet and various other foldings. At the end of the month of April, which is also known as ASD awareness month, the students helped promote a lecture aimed at teachers and students entitled Identification and assistance for people with ASD. All activities brought good results for the students, as they promoted self-knowledge and empathy on the part of their colleagues.</p> <p>Keywords: Autism Spectrum Disorder. Federal Institute of Santa Catarina. Educational Accessibility Center.</p>
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Title	ATP-dependent mechanism of low-glucose sensitivity in neurons from nucleus of the solitary tract from young rats
Authors	Zarpellon, P. S. B., Leão, R. M.
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Session	

Abstract, Ethics Committee Number*, and Keywords	<p>Introduction: Glucose is the primary energy source for the Central Nervous System (CNS). Nucleus of the solitary tract (NTS) in the brainstem express neurons that are sensitive to changes in extracellular glucose. We showed that a significant fraction of subpostremal NTS (spNTS) neurons from young rats depolarize in low glucose. Nevertheless, the specific mechanisms underlying this sensitivity remain unknown.</p> <p>Objective: We aim to investigate the mechanisms triggering the depolarization induced by low glucose in spNTS neurons.</p> <p>Methodology: We used the spNTS slice of Rats Wistar Hannover (males, 3 to 6 weeks). All animal procedures were approved by the Ethics Committee on the Use of Animals of the FMRP-USP (protocol No 189/2020). The Patch-Clamp was used to recording the neurons. Statistical analyses were performed using GraphPad Prism 8.0 software, with paired or unpaired two-tailed Student t-test and one-way ANOVA. Significant levels were established with $p < 0.05$. $n =$ neurons.</p> <p>Results: Majority of NTS neurons depolarized ($n=9$) and decreased input resistance (R_{input}) in low glucose (0.5mM). We investigate whether the effects of low glucose could be replicated by reducing mitochondrial ATP synthesis using an uncoupler agent CCCP (1 μM). CCCP replicated the effects of low glucose: membrane depolarization (10.1 ± 2.4 mV, $n=11$), input current increasing (-95.89 ± 9.53 pA at -75 mV, $n=5$), decreased R_{input} (-100 ± 42 MΩ, $n=9$). Na^+/K^+-ATPase inhibiting with ouabain 10 μM depolarized the membrane (13.63 ± 3.14 mV, $n=8$), but had no effect on R_{input} ($n=6$). We tested if the activation of AMPK by AICAR mimicked the low-glucose effects, but we did not observe significant effects on membrane potential.</p> <p>Conclusion: Perfusion of spNTS neurons with a low glucose solution result in membrane depolarization very likely caused by a decrease in metabolic ATP, as these effects are reproduced by CCCP. The depolarization does not seem to be caused by the activation of AMPK or a reduction pump activity.</p> <p>Funding: FAPESP, CNPq and CAPES.</p>
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Title	The impact of music on children's brain development: An integrative review
Authors	Aline Ananias de Lima ^{1,5} ; Leonilson Oliveira Silva Paz ² ; Karollainy Gomes da Silva ^{1,7} , Maria Eduarda Rodrigues Alves dos Santos ^{1,7} , Ana Patrícia da Silva Souza ^{1,7} , Ana Beatriz Januário da Silva ^{1,7} , José Maurício Lucas da Silva ⁷ , Mayara Luclécia da Silva ⁷ , Priscyla Evelyn da Silva Albuquerque ⁷ , Robson Feliciano da Silva ⁷ , Erica Helena Alves da Silva ⁷ , Antonietta Claudia Barbosa da Fonseca Carneiro ¹ , Waleska Maria Almeida Barros ^{6,7} , Rosana Christine Cavalcanti Ximenes ^{1,3} Sandra Lopes de Souza ^{1,4}
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Session	34 - Neuroscience

Abstract, Ethics Committee Number*, and Keywords	<p>Music plays a significant role in human lives and minds, influencing emotions and behavior. This impact is even more noticeable in children due to the intense brain plasticity at this stage. The ability of the infant brain to adapt to new experiences makes music a powerful tool for neuronal stimulation, providing fertile ground for child development. This is an integrative review study of the last five years, with no language restrictions. The databases used were PubMed and Science Direct, using the descriptors "Cerebrum", "Music" and "Child", with the Boolean operator AND (MeSH). Epidemiological studies exploring the impact of music on child development were included. Case studies, literature reviews and animal experiments were excluded, as were studies that were not directly related to the proposed topic. Out of a total of 67 articles found, 7 met the inclusion criteria for this study. Studies highlight the significant influence of music and sensory experiences on children's brain development. Evidence of neural plasticity is consistently observed, with musical training reshaping brain structures over time. Specific areas of the brain, such as the auditory cortex, the right inferior frontal gyrus and the posterior temporal cortex, emerge as protagonists in the complex cognitive activities associated with music perception, comprehension and</p>
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processing. Correlations between brain activity and musical abilities indicate a close relationship between keen musical perception and specific underlying brain processes. Children's brain development is strongly influenced by brain areas such as the right inferior frontal gyrus, the auditory cortex and the posterior temporal cortex, which play crucial roles in the analysis of complex structures and musical perception.

Keywords: Music; Brain; Child Development.



Title	Maternal high sucrose diet may impair offspring cognition and memory behavior through mechanisms mediated by fructose action within the hippocampus
Authors	Mayara N. Baqueiro ¹ , Suleyma O. Costa ¹ , Luciana C. P. Nascimento ¹ , Aline F. Campos ¹ , Maiara J. Silva ¹ , Lavinia V. G. Alves ¹ , Marcio A. Torsoni ¹ , Michael G. Ross ² ; Mina Desai ² ; Adriana S. Torsoni ¹ .
Affiliations	¹ School of Applied Sciences, University of Campinas (UNICAMP); Limeira, SP, Brazil ² Department of Obstetrics and Gynecology, David Geffen School of Medicine, University of California Los Angeles at Harbor-UCLA, Torrance, CA, USA
Session	Neurociência

Abstract, Ethics Committee Number*, and Keywords	<p>Sucrose is prevalent in the Western diet, and its excessive consumption is linked to neuroinflammation. Fructose, a component of sucrose, can disrupt metabolism, cognitive function, and hippocampal activity—an essential region for learning and memory. This study aimed to assess the effects of maternal high-sucrose diet on offspring memory and explore the potential influence of fructose treatment on the proliferation and differentiation of hippocampal cells. CEUA 5636-1/20. C57BL/6 female mice were fed a control diet (CT 10% fat, 7% sucrose n=6-9), high sucrose diet (HS 10% fat, 35% sucrose n=13-18), or high-fat diet (HF 45% fat, 17% sucrose n=5-6). The offspring of this females were weaned on control diet. At 12 weeks, the novel object recognition test (NOR) was performed. Neuroprogenitor cells (NPCs) were cultured from the hippocampus of 36 neonatal mice. NPCs were treated with glucose-free DMEM/F12 containing 5mM and 12.5mM D-Fructose for 48 hours. Regular medium with D-Glucose was used as a control. MTT was used to determine proliferation after treatment. Differentiation was induced for 7 days. Immunofluorescence was performed on differentiated NPCs. Female high-sucrose offspring (HS-O) exhibited increased distance covered and a trend towards an increased time to explore a novel object, compared to CT offspring (CT-O). The discrimination rate was lower in female from HS-O compared to CT-O while male high-fat offspring (HF-O) displayed a decreased discrimination rate compared to CT-O and HS-O. Exploration time of the familiar and novel object was shorter in female HS-O compared to CT-O. In NPCs, treatment with fructose (12.5mM) resulted in reduced proliferation, greater labeling of GFAP+ compared to NeuN+ and decrease in DNMT3a+. The findings suggest that maternal HS diet affects offspring memory. This effect may be attributed to fructose-induced changes such as increased glial cells at the expense of neurons and decreasing proteins associated with cognition.</p> <p>Keywords: Cognition, Fructose, Hippocampus, Memory, Sucrose.</p>
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Title	Melatonin absence alters cervical brown adipose tissue clock genes and thermogenic molecular machinery gene expression
Authors	Rafael Américo Pitanga dos Santos Caroline Aparecida Pereira de Souza Fernanda Akane Nishino Fernanda Gaspar do Amaral
Affiliations	Pineal Neurobiology Lab, Department of Physiology, Federal University of São Paulo
Session	Neurociência

Abstract, Ethics Committee Number*, and Keywords	<p>Pineal melatonin is shown to regulate interscapular brown adipose tissue (BAT) thermogenesis daily and seasonally. Cervical BAT (cBAT) is crucial for blood thermoregulation in the SNC due to its anatomical location. Although melatonin is shown to act as a photoperiod signal controlling the thermogenesis molecular machinery and the clock genes expression in interscapular BAT, there is a lack of information on cBAT. Control (C), Pinealectomized (Pinx), and Melatonin-replaced Pinealectomized (Pinx/Mel, daily 1mg/Kg in the drinking water) male Wistar rats (200-250g) were housed under a 12h/12h light/dark cycle with water and food <i>ad libitum</i>. cBAT samples were collected every 3h over 24h (n=4/time point/group), 13 weeks after surgery (CEUA-UNIFESP #8074220415). Real time polymerase chain reaction (PCR) was used to evaluate Clock (NM_021856.1), Bmal1 (NM_024362.2), Cry1 (NM_198750.2), Cry2 (NM_133405.1), Per1 (NM_001034125.1), Per2 (NM_031678. 1), Rev-Erba (NM_145775.1), Dbp (NM_012543.2), Adrβ3 (NM_013108.2), Dio2 (NM_031720.4), Ucp1 (NM_012682.2), G-actin (NM_001127449.1) and RPL37a (NM_001108801) gene expression. Statistical analyses were carried out using Prism v 6.01 software (p<0.05). Pinx animals cBAT showed altered daily rhythmicity of Clock, Per1, Per2, Cry1, Cry2, and Dbp gene expression. Bmal1 gene expression was not changed in comparison to C rats. Adrβ3, Dio2, and Ucp1 daily gene expressions were also disrupted in Pinx animals. The oral melatonin replacement timing used in the present experiment may have impaired adequate prevention of the rhythmic alterations, as melatonin was available in the drinking water for 14h over the dark phase. Further analyses are ongoing and may help foster a broader understanding of melatonin's role in controlling clock genes and the thermogenesis molecular machinery cBAT gene and protein expression. Keywords: Cervical Brown Adipose Tissue, Melatonin, Clock Genes, Thermogenesis, Rhythmicity.</p>
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XXXVIII REUNIÃO ANUAL DA FESBE
XXII REUNIÃO ANUAL DA BRAVO
XVIII CONGRESSO DA SBCAL
III CONGRESSO DOHAD BRASIL
II CONGRESSO DA SBBA
2 A 5 DE JULHO 2024, CAMPINAS/SP
FRONTEIRAS DAS CIÊNCIAS BIOMÉDICAS E SEUS DESAFIOS

Title	Neuroprotective and anxiolytic effects of <i>Tradescantia spathacea</i> aqueous extract
Authors	Margarete Zanardo Gomes Lorena Emília Sena Lopes Eduarda Portugal Freitas Santos Klebson Silva Santos
Affiliations	Tiradentes University
Session	40 - Neurociência

Abstract, Ethics Committee Number*, and Keywords	<p>Phytochemicals with antioxidant properties can be a strategy to protect dopaminergic neurons in Parkinson's disease. The aim of this work was to evaluate the effects of the treatment with aqueous extract of <i>T. spathacea</i> (AETS) on the 6-hydroxydopamine-induced striatal lesion in rats. The total yield, chemical profile and antioxidant activity (DPPH) of AETS were assessed. Adult male Wistar rats (ethics committee number 020517, n = 8/group) received a striatal microinjection 6- hydroxydopamine (6-OHDA, 20 µg/3 µL) or vehicle. They were treated with vehicle (0.5 mL, v.o.) or AETS (30 and 100 mg/kg) for 30 days from the surgical procedure. Behavioral analysis consisted of the open field test (crossing, rearing and anxiety parameters, after 15 days) and apomorphine-induced rotational behavior (0.5 mg/kg, 30th day, to evaluate the lesion extent). Then, the euthanasia was performed and sections of the substantia nigra pars compacta (SNc) and striatum were obtained and stained by immunohistochemistry for tyrosine-hydroxylase (TH) and glial fibrillary acidic protein (GFAP). Comparisons were made by one-way ANOVA followed by Bonferroni's post-hoc. The yield of AETS was 21.7%, with 31.7 mg/g of phenolic compounds, 35.4 mg/g of total flavonoids, and presented an IC50 of 16.7 ± 1.9 µg/mL. The treatment with AETS at 30 mg/kg significantly increased horizontal (F4-35 = 9.271; p<0.0001) and vertical (p<0.01) exploratory activity in the open field when compared to 6-OHDA untreated group, while 100 mg/kg reduced rotational behaviour (25.1±4.4 X 7.8±3.1) and defecation (p<0.001). Both doses significantly counteracted TH loss in the SNc (F4-25=91.07; p<0.0001) and striatum (p<0.0001), and the increase of the astrocytic reaction (p<0.0001). Thus, the results indicate a neuroprotective, anti-neuroinflammatory and anxiolytic effect of AETS.</p> <p>Keywords: Functional drink; flavonoids; 6-hydroxydopamine.</p>
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Title	Influence of postbiotics on α-synuclein aggregation in yeast model
Authors	Luanna D'Luck Ferreira Gomes ¹ Rosa Helena Luchese ² Cristiano Jorge Riger ² Edlene Ribeiro Prudencio de Souza ¹
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Session	Neuroscience

Abstract, Ethics Committee Number*, and Keywords	<p>Parkinson's disease (PD) is traditionally viewed as a central nervous system disorder, yet emerging evidence suggests microbial influence on neurological function. Dysbiosis signatures found in PD patients include increased <i>Akkermansia</i>, <i>Lactobacillus</i>, and <i>Bifidobacterium</i>. Conversely, probiotic supplementation, particularly with <i>Lactobacillus</i> and <i>Bifidobacterium</i>, is associated with improved non-motor symptoms. To explore the impact of probiotic metabolites on α-synuclein aggregation, a hallmark of PD, <i>Saccharomyces cerevisiae</i> expressing α-synuclein was used. The causative relationship between microbiota composition and symptoms remains unclear. To explore the impact of probiotic microbial metabolites on α-synuclein protein aggregation, a key etiological marker of PD, <i>Saccharomyces cerevisiae</i> modified to express galactose-induced α-synuclein was utilized. This expression induces physiological dysfunctions akin to those observed in PD-affected neurons, ultimately leading to cell death. <i>Bifidobacterium animalis</i> and <i>Lactobacillus paracasei</i> strains, either isolated or co-cultured (2×10^5 CFU/mL), were cultured in Minimal Lactobacilli Medium at 37°C for 168h. Subsequently, they were supplemented with SGAL medium components and filtered (0.22μm). Transformed yeast cells (α-syn) and control (P426), at OD600 0.5, were treated with cell-free metabolites for 24h, successively diluted 10,000x, and plated in spots. Colony density of α-syn-treated yeast cells with metabolites from <i>L. paracasei</i>, <i>B. animalis</i>, and the co-culture, compared to the P426 strain, remained similar across all dilutions, indicating that the metabolites, at the concentration tested, did not impact α-syn aggregation. These findings suggest that the tested probiotic strains, albeit belonging to the <i>Lactobacillus</i> and <i>Bifidobacterium</i> genera, either individually or in co-culture, did not associate with promoting α-syn aggregation. Quantitative and imaging assays are ongoing.</p> <p>Probiotic, Parkinson, Neuroprotection</p>
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Title	Astrocytes and microglia evaluation in the brain of iNOS knockout mice after contextual fear conditioning
Authors	Karen Barbosa ¹ Erika Meyer ¹ , Sabrina F. S. Lisboa ¹
Affiliations	1-Department of Biomolecular Sciences, School of Pharmaceutical Sciences of Ribeirão Preto, USP – SP, Brazil
Session	34- Neuroscience

Abstract, Ethics Committee Number*, and Keywords	<p>Introduction: Post-traumatic stress disorder can develop after a very stressful experience, resulting in symptoms such as difficulty in extinguishing aversive memories. Knockout mice for the inducible nitric oxide synthase isoform (iNOS) present impaired contextual conditioned fear extinction. The involvement of glial activation may be crucial in understanding the extinction deficits observed in these mice. Aim: to investigate the possible microglia and/or astrocytes activation in the prefrontal cortex (PFC) of iNOS KO and wild-type mice submitted to inescapable footshocks. Methods: Female C57Bl/6J and iNOS KO mice (n=23) were submitted to fear conditioning or habituation sessions consisting of three inescapable footshocks (0.75 mA, 2s each). Three hours after the session, the animals were euthanized for immunofluorescence analyses of Iba-1 and GFAP expression in the pre- and infralimbic cortex. Experimental procedures were approved by the Ethical Review Committee of the Pharmaceutical Sciences School of Ribeirão Preto (CEUA nº. 22.1.732.60.9). Results: iNOS KO mice presented a decreased expression of Iba-1 in both analysed PFC regions. After the aversive stimulus an augmented Iba-1 expression was detected in iNOS KO mice, while no similar effect was observed in wild-type mice. GFAP expression did not differ among the experimental groups in neither of the investigated PFC regions. Conclusion: Our results suggest that the microglial cells in iNOS KO mice may stay at a more passive state under basal conditions. However, the prompt response of these cells to a stimulus seems to be maintained, possibly operating by compensatory mechanisms.</p> <p>Keywords: iNOS KO mice, fear conditioning, microglia, astrocytes</p>
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Title	Aged mice have fewer 5-HT neurons in raphe nuclei important for cognition
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Session	Neuroscience

Abstract, Ethics Committee Number*, and Keywords	<p>5-HT neurons located in the dorsal (DRN) and median (MRN) raphe nucleus project to regions of the brain that control cognition. During aging, neurodegeneration can occur in brain regions, affecting neurons of different phenotypes. It is possible that 5-HT neurons are among the neurons affected during the aging process. To evaluate whether aged animals would present reductions in 5-HT neurons in regions such as the DRN and MRN. And whether there would be any difference in these variations in relation to sex. Adult (3 months) and aged (19 months) male and female mice (C57 isogenic mice) were used (CEUA 2623110719). Immunohistochemistry, microscopic analysis, and treadmill running were performed. In the DRN of male mice we observed an average of 170.70 ± 14.04 5-HT neurons in aged animals, and 227.40 ± 17.77 5-HT neurons in young animals ($p < 0.05$). In the DRN of females, we observed an average of 119.70 ± 10.74 5-HT neurons in aged animals, and 220.00 ± 12.59 5-HT neurons in young animals ($p < 0.0001$). We discovered that aged females showed a more significant delta reduction in 5-HT neurons ($\Delta = -100.3$) compared to aged males ($\Delta = -56.7$) ($p = 0.005$). In the MRN of males we observed an average of 44.97 ± 2.73 5-HT neurons in aged animals, and 69.20 ± 2.33 5-HT neurons in young animals ($p < 0.0001$). In the MRN of females we observed 39.46 ± 2.53 5-HT neurons in aged animals, and 74.86 ± 1.82 5-HT neurons in young animals ($p < 0.0001$). Aged females showed a delta reduction in 5-HT neurons ($\Delta = -24.23$) similar to that observed in aged male mice ($\Delta = -35.40$) ($p > 0.05$). We show that in mice, both males and females, there are damages in the 5-HT system in aging, such as reductions in the number of 5-HT neurons originating from regions involved in cognition, such as the DRN and MRN. There is a difference in relation to sex, as in the DRN region of females the reduction of number of 5-HT neurons during aging was more significant.</p> <p>Keywords: 5-HT neurons, raphe nuclei, aging</p>
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Title	Short-term high-fat diet induces anxiolytic-like effect in C57BL/6 mice depending on inflammatory and neuroplasticity markers
Authors	Silote, G.P.1 Santos, N.J.1 Martins, I.C.A.1 Lopes, P.K.F.1 Torsoni, A.S. 1,2 Torsoni, M.A.1,2
Affiliations	1 – Laboratory of Metabolic Disorders (Labdime), School of Applied Sciences, University of Campinas (UNICAMP), Limeira, Brazil. 2 – Obesity and Comorbidities Research Center, University of Campinas (UNICAMP), Campinas, Brazil.
Session	34. Neurociência

Abstract, Ethics Committee Number*, and Keywords	<p>Obesity, depressive, and anxiety disorders are considered serious health problems. Several studies indicated that immune and inflammatory aspects are involved in both conditions. Here, we investigated the effect of short-term HFD on depressive and anxiety-related behavior and the involvement of inflammatory and neuroplasticity markers in the prefrontal cortex (PFC) and hippocampus (HPC).</p> <p>Adult male C57BL/6 mice (8-10 weeks) 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 days. On 3rd 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 glucose. Then, they were euthanized to dissect the PFC and HPC to evaluate gene expression of <i>Chrna7</i>, inflammatory, and neuroplasticity markers.</p> <p>The exposition to short-term HFD increased the body weight (Bonferroni: $p < 0.0001$), cumulative body weight gain (t-test: $p < 0.0001$), and fasting blood glucose (t-test: $p < 0.0001$) compared to Ctrl group. The HFD group increased the time in the central zone of the OFT (t-test: $p = 0.0017$), suggesting an anxiolytic-like effect. No changes were found in the EPM, FST, and NOR. HFD group increased the expression of <i>Il6</i> (t-test: $p = 0.0158$), and decreased <i>Syp</i> (t-test: $p = 0.0176$) and <i>Dlg4</i> (t-test: $p = 0.0587$) in the PFC. While, in HPC, HFD diet increased <i>Bdnf</i> mRNA (t-test: $p = 0.0583$).</p>
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The results indicate that short-term HFD induces changes in murimetric parameters and an anxiolytic-like effect in C57BL/6 mice exposed to OFT. The effect is associated with increased transcript levels of Bdnf in HPC, and, an increment in Il6 and decreased Syp and Dlg4 in the PFC. Further investigations on the protein levels are needed to understand better the molecular mechanism involved in our results.

Keywords: High-fat diet; Anxiety; Neuroplasticity markers; Inflammation.



Title	Metabolic phenotyping of transgenic mice lacking neuropeptide y (NPY) in tyrosine hydroxylase neurons
Authors	Gabriela Batitucci ^{1,2} Erika Anne Roman ¹ Ester dos Santos Alves ^{1,2} Leticia Pires ¹ Evelin da Silva Matai ^{1,2} Ana Domingos ³ Licio Augusto Velloso ^{1,2}
Affiliations	¹ Laboratory of Cell Signaling, Obesity and Comorbidities Research Center, University of Campinas, Brazil; ² School of Medical Sciences, Department of Medical Pathophysiology, University of Campinas, Brazil; ³ Dept. Physiology, Anatomy, Genetics at University of Oxford
Session	Oral

Abstract, Ethics Committee Number*, and Keywords	<p>The hypothalamus integrates central and peripheral signals to control several autonomic functions, including the body's energy balance. A fraction of this regulation is exerted through sympathetic connections with systemic tissues regulating energy homeostasis and glucose metabolism. There is a subpopulation of autonomic neurons, the tyrosine hydroxylase (Th) neurons which express NPY and innervate the brown adipose tissue (BAT) controlling thermogenesis; however the role played by these autonomic fibers in regulating systemic metabolism is currently unknown. Here, we evaluated a transgenic mouse model that does not express NPY specifically in neurons Th (Thcre/Npyflox). Metabolic phenotyping of chow-fed males and females (n=8/group/sex) was carried out over 16 weeks and included control (CT) and knockout (KO_NPY) mice groups, ethics committee n°6273-1/2023. The results indicate that in males the KO_NPY group has smaller body mass than the CT group at the end of 16W ($P=0.016$); food intake of the KO_NPY group is lower than that of the CT group at 9W ($P=0.009$), 14W ($P=0.021$) and 15W ($P=0.009$). Furthermore, the KO_NPY group had greater glucose intolerance only at 16W ($P = 0.003$) and greater fasting glycemia ($P<0.0001$) compared to the control, as well as, lower lean mass (g) ($P=0.013$), without changes to the other parameters. Regarding females, there were no changes in the variables evaluated, except for the fact that the KO_NPY group also showed reduced lean mass ($P=0.020$). The 12h fasting tolerance test (FTT) and pyruvate tolerance test (PTT) after 6h of fasting and a dose of 2g/kg of pyruvate, were performed and there was no difference in the PTT between the groups for males and females, or in the FTT for females. However, KO_NPY males showed greater intolerance to prolonged fasting ($P=0.007$) compared to the control group. Thus, the study demonstrates that NPY deletion in peripheral neurons can alter metabolic regulation under normal metabolic conditions.</p> <p>Keywords: Neuropeptide Y, peripheral neurons, metabolic regulation.</p>
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Title	Effects of dapagliflozin on cellular viability in aortic endothelial cells treated with doxorubicin
Authors	Beatriz R. Goes-Santos ¹ Juliana Q. Rocha ² Leticia Barssotti ² Ana Paula Davel ² Ligia M. Antunes-Correa ¹
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Session	Biologia e Doenças Cardiovasculares

Abstract, Ethics Committee Number*, and Keywords	<p>Cancer is the second leading cause of death in the world and Brazil. Breast cancer (BC) is the most prevalent type of cancer among women, excluding nonmelanoma skin. Over the last decade, BC treatment markedly improved survival rates, despite doxorubicin's (DOXO) toxic effects. In vitro and in vivo studies have demonstrated that DOXO treatment increases apoptosis and oxidative stress while reducing cell viability, leading to endothelial dysfunction. Conversely, sodium-glucose co-transporter-2 inhibitors (SGLT2i), such as dapagliflozin (DAPA), have gained popularity as drugs for vascular protection. Studies have shown that SGLT2i reduces apoptosis, oxidative stress, and increases endothelial function. We hypothesize that DAPA could attenuate the effects of DOXO on endothelial cells, thereby improving cell viability. For this, we use bovine aortic endothelial cells (BAEC) in DMEM (10%FBS) and evaluate cell viability using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. ANOVA one or two-way was used for statistical analysis. To define the dose of DOXO, we treat cells with 0.1, 0.5 e 1.0µM of DOXO for 24h. Subsequently, we treated cells with 1.0, 5.0, and 10.0µM of DAPA, with or without 0.1µM of DOXO for 24 hours. In addition, we test DAPA pre-treatment, using 1.0, 5.0, and 10.0µM of DAPA for 24 hours before adding DOXO for more 24 hours. As results, DOXO reduced cell viability dose-dependently compared to the control ($p < 0.001$). No effects of any dose of DAPA (1.0, 5.0, and 10.0µM) were observed in endothelial cells viability, when compared to either the DOXO-</p>
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only or control group for 24h ($p=0.010$; $p=0.005$ and $p=0.021$, respectively), or even when cells were pretreated with DAPA ($p<0.001$, $p<0.001$ and $p=0.005$, respectively). In conclusion, high doses of DAPA did not attenuate the reduced cell viability induced by DOXO in endothelial cells.

Key-Words: cardiotoxicity; endothelial cells; SGLT2i; dapagliflozin; doxorubicin.



Title	Human apoCIII overexpression does not affect blood pressure and cardiovascular parameters in transgenic mice
Authors	1 – Nilton Rodrigues Teixeira Junior; 2 - Anna Rebeka Oliveira Ferreira; 3 – Kesia Gemima Palma Rigo Wutzow; 4 - Paulo César de Freitas Mathias; 5 – Jairo Augusto Berti;
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Session	04 – Biologia e doenças cardiovasculares

Abstract, Ethics Committee Number*, and Keywords	<p>Hypertriglyceridemia is a serious dyslipidemia caused by an increase in plasma triglyceride (TG) concentrations. It has many causes and can be linked to poor eating habits, a sedentary lifestyle and genetic alterations. In addition, dyslipidemia is considered an isolated factor in the development of disorders in the cardiovascular system, such as hypertension. Previous studies show that dyslipidemic human apoCIII transgenic (CIII) mice have hypertriglyceridemia, and atherosclerosis propensity. This study aimed to evaluate the blood pressure and heart rate in aged CIII mice. The procedures followed the approvals from the Ethics Committee on Animal Use (CEUA N. 7925010719) of the UEM, where were used 17 male C57Bl/6 mice, with subclassification into non- transgenic (NTG, triglyceridemia <100 mg/dL) or transgenic (CIII, triglyceridemia >200 mg/dL) groups. To the experiments, direct blood pressure and heart rate were recorded in anesthetized (ketamine (100mg/Kg) and xylazine (20mg/Kg)) in eight months-old mices, CIII (n=9) and NTG (n=8) mice. Data analysis used GraphPad Prism[®] software. Student's t-test was employed and the results were presented as mean \pm standard deviation, with significance set at $p < 0.05$. No statistical differences were found in mean arterial pressure (CIII: 92 ± 1.93 vs. NTG: 90 ± 1.21 mmHg; $p=0.507$), systolic arterial pressure (CIII: 113 ± 1.49 vs. NTG: 111 ± 0.84 mmHg; $p=0.272$), diastolic arterial pressure (CIII: 75 ± 2.20 vs. NTG: 74 ± 1.39 mmHg; $p=0.621$) and heart rate (CIII: 158 ± 7.29 vs. NTG: 158 ± 10.20 mmHg; $p=0.950$) between CIII and NTG animals. In conclusion, despite the human apoCIII overexpression induces hypertrygliceridemia, that metabolic dysfunction on the TG mice does not changes the basal blood pressure and heart rate on those animal model.</p> <p>Keywords: Metabolism; apoCIII; Hypertension.</p>
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Title	Investigating the interaction between PTK2 and PARP1 in the DNA damage response in cardiomyocytes treated with doxorubicin
Authors	Pedro Víctor-Carvalho ¹ ; Ingridi Rafaela de Brito ¹ ; Mariana Conceição da Silva ¹ ; Maria das Graças Souza Carvalho ¹ ; Isabela Aparecida Moretto ¹ ; Aline Mara dos Santos ^{1,2} ; André Alexandre de Thomaz ^{1,3} .
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Session	04 - Biology and Cardiovascular Diseases

Abstract, Ethics Committee Number*, and Keywords	<p>Cardiomyopathies resulting from antineoplastic therapies have become a significant concern in public health, particularly given the notable rise in cancer survival rates in recent years. The cardiotoxicity induced by chemotherapeutic agents, such as doxorubicin (dox), poses a severe risk that may lead to chronic cardiomyopathy, congestive heart failure, and, in extreme cases, casualties. Studies dedicated to investigating the signaling pathways triggered by antineoplastic therapies have underscored the importance of protein tyrosine kinase 2 (PTK2, also known as focal adhesion kinase - FAK) in cell survival and resistance to these treatments. Nevertheless, there are still gaps in our understanding of this signaling pathway that require further exploration. This work aims to deepen our comprehension of the molecular mechanisms underlying cell survival and resistance by examining the interaction between PTK2 and proteins involved in DNA damage repair, such as PARP1, in H9c2 cardiomyocytes. Our immunoprecipitation (IP) results have unveiled an interaction between PTK2, PARP1, and poly(ADP-ribose) (PAR chains added by PARP1 to target proteins). Western blotting analyses have indicated that treatment with doxorubicin increases the protein expression of PTK2. Analysis of co-localization using structured illumination microscopy (SIM) fluorescence images has revealed a reduction in the presence of PTK2 at DNA damage sites under PARP1 inhibition. These findings suggest a significant interaction between PARP1 and PTK2, particularly at DNA damage sites (γH2AX foci). Our results indicate an interaction between PTK2 and PARP1, with the addition of PAR chains on PTK2 being an important event for the positioning of the latter at γH2AX foci.</p>
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Title	Unraveling the impact of a Brazil nut-enriched diet on vascular calcification in CKD rats
Authors	Da Cruz BO ¹ , Silva-Costa N ¹ , Araujo JR ² , Almeida PP ² , Melo MFS ¹ , Lima AL ³ , Autran LJ ⁴ , Magliano DC ⁵ , Soulage C ⁶ , Mebarek S ⁷ , Brizuela L ⁷ , Cardozo L ¹ , Stockler-Pinto MB ^{1,2}
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Session	Biology and Cardiovascular Diseases

Abstract, Ethics Committee Number*, and Keywords	<p>Vascular calcification (VC) is an independent risk factor for cardiovascular mortality. Food bioactive compounds and nutrients have been tested as a promising tool to modulate VC. This study aimed to investigate the effects of a Brazil nut-enriched diet on vascular calcification in a CKD animal model. VC was evaluated in an animal model of CKD induced by 5/6 nephrectomy, where 23 male <i>Wistar</i> rats were divided into 3 groups: Sham group (n=6), CKD group (n=9), and Brazil nut (BN) group (n=8). To induce VC, the CKD and BN animals received a diet with high phosphate and an injection of 20ng calcitriol 3 times a week. For 4 weeks, animals in the BN group received a diet enriched with 10% of Brazil nuts. After 4 weeks, the animals were euthanized, and blood, aorta, and kidney were collected. Serum concentrations of urea and creatinine were determined. To analyze the effects of Brazil nuts on the VC, gene expression of Runx2, Nrf2, and NF-κB was assessed by RT-qPCR. TBARS and GPx activity were measured on plasma samples. Histomorphology analyses on the aorta and kidney were carried out. As expected, renal function markers were significantly higher in the CKD group compared to the Sham group (Creatinine: 0.38mg/dL vs 0.78mg/dL, p=0.0003; Urea: 40.33mg/dL vs 75.88mg/dL, p=0.0002). Furthermore, the Brazil nut-rich diet significantly reduced creatinine concentrations in the BN group compared to the CKD group (0.59mg/dL vs 0.78mg/dL, p=0.05). Also, BN reduced glomerular dilation compared to the CKD group. The BN diet also modulated aorta thickness compared to the CKD diet (123.1µm vs 156.8µm, p=0.0038). No significant differences were shown in other parameters. Brazil's nuts-enriched diet attenuates kidney damage in CKD rats. In addition, the Brazil nut diet modulated the aorta's thickness. These</p>
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	<p>results indicate that Brazil nut promise to attenuate VC and contribute to controlling kidney disease progression.</p> <p>Ethics committee: CEUA/UFF 3994100220</p> <p>Keywords: Brazil nut, vascular calcification, chronic kidney disease</p>
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Title	Bradykinin inhibitors mitigate vascular alterations in aging mice exposed to extracellular vesicles from severe COVID-19 patients
Authors	Natália Ribeiro Cabacinha Nóbrega, Juliana Quinholi Rocha, Geiza Rafaela Bobato, Daniele Mendes Guizoni, Ana Paula Davel.
Affiliations	Laboratory of Vascular Biology (LaBiVasc)- Biology Institute (IB)- Universidade Estadual de Campinas (UNICAMP)
Session	Biology and Cardiovascular Diseases

Abstract, Ethics Committee Number*, and Keywords	<p>COVID-19 pandemic led to ~7 million deaths worldwide, with the main risk group being the aging. Treatment of patients during the acute phase of the disease with B2 receptor antagonist (Firazyr – F) and C1 esterase inhibitor (Berinert – B) demonstrated an improvement in the pulmonary CT score and increase in eosinophils. As extracellular vesicles (EV) may contribute to the worsening of COVID-19 in the long term, we hypothesized that F and B treatments influence EV signaling in the vasculature, especially in aging. The aim was to evaluate the impact of EV isolated from patients hospitalized by COVID-19, who received or not treatment with F or B, on the vascular function of aging mice. Plasma EV were isolated from patients with severe COVID-19 treated or not with F or B, 7 days after hospital discharge (ITHACA-PMID clinical trial 33472675). Aorta and mesenteric resistance arteries (MRA) from male C57BI/JUnib mice (3 or 16 months aged) were incubated ex vivo with vehicle (Veh, PBS) or EV (10⁶ EV/mL, in DMEM, at 37°C, 24 h). Cumulative concentration-response curves to phenylephrine (Phe) and acetylcholine (ACh) were performed (CEUA 6426-1/2024). EV from non-treated post- COVID patients reduced the Phe-induced contraction in MRA (Rmax: Veh:2.49 ±0.16 vs. EV=1.61 ±0.16 mN/mm p<0,05) while increased it in aorta from aged mice (Rmax: Veh= 2.42 ±0.35 vs. EV: 3.96 ± 0.06 mN/mm p<0.05). However, this effect of EV was attenuated by F or B treatment in both vessels. The relaxation response induced by ACh was not changed in aorta and reduced in MRA by EV from non-treated patients, which was partially reversed by B or F. Vascular reactivity of young mice was not affected by these EV. The data suggest that EV impact vascular function in aging depending on the vessel type, which seems to be attenuated by the treatment with bradykinin inhibitors.</p> <p>Keywords: COVID-19, aging, extracellular vesicles, vascular function, aorta, mesenteric arteries.</p>
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Title	Aging exacerbates hypertriglyceridemia and does not affect cholesterolemia and glycemia in human apociii transgenic mice
Authors	1 – Nilton Rodrigues Teixeira Junior; 2 – Smyrna Cele Sales; 3 – Giovanna Sachelli Peres 4 – Carmem Patrícia Barbosa 5 – Jairo Augusto Berti;
Affiliations	Universidade Estadual de Maringá, Maringá, Paraná, Brazil
Session	07 – Nutrição e metabolismo

Abstract, Ethics Committee Number*, and Keywords	<p>Hypertriglyceridemia is a dyslipidemia characterized by a marked elevation of circulating triglyceride (TG) levels. Factors such as inadequate diet, sedentary behavior, genetic predisposition, and aging can lead to this condition. Human apolipoprotein CIII (apoCIII) transgenic mice serve as a model for investigating lipid metabolism since apoCIII overexpression induces hypertriglyceridemia. We investigated the metabolic consequences of aging and apoCIII-induced hypertriglyceridemia on the lipidic and glycemic profiles of both young and aged mice. The procedures followed approvals from the Ethics Committee on Animal Use (CEUA N. 7925010719) of the UEM. We used 29 male C57Bl/6 mice, subclassified into non-transgenic (NTG, triglyceridemia <100 mg/dL) and transgenic (CIII, triglyceridemia >200 mg/dL) groups. Euthanasia was performed on the animals at 5 and 22 months of age, resulting in four groups (NTG Young, n=8; CIII Young, n=8; NTG Aged, n=5; CIII Aged, n=8). Blood samples were collected via tail vein and retro-orbital methods for biochemical assays. Data analysis was conducted using GraphPad Prism® software. Two-way ANOVA followed by Tukey's post-test was employed, with results presented as mean ± standard deviation, and significance set at p<0.05. The impact of apoCIII overexpression on TG levels was significant (p<0.0001), clearly reflected between the NTG and CIII groups (NTG Young 67±16 vs. CIII Young 260±52). Furthermore, aging exacerbated TG levels, particularly in the CIII group (p=0.0002) (CIII Young 260±52 vs. CIII Aged 446±126). Additionally, apoCIII overexpression significantly (p<0.0001) elevated plasma cholesterol levels (NTG 83±13 vs. CIII 132±31); however, glycemic levels remained consistently within the normal range across all experimental groups. This study demonstrates that aging increases hypertriglyceridemia in transgenic animals overexpressing apoCIII, yet it is not sufficient to alter cholesterol and glucose homeostasis.</p> <p>KeyWords: Dyslipidemia; apoCIII; Metabolism.</p>
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Title	PM20D1 plays a role in the thermogenic response to cold exposure
Authors	Marcela R. Simoes ^{1*} , Bruna Bombassaro ¹ , Ana Luisa Ferraz ¹ , Fernando Valdivieso-Rivera ^{1,2} , Ariane M. Zanesco ¹ , Guilherme A. S. Nogueira ¹ , Milena Monfort-Pires ³ , Licio A. Velloso ¹
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Session	7: Nutrição e Metabolism

Abstract, Ethics Committee Number*, and Keywords	<p>Currently, obesity is one of the most prevalent medical conditions in the world, as it is a multifaceted disease and can be affected by both environmental and genetic factors. In a previous study, we showed that metabolism, thermogenesis and brown adipose tissue (BAT) activity are different in three mice strains. Balb/c had better glycemic control and, after acute cold, had higher mitochondrial oxygen consumption rate (OCR), core and BAT temperature, indicating an increased BAT activity and thermogenic process. In this study, we investigated which mechanisms could explain these differences. We exposed Balb/c, C57BL and Swiss mice to thermoneutrality, room temperature, acute cold, chronic cold, and prolonged fasting (CEUA 6139-1/2022). After acute cold, BAT Pgc1a was higher in Balb/c ($p=0.04$: balb vs. c57; $p=0.007$: balb vs. swiss, one-way ANOVA, $n=5-6$/group), however there was no difference in Ucp1. Balb/c had higher BAT Pm20d1 expression ($p<0.04$: balb vs. c57 and swiss, one-way ANOVA, $n=5-6$/group) and serum PM20D1 ($p=0.02$: balb vs. c57; $p=0.06$: balb vs. swiss, one-way ANOVA, $n=6$/group). In Balb/c, an immunoneutralization of PM20D1 worsened its thermic control ($p<0.05$: control vs. treatment, unpaired t-test, $n=5-6$) and decreased its BAT's mitochondrial OCR ($p=0.03$: control vs. treatment, unpaired t-test, $n=5-6$) after acute cold. We also showed that these mice strains adapt differently to conditions where there is a shift in energy demand, such as prolonged fasting, thermoneutrality and chronic cold. In humans (CAEE 60698716.1.0000.5404), we found a negative correlation of serum PM20D1 levels and BAT glucose uptake ($p=0.02$, Pearson's, $n=40$), suggesting that a higher BAT activity might be recruiting this enzyme from the blood. Together, these findings suggest that BAT activation via the enzyme PM20D1, can have a role on metabolism and thermogenesis, being a potential therapeutic target in the treatment of obesity.</p> <p>Keywords: obesity, metabolism, energy expenditure</p>
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Title	Pediatric Drone: Estimating morphometric measurements in spinner dolphin (<i>Stenella longirostris</i>) calves based on aerial photogrammetry
Authors	Raul Rio
Affiliations	Federal University of Juiz de Fora, Departamento de Medicina Veterinária, Minas Gerais, Brazil; Ocean Sound, a nongovernmental organization (NGO), https://www.oceansound.org , Brazil.
Session	15 Tecnologias Ópticas e Mecânicas para a Saúde

Abstract and Keywords	<p>Monitoring and assessing cetaceans through non-invasive methods may be used as shortcut to help improving knowledge about these species; many of them are endangered species. The current study was the first to collect and analyze aerial morphological measurements taken from odontocete calves and spinner dolphin (<i>Stenella longirostris</i>) species. Aerial photogrammetry data were collected from oceanic spinner mother-calf pairs swimming together, at a shallow rest bay called "Biboca", around Fernando de Noronha Archipelago, Brazil. Estimated body length (EBL), anterior width of pectoral fin (PEC), width at the axilla (AXI) and body condition indices (PEC_{idx} and AXL_{idx}) were the morphometric measurements adopted. The herein adopted aerial protocol was accurate in identifying temporal body condition variations in calves over 106 days without any disturbing behavior potentially associated with the presence of the drone registered throughout the study. The results added new limits to the current morphological local knowledge by registering a 201 cm-long adult individual and a 78 cm-long newborn dolphin. The aerial protocol can be used to assess calf growth patterns in small cetacean species, which, in their turn, can be indicators of cetaceans' individual and population health, mainly in environments threatened by human activities, like the one in Fernando de Noronha Archipelago.</p> <p>Ethics Committee Number: Chico Mendes Institute for Biodiversity Conservation (ICMBio; SISBIO #75705-9) and Ethics Committee on the Use of Animals (CEUA- UFJF) no 026/2020].</p> <p>Keywords: aerial photogrammetry; morphometric measurements; body condition; <i>Stenella longirostris</i>; spinner dolphin; drones; UAV; Fernando de Noronha; Brazil.</p>
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Title	Data normalization of plasma mirna profiling from COVID-19 patients
Authors	Julia Tiemi Siguemoto ¹ , Carolini Motta Neri ¹ , Nadine de Godoy Torso ² , Aline de Souza Nicoletti ² , Marília Berlofa Visacri ³ , Carla Regina da Silva Correa da Ronda ¹ , Mauricio Wesley Perroud Junior ² , Leonardo Oliveira Reis ² , Luiz Augusto dos Santos ⁴ , Nelson Durán ² , Wagner José Fávoro ² , Eder de Carvalho Pincinato ² and Patricia Moriel ¹
Affiliations	<ol style="list-style-type: none"> 1- Faculty of Pharmaceutical Sciences, Universidade Estadual de Campinas (Campinas – SP) 2- School of Medical Sciences, Universidade Estadual de Campinas (Campinas – SP) 3- Faculty of Pharmaceutical Sciences, University of São Paulo (São Paulo – SP) 4- Hospital Municipal de Paulínia (Paulínia – SP)
Session	16 - Terapia Gênica e Celular, Biologia Omics

Abstract, Ethics Committee Number*, and Keywords	<p>When using the RT-qPCR technique for quantitative assessment of microRNA (miRNAs) expression, it is essential to normalize data by a stable endogenous gene; however, there is no universally adequate reference gene. Therefore, this study aimed to determine, via the RNA-Seq technique, the most adequate endogenous normalizer for use in the expression assessment of plasma miRNAs from Coronavirus Disease 2019 (COVID-19) patients. Two massive sequencing were performed: a) to identify differentially expressed miRNAs between patients with COVID-19 and healthy volunteers (n = 12); and b) to identify differentially expressed miRNAs between patients with severe COVID-19 and those with mild COVID-19 (n = 8). The endogenous normalizer candidates were selected according to the following criteria: (1) the miRNA must have a fold change = 1; (2) the miRNA must have a p-value > 0.990; and (3) select those miRNAs that were discovered the longest ago. Four miRNAs (hsa-miR-34a-3p, hsa-miR-194-3p, hsa-miR-17-3p, and hsa-miR-205-3p) met all the criteria and were chosen for validation by RT-qPCR in a cohort of 125 patients. Of them only hsa-miR-34a-3p and hsa-miR-205-3p were shown to be eligible endogenous normalizers in the context of COVID-19 disease, because their expression was shown to be stable between the compared groups.</p> <p>Keywords: microRNA; COVID-19; endogenous normalizer; reference gene</p>
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Title	Differential expression of HERBB2/B3 genes in women diagnosed with COVID-19, and its relationship with genomic instability
Authors	Maria Eduarda Ribeiro da Silva ² Luis Fernando Leite de Jesus ¹ Samara Ahmad Fayad Pires ¹ Estela Guedes Franco Lima ¹ Luiz Vinicius Pena Cardozo ¹ Gabriel Nunes Rodrigues ¹ João Donizetti Gomes da Silva Filho ¹ Sabine Mai ¹ Edlaine Villela ¹ Keilah Valéria Naves Cavalcante ¹ Fábio Morato de Oliveira ¹
Affiliations	¹ Faculdade Medicina, Universidade Federal de Jataí, Goiás, Brasil ² Instituto de Ciências Biológicas, Universidade Federal de Goiás, Goiás, Brasil
Session	16 - Terapia Gênica e Celular, Biologia Omic

Abstract, Ethics Committee Number*, and Keywords	<p>ErbB2 and ErbB3 receptors belong to the ErbB tyrosine kinase receptor family, whose activation by the binding of epidermal growth factors (EGF) activates an intracellular signaling cascade and regulates multiple functions related to cell growth, proliferation and survival. Previous studies show that these ErbB receptors are essential for infection by the Sars-CoV-2 virus, as they act in the mechanism of internalization of the virus into the cell, which leads to an increase in its gene expression. Therefore, the present study aimed to evaluate the different expressions of the <i>ErbB2</i> and <i>ErbB3</i> genes in women diagnosed with COVID-19. A survey questionnaire was researched to 84 female patients who sought the Emergency Care Unit (Jataí-GO; CONEP 30343820.9.0000.0008) with a possible CD19 condition. We collected data such as: age, pregnant or not, high blood pressure, diabetes mellitus, and whether they were reinfected by CD19. Blood samples were collected to perform the qPCR method to analyze the <i>ERBB2</i>, <i>ERBB3</i> and <i>GAPDH</i> genes (endogenous control). Pearson r was used for correlation analysis. Due to diabetes mellitus (DM) in pregnant women (MG; n=35), we observed a strong positive correlation with an increase in the expression of <i>ERBB2</i> (r= 0.82; p<0.05) and <i>ERRB3</i> (r= 0.63; p<0.05) and DM. Furthermore, diabetic MG has a strong positive correlation with COVID-19 reinfection (r=0.89; p<0.05). Non-pregnant women (MNG; n=49) have a strong positive correlation only with genomic alteration and covid reinfection (<i>ERBB2</i> r=0.86, p<0.05; <i>ERBB3</i> r=0.86, p<0.05). Given these results, we observed that</p>
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women who have DM, especially pregnant women, are more likely to have reinfection with COVID-19 with genomic instability in the *ERBB* genes, especially in the *ERBB2* gene, which plays a role in cell survival.

Keywords: Pregnant; COVID-19; ERBB; Diabetes mellitus.



Title	Differential expression of SMYD2/D3 genes in patients with COVID-19: What we can expect about genomic instability?
Authors	Estela Guedes Franco Lima ¹ Samara Ahmad Fayad Pires ¹ Luís Fernando Leite de Jesus ¹ João Donizetti Gomes da Silva Filho ¹ Thaíz Furtado Silva ³ Maria Eduarda Ribeiro da Silva ² Gabriel Nunes Rodrigues ¹ Pedro de Paiva Moura ¹ Sabine Mai ¹ Edlaine Villela ¹ Keilah Valéria Naves Cavalcante ¹ Fábio Morato de Oliveira ¹
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Session	16 - Terapia Gênica e Celular, Biologia Omic

Abstract, Ethics Committee Number*, and Keywords	<p>Lysine methyltransferases participate in the regulation of gene and epigenetic expression, as well as in the activation of macrophages and the polarization of immune cells. Therefore, changes in the immune system can lead to the risk of possible reinfections. Studies also point to a possible correlation between SMYD proteins and the development of gestational diabetes, as well as high blood pressure, since they act on vascular smooth muscle cells. Therefore, the objective of this study is to promote a better understanding of the relationship between diabetes, pregnancy, high blood pressure and COVID-19 (CD19) reinfection, from the perspective of genomic instability. A survey questionnaire was research to 84 female patients who sought the Emergency Care Unit (Jataí-GO; CONEP 30343820.9.0000.0008) with a possible CD19 condition. We collected data such as: age, pregnant or not, high blood pressure, diabetes mellitus, and whether they were reinfected by CD19. Blood samples were collected to perform the qPCR method to analyze the <i>SMYD2</i>, <i>SMYD3</i> and <i>GAPDH</i> genes (endogenous control). Pearson r was used for correlation analysis. Non-pregnant women (NPW; n=49) showed increased expression of <i>SMYD2</i> and <i>SMYD3</i>. Likewise, pregnant women (PW; n=35) also show an increase in the expression of both genes. The increase in these genes is positively correlated with diabetic PW for both <i>SMYD2</i> (r=0.8) and <i>SMYD3</i> (r=0.9). Furthermore, the</p>
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SMYD3 gene is positively associated with hypertensive PW ($r=0.6$). Due to the search for patients in the ECU with possible COVID-19, we associated the genomic alteration with covid reinfection and found a strong positive correlation for PW (*SMYD2* $r=0.9$; *SMYD3* $r=0.9$) and for NPW (*SMYD2* $r=0.8$; *SMYD3* $r=0.8$). Summary, PW with DM, and hypertension are prone to reinfection with COVID, in addition to presenting gene alterations in *SMYD2/D3* genes in both populations studied. However, more studies are still needed to emphasize this pathway.

Keywords: Hypertension; SMYD genes; COVID; Women;



Title	Region-specific regulation of matrix metalloproteinases in the mouse epididymis to LPS-induced inflammation
Authors	Martini, P.V. ¹ Camargo, I.A. ¹ Andrade, A.D. ¹ Ferreira, C.T. ¹ Portela, L.M.F. ² Kushima, H. ¹ Junior, L.A.J. ² Fernandes, C.J.C. ¹ Silva, E.J.R. ¹
Affiliations	¹ Department of Biophysics and Pharmacology, Institute of Biosciences, São Paulo State University "Júlio de Mesquita Filho" (UNESP), Botucatu-SP, Brazil. ² Department of Structural and Functional Biology, Institute of Biosciences, São Paulo State University "Júlio de Mesquita Filho" (UNESP), Botucatu-SP, Brazil.
Session	Farmacologia Básica e Clínica (17)

Abstract, Ethics Committee Number*, and Keywords	<p>Epididymitis is a common inflammatory disease of the male urogenital tract and a cause of male infertility. Epididymitis may lead to interstitial fibrosis, which may cause loss of epididymal function. Matrix metalloproteinases (MMPs) are enzymes involved in the inflammatory and fibrotic process; however, their involvement in the pathophysiology of epididymitis remains poorly explored. We aimed to investigate whether inflammation of the epididymis modifies the expression and activity of MMPs (<i>Mmp2</i>, <i>Mmp7</i>, <i>Mmp9</i>, and <i>Mmp13</i>) transcripts and their tissue inhibitors <i>Timp1-4</i>. We induced epididymitis in adult C57BL/6 mice (90-120 days old; CEUA n°9371270522) by injecting ultrapure LPS from <i>E. coli</i> (50 µM) into the interstitial compartment of the initial segment (IS) or cauda epididymis (CD). Mice were euthanized 6, 24, 72 h and 7 days after induction; their IS and CD were dissected and processed for RT-qPCR and Zymography assays. Results were analyzed either by ANOVA followed by Bonferroni test (RT-PCR) or Student's t-test (Zymography); $p < 0.05$ was considered significant. LPS increased the <i>Mmp13</i> levels in the IS ($p < 0.0001$) and CD (0.0006) after 6 h. Moreover, LPS increased <i>Mmp2</i> ($p = 0.0001$), <i>Mmp9</i> ($p < 0.0001$), <i>Timp1</i> ($p = 0.021$), <i>Timp3</i> ($p = 0.0001$), and <i>Timp4</i> ($p < 0.0001$) levels at 72 h in the IS, and only <i>Mmp9</i> ($p = 0.007$) at 72 h in the CD. LPS reduced the activity of MMP2 zymogens after 24 h ($p = 0.008$) but increased their activity after 7 d ($p = 0.029$) in the CD only. In parallel, LPS reduced the activity of MMP9 zymogens at 6 h in the IS ($p = 0.049$) and CD (0.002) and at 24 h in the CD only ($p = 0.048$), increasing their activity at 72 h in the IS ($p = 0.003$) and CD (0.007). The activity of MMP9 zymogens remained elevated at 7 days in the IS only ($p = 0.047$). Thus, LPS-induced inflammation differentially modulated MMP expression and activity in the proximal and distal epididymis, supporting the hypothesis that they are downstream players during bacterial epididymitis.</p> <p>Keywords: epididymis, inflammation, MMP</p>
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Title	Influence of JAK inhibition on periodontitis progression in rats
Authors	Lucas Nogueira Ramos ¹ Mariely Araújo de Godoi ¹ Fábio Renato Manzolli Leite ² Angelo Constantino Camilli ¹ Vitória Bonan Costa ¹ Iolanda Augusta Fernandes de Matos ¹ Morgana Rodrigues Guimarães-Stabili ¹
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Session	Immunology

Abstract, Ethics Committee Number*, and Keywords	<p>Periodontitis involves the activation of cytokines that are modulated by intracellular signaling pathways, including the Janus kinase/Signal Transducers and Activators of Transcription (JAK/STAT) pathway. The aim of this study was to evaluate the effect of systemic administration of JAK1-3 and JAK3 inhibitors on the progression of periodontitis in rats. After approval by the Animal Use Ethics Committee (number 27/2020), the animals were subjected to ligature placement around the mandibular first molars to induce periodontitis and received, concomitantly with the progression of the disease, JAK1-3 or JAK3 inhibitors, orally for 7 days. Animals in the positive control group (with ligatures), and negative control group (without ligatures), received only distilled water (orally). At the end of the experimental period, the mandibles containing gingival tissue around the first molar were collected for evaluation: (alveolar bone resorption (by uCT); quantification of the number of osteoclasts (morphometry); gene expression (RT-qPCR), and protein (ELISA); expression of inflammatory cells (CD45 and CD3, by immunohistochemistry); and quantification of cellular elements (stereometry). The ANOVA test, followed by Tukey's test, was used for multiple comparisons between groups with a 95% confidence level ($P < 0.05$). Both inhibitors were shown to prevent periodontitis-induced bone loss ($p < 0.05$), and reduced the amount of osteoclasts. Significant reduction of cell infiltrate, as well as an increase in collagen matrix and blood vessels were found in the animals treated with the inhibitors ($p < 0.05$). JAK inhibitors also reduced the amount of CD45+ cells in the gingival tissue and expression of inflammatory markers (RANKL, IL-6, TNF) at the gene and protein level (TNF). The data indicate the relevance of pathway modulation as a therapeutic alternative to periodontitis.</p> <p>Keywords: Periodontitis. Janus Kinase inhibitors. Signal transduction. Immunomodulation.</p>
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Title	Search for associations between genomic and metabolic alterations in pediatric osteosarcomas
Authors	Juliana Silveira Ruas ¹ Edilene S Siqueira-Santos ³ Izilda A. Cardinalli ² Mayra Troiani ² Alejandro Cassoni ² Roger F Castilho ³ Mariana Maschietto ¹
Affiliations	1 – Research Center, Boldrini Children's Hospital, Campinas, São Paulo, Brazil. 2 – Boldrini Children's Hospital, Campinas, São Paulo, Brazil. 3 – Clinical Patology Department, Medical Sciences College, State University of Campinas, Campinas, SP, Brazil
Session	22

Abstract, Ethics Committee Number*, and Keywords	<p>Pediatric osteosarcomas (OSs) are primary bone tumors of mesenchymal origin that usually arise near the adolescent age. Genomic sequencing uncovered a high incidence of structural variations in OS, marked by numerous somatic mutations and copy number alterations, albeit with few recurrent mutations. The molecular alterations instigate changes in cellular. Tumor cells typically harbor functional mitochondria and engage in oxidative phosphorylation, despite exhibiting the Warburg effect phenotype. The mitochondrial bioenergetic function sustains tumor cell viability and proliferation. The relation between energy metabolism and molecular alterations is poorly explored in OSs. This study aimed to investigate the interplay between molecular aberrations in OS and their influence on metabolic adaptations, to elucidate their roles in tumor progression. Following the ethical committee approval (CAAE 22737219.1.0000.5376), the 2D primary cell culture of OS tissues was established and characterized in relation to cellular viability. The MTT assay revealed increase in proliferation, with a 350% rise between 24-48 hours, and 1,550% increase from 24-72 hours, suggesting an adaptation of the cells in the culture that can be related to the efficiency of cellular machinery changes. The investigation of O₂ consumption in intact cells indicated the presence of a functional mitochondrial apparatus in OS cells, contributing to the current metabolic demand. Basal respiration accounts for 30% of the maximum oxygen capacity, with the mitochondrial fraction responsible for ATP synthesis constituting 40% of basal respiration. In conclusion, our study successfully established primary cultures of OS cells, demonstrating normal mitochondrial bioenergetic function. This experimental model holds promise for investigating the impact of somatic mutations on OS cell metabolism. Funding: FAPESP 21/06782-7</p> <p>Key Words: Genome instability, Metabolism, Mitochondria, Mutations, Osteosarcoma.</p>
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Title	Chromosomal stability of stem cells from human exfoliated deciduous teeth
Authors	Mateus de Oliveira Lisboa Valderez Ravaglio Jamur Agner Henrique Dorigo Hochuli Gabriel Silva Morais Paulo Roberto Slud Brofman Letícia Fracaro
Affiliations	Core for Cell Technology, Pontificia Universidade Católica do Paraná (PUCPR), Curitiba, Brazil
Session	24 - Medicina Regenerativa e Biologia do Desenvolvimento

Abstract, Ethics Committee Number*, and Keywords	<p>Stem cells from human exfoliated deciduous teeth (SHED) comprise valuable source of stem cells in regenerative medicine. SHED are relatively easy to obtain eliminating invasive procedures for obtaining mesenchymal stem cells (MSCs), such as bone marrow aspiration. Like other types of MSCs, SHED have immunomodulatory properties and differentiation capacity in several cell types. However, because of its ectodermal origin, SHED are more likely to differentiate into neuronal-like cells when compared to other MSCs. These differentiation capabilities make SHED a promising candidate for cell-based therapies for neurological disorders. However, in vitro expansion is necessary to obtain enough cells, which can increase the risk of genetic instability. Genetic instability manifesting as mutations and chromosomal abnormalities, can affect their therapeutic abilities and raise tumorigenesis risks. Unlike bone marrow MSCs, whose genetic stability is well-documented, the genetic stability of SHED is less understood, underscoring the need for further research in this area. This study aimed to verify the chromosomal stability of SHED. After approval by the Local Research Ethics Committee (09537119.0.0000.0020), healthy teeth from five donors (8.8 ± 1.8 years) were collected, and SHED were isolated and expanded. Cells were characterized by immunophenotyping and differentiation in adipogenic and osteogenic lineages. Banding cytogenetics analyses were performed in passage four using G-banding karyotyping. SHED showed homogeneity for the characteristics of MSCs. All samples were devoid of clonal chromosomal abnormalities. The absence of abnormalities suggests the biosafety in using these cells, which may guarantee that these cells will perform their expected function and diminish the risk of adverse outcomes in patients.</p> <p>Keywords: Dental pulp, mesenchymal stem cells, cytogenetics.</p>
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Title	Scientific Dissemination as a tool for disseminating knowledge about obesity
Authors	1- Tatiane Ramos dos Santos Silveira 2- Licio Augusto Velloso
Affiliations	1 - 2 – Faculty of Medical Sciences (FCM), University of Campinas (UNICAMP), Campinas-SP, Brazil
Session	Education, History and Philosophy of Science, Science Communication

Abstract, Ethics Committee Number*, and Keywords	<p>Obesity is a disease characterized by the excessive accumulation of adipose tissue capable of causing damage to health. The prevalence of this condition has increased at an alarming rate in practically every country in the world since the 1970s. Poor diet and a sedentary lifestyle are the main risk factors for the development of obesity, which entails other health problems such as: impaired sense of well-being and quality of life, numerous metabolic complications, high frequency of sick leave, early retirement and, ultimately, increased mortality. Health-related complications are mainly due to the increase in the proportion of body fat, with a concomitant increase in mechanical load, associated with endocrine/metabolic function disorders. In this context, acting to prevent this disease must be a continuous and collective effort on the part of the scientific community in relation to society. As a result of this study, 9 educational videos were created, produced on the basis of scientific knowledge, in a format and language accessible to the general public. The videos cover topics related to health - diet and physical activity - as well as obesity. Their titles are: Unraveling Obesity and its Comorbidities, Unraveling Healthy Eating and Oral Health, Unraveling the Different Types of Fats, Unraveling Excessive Screen Time and Obesity, Unraveling Healthy Eating, Unraveling Routine and Physical Activity, Unraveling Sleep and Obesity, Unraveling Breastfeeding and Unraveling the Importance of Play. In computerized societies such as the West, educational videos have gained prominence as a powerful tool for disseminating scientific information. The videos were created using Canva and Video Scribe software, based on the reading of scientific articles, and went through: i) pre-production - the planning phase for the videos to be created, taking into account their specific objectives; ii) the creation of a script for each of the subjects according to the target audience of the research; iii) the organization of the video recording material into plans and sequences; iv) the production of the videos and v) post-production, which involves editing and finalizing the video produced and, finally, vi) the dissemination of the recorded material on Youtube and Instagram. Educational videos and scientific dissemination have the potential to revolutionize health teaching, especially when it comes to complex issues such as obesity and its comorbidities. Approaches based on learning theories, combined with the visual and narrative advantages of videos, make this tool an effective choice for scientific dissemination. As evidenced by the studies, educational videos can play a key role in promoting awareness, prevention and management of obesity and its comorbidities. Keywords: Obesity and Comorbidities, Scientific Dissemination, Educational Videos.</p>
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Title	Maternal low-protein diet increases autophagy in the tooth germ of 14-day fetuses
Authors	Bruno Calsa ¹ , José Antônio Rocha Gontijo ¹ , Milton Santamaria-Jr ² , Patrícia Aline Boer ¹ .
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.
Session	28 – DOHaD e Desnutrição

Abstract and Keywords	<p>Autophagy is a cellular process that promote intracellular components degradation to maintain intracellular homeostasis and has a key role during development. Herein, we aimed to evaluate the effects of gestational protein restriction on tooth germ autophagy flux using a transgenic mouse model. The study was approved by ethic committee (5923-1/2021). CAG-RFP-EGFP-LC3 female mouse at 8 weeks of age were mated and randomized into two groups that received: NP (normal protein, 17% casein, n=4) or LP (low protein, 6% casein, n=4) diet. At 14th gestational day, the embryos were collected, weighed, and their heads cryosectioned for autophagic assessment. The results showed an increase of body mass in males LP fetus compared to NP. The placental mass did not change. Regarding autophagic assessment on tooth epithelium, was found an increase in autophagosomes number, however no change in autolysosomes, autolysosomes with lysosomes and total number of autophagic vesicles was found. On tooth mesenchyme, the number of autophagosomes decreased in LP group compared to NP group. The autolysosomes increased in LP compared to NP. On the other hand, autolysosomes with lysosomes marker decreases and the total number of autophagic vesicles increase. The cleaved caspase 3 expression in LP fetus increased in dental epithelium and mesenchyme. In conclusion, the low-protein diet during gestation increased the autophagy on tooth mesenchyme, moreover enhanced apoptosis in both dental epithelium and mesenchyme. We can assume that the lack of amino acids due to malnutrition is leading to an increase in autophagy to release fundamental units for development. This process would allow development at the expense of cell multiplication resulting in smaller structures.</p> <p>Keywords: undernutrition, tooth development, autophagy, apoptosis.</p>
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Title	Altered pathways of lung branching morphogenesis of 14-day- old male rat fetuses programmed by low protein diet
Authors	Gomes JS, Calsa, B, Folguieri, MS, Boer PA and Gontijo JAR
Affiliations	Fetal Programming and Hydroelectrolyte Metabolism Laboratory, Nucleus of Medicine and Experimental Surgery, Department of Internal Medicine, FCM, Campinas, São Paulo, Brazil.
Session	

Abstract, Ethics Committee Number*, and Keywords	<p>Introduction: Gestational malnutrition promotes permanent changes in the fetal lungs that may raise susceptibility to pulmonary diseases during adulthood. In rats maternal protein restriction during gestation causes lung hypoplasia and promotes inflammation and oxidative stress, simplifying the lung structure in mice. Although these influences on organogenesis have been described previously, signaling pathways that control lung branching morphogenesis still need to be investigated.</p> <p>Aims: Thus, in the present study, we investigate gene expression and proteins of key developmental signaling pathways in the lung of male fetuses from mothers submitted or not to protein restriction. The C57BL/6 mice (8–10 weeks) were used for breeding. After pregnancy confirmation, female mice were randomly divided into a normal-protein (NP 17% casein) or low-protein (LP 6% casein) diet group. On the 14th gestational day (14GD) the lungs from male fetuses were processed for immunofluorescence or PCR-array of 84 growth factors. Ethic committee number 6029-1/2022.</p> <p>Results: Immunofluorescence shows lower expression of AMPK and mTOR in lungs of 14th gestational day LP fetus when compared to the control animal. PCR array shows that the lungs of 14GD LP fetus presented significant <i>Tgfa</i>, <i>Tgfβ</i>, <i>Fgf11</i>, and <i>Csf1</i> down-regulated genes compared to age-matched NP progeny. Discussion and Conclusion: These are essential pieces for the developmental mechanisms that control lung branching morphogenesis. TGF-β promotes elongation at the expense of branching in embryonic cultures. FGF signaling via the ERK/MAPK pathway is central to controlling lung branching morphogenesis, as it is necessary and sufficient to induce lung branch. mTOR is an essential key to the permanency of lung pluripotent cells that are associated to cell proliferation and, consequently, lung branching. AMPK activation is linked to normal lung development and your decrease to impaired organogenesis. These results suggest an impaired branching morphogenesis with a potential role in the genesis of lung hypoplasia in this model.</p> <p>Keywords: Gestational protein restriction, Fetal Programming, Lung development, Growth factor.</p>
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Title	Maternal exposure to polystyrene nanoparticles during gestation and lactation: preliminary data
Authors	Iala Milene Bertasso; Nathália Medeiros Nehme; Nelyana Oliveira Serpa; Nathália Pereira Dellatorre; Nícolas de Abreu Marinho Lopes; Beatriz Souza da Silva; Luana Lopes de Souza; Rosiane Aparecida Miranda; Egberto Gaspar de Moura; Patrícia Cristina Lisboa.
Affiliations	Laboratório de Fisiologia Endócrina, Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro, Brazil.
Session	Abordagens Pré-clínicas em DOHAD

Abstract, Ethics Committee Number*, and Keywords	<p>Plastic waste of various sizes dispersed in air, soil, water, and food have been found in the placenta and breast milk and associated with impacts on the progeny endocrine and cognitive systems. In this context, we evaluated the impacts of exposure to nanoplastics during gestation and lactation on the endocrine-metabolic profile of male and female offspring. The experiments were approved by the Animal Care and Use Committee of the Biology Institute of the State University of Rio de Janeiro (CEUA 033/2022). For this, pregnant Wistar rats received filtered water (Control group, n=9) or 100 nm polystyrene nanoparticles (25 µg/kg of body mass/day; NP group, n=8) from gestational day 7 until the end of lactation by intragastric gavage. Biometrics and biochemical parameters of dams, male (M) and female (F) offspring at birth and PN21 (postnatal day) were studied. T-test was used for statistical analyses. NP dams showed decreased body mass during gestation (-19%, P=0.0098) and plasma total cholesterol at weaning (CHOL, -31%, P=0.0243). Whereas, cumulative food intake (P=0.6137), visceral fat mass (P=0.8662), plasma glucose (P=0.7475), triglycerides (TG, P=0.5297), leptin (P=0.8874) and insulin (P=0.1921) were unchanged at weaning. At birth, pups were slightly heavier (M: +5%, P=0.0370; F: +8%, P=0.0005) and larger (M: +3%, P=0.0186; F: +5%, P<0.0001). Until weaning, the NP pups had unchanged body mass gain (M: P=0.4048; F: P=0.8562), naso-anal length (M: P=0.2306; F: P=0.8953), percentual of fat (M: P=0.8603; F: P=0.1784) and lean mass (M: P=0.3691; F: P=0.4294). At weaning, only males NP showed higher TG (M: +76%, P=0.0254; F: P=0.5457). Other plasma parameters such as glucose (M: P=0.5069; F: P=0.7837), total CHOL (M: P=0.8367; F: P=0.3899), leptin (M: P=0.7307; F: P=0.9293) and insulin (M: P=0.3741; F: P=0.7525) were unaltered. These data are preliminary and other markers are being analyzed to better understand the consequences of maternal exposure to NP.</p> <p>Polystyrene, nanoparticles, lactation, gestation, endocrine-metabolic profile.</p>
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Title	Puberty as an ontogenetic window: Childhood obesity together with the autophagy pathway modulates brown adipose tissue in older adults
Authors	Cavalcante, KVN ¹ Matos, MO ¹ Soares, RBA ¹ Ferreira-Junior, MD ¹ Ramiro, MN ¹ Boer, PA ² Gomes, RM ¹
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Session	27 - Abordagens Pré-clínicas em DOHAD

Abstract, Ethics Committee Number*, and Keywords	<p>Puberty is an ontogenetic window in which important changes in metabolism can be imprinted and impact health outcomes throughout life. Considering the worldwide rates of childhood obesity and the increasing diet among adolescents, several studies demonstrate the nutritional influences in key periods of development on the emergence of diseases later in life, in addition to the fact that puberty is a window-sensitive ontogenetic factor. The present work aims to investigate the long-term effects on brown adipose tissue of ingesting a diet high in sugar and fat during puberty in mice, focusing on the autophagy pathway. We used a juvenile obesity model to analyse metabolic parameters in male and female C57BL/6 mice (CEUA 116/22). To do this, we separated the animals into 2 groups: Control (CO); and high sugar + high fat (HSHF). The dietary intervention was performed from postnatal day 30 (PN30) to PN60. The assessments were made at PND180. In a cold exposure protocol, which activates the thermogenic function, we observed that HSHF males showed an increase in brown adipose tissue (BAT; CO 0.19±0.009 vs HSHF 0.24±0.02) and retroperitoneal adipose tissue (WAT; CO 0.29±0.03 vs HSHF 0.44±0.05). And HSHF females showed an increase in BAT (CO 0.15±0.01 vs HSHF 0.20±0.01) without changing the white fat stock. In the cold exposure protocol with administration of colchicine, a drug that inhibits the autophagy pathway, we found a reduction in WAT (CO 0.40±0.04 vs HSHF 0.21±0.06) and BAT (CO 0.24±0.01 vs HSHF 0.17±0.01). In females we found no significant differences. Given these results, we are finalizing the morphological analysis of the BAT of both protocols. In summary, we observed that brown adipose tissue in obese</p>
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people can be modulated, which is still under analysis as it could be a modification due to either bleaching or darkening, and we showed that the autophagy pathway directly influences this tissue.

Keywords: Childhood obesity; Brown Adipose Tissue; Autophagy;



Title	Maternal diet with interesterified palm oil might lead to metabolic changes in young and adult offspring
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Session	Abordagens Pré-clínicas em DOHAD

Abstract, Ethics Committee Number*, and Keywords	<p>There is evidence regarding the consumption of interesterified fats and the development of metabolic disorders, but there is still a lack of the effects of maternal consumption on their offspring's metabolism. Female C57BL/6J mice were assigned to a normocaloric diet with natural (P) or interesterified (I) palm oil for four weeks, mating, pregnancy, and lactation. Offspring were weaned onto a natural palm oil diet from day 21 until euthanasia (d30) or day 42 (d98). The d98 offspring were split into four groups based on dam's diet and the diet they received during the following 8 weeks until euthanasia, natural (P) or interesterified palm oil (I): PP, PI, IP, and II. Results were expressed as mean \pm SD, sample size of 32 for dams and 6-10 for the offspring. Dams' from group I were heavier on week 2 and 3 when compared to P dams (I-19,01\pm1,28 vs P-18,20\pm1,63-w2; I-19,66\pm1,41 vs P-19,05\pm1,22-w3), in accordance to weekly food intake (I-24,51\pm1,73 vs P-22,82\pm3,11-w2; I-24,45\pm1,75 vs P-22,79\pm2,56-w3; I-24,60\pm2,80 vs P-23,20\pm2,33-w4). Offspring's analyses indicate a reduction in pAkt in the soleus of I male and female young offspring (d30) compared to P group, as well as in the adult offspring's (d98) PI and II compared to PP and IP groups, but only male offspring showed a similar pattern in the liver. PI, IP and II female adult offspring showed an increase in PTP1B in the soleus when compared to PP. Oxidative stress analyzes show increased SOD activity and MDA concentration in the liver of female IP (0,26\pm0,02-sod; 0,14\pm0,03-mda) compared to PP (0,19\pm0,033-sod; 0,09\pm0,03-mda), and of II (0,29\pm0,037-sod; 0,17\pm0,04-mda) compared to PI (0,23\pm0,06 -sod; 0,11\pm0,06 -mda), whereas, in the female I young offspring there was a decrease of MDA concentration (I-</p>
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0,07±0,01 v P-0,10±0,02). In summary, a diet containing interesterified palm oil can impact dams' murinometric parameters and induce molecular changes in the liver and muscle of the young and adult offspring.

CEUA/Unicamp nº 5764-1/2021

Keywords: DOHaD; Interesterified Fat; Metabolism.



Title	Effects of gestational protein restriction on mandible development
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Session	28 – DOHAD e Desnutrição

Abstract and Keywords	<p>This study aims to assess the impact of gestational protein restriction on mandible osteogenesis. (Ethic committee #5923-1/2021). C57BL/6J and CAG-RFP-EGFP-LC3 female mouse were mated and randomized into two groups that received diet with normal protein (NP, 17% casein, n=5) or low protein (LP, 6% casein, n=5) content. On the 18th gestational day (GD) male embryos were collected for microtomography (μCT), scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDS), PCR array of 84 growth factor genes and autophagy dynamic analyses. LP offspring exhibited lower body mass than the NP group, with μCT analysis revealing no head volumetric differences. In LP offspring the EDS demonstrated lower percentage of calcium and higher percentage of phosphate in mandibles. The SEM showed lower hydroxyapatite crystals in mandible surface. The collagen fibers visualized through second harmonic generation microscopy increases in bone trabeculae of LP fetus. The PCR array showed that 27 genes were differentially expressed: 24 down-regulated genes (<i>Bdnf</i>, <i>Bmp2</i>, <i>Bmp3</i>, <i>Bmp4</i>, <i>Bmp6</i>, <i>Bmp7</i>, <i>Cxcl12</i>, <i>Ereg</i>, <i>Fgf2</i>, <i>Fgf10</i>, <i>Fgf11</i>, <i>Fgf22</i>, <i>Gdf10</i>, <i>Gdnf</i>, <i>Il18</i>, <i>Inha</i>, <i>Inhbb</i>, <i>Kitl</i>, <i>Ntf3</i>, <i>Ntf5</i>, <i>Tgfa</i>, <i>Tgfb2</i>, <i>Vegfc</i> and <i>Vegfd</i>) and 3 up-regulated genes (<i>Fgf4</i>, <i>Gdf5</i>, and <i>Spp1</i>). Moreover, mRNA levels of Akt1, Mtor, Nfkb and Smad1 were increased in LP group. The LP diet during gestation decreases the autophagy on bone mesenchyme, moreover impairs mineralization of mandible. There a downregulation of BMP-TGFB ligands that possibly drives the mandible hipomineralization status. In conclusion, the results suggests that gestational protein restriction anticipated bone differentiation in utero, prior to 18GD where these process are reduced compared to the control, leading to the reduction in bone area at 15 days of life previously observed.</p> <p>Keywords: undernutrition, osteogenesis, autophagy</p>
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Title	Maternal acephate pesticide exposure in Wistar rats induces intrauterine growth restriction and affects the placental transcriptome by a sex dependent manner
Authors	<u>Mariana de Souza Pomacena</u> ¹ ; Pedro Vinícius Gonçalves Martins ¹ ; Yasmin Vitória Rezende Avelino dos Reis ¹ ; Manoelle Lacerda Miranda Pereira ¹ ; Beatriz Souza da Silva ¹ ; Nathália Medeiros Nehme ¹ ; Iala Milene Bertasso ¹ ; Luana Lopes de Souza ¹ ; Egberto Gaspar de Moura ¹ ; Vitor Lima Coelho ² ; Patrícia Cristina Lisboa ¹ ; Rosiane Aparecida Miranda ¹ .
Affiliations	1 – Laboratory of Endocrine Physiology, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil. 2 – SENAI-Cetiqt , Rio de Janeiro, Brazil
Session	Abordagens Translacionais e Clínicas em DOHAD

Abstract, Ethics Committee Number*, and Keywords	<p>Acephate is the most insecticide used in Brazil, despite has already been banned in some countries. This pesticide acts as a endocrine disruptor due to its impact on glyceimic homeostasis and reproductive function; but in utero exposure was not fully investigated. We hypothesize that acephate exposure during pregnancy alter placental function, inducing changes in the offspring in a sex-specific manner. Thus, we exposed pregnant Wistar rats from 6.5th to 18.5th day of gestation, via gavage with filtered water (Control) or acephate (4.5 mg/kg body weight; ACE). Protocol approved: CEUA/012/2022. A cesarean was performed on the 18.5th day to obtain the placentas (n=8 dams/group) and fetuses. We evaluated the dams' body weight, liver and spleen weight, embryonic resorption and percentage of fetuses/dam and dams' plasma pro-inflammatory cytokines (GRO/KC, IL-β, TNF-α and MCP1). Placental efficiency was calculated (fetuses weight/placenta weight). Statistical analysis: Student's t test ($p < 0.05$). Placental fragments were used for total RNA extraction, sequencing and transcriptomic analyzes. Acephate exposure did not alter maternal body weight gain, but increased liver weight (14%) and MCP1 levels (95%). ACE dams showed resorption points. Fetuses from both sexes showed intrauterine growth restriction (13%); but males showed a reduction (ACE, -10%), while females showed an increase in placental efficiency (ACE, 20%). Placental transcriptomic analyzes presented that ACE females have 276 up-regulated and 185 down-regulated genes ($FDR < 0.01$; $FC > 2$) while males does not presented differentially expressed genes. Most of the up-regulated genes are associated with transport, growth and differentiation while the down-regulated genes are associated with cellular activity, movement, secretion and enzyme production. Intrauterine exposure to acephate compromises the placental function by a sex-dependent manner. Current data highlight possible acephate effects in human placenta. Keywords: Acephate, placenta, fetus, transcriptome, sexual dimorphism.</p>
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Title	Glyphosate herbicide induces sex-dependent intrauterine growth restriction and placental transcriptomic changes in offspring of Wistar rats
Authors	Pedro Vinícius Gonçalves Martins ¹ ; Yasmin Vitória Rezende Avelino dos Reis ¹ ; Mariana Pomacena de Souza ¹ ; Yasmim Petronilho de Souza ¹ ; Beatriz Souza da Silva ¹ ; Nathália Medeiros Nehme ¹ ; Iala Milene Bertasso ¹ ; Luana Lopes de Souza ¹ ; Egberto Gaspar de Moura ¹ ; Vitor Lima Coelho ² ; Patrícia Cristina Lisboa ¹ ; Rosiane Aparecida Miranda ¹ .
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Session	Abordagens Translacionais e Clínicas em DOHAD

Abstract, Ethics Committee Number*, and Keywords	<p>Glyphosate, the most used pesticide in the world, is associated with reproductive and metabolic diseases; but it is unclear how it can affect intrauterine development. We studied the impact of exposure to low-dose glyphosate on intrauterine development, considering sexual dimorphism and placental transcriptome. Protocol approved: CEUA/013/2022. From 6.5th to 18.5th day of gestation, rats were exposed by gavage with water (Control) or glyphosate (0.5 mg/kg body weight; bw; GLY). On the 18.5th day, placentas (n=8 dams/group) and fetuses were obtained via cesarean section. In dams, we evaluated bw gain, liver and spleen weight, embryonic resorption and the percentage of fetuses/dam. Placentas and fetuses were weighed and measured and placental efficiency was calculated. Pro-inflammatory cytokines (GRO/KC, IL-β, TNF-α and MCP1) were measured. Statistic: Student's t test ($p < 0.05$). Placental fragments were used for total RNA extraction, sequencing and transcriptomic analyzes. Glyphosate does not alter bw gain, but increases liver weight (10%) and MCP1 levels (79%) in dams. GLY group showed increased percentage of resorption points and fetal deaths (25%). Male GLY fetuses showed intrauterine growth restriction (10%). Males showed a reduction (12%), while females showed an increase in placental efficiency (13%). Analysis of the placental RNA-Seq experiments demonstrated that male GLY fetuses have no differentially expressed genes while females presented 434 up-regulated and 178 down-regulated genes. In general, up-regulated genes are associated with transport, growth and differentiation while down-regulated genes are associated with cellular metabolism, cytokine-mediated signaling pathway, DNA repair</p>
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mechanisms and enzyme production. Maternal exposure to glyphosate compromises placental function suggesting the origins of reproductive and metabolic dysfunction mainly in female offspring. These lessons in rodents can be useful to understand placental dysfunction in humans.

Keywords: Glyphosate, placenta, transcriptome, sexual dimorphism.



Title	Using functional genomics to understand the interaction between genes and birth weight on infant body mass index
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Session	Abordagens Translacionais e Clínicas em DOHAD

Abstract, Ethics Committee Number*, and Keywords	<p>Poor fetal growth, assessed by birth weight, is one of the most frequent prenatal adversities, being associated with numerous neurometabolic alterations, including altered eating behavior and increased obesity risk later in life. However, interindividual differences make obesity development a variable outcome among children exposed to such adversity. Aiming to identify markers of risk, we used data from a recently described discovery epigenetic wide association study (EWAS) of adult body mass index (BMI) to calculate a methylation score for children included in three cohorts [MAVAN (Canada), n= 87; BIBO (Netherlands) n= 124; GUSTO (Singapore), n=289]. Genotype information from those cohorts was used to calculate the expression-based polygenic risk scores (ePRS), based on nucleus accumbens (NAcc) and white adipose tissue (WAT) gene expression from GTEx data, according to the genes mapped from the EWAS significant CpGs. Next, we investigated if ePRS moderated the association between birth weight and BMI, and how methylation score contributes to the models. In MAVAN, NAcc ePRS interacted with birth weight in association to BMI at 6 y.o. ($\beta=-0.0008$, $p<0.05$), with or without the methylation score, but not WAT ePRS. The same was found for BIBO ($\beta=0.0007$, $p<0.05$). On the other hand, in GUSTO, the interaction between WAT ePRS and birth weight associated with BMI at 6 ($\beta=-0.0004$, $p<0.05$) and 12 months old ($\beta=-0.0002$, $p<0.05$), with or without the methylation score, but not NAcc ePRS. Thus, using functional genomics approaches to investigate individual differences in responsivity to prenatal adversity may inform the development of precision preventive measures regarding BMI, although tissue-specific score interaction with populations can vary.</p> <p>Keywords: functional genomics; ePRS; birth weight; BMI; epigenetic; DOHAD.</p>
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Title	Socioeconomic determinants of low birth weight and their association with peripubertal obesity in Brazil
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Session	Abordagens de Saúde Coletiva em DOHAD

Abstract, Ethics Committee Number*, and Keywords	<p>Low birth weight (LBW) is an early life adversity associated with several risk factors and metabolic dysfunction late in life. However, it is unclear how socioeconomic determinants interact with population-based risk factors for LBW, and how LBW impacts peripubertal health, specially in low- and middle-income countries. Thus, we sought to investigate the association of population-based and socioeconomic risk factors with LBW in Brazil. State level data were collected from the Global Health Data Exchange and official sources of Brazilian government, from 1995 to 2017. First, we tested the summary exposure value (SEV) of reproductive age (15-49 y.o.) population risk factors as exposures in association with 1-year lagged SEV LBW as outcome. Next, we tested the association between SEV LBW as exposure and ten years-lagged high body mass index (HBMI) in peripuberty (10-14 y.o.) as outcome. For both associations, fixed-effects multivariable linear regression models were constructed, adjusting for socioeconomic covariates together and separately. Reproductive-age population exposure to smoking (0.31 SEV, 95%CI 0.18-0.43), alcohol (0.71 SEV, 95%CI 0.23-1.19), high systolic blood pressure (0.23 SEV, 95%CI 0.11-0.35) and HBMI (0.15 SEV, 95%CI 0.04-0.27) were positively associated with SEV LBW. Diet high in sugar-sweetened beverages was also positively associated with LBW (0.23 SEV, 95%CI 0.11-0.35). However, this association was no longer significant after adjusting for GDP per capita and access to primary care. Regarding the repercussions of LBW, a 1-point increase in SEV LBW was positively associated with increases in peripubertal HBMI (1.6 SEV, 95%CI 0.66-2.55), which was also lost after adjusting for GDP per capita and access to primary care. Our study suggests that GDP per capita and access to primary care play a capital role in determining poor birth outcomes associated to the tested risk factors and to mid-term consequences of LBW in Brazil.</p> <p>Keywords: Low birth weight; GDP per capita; access to primary care; high body mass index; LMIC;</p>
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Title	<i>Acanthosis nigricans</i> in children and adolescents is associated with overweight, high waist-to-stature ratio, hepatic steatosis, high blood pressure and low HDL-c
Authors	Ellen Carine Soares Xavier - 1 Roseli Oselka Saccardo Sarni - 2 Fabiola Isabel Suano de Souza - 2 Camila Augusta Victorino – 2 Fernando Luiz Affonso Fonseca - 3 João Carlos Pina Faria - 2 Sonia Hix - 1
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Session	DOHAD e Hipernutrição

Abstract, Ethics Committee Number*, and Keywords	<p>Obesity is a chronic disease that increases the risk of several diseases and has been on the rise in childhood. Excess weight is associated with insulin resistance, which can favor development of Acanthosis Nigricans (AN) - hyperpigmented spots usually present on the neck, armpits, and elbow. The aim of this study was to evaluate the presence of acanthosis nigricans (AN) and its association with anthropometric and biochemical parameters. A cross-sectional study with 170 children and adolescents aged between 4 and 15 years, 106 (62,4%) with normal body mass index (BMI); 39 (22,9%) overweight; 25(14,7%) obese from a public institution in Santo André-Brazil was performed. We evaluated: weight, height, waist circumference, waist-to-stature ratio (WSR), blood pressure, presence of AN and hepatic steatosis (HS), lipid profile; apo A1, apo B, glycemia and insulin (HOMA-IR); liver enzymes: AST and ALT and high-sensitivity C-reactive protein (hsCRP). AN was detected in 29 (17,1%) of 170; in 9 of 105 (8,5%) normal BMI; 10 of 39 (25,6%) overweight; 10 of 25 (40,0%) obese individuals. AN had a statistically significant association with ZBMI ($p=0,000$), WSR ($p=0,029$), HS ($p=0,002$), systolic blood pressure ($p=0,000$) and diastolic blood pressure ($p=0,013$). Even though no statistically significant associations were found between AN and lipid profile, HDL-c levels showed a tendency to be lower ($p = 0,065$). AN did not associate with the other evaluated parameters. The data point to a significant presence of AN in children and adolescents, with a higher frequency in overweight and</p>
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	<p>obese subjects, with higher WSR, blood pressure and lower HDL-c. The periodic monitoring of children and adolescents might prevent the development of diabetes mellitus, dyslipidemia, liver diseases, and other medical conditions in adulthood. Statistics: SPSS 24.0. CAAE: 02670518.7.0000.0082 key words: Obesity, overweight, acanthosis nigricans, WSR, HDL-c</p>
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