ANAIS

IV SIMPÓSIO INTERNACIONAL DE ESTRESSE OXIDATIVO E DOENÇAS CARDIOVASCULARES

25 de Setembro de 2015

Universidade Federal do Rio Grande do Sul

Porto Alegre, Brasil

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IV Simpósio Internacional de Estresse Oxidativo e Doenças Cardiovasculares

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SUMÁRIO

APRESENTAÇÃO

A Fisiologia Cardiovascular é uma ciência em contínua transformação e novas descobertas científicas são publicadas diariamente, tornando necessária a revisão constante dos conteúdos, tanto por docentes como por discentes. Neste sentido, foi criado o evento denominado “Simpósio Internacional de Estresse Oxidativo e Doenças Cardiovasculares”, com sua primeira edição em 2012. Com ocorrência anual, até o momento foram realizadas quatro edições.

O IV Simpósio Internacional de Estresse Oxidativo e Doenças Cardiovasculares, realizado no dia 25 de setembro de 2015, teve como objetivo divulgar atualidades científicas relativas ao estresse oxidativo e sua influência na enfermidade cardiovascular, correlacionando aspectos básicos e clínicos. Também teve como intenção divulgar o trabalho realizado por diferentes grupos de pesquisa, tanto do Rio Grande do Sul como de outros estados, que realizam investigação científica nesta área, fomentando a integração entre estes e com os palestrantes internacionais presentes. Além disso, o evento proporcionou um incentivo aos alunos de graduação e pós-graduação a apresentarem seus trabalhos e oportunizar sua discussão com eminentes cientistas nesta área de concentração. Este incentivo ocorreu por meio de exposição de pôsteres, sendo que, após uma seleção realizada pela comissão científica, os cinco melhores resumos foram apresentados oralmente, a fim de concorrerem ao “Prêmio Prof. Antonio Belló”, que foi entregue ao melhor trabalho apresentado.

Desta forma, a presente produção bibliográfica é uma união de todos os resumos dos pôsteres apresentados no IV Simpósio Internacional de Estresse Oxidativo e Doenças Cardiovasculares.

A todos, uma boa leitura!

Profa. Dra. Adriane Belló-Klein

TRABALHOS APRESENTADOS

IV SIMPÓSIO INTERNACIONAL DE ESTRESSE OXIDATIVO E DOENÇAS CARDIOVASCULARES

ACUTE EXPOSURE TO ALUMINUM CHLORIDE DECREASES VASCULAR REACTIVITY AND INCREASES OXIDATIVE STRESS IN WISTAR RATS

Schmidt, P.M.[1]; Torres, J.G.D.[1]; Martinez, C.S. [1]; Escobar, A.G.[1]; Kunz, S.N. [1]; Vassallo, D.V.[2]; Peçanha, F.M.[1]; Wiggers, G.A.[1]

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Introduction: Aluminum is the most common metal and its high applicability points to an important environmental contaminant. Aluminum can be accumulated in several organs and has been associated with diseases, mainly neurological disorders. At cardiovascular system, there are not enough evidences of Al-induced dysfunction. **Objectives:** To investigate the effects of acute exposure to aluminum chloride ($AlCl_3$) on blood pressure, vascular reactivity and oxidative stress. **Material and Methods:** Male *Wistar* rats were divided into groups: *Untreated*: vehicle (ultrapure water, *ip*) and *$AlCl_3$*: single dose of $AlCl_3$ (100 mg/kg *ip*). Systolic and diastolic blood pressure was assessed in anesthetized rats through cannulation of carotid artery immediately before aluminum administration and after one hour. Nitric oxide (NO) released was studied in arteries; reactive oxygen species (ROS), malondialdehyde, non-protein thiol (NPSH) levels, superoxide dismutase (SOD), catalase, glutathione peroxidase and glutathione-S-transferase enzyme activities were verified in plasma. Aorta reactivity was realized in isolated organ bath and concentration-response curves to acetylcholine, sodium nitroprusside and phenylephrine were performed in presence and absence of endothelium, NO synthase inhibitor (L-NAME), potassium channels blocker (TEA), NADPH-oxidase inhibitor (apocynin), and superoxide scavenger SOD. Results were expressed as mean±SEM and analyzed by t test or two-way ANOVA followed by Bonferroni *post-hoc* (*P<0.05). **Results:** Aluminum concentration after one hour of $AlCl_3$-exposure reaches 147.7±25.0 µg/L, besides increased ROS, malondialdehyde and NPSH levels, glutathione-S-transferase and catalase activities, instead Al reduced SOD activity. $AlCl_3$-exposure did not change blood pressure however decreased vasoconstrictor response to phenylephrine (Emax - *Untreated*: 114.5±1.4 *vs* *$AlCl_3$*: 91.0±4.3* %KCl), with increased negative endothelial modulation and ROS production from NADPH-oxidase, mainly superoxide anions. Aluminum exposure has not changed the endothelium-dependent and independent relaxation. **Conclusion:** Acute $AlCl_3$-exposure promotes oxidative stress and reduces the vasoconstrictor response in

aorta, through nitric oxide pathway as a potential responsible mechanism. Registration number 028/2013 (CEUA/UNIPAMPA).

Key-Words: Aluminum, cardiovascular system, oxidative stress.

Acknowledgements: CNPq, Unipampa.

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ALTERATIONS ON THE LEVELS OF PROTEINS INVOLVED IN BRAIN OXIDATIVE STRESS INDUCED BY NEONATAL HYPERGLYCEMIA IN RATS

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Introduction: Recently, the consequences of diabetes on the central nervous system have received great attention. However, the mechanisms by which hyperglycemia affect the central nervous system remain poorly understood. In addition, recent studies have shown that hyperglycemia induces oxidative damage in the adult rat brain. In this regard, no study has assessed oxidative stress as a possible mechanism that affect the brain normal function in neonatal hyperglycemic rats. Furthermore, our recent studies have shown that neonatal hyperglycemia induces oxidative damage in rat brain. **Objectives:** We assessed protein levels that probably are involved in the role of oxidative stress in the brain of rats with neonatal hyperglycemia. **Material and Methods:** Seven-day-old Wistar rats were subject to a single administration of streptozotocin (100mg/Kg body weight) and rats with glycemia above 200mg/dL are considered hyperglycemic, while controls received saline. Five days after injection of streptozotocin, animals were killed. We assayed catalase, gluthatione peroxidase, superoxide dismutase, Nrf2, total Akt and phosphorylated Akt protein levels by western blot method. T test was used to statistical analyses. **Results:** No significant changes were detected in superoxide dismutase, glutathione peroxidase and total Akt protein levels. On the other hand, neonatal hyperglycemic rats presented increased catalase and decreased Nrf2 and p-Akt protein levels in the brain when compared to control group. **Conclusion:** Neonatal hyperglycemia was able to alter catalase protein levels, an important enzyme involved in the brain redox status. Also, neonatal hyperglycemia promoted alterations in brain signaling pathways. So, these results suggest that oxidative stress could represent a mechanism to explain the harmful effect of neonatal hyperglycemia on the central nervous system. Protocol number CEUA: 25395.

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ANALYSYS OF OXIDATIVE STRESS MARKERS OF RECREATIONAL RUNNERS AFTER A 10-KM OUTDOOR RUNNING

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*** Centro Universitario Metodista do sul – IPA**

INTRODUCTION: Oxidative stress could be increased following acute exercise and it could lead to lipid, protein and deoxyribonucleic acid damage. However, chronic exercise could increase antioxidant capacity. **OBJECTIVE:** To evaluate and compare the acute effects of a 10-km race on the oxidative profile in physically active adults. Method: thirteen male volunteers, (age 38,31±7,00 years) participate the study. After the clarification of the procedures, they signed the free and informed consent, answered the international physical activity questionnaire - ipaq questionnaire and were submit to the anthropometric evaluation following international society for the advancement of kinanthropometry (isak) standards. This study was approved by ethics research committee of the methodist university center - ipa under protocol number 809.115/2014 and assessment procedures were performed in the laboratory of biochemistry and exercise physiology laboratory, of the methodist university center - ipa, in porto alegre. The evaluation of oxidative stress was perform by measuring the levels of malondialdehyde by tba method. It was also evaluated catalase active and sulfhydryls. The normally data was analyzed by shapiro-wilk test, the comparison of the variables pré and post was analyzed by test-t student considering a significance level $p<0,05$. **RESULTS:** There was a significant difference of sulfhydryls levels between pre and post running moment ($p < 0.05$). There were no significant differences between the pre- and post-race 10-km for catalase values and the mda. **CONCLUSION:** The running of 10-km altered protein oxidation (sulfhydryl) without changing the levels of lipid peroxidation and the antioxidant activity of catalase.

Support : CAPES

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BRAZILIAN BERRY MODULATES MITOCHONDRIAL FUNCTION AND REDUCES OXIDATIVE STRESS IN HUMAN FIBROBLASTS CELLS (MRC5)

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Introduction: Jaboticaba (*Plinia* sp.), termed the Brazilian berry, has emerged as a new functional food with potential health benefits, mainly because of their significant levels of phenolic compounds, primarily anthocyanins and flavonols*. Plinia trunciflora* (O. Berg) Kausel is one of the main species of jaboticaba that is naturally occurring and cultivated in Brazil. **Objective:** Thus, the aim of this study was to evaluate the antioxidant activity of jaboticaba peel extract (JPE) and its capacity to modulate oxidative stress as well as mitochondrial function in human lung fibroblast cells (MRC-5). Besides, macronutrient and phenolic composition of *P. trunciflora* peel were determined. **Materials and Methods:** MRC-5 cells were exposure to the JPE for 1 hour and then challenged with 700µM H_2O_2 during 26 hour. After treatment, the JPE ability to reduce oxidative stress was evaluated though lipid peroxidation and nitric oxide levels. Mitochondrial function was assessed through the quantification of ATP biosynthesis and determination of complex I activity. Cell viability was evaluated using MTT assay. Chemical composition was determined by Folin Ciocalteau assay and high resolution mass spectrometry (HRMS). For determination of anthocyanin content was used pH differential method. **Results:** JPE was able to reduce the decreases in complex I activity and the ATP levels induced by H_2O_2 and thereby decreased the oxidative stress in MRC-5 cells. Besides, the macronutrient found to have the highest level in both the peel and pulp was carbohydrates, followed by the high fiber content of the peel. The total phenolic compounds and anthocyanin levels were higher in the peel than in the pulp. HRMS showed the presence of cyanidin-3-*O*-glucoside and kaempferol in JPE. **Conclusion:** These findings show a new role for jaboticaba, a berry rich in phenolic compounds, as a mitochondrial protectant in pathological conditions where mitochondrial dysfunction is involved.

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CAN PHENOLIC *PLEUROTUS ALBIDUS* EXTRACT PROTECT ENDOTHELIAL CELLS AGAINST OXIDATIVE STRESS INDUCED BY HYPERGLYCEMIA?

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#Laboratório de Enzimas e Biomassas, Instituto de Biotecnologia, Universidade de Caxias do Sul (UCS), 95070-560, Caxias do Sul RS, Brazil

Introduction: Diabetes mellitus (DM) is a chronic disease for which the prevalence has risen to epidemic proportions worldwide. It is characterized by elevated blood glucose. There is growing evidence that hyperglycemia cause damage in a variety of tissues due to increased production of reactive oxygen species. Endothelial dysfunction lead both micro- and macrovascular diabetes complications. Studies have shown that phenolic extracts appear to provide protection from diabetes. *Pleurotus albidus* is an edible mushroom recently described, so there is no enough biological studies. **Objectives**: Therefore, the aim of this study was to evaluate *P. albidus* dry extract *in vitro* antioxidant activity, to determine phenolic content and measure its impact in EA.hy926 endothelial cell line under hyperglycemic condition. **Method**s: *In vitro* antioxidant activity was assessed by 2,2 diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assays. Folin-Ciocalteu method was assayed to determine phenolic content. Endothelial cells were exposed to hyperglycemic condition (glucose 35 mM) for 5 days and then the oxidative stress was evaluated. **Results**: It was observed that the extract presented high level of phenolic content (742.80 ± 0.26 mg% of gallic acid equivalent). This agrees with DPPH (IC_{50} = 21.62 ± 1.02 mg of extract) and ABTS (IC_{50} = 12.47 ± 0.16 mg of extract) radical scavenger activity. *P. albidus* extract was able to reduce the lipid peroxidation and carbonyl content induced by hyperglycemia. Besides, with extract treatment, the imbalance in antioxidant enzymes superoxide dismutase and catalase were normalizes. An important find was the ability to modulate the electron transport chain dysfunction in hyperglycemic endothelial cells by the extract intervention. **Conclusion**: Although another studies are need, our data showed that *P. albidus* extract present promising anti diabetic activity by endothelial protection of oxidative stress.

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CHRONIC TREATMENT WITH ORALLY ACTIVE ANGIOTENSIN-(1-7) FORMULATION DECREASES OXIDATIVE DAMAGE AND IMPROVES HEMODYNAMIC PARAMETERS IN CARDIAC TISSUE OF SPONTANEOUSLY HYPERTENSIVE RATS

Trabalho selecionado para concorrer ao prêmio Prof. Antonio Belló

Daniela Ravizzoni Dartora[1], Paula Rohr[1], Mariane Bertagnolli[2]; Ademir Nascimento[3], Danielle da Silva Dias[4], Karina Rabello Casali[1]; Kátia De Angelis[4], Maria Cláudia Irigoyen[1,3,5], Robson A. Santos[1,5].

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Background: Renin-angiotensin (RAS) system has been implicated in the pathogenesis of cardiovascular diseases. The cardioprotective Angiotensin-(1–7)/Mas axis has an important role in cardiovascular regulation once that Ang-(1-7) exerts beneficial effects mainly due to antihypertensive and antioxidant properties. Considering that oxidative stress seems to be an important mechanism in hypertension, Ang-(1-7) administration may have some cardiovascular benefits. **Purpose:** To assess the effect of a chronic treatment with orally active formulation of Angiotensin-(1-7) (HPβCD/Ang-(1-7)) in cardiac oxidative stress and hemodynamic parameters in SHR. **Methods:** Male SHR (15 weeks) treated by gavage with tap water or HPβCD/Ang-(1-7) (30 ug/Kg) once a day for 10 weeks (n=8/group).Systolic (SAP), diastolic (DAP) and mean arterial pressure (MAP) and heart rate (HR) were directly recording by a data acquisition system (Windaq/2Hz). Hearts were processed for oxidative stress assays: lipid peroxidation (CL) initiated by t-BOOH, hydrogen peroxide (H2O2) and superoxide anion (O2-) analysis; superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities; and concentrations of reduced (GSH) and oxidized glutathione (GSSG). **Results:** Chronic treatment with the Ang-(1-7) reduced SAP, DAP, MAP and HR in treated group (SAP=216.2±5.09 and 195.4±4.15, DAP= 147±1.90 and 134.6±3.17, MAP=180.2±3.48, and 162±3.18mmHg, HR=353.5±9.95 and 299,9±6,54 bpm in SHR and SHR+Ang-(1-7) respectively; $P<0.05$). CL and O2- were decreased in Ang-(1-7) treated SHR (CL=4870±968 and 1693±622.7 cps/mg protein and O2-: 9.48±0.40 and 5.40±0.43 mmoles/mg protein $P<0.05$). Regard to H2O2 and SOD, CAT and GPx activities there were no significant differences between

groups The redox status, indicated by GSH/GSSG, was reduced in the treated group (7.12±1.63 and 8.97±1.20). **Conclusion:** Our results demonstrated that the chronic oral treatment with Ang-(1-7) was able to improve relevant cardiovascular parameters which are altered in hypertension, such as oxidative damage and arterial pressure values, reinforcing the pharmacological potential of orally active Ang-(1-7) in hypertension therapy.

Ethic Committee Approval (UP.2546/09)

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Financial Support: INCT Nanobiofar, Capes, Fapergs,

42-DAY CHRONIC EXPOSURE TO ALUMINUM CHLORIDE CAUSE OXIDATIVE STRESS AND ENDOTHELIAL DYSFUNCTION IN RESISTANCE ARTERIES

Trabalho selecionado para concorrer ao prêmio Prof. Antonio Belló

Martinez, C.S.[1], Piagette, J.[1], Escobar, A.G.[1], Vassallo, D.V.[2], Alonso, M.J.[3], Salaices, M.[4], Peçanha, F.M.[1], Wiggers, G.A.[1].

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Introduction: Aluminum (Al) is a ubiquitous element found in drinking water and in food product. This high bioavailability points to this metal as an important environmental contaminant. Moreover, Al has been related with several diseases, mainly age-related neurological changes which oxidative and inflammatory disorders are the postulated toxicity mechanisms. At cardiovascular system, there is not enough evidences of Al induced toxicity. **Objectives:** Investigates the effects of chronic exposure of aluminum chloride ($AlCl_3$) on vascular reactivity and oxidative stress. **Materials and Methods:** 20 three-month-old male *Wistar* rats (± 300 g) were divided into two groups and treated daily for 42 days with: a) Control - normal drinking water; b) $AlCl_3$ - aluminum at a dose of 100 mg/kg bw (Basic & Clin. Pharm. & Toxicol. 105; 98–104, 2009). Mesenteric resistance arteries (MRA) segments (2 mm in length) were mounted in a small-vessel dual chamber myograph according to Mulvany and Halpern (1977). Concentration-response curves to acetylcholine (Ach) and sodium nitroprusside (SNP) were performed. Vasoconstrictor response to PHE in presence and absence of endothelium and in presence of NOS inhibitor (L-NAME), were analyzed. Systemic and local lipid peroxidation and reactive oxygen species (ROS), were measured. Results were expressed as mean and SEM, compared by t–test and ANOVA followed by Bonferroni test (*P<0.05). **Results:** $AlCl_3$ exposure for 42 days decreased ACh induced concentration-dependent relaxation, increased vasoconstrictor response to PHE (Emax % to KCl Ct: 109,1 ± 3,1% vs $AlCl_3$: 120 ± 2,7%*, n=10), decreased the endothelium vasoconstrictor - modulation and nitric oxide (NO) bioavailability. Vascular and plasmatic ROS production as well as lipid peroxidation increased after Al treatment. **Conclusion:** Our results demonstrate that a 42-day chronic exposure to $AlCl_3$ leads to vascular dysfunction, which appear to be related with reduced NO bioavailability and increased oxidative stress. Ethics Committee Approval 028/2014 - Unipampa.

Financial support: Unipampa, CNPQ and CAPES Email: caroline.s.martinez@gmail.com

EFFECT OF GRAPE JUICE CONSUMPTION DURIGN THE PREGANCY AND LACTATION PERIODS ON ENZYMATIC ANTIOXIDANTS DEFENSES IN LIVER AND SERUM MALE OFFSPRING *WISTAR*

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Introduction: Environmental exposure to oxidants and / or enzymatic changes can cause the oxidative stress and this situation provokes cellular changes, causing important damages to the cells. Several studies demonstrated that the purple grape juice presents antioxidant properties. These effects were attributed to the phenolic compounds presence. The grape is considered a major source of these compounds on different beverages. **Aim:** The aim of this study was evaluated the influence of purple grape juice consumption during the fetal and lactation periods on antioxidant enzymes activities in liver and serum from adult male rats. **Material and methods**: This experimental study was performed with 48 adulthood male rats from 14 female Wistar which received purple grape juice or water by gavage (7 µL/g), daily during 42 (pregnancy and lactation). After the lactation, the offspring male rats were separated in other two groups, water or grape, they received by gavage (7 µL/g) daily during 30 days. The animals were euthanized by guillotine and the liver and blood were collected. The antioxidant activities enzimes, superoxide dismutase and catalase, were performed in the tissue homogenate and the serum. $p<0,05$ was statistically different.**Results**: The purple grape juice consumption didn´t modify the superoxide enzyme activity in the liver and serum of offspring. However, the grape juice consumption during the pregnancy increased the catalase enzyme activity on liver. In serum, the grape juice consumption did not alters**Conclusion**: We suggest that the consumption of purple grape juice during the pregnancy and lactation can help to the oxidative balance in liver, and this effect could contribute to reduce the damage provoked by the pregnancy oxidative stress. CEUA / IPA: 007/2014.

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EFFECT OF INTRADIALYTIC FUNCTIONAL ELECTRICAL STIMULATION ON DAMAGE TO THE DNA OF PATIENTS WITH CHRONIC RENAL FAILURE: RANDOMIZED CONTROLLED TRIAL

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Introduction: Patients with chronic renal failure (CRF) requiring renal replacement therapy to survive and can make it through hemodialysis (HD). The CRF leads to accumulation of uremic toxins in the organism causing oxidative stress, which leads to oxidation of proteins, lipids and DNA. **Objectives:** To assess the effect of intradialytic functional electrical stimulation on damage to the DNA of patients with CRF. **Material and Methods:** Twenty-one patients with CRF on HD recruited at Policlínica Santa Clara of Irmandade Santa Casa de Misericórdia de Porto Alegre (ISCMPA) were randomized into control group (CG = 10) and functional electrical stimulation group (FESG = 11). The groups were evaluated prior to follow-up, after 4 and 8 weeks for analysis of DNA damage in the blood of patients and it was done by alkaline comet assay. The blood samples were previously collected at the 2nd weekly HD session. Only FESG received electrical stimulation on the quadriceps muscle during hemodialysis for 8 weeks. Data were analyzed by one-way ANOVA, post hoc Tukey, expressed as mean ± SEM, with $p < 0.05$. **Results:** There was reduction of DNA damage in FESG after 4 (99.5±6.6 vs 47.9±4.3; $p < 0.05$) and 8 (99.5±6.6 vs 19.8±2; $p<0.05$) weeks of FES from the previous analysis to the following one, and this difference was also significant when it was compared to CG for the same intervention periods respectively (CG: 84±15 vs FESG: 47.9±4.3 and CG: 76.4±11.7 vs 19.8±2; $p<0.05$). **Conclusion:** Intradialytic functional electrical stimulation reduces damage to the DNA in the blood of patients with CRF.

Approval number of Ethics Committees in search ISCMPA and UFCSPA: 436.347/467.789

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EFFECT OF *OLEA EUROPAEA L.* ON RATS' LIVER AND KIDNEY IN ACUTE TOXICITY STUDY BY TBARS ASSAY

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Introduction: *Olea europaea* L., traditionally known as olive, is a plant of the Oleaceae family largely grown in the Mediterranean basin. Due to its use in popular medicine, this plant has been the target of several studies regarding its pharmacological properties, like antioxidant, antihypertensive, hypoglycemic and anti-inflammatory. **Objective:** evaluate the acute toxicity of olive's tincture (OT) by thiobarbituric acid reactive substances (TBARS) method. **Methodology:** OT was purchased from FLORIEN – Flores e Ervas Com. Farm. Ltda – EPP. Adult female and male Wistar rats were obtained from the animal house of Universidade Federal de Santa Maria (UFSM). Animals received a single dose of 2000 mg/kg of OT by oral gavage; control group was treated with ethanol 51% (vehicle of OT-10 mL/kg). The acute toxicity study was developed following the guidelines of OECD 423. After 14 days of administration, animals were subjected to a fasting period of 8h and then were euthanized by cardiac puncture. All animals were used according to Committee on Care and Use of Experimental Animal Resources from UFSM, Brazil (number 121/2014). Oxidative damage on kidney and liver was assessed by TBARS. This assay quantifies the malondialdehyde levels in the organs' samples. Data were analyzed by one-way ANOVA followed by Tukey *post-hoc*. Differences between the groups were considered significant when $p<0.05$. **Results:** the tincture did not alter the redox status of investigated organs in toxicity assay. Malondialdehyde is a product of the lipid peroxidation produced when the cells' plasmatic membrane is damaged. Its increase can be used as a toxicity marker. **Conclusion:** the result infers that there was no oxidative damage when the rats were treated with a single dose of OT. However, more tests must be assessed to evaluate olive's toxicity.

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Financial Agencies: CAPES, UFSM

EFFECTS OF ACUTE EXERCISE AND PERIODISED TRAINING PERFORMED IN DIFFERENT ENVIRONMENTS ON OXIDATIVE STATUS PARAMETERS IN PATIENTS WITH TYPE 2 DIABETES

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Introduction. The effect of exercise on oxidative status in patients with type 2 diabetes has been evaluated, however the results are contradictory pointing to a rather complex relationship to link exercise and the type 2 diabetes. In addition, an individualized protocol, specifically the periodized exercise, performed in different environments yet remains poorly studied. **Aims:** Our purpose was to elucidate the acute and chronic effects of aerobic periodized training performed in aquatic and land environments on oxidative status parameters in patients with type 2 diabetes. **Material and methods.** Twenty-one diabetes patients were randomized and submitted to exercise in water or dry land environments by 12 weeks of periodized training consisting of four mesocycles. The intensities were determined by individual second ventilatory threshold, obtained through maximal effort test, performed in their specific training environment. Blood samples were collected before and after both first and last exercise sessions. We evaluated the reactive species content (evaluated by the DCF test), lipid peroxidation (assessed by 8-isoprostane levels and water-soluble fluorescent substance formation) and the antioxidant enzyme activities (CAT, GPX, SOD). The total reactive antioxidant potential (TRAP) was measured in both plasma and erythrocytes samples. **Results and Conclusion.** The periodized exercise in both environments reduced acutely, after first and last session, the reactive species content and 8-isoprostane levels, suggesting that benefits on oxidative parameters can occur in both sedentary and trained patients with type 2 diabetes. Exercise reduced

antioxidant enzyme activities, without any effect on TRAP levels, we could suppose that lesser antioxidant activity may mask increased unidentified antioxidant content. Another finding that emerged from our study was the similar effect of periodised exercise performed in water and dry land environments. Our results disagree the hypothesis that acute exercise increases free radical levels and improves the antioxidant system in type 2 diabetes.

The Local Ethics Committee (CEP/UFRGS) approved the protocol of this study (nr. 108997).

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EFFECTS OF TRAPIDIL IN MONOCROTALINE-INDUCED PULMONARY ARTERIAL HYPERTENSION IN RATS

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Introduction: Pulmonary arterial hypertension (PAH) is a progressive disease characterized by elevated pulmonary arterial pressure and pulmonary vascular resistance, leading to right ventricular failure and death. PAH's disturbances between vasodilators and vasoconstrictors and induction of inflammatory response are consequences aggravated by redox imbalance present in the disease. Treatments that aim not only the outcomes, but also act as antioxidants may have better efficacy. Trapidil is an antiplatelet, antimitogenic and phosphodiesterase inhibitor drug currently used in clinics to prevent restenosis. Recent studies also describe Trapidil's capacity to suppress inflammatory responses, inhibit metalloproteinases activation and improve redox balance by decreasing lipoperoxidation and superoxide formation. **Objective:** To verify the effect of Trapidil in the pulmonary hypertension monocrotaline-induced model, evaluating echocardiographic and haemodynamics parameters. **Material and Methods:** UFRGS Ethics Committee in Animal Experimentation approved this study (code 28515). Rats were divided into four groups: Control, Control + Trapidil, Monocrotaline and Monocrotaline + Trapidil. PAH was induced by a single intraperitoneal injection of monocrotaline 60 mg/kg at day 0. Trapidil treatment started at day 7 and a single injection of 2.5 mg/kg was administrated once day until day 14, when the animals were euthanized after echocardiography and right ventricle catheterism. Statistical analysis using Two-way ANOVA with Tukey's post hoc was carried. **Results:** Monocrotaline animals exhibited elevated diastolic (2.36 ± 0.21 mmHg) and systolic (43.5 ± 7.3 mmHg) pressures in RV as well as increased diastolic (0.290 ± 0.029 cm) and systolic (0.242 ± 0.045 cm) diameters. Trapidil attenuated diastolic pressure (0.64 ± 0.34 mmHg) in RV and reduced RV diastolic (0.266 ± 0,034 cm) and systolic (0,202 ± 0,024 cm) diameters in monocrotaline rats. **Conclusion:** Trapidil seems to improve morphofunctional parameters in PAH. Its antioxidant and anti-inflammatory described properties still need to be checked, what may turn it in a promisor drug in PAH treatment.

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EPICATECHIN REDUCES OXIDATIVE STRESS INDUCED BY AMIODARONE ON HUMAN LUNG FIBROBLASTS CELLS (MRC-5)

Trabalho selecionado para concorrer ao prêmio Prof. Antonio Belló

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Introduction: Amiodarone (AMD) is an antiarrhythmic drug which causes serious adverse effects such as pulmonary toxicity. Mitochondrial dysfunction and oxidative stress may play a role in the mechanism of AMD toxicity. Aim: To evaluate the ability of the polyphenol epicatechin in reversing mitochondrial dysfunction and oxidative damage caused by AMD in human lung fibroblast cells (MRC-5). **Materials and methods:** MRC-5 cells were treated with 10, 100 and 500 µM of epicatechin for 30 minutes, then exposed to 100 µM of AMD for 24h. Activity of mitochondrial complex I and ATP biosynthesis were assessed by kits. Cell viability was evaluated using MTT assay. Superoxide dismutase and catalase activities were evaluated spectrophotometrically. Protein and lipid oxidative damage were determined by protein carbonyl and thiobarbituric reactive substances, respectively. Nitric oxide (NO) levels were evaluated using Griess reactive method. Data were expressed as mean and standard deviation and analyzed by one way analysis of variance (ANOVA) followed by Duncan's multiple range test using the software SPSS 21.0 for Windows. Values of $p<0.05$ were considered as statistically significant. **Results:** Results showed that AMD was able to inhibit mitochondrial complex I activity (53%) and ATP biosynthesis (9,47%) in MRC-5 cells. Lipid (12,69±0,62 nmol of TMP / mg of protein) and protein (6,93±0,81nmol of DNPH / of protein) oxidative stress markers and cell death (50,5%) were increased by amiodarone, while superoxide dismutase activities (2,58±0,14USod / mg of protein) and catalase activities (1,79±0,13 UCat / mg of protein) and NO production (2,83±0,02 nmol of nitrite / mg of protein) were decreased in AMD treatments. Epicatechin avoided the mitochondrial dysfunction, and reduced oxidative damage and cell death in AMD treated cells. **Conclusion:** This study shows that the toxicity of AMD may be associated with mitochondrial dysfunction. Epicatechin could prevent mitochondrial damage induced by AMD in lung cells.

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EVALUATION OF SUB-ACUTE AND SUB-CHRONIC TOXICITY OF OZONE GENERATED BY AIR PURIFIER IN RATS

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Introduction: Ozone is a gas with high oxidative and microbicide power. There are reports indicating that at high concentrations and inhaled for long periods the gas may cause adverse health effects, such as oxidative damage and reduction in pulmonary function. The air purifiers commercially available aim the improvement of air quality and, between these, there are the ozone generators. In Brazil, the Ministry of Labor indicates a maximum exposure to ozone equal to 0.08ppm for 8h/day. The commercially ozone generators produce quantities lower than 0.05ppm and although they ensure the governmental regulations, it is necessary to evaluate their toxicity. **Objective**: To evaluate the toxicity of ozone generated by air purifier in rats. The rats were divided into three groups (twelve per group): control (no ozone exposure), 3h and 24h of ozone exposure. **Methods**: Weight, food and water consumption changes and oxidative stress markers (non-protein thiols, lipid peroxidation – TBARS, nitric oxide) were analyzed after 14 days (sub-acute toxicity) and 28 days (sub-chronic toxicity). **Results**: All data were expressed as mean ± standard deviation (n=6). The results were submitted to Kolmogorov-Smirnov test to verify the data distribution. One-way ANOVA test (for parametric data) followed by Tukey's test ($p < 0.05$) were used for mean comparison of the oxidative stress markers. The food and water consumption and weight changes were evaluated using two-way ANOVA test followed by Tukey's test. There were no statistical differences between the studied groups considering the tests and periods of time analyzed. **Conclusion:** The

ozone generated by the air purifier is safe for the tested concentrations and does not generate reactive oxygen and nitrogen species.

The study was submitted to the Ethics Committee on Animal Use (CEUA) in research at the University of Passo Fundo and approved under n ° 032/2012.

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EXERCISE TRAINING DECREASES OXIDATIVE STRESS IN PEOPLE LIVING WITH HIV/AIDS – A PILOT STUDY

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Background and Purpose: Exercise training has been shown to be an effective strategy to improve the oxidative stress status; however, this is little explored in people living with HIV/AIDS (PLWHA). Objective: This study evaluated the effects of exercise training in oxidative stress in PLWHA undergoing antiretroviral therapy. **Material and Methods:** Virologically suppressed patients performed 24 sessions (3 times a week, during 8 weeks) of either Aerobic (AT) or Resistance (RT) or Concurrent Training (CT). AT consisted of 40 min on a treadmill, RT comprised 3 sets of 10-12 repetitions of 6 resistance exercises and CT consisted of 20 min on a treadmill in addition to a single set of the same exercises used in RT. Oxidized to reduced glutathione ratio (GSSG/GSH) and thiobarbituric acid–reactive substances (TBARS) were assessed in circulating erythrocytes and plasma, respectively, as oxidative stress markers. Fourteen PLWHA started the training protocol and eight completed it (AT=3, RT=3, CT=2). The GSSG/GSH and TBARS values were logarithmically transformed to approximate a normal distribution. A paired Student *t*-test was used to determine the differences between baseline and post-training values. **Results:** A marked improvement of redox status was observed in all the test groups after exercise training protocol (log GSSG/GSH = -1.26±0.57 *vs* -1.54±0.65 p=.01 and log TBARS = 0.73±0.35 *vs* 0.43±0.21 p=.01). This was paralleled by a rise in maximal oxygen uptake (VO_{2peak} = 29.14±5.34 *vs* 32.48±5.75 $mL.kg^{-1}.min^{-1}$ p=.04). All the subjects who performed resistance exercises showed an average gain of 40±11% in muscle strength with no difference between performing single or multiple sets in terms of muscle strength

gain. **Conclusion:** The results of this study confirm the benefits of exercise training on physical fitness as well as the decrease of oxidative stress, reinforcing that exercise training may be an effective antioxidant strategy in PLWHA. (CEP UFCSPA 951/09)

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HIGH-FAT DIET DOES NOT INDUCE STRUCTURAL CHANGES OR OXIDATIVE STRESS IN HEART OF WISTAR RATS

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Introduction: Cardiovascular events have been linked with nutritional habits and the consumption of high fat diets is considered to increase the risk of events. **Objective:** To evaluate the influence of high-fat diet on heart morphological and functional parameters, molecular markers of heart failure, and oxidative stress. **Methods:** Adult Wistar rats were fed with standard rat chow (control) (n=18) or high-fat diet (HFD) (n=19) for 21 weeks. The animals were weighed, tested for insulin tolerance (ITT) and oral glucose tolerance (OGTT). The morphological and functional parameters were analyzed by echocardiography. After, the rats were killed and had the left ventricle (LV) dissected, weighted, and stored (-80°C) for further evaluation of biochemical, molecular, and inflammatory parameters. Data were analyzed by t-test. **Results:** The animals with HFD presented no difference in body mass when compared with those receiving standard diet. However, HFD caused an insulin resistance, evidenced by a 12% increase in area under the curve (AUC) of OGTT (p=0.002) and 16% increase AUC of ITT (p=0.001). HFD did not influence ventricular weight, diastolic diameters or ejection fraction, evidenced by echocardiography. Also, there were no disturbances in antioxidant enzymes, catalase and superoxide dismutase, or oxidative stress marker carbonyl. However, protein sulfhydryl oxidation could be found in heart from HFD fed group when compared with control group (7 nmol/mg prot vs. 4 nmol/mg prot, p=0.03). The expression of Nppa gene was not induced by HFD. The fetal gene Myh7 (30%, p=0,04) and the relationship between Myh7/Myh6 (63%, p=0,001) was decreased in HFD group. **Conclusion:** Despite clear influence on insulin resistance, high-fat diet did not caused any molecular imbalance that could indicate inflammation or damage. In the same way, cardiac architecture and function seemed to be preserved. Taken together, these data suggest that the heart is resistant to the early metabolic alteration caused by HFD.

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INFLUENCE OF AEROBIC EXERCISE ON THE PROTEIN IMMUNOCONTENT INVOLVED WITH ANGIOGENESIS IN MUSCLE SOLEUS OF RATS WITH *COR PULMONALE*

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Introduction: *Cor pulmonale* is a disease characterized by evident structural and functional changes in the right ventricle due to a primary pulmonary dysfunction. **Objectives:** to analyze the encroachments of aerobic exercise on the proteins immunocontent involved in angiogenesis and the structural changes of the soleus muscle of rats with Cor pulmonale. **Methods:** 19 Wistar rats were divided into four groups: sedentary control (CS), sedentary monocrotaline (MS), trained control (CT) and trained monocrotaline (MT). After two weeks of pre-training on a treadmill, the MS and MT mice were given a single intraperitoneal dose of MCT (60 mg / kg). After drug administration, the animals of CT and MT groups underwent three weeks of aerobic exercise. Soleus muscle was removed and frozen in order to develop the following analyzes: vessels, interstitium percentage, and larger diameter of muscle fibers as well as immunocontent growth factor of vascular endothelial (VEGF), angiopoietin (Ang-1) and Tie-2 receptor. Molecular results were expressed as mean ± standard deviation, and after using two-way ANOVA, they were complemented by Bonferroni's test, $p = 0.05$. **Results:** Only has the Ang-1 immunocontent been increased in monocrotaline animals when compared with control animals ($p = 0.05$). The other results (immunecontents and histological outcomes) showed no alterations between the groups. **Conclusion:** It is suggested that aerobic exercise has influence on the immunocontent of Ang-1 as well, is able to promote the stabilization of new vessels in the soleus muscle of rats with *Cor pulmonale* at this stage.

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L-CARNITINE SUPPLEMENTATION DECREASES DNA DAMAGE IN TREATED MSUD PATIENTS

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Introduction: Maple syrup urine disease (MSUD) is an inherited disorder of branched-chain amino acids (BCAA). Patients generally present psychomotor delay, mental retardation and brain abnormalites. Studies have shown that oxidative stress may be involved in neuropathology of MSUD. In this regard, it was recently reported that MSUD patients have L-carnitine (L-car) deficiency, a compound with antioxidant properties that is used as adjuvant therapy in some inborn errors of metabolism. **Objective:** we evaluated the role of dietary L-car supplementation on DNA damage in whole blood leukocytes from MSUD patients and the relationship between DNA lesions, malondialdehyde (MDA) levels and L-car concentrations in plasma from MSUD patients. **Material and methods:** Six MSUD patients admitted at the Hospital de Clinicas de Porto Alegre (mean age 8.28 ± 2.87 years) were supplemented with a BCAA-restricted diet and L-car (50 mg kg−1 day−1). The DNA damage (determined by the alkaline comet assay), as well as MDA, a marker of lipid peroxidation, and L-car concentrations were determined in blood of MSUD patients before, after 1 month and 2 months of L-car supplementation and compared to control group (six age-matched healthy individuals (mean age 6.0 ± 3.12 years)), using repeated measures of ANOVA, followed by the Tukey multiple range test). **Results:** We observed a significant increase of DNA damage index (DI) in leukocytes from MSUD patients under BCAA-restricted diet as compared to controls and L-car supplementation significantly decreased DNA DI levels ($P<0.05$). It was also found a positive correlation between DI and MDA content ($r = 0.93$; $p < 0.05$), and an inverse correlation between DI and L-car levels ($r = -0.82$; $p < 0.05$). **Conclusion:** Our results suggest the involvement of oxidative stress in DNA damage in this disorder. Since L-car reduced DNA damage, it is presumed that dietary supplementation of this compound may serve as an adjuvant therapeutic strategy for MSUD patients. The study was approved by the Ethics Committee of HCPA (project 140191). Financial support: FAPERGS, CNPq and FIPE/HCPA-Brazil.

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LOW INTENSITY EXERCISE TRAINING IMPROVES CARDIOVASCULAR PARAMETERS IN PULMONARY HYPERTENSION

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Introduction: Pulmonary hypertension (PH) is characterized by increasing in pulmonary vascular resistance, leading to right ventricle (RV) hypertrophy and failure, and autonomic nervous system (ANS) dysfunction. There was a reduction in heart rate variability (HRV) and an imbalance of the renin angiotensin system (RAS), increasing the vasoconstrictor axis, represented by the angiotensin converting enzyme (ACE)-angiotensinII-AT1 receptor (AT1R). It remains unclear the ideal intensity of exercise training (ET) in PH. **Objective**: We evaluate the effect of low and moderate ET on the ANS and RAS in the heart of PH-rats. **Methods:** PH was induced by a single injection of monocrotaline (MCT; 50mg/kg). Groups: control (CO), MCT-sedentary (MCT-S), MCT-low (MCT-L; 40%), and MCT-moderate exercise training (MCT-M; 60% of the maximal speed). ET was performed on treadmill for 50 minutes/5 times/week/3weeks. ANS was analyzed through HRV; RAS by the ACE, ACE2, AT1R and AT2R, and Mas receptor expression by western blot in the RV. Data were compared by one-way ANOVA follow by Student-Neumann-Keuls test ($P<0.05$). **Results:** The weight of RV was higher in MCT-S *vs* all groups, and systolic pressure (RVSP) 120% higher than CO. HRV was significantly higher in the ET-groups than in sedentary groups, and MCT-L was 47% higher than CO. ACE expression was significantly decreased in MCT-L (46%) and MCT-M (44%) compared with MCT-S, but ACE2 was significantly increased in MCT-M (133%) *vs* CO. On the other hand, AT2R and Mas were decreased in MCT-S and Mas was 38% higher than CO. Regarding to AT1R, there was no difference between groups. **Conclusion:** Collectively, our results indicate that the low

intensity of ET was more effective in improving the ANS in favor to parasympathetic nervous system, and RAS balance in favor to the vasodilator axis.

*All procedures were approved by the Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA) Ethics and Research Committee (protocol number 115/13).

MYOSTATIN AND AUTOPHAGY EXPRESSION IN PHYSIOLOGICAL CARDIAC HYPERTROPHY AND ITS RELATION WITH MIRNA-MEDIATED REGULATION.

Trabalho selecionado para concorrer ao prêmio Prof. Antonio Belló

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Introduction: Myostatin and autophagy are involved in muscle growth regulation. However, there are few studies exploring their role in physiological cardiac hypertrophy. **Aim:** Evaluate myostatin and autophagy in mice subjected to a swimming protocol to induce physiological cardiac hypertrophy. **Methods:** Adult (8 weeks-old) male BALB/c mice (*n*=52) divided in sedentary (S) and trained (T) groups were evaluated in 7 (S7 or T7) and 28 (S28 or T28) days. Left ventricular/tibial length ratio (LV/TL) and cardiomyocyte diameter were used to assess cardiac hypertrophy. Gene expression was evaluated by RT-qPCR, while protein expression was analyzed by western blot. Bioinformatics analysis was performed by TargetScan to predict potential miRNAs' targets and Genemania to create an interaction network between miRNAs and genes. All results are expressed as mean ± SEM and comparisons were performed with Student T test. **Results:** Myocardial hypertrophy was confirmed in trained group either by the increase in LV/TL ratio in 28 days (13%, p=0.0001) and cardiomyocyte diameter in 7 days (20%, p=0.04) and 28 days (30%, p=0.002). There was a reduction in myostatin levels only in T7 compared to S7 (0.8 ± 0.1 *vs* 1.2 ± 0.1, p=0.01). Conversely, mTOR was increased only in T28 compared to S28 (397±95 *vs* 90±23 AU; p=0.02). Autophagic genes showed reduced levels in T7 and T28 (19% and 10% for *Lc3* and *Beclin1,* 22% and 11% for *P62* in T7 and T28; p<0.05 compared to sedentary), but there was no difference at protein levels. Bioinformatics analysis showed that miR-30a, - 221, -27a/b and 208a/b potentially regulate autophagic and myostatin genes. **Conclusions:** Taken together, reduced myostatin during initial hypertrophy and increased mTOR phosphorylation in the established hypertrophic phenotype might favor muscular growth and reduce autophagy. Candidate miRNAs identified might be regulating this process and should be further validated in this scenario.

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ORAL SUPPLEMENTATIONS WITH L-GLUTAMINE OR L-ALANYL-L-GLUTAMINE DO NOT CHANGE METABOLIC ALTERATIONS INDUCED BY LONG TERM HIGH-FAT DIET IN THE B6.129F2/J MOUSE MODEL OF INSULIN RESISTANCE

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Introduction Obesity and diabetes are major worldwide causes of cardiovascular disease which is associated with a general state of low-grade inflammation. Conversely, some nutrients, such as L-glutamine (GLN) and L-alanyl-L-glutamine dipeptide (DIP) have been shown to potentiate the expression of the 70 kDa family of heat shock proteins (HSP70), which are strongly anti-inflammatory. **Materials and Methods** In this work we aimed to investigate the effects of long-term supplementations with GLN or DIP, in the high-fat diet (HFD)-fed B6.129SF2/J mouse model, over insulin sensitivity and signaling, oxidative stress markers, metabolism and HSP70 expression. Mice were fed in a standard low-fat diet (STA) or a HFD for 20 weeks. In the 21th week, mice from the HFD group were allocated in five groups and supplemented for additional 8 weeks with GLN, DIP or its constituint amino acids: 1) HFD controls, 2) HFD+DIP group, 3) HFD+L-alanine group, 4) HFD+GLN group or 5) HFD L-alanine+L-glutamine group. Procedures were approved by the Federal University of Rio Grande do Sul Ethics Committee on Animal Experimentation (CEUA #21293/2011). **Results** HFD induced higher body weight, fat pad, fasted glucose and total cholesterol in comparison with STA group. Amino acid supplementations did not induce any modifications in these parameters. Although insulin tolerance tests indicated insulin resistance in all HFD groups, any amino acid supplementation could improve insulin sensitivity in the model. There were also no significant differences in the immunocontents of insulin receptor. Notably, HSP70 contents in the liver were markedly increased in HFD controls as compared to STA group, which suggest that insulin resistance is only in the beginning. **Conclusion** Apparently, B6.129SF2/J mice are more resistant to the harmful effects of HFD through a mechanism that may include gut

adaptation, reducing the absorption of nutrients, including amino acids, which may explain the lack of improvements in our intervention.

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OXIDATIVE BIOMARKERS IN POSTMENOPAUSAL WOMEN

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Introduction: In postmenopausal women it is observed a marked reduction in serum levels of estrogen, which causes many women suffer from symptoms inherent from this period. Furthermore, studies have shown that estrogen has an antioxidant role ant its reduced levels may induce oxidative stress and consequently trigger damage to cellular macromolecules, especially lipids and proteins. **Aims:** To evaluate the levels of reactive substances thiobarbituric acid (TBARS) and protein carbonyls (PCs) in the plasma of postmenopausal women. **Methods:** The study population was 50 women, of these, 25 were postmenopausal and 25 in the reproductive period (control group), they are part of institutional research "Study of the Aging Female" of UNIJUÍ. The blood samples of the subjects were obtained using vacutainers containing *ethylenediamine tetraacetic acid* (EDTA) and subsequently the samples were centrifuged at 3000 rpm for 10 min and plasmas stored to perform analytical determinations. The determination of TBARS levels was performed according to the technique described by Jentzsch et al. (1996) and PCs levels it was realized from the technique described by Levine et al. (1990). Data were analyzed using the Student t test for parametric variables, and values of $p < 0,05$ were considered statistically significant. **Results:** The mean age of women in the postmenopausal and fertile was 56,3 ± 6,5 and 44,2 ± 5,7, respectively. It was found that TBARS and PCs were significantly higher ($p = 0,0001$) in postmenopausal women when compared to women in childbearing age. **Conclusion:** The results showed that postmenopausal women present damage to lipids and proteins when they are compared to fertile women, which can play an important role in the pathogenesis of various diseases at this stage of life.

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OXIDATIVE STRESS AND STROKE IN PATIENTS WITH OBSTRUCTIVE SLEEP APNEA

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INTRODUCTION: Obstructive sleep apnea (apnea) has been shown to be an independent risk factor for stroke. The literature point out as risk factors for stroke the atherogenesis, oxidative stress and inflammation. These risk factors are significantly higher in patients with apnea. **AIM:** Evaluate oxidative stress pathophysiology mechanisms in patients with apnea and its relation with cerebrovascular accident risk. **METHODS:** Structured review results 48 articles in Pubmed database. 22 were selected for review based in title and abstract criteria. **RESULTS:** The apnea elicits the increased production of reactive oxygen species (ROS) due the repetitive episodes of hypoxia/reoxygenation. ROS may activate redox-sensitive signaling pathways initiating inflammatory or adaptive responses. Oxidative stress destabilizes and reduces endothelial nitric oxide synthase(NOs). The NOs is a main enzyme of NO production. The lipid peroxidation also is an important step in triggering atherogenesis by ROS mechanism of low density lipoproteins (LDL). ROS peroxidation of LDL size is able to go through the endothelial barrier leading to the typical atherosclerotic plaques. In the plasma an increase antioxidants markerz occurs. All this mechanisms leads brain and carotid endothelial damage. Such injury, increasing the risk of stroke events in patients with apnea. The recommended treatment observed in this review is the continuous positive airway pressure (CPAP). The allopurinol daily administration is considered as an alternative treatment in 2 articles. This drug may inhibit the production of ROS by xanthine oxidase reducing oxidative stress and increasing NO bioavailability. **CONCLUSION:** Oxidative stress responses are related to cerebrovascular accident due multiple risk factors apnea- related. The decreased levels of NO production and lipid peroxidation may be mentioned as risk factors for increasing risk for stroke.

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OXIDATIVE STRESS IN HUMAN AORTAS FROM OCCLUSIVE AND ANEURYSMAL DISEASES.

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Introduction: Oxidative stress have been implicated in the atherosclerosis and development of aortic aneurysms. **Objective:** We evaluated the role of reactive oxygen species (ROS) and antioxidant enzymes in human aortas from patients with abdominal aortic aneurysm (AAA) or abdominal aortic occlusive disease (AO). **Material and methods:** Demographic and risk factors of 30 patients (16 with AO and 14 with AAA) submitted to aortic surgery were revised and their aortic samples were homogenized and analyzed for reactive oxygen species (ROS) levels, NADPH oxidase, superoxide-dismutase (SOD), and catalase (CAT) activities, as so as tissue levels of nitrites. We performed exact Fisher test for risk factors analysis and Student's t test for others variables. **Results:** NADPH oxidase activity and ROS levels were higher in the AO group when compared to AAA group ($p<0,05$). Nitrites tissue levels also higher in the AO group ($0,099\pm0,073$ vs $0,045\pm0,02$ mML^{-1}; $p<0,05$). Furthermore, SOD activity was higher in the AAO group, while CAT activity was higher in the AAA group. **Conclusions:** Oxidative stress was more relevant in patients with aortic occlusive disease than with aortic aneurysmal disease. Besides, antioxidant enzymes seem to express themselves differently in these two groups of aortic pathologies, being the SOD more important in the occlusive disease and CAT more relevant in the aneurysmal group.

Protocolo Comitê de Ética da Santa Casa de Porto Alegre: 3435/10

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QUANTIFICATION OF DNA DAMAGE IN DIFFERENT TISSUES IN CHRONIC HEART FAILURE RATS

Ganhador do Prêmio Professor Antonio Belló* – Melhor trabalho

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Introduction: Chronic heart failure (CHF) is a complex syndrome, which comprises structural and functional alterations in the heart to maintaining the adequate blood demand to all tissues. Few investigations sought to evaluate oxidative DNA damage in CHF, however, it has not been characterized DNA damage in distinct tissues in CHF. **Aim:** Quantify the DNA damage using the comet assay in left ventricle (LV), lungs, diaphragm, gastrocnemius and soleus muscles in rats with CHF. **Methods:** Twelve male Wistar rats (300 to 330 g) were selected for the study: Sham (n=6) and CHF (n=6). The animals underwent myocardial infarction by the ligation of the left coronary artery. After six weeks, the animals were euthanized. It was performed a cell suspension of the tissues. The comet assay was performed to evaluate single and double strand breaks in DNA. **Results**: The CHF group showed higher values of left ventricle end-diastolic pressure (LVEDP), pulmonary congestion, cardiac hypertrophy and lower values of maximal positive and negative derivatives of LV pressure, LV systolic pressure ($P<0.05$). CHF group showed higher DNA damage (% tail DNA, tail moment and Olive tail moment) compared to Sham ($P<0.001$). The tissue with the highest damage was the soleus, compared to LV and gastrocnemius in CHF group ($P<0.05$). DNA damage was positively correlated with LVEDP in all tissues ($P<0.05$). **Conclusion:** Our results indicates that the CHF affects all

tissues, both centrally and peripherically, being more affected in skeletal muscle (soleus) and is positively correlated with LV dysfunction.

This study was approved by CEUA/UFCSPA, under the protocol number 114/13.

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Apoio financeiro:CAPES and CNPq

*Patrocinado pela empresa ALESCO.

RADIOPROTECTOR EFFECTS OF GRAPE JUICE AGAINST RATS' MANDIBLE EXPOSED TO CRANIAL IRRADIATION

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Introduction: Radiotherapy is based on the direct interaction of ionizing radiation with cellular DNA or indirect through water's radiolysis, culminating with the production of free radicals and reactive oxygen species. It is used on the treatment of brain tumors, but it has side effects, like osteoradionecrosis (ORN), being necessary searching for radiomodifier compounds which can minimize these deleterious effects. **Objectives:** Analyze the radiomodifier effects of organic grape juice on the prevention of ORN on cranial irradiation on rats through mandibular morphometric measurements. **Material and methods:** The grape juice was produced by Econatura®. For the experiments, 40 *Wistar* rats were divided in 4 groups: (NG) non-irradiated, placebo; (NJ) non-irradiated, juice; (RG) irradiated, placebo; (RJ) irradiated, juice. The supplement (juice or placebo) was administered 4 days, during and 4 days after the irradiation (1 mL/200 g/i.g.). Each irradiated rat received 8 fractions of 4 Gy of x-rays on the cranium, during 2 weeks. After anesthesia with pentobarbital (6%/i.p.), the lower mandibles were dissected and 4 points were used for mandibular morphometric measurements. Data were analyzed with two-way ANOVA, followed by Bonferroni's test. The project was approved by the Ethics Committee of Leon's University. **Results:** Occurred a drop on the three sagittal measurements analyzed on this study for the irradiated rats. Grape juice was capable of increasing on 5,06% the mandibular length 1 compared to RG group. The cranial irradiation decreased on 14,13% the length of the mandibular shape (CCM) in comparison to NG. When compared RG with RJ, is seen an 4,92% raise on CCM for the RJ group. **Conclusion:** Therefore, was possible to hypothesize that irradiated rats and supplemented with the juice were more protected than the irradiated and supplemented with placebo, once the antioxidant compounds prevented cellular damage on bone cells.

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REDUCED GLUTATHIONE LEVELS IN POSTMENOPAUSAL WOMEN

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Introduction: In postmenopausal occurs depletion of estrogen levels and as a consequence, may occur oxidative stress, characterized by an imbalance between operation of the antioxidant defense system and the generation of reactive species (REs). Thus, this condition leads to the increase ER levels resulting in injuries macromolecules and cellular structures. To counteract these adverse effects, the human body is equipped with a variety of antioxidant molecules, among which stands out the reduced glutathione (GSH). **Aims:** To evaluate the levels of Glutathione Reduced (GSH) in plasma of postmenopausal women. **Methods:** The study population was 50 women, of these, 25 were postmenopausal and 25 in the reproductive period (control group), they are part of institutional research "Study of the Aging Female" of UNIJUÍ. The blood samples of the participants were performed using vacutainers containing *Ethylenediamine tetraacetic acid* (EDTA) and subsequently the samples were centrifuged at 3000 rpm for 10 min and plasma stored for performing the determinations of GSH levels, according to the technique described by Ellman (1959). Data were analyzed by t-student test for parametric variables, and values of $p < 0.05$ were considered statistically significant. **Results:** The mean age of women in the postmenopausal and fertile was 56,3 ± 6,5 and 44,2 ± 5,7, respectively. It was found that GSH levels were significantly higher ($p = 0,0001$) in fertile women when compared with women in the postmenopausal period. **Conclusion:** The results suggest that the main endogenous antioxidant was consumed in postmenopausal women, which possibly shows an attempt by the organism to reverse macromolecular damage caused by estrogen decline observed in this period of life.

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STRENGTH OF THE LEVELS OF LIPID PEROXIDATION BY METHOD TBARS AFTER SUB-ACUTE TREATMENT WITH *BACCHARIS TRIMERA*

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INTRODUCTION: *Baccharis trimera* (Less) DC, popularly known as “carqueja” (broom), native to South America, it is cultivated mainly in Brazil, Argentina, Paraguay and Uruguay. Its infusion is popularly used in the treatment of hepatic and digestive problems. **OBJECTIVE**: Asses if subacute treatment with *B. trimera* tincture influence on the levels of lipid peroxidation liver and kidney of rats of both sexes. **METHODS:** The tincture of *B. trimera* used in the experiments was purchased from Flores e Ervas Com. Farm. Ldta. (Piracicaba, São Paulo, Brazil). This study was developed following the guidelines OECD 407. Adult male and female Wistar rats, were supplied by the production facility of the Biotério Central da UFSM. Animals were divided according to gender in the eight groups treated, by gavage once a day for 28 days, with the following doses: I: served as control and received 63% ethanol (10 mL/kg) (tincture vehicle); II: *B. trimera* (100 mg/kg); III: *B. trimera* (200 mg/kg) and IV: *B. trimera* (400 mg/kg). Animals were sacrificed from the cardiac puncture. Oxidative damage on kidney and liver was assessed by thiobarbituric acid reactive substances (TBARS). Results were analyzed by ANOVA followed by Tukey. Differences between the groups were considered significant when $p<0.05$. **RESULTS:** TBARS method quantifies the malondialdehyde substance, the end product of oxidation of fatty acids, as an indirect measure of the formation and action of highly reactive substances and oxidants. No change was observed in TBARS levels in liver and kidney of male and female rats exposed to different doses of *B. trimera* tincture after 28 days treatment. **CONCLUSION:** *B. trimera* when administered per 28 days did not show apparent liver and renal damages. Work approved by the Ethics Committee on Animal Use UFSM number 050/2014.

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SYSTOLIC BLOOD PRESSURE INCREASE CAUSED BY MERCURY-INDUCED OXIDATIVE STRESS IS PREVENTED BY EGG WHITE HYDROLYSATE

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Introduction: Mercury-induced oxidative stress promotes endothelial dysfunction, vascular reactivity and arterial blood pressure increase in cardiovascular system. In this context, bioactive peptides from egg proteins hydrolysis (EWH) have shown antioxidant activity, and may be potentially useful on cardiovascular toxicity. **Objective:** To investigate the effects of EWH on the vascular damage caused by chronic exposure to Hg. **Material and methods**: Four groups of 8-week-old *Wistar* male rats (200 g) were treated for 60 days with: a) Untreated (saline solution, i.m., n=8); b) Mercury ($HgCl_2$, i.m., 1st dose 4.6 µg/kg, subsequent doses 0.07 µg/kg/day - Wiggers et al., 2008, n=8); c) Hydrolysate (1 g/kg/day, gavage - Miguel et al., 2007, n=8); d) Mercury plus Hydrolysate (n=8). Indirect Sistolic Blood Pressure (SBP) was assessed by tail-cuff plethysmografy. Lipid peroxidation and antioxidant capacity in plasma were analyzed by the TBARS (Ellman, 1952) and ORAC (Ou et al., 2001). Data were analyzed by Analysis of Variance (ANOVA) followed by a Bonferroni test. **Results**: Hg treatment increased SBP (Ct: 120,1 ± 2,0; Hg: 135,2 ± 2,8*; Pep: 124,5 ± 1,5; PepHg: 122,0 ± 2,2$^{\#}$; mmHg, n=6, *p<0,05vsCt; $^{\#}$p<0,05vsHg) and MDA levels (Ct: 0,75 ± 0,03; Hg: 0,87 ± 0,05*; Pep: 0,67 ± 0,04; PepHg: 0,69 ± 0,03$^{\#}$ µmol/ml plasma, n=8) and reduced antioxidant capacity (Ct: 12,7 ± 0,3; Hg: 11,3 ± 0,3*; Pep: 13,3 ± 0,4; PepHg: 12,7 ± 0,6$^{\#}$ µmol Trolox/ml plasma, n=8). EWH prevented the increase in SBP and MDA and the reduction in antioxidant capacity. **Conclusions:** Hg

exposure at low doses leads to an increase in SBP and oxidative stress and EWH provides cardiovascular protection against this damage.The research was approved by the Ethics Committee on Animal Use at the UNIPAMPA - RS, Brazil (protocol number 005/2014).

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THE CONSUMPTION OF PURPLE GRAPE JUICE DURING FETAL AND LACTATION PERIODS EFFECTS THE ACTIVITIES OF OXIDATIVE ENZYMES ON BRAIN TISSUES IN ADULTHOOD OF RATS

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Introduction: Several neurodegenerative diseases showed their appearance associated with the oxidative damage. Purple grape juice showed different health benefits, among them the antioxidant effect is the most studied. However, heretofore few studies showed the pregnancy consumption benefits to the pregnant women and their offspring. **Aim**: The aim of this study was to evaluate the influence of purple grape juice consumption during the fetal and lactation periods on antioxidant enzymes activities in adult rats. **Material and methods**: This experimental study was performed with 48 adult male rats from 14 female *Wistar* rats which received purple grape juice or water by gavage (7 µL/g), daily during 42 days (pregnancy and lactation). After the lactation, the offspring male rats were separated in other two groups, water or grape juice; they received 7 µL/g by gavage daily during 30 days. The animals were euthanized by guillotine and the cerebellum, cerebral cortex and hippocampus were collected. The activities of the antioxidant enzymes, superoxide dismutase and catalase, were performed in the tissues homogenate. $p<0.05$ was statistically different. **Results:** The purple grape juice consumption during the pregnancy and lactation periods increased the superoxide dismutase activity in cerebral cortex and hippocampus. The purple grape juice consumption during the pregnancy and lactation periods also increased the catalase activity in hippocampus; however, this consumption reduced the catalase activity in cerebral cortex. In cerebellum, we did not observe statistical differences. **Conclusion**: We can suggest that the grape juice consumption during pregnancy and lactation influences in antioxidant enzyme activities. However, more studies are necessaries to show the pathways this effect occurs. CEUA/IPA: 014/2014.

Apoio Financeiro: Capes, CNPq e Fapergs

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THE INFLUENCE OF AN ACUTE SESSION OF EXERCISE WITH OR WITHOUT RESTRICTION OF BLOOD FLOW OVER THE PARAMETERS OF OXIDATIVE STRESS

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Introduction: The low intensity resistance exercise offers similar gains as the traditional strength training. People that has a limitation factor for the use of high weights to gains of strength and hypertrophy could be benefit of that kind of training. **Objective:** To compare markers of oxidative stress between exercises with or without restriction of blood flow. **Methodology:** The sample was based in health males with ages 23.72 ± 3.49 years . The distribution of the sample was done into 3 groups: I low intensity exercise (30% 1 RM) with moderate vascular occlusion(n=11); II high intensity exercise (80% 1 RM) (n=10); III low intensity exercise (30% 1 RM) without occlusion (n=8). The samples were collected before and immediately after, 24 and 48 hours after the acute protocol. The levels of lipid peroxidation were evaluated by the test of reactive substances to the thiobarbituric acid (TBARS) and the levels of protein oxidation by the test of oxidative carbonyls. The Anova test was used to evaluate repeated measures with Tukey's Post-Hoc. The present study was approved by Ethics Committee of Methodist University Centre - IPA (n. 364.202). **Results**: TBARS: Group I, 7.25± 1.03 nmol/mL, 6.05±0.72 nmol/mL, 5.26±1.04 nmol/mL and 6.34±1.39 nmol/mL; Group II, 6.4±1.07 nmol/mL, 6.29±1.31 nmol/mL, 6.02±1.18 nmol/mL and 6.94±1.85 nmol/mL; Group III, 6.77±0.93 nmol/mL, 6.47±1.03 nmol/mL, 4.51±2.24 nmol/mL and 6.06±2.27 nmol/mL, before, after, 24 and 48 hours respectively. The variable of TBARS presented significant difference on Group I between the moment pre, post and 24 h and in Group III in the moment pre and post to moment 24 h. The levels of carbonyls have not present significant differences. **Conclusion**: The vascular occlusion during the exercise with low intensity generated significant decrease on levels of lipid peroxidation without alteration of carbonyls levels.

Support: CAPES/FAPERGS, CNPQ

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THE INFLUENCE OF THYROID HORMONES ON PERIPHERAL VASCULATURE AFTER ACUTE MYOCARDIAL INFARCTION

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Introduction: The treatment with thyroid hormones (TH) have presented positives effects over the myocardium after acute myocardial infarction (AMI), but there is no data regarding the influence of AMI and this treatment over the peripheral vasculature. **Objective**: The main goal was to analyze the influence of these factors on the peripheral vasculature through parameters of oxidative stress, angiogenesis and TH receptors (TRα,TRβ) and endothelial nitric oxide synthase (eNOS) expression. **Methods**: Male Wistar rats (~350g)(n=11-16/group) were divided into four groups: Sham-operated (SHAM), infarcted (AMI), sham-operated + TH (SHAMT) and infarcted + TH (AMIT). During 12 days, the animals received T3 and T4 (2 and 8µg/100g/day) by gavage. After, the rats were submitted to echocardiographic analysis. The aorta was collected to molecular analysis. Statistical analyses: two-way ANOVA with Student-Newman-Keuls post test. **Results**: The vascular endothelial growth factor, hypoxia-inducible factor 1α, TRα and TRβ receptors expression increased in AMIT compared to AMI. The reactive oxygen species production, NADPH oxidase activity and eNOS expression decreased in AMI compared with SHAMT, while there is no deference between AMIT and AMI. **Conclusion**: The TH presented an action over angiogenesis and TRα and TRβ expression on peripheral vasculature in a post-AMI period. These may indicate an improvement in angiogenesis and a better responsiveness of peripheral vasculature to HT.

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THE LOW-INTENSITY RESISTANCE TRAINING WITH BLOOD FLOW RESTRICTION PROMOTES SIMILAR VASCULAR ADAPTATION TO HIG-INTENSITY RESISTANCE TRAINING IN YOUNG HEALTHY MALES

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Background: Resistance training with blood flow restriction (BFR) can increase muscle size and strength like a traditional high-intensity resistance training (HIRT). However, few studies investigated the vascular response to resistance training with BFR. **Aim:** Evaluate the vascular response of healthy young males submitted to resistance training with BFR or HIRT. **Methods:** The study was conducted with humans. Twenty-eight male individuals (23.96 ± 2.67 years) were randomized in two groups, high-intensity (HI) and Low-Intensity with BFR (LI-BFR). The loads were determined at 80% of 1RM for the HI group and 30% of 1RM for the group LI-BFR. The vascular response was assessed by the flow mediated dilatation (FMD), dosage of nitric oxide by-products nitrite and nitrate (NOx), and antioxidant capacity by superoxide dismutase (SOD) activity. Data was compared by independent t-test between groups and significance was $p < 0.05$. **Results:** NOx concentration was 104.4 ± 6.5 µM vs 120.2 ± 9.3 µM for HI group; 101.1 ± 8.5 µM vs 119.9 ± 10.3 for LI-BFR, pre and post training respectively. There was no significant difference between groups. However, after eight weeks of training NOx was significantly greater in both groups HI and LI-BFR. There was no significant difference in pre and post-study FMD (5.4 ± 2.6 %; 6.8 ± 1.8 %) for HI group, and for LI-BFR group (4.1 ± 1.0 %; 6.5 ± 1.9 %). Between the groups there was no significant difference. The activity of SOD in the OC group (pre training) showed a significant difference between the moments pre and post of acute session. Comparing the groups OC and HI there was a significant difference on pre training for the moments post to the group HI. **Conclusion:** The BFR training seems to promotes similar adaptation to HIRT. Study approved by Ethics Commission at Methodist University Center (364.202.)

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FAPERGS

THE ROLE OF DIFFERENT NADPH OXIDASE INHIBITORS ON VASCULAR REACTIVITY - DIAPOCYNIN VS APOCYNIN

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Introduction: Diapocynin is a dimer of apocynin obtained synthetically and both compounds are inhibitors of NADPH oxidase. Diapocynin showed better effects against oxidative stress derived by NADPH oxidase in models of asthma, inflammatory and neurodegenerative diseases, when compared to apocynin. (J.Inflamm (Lond) 13: 9. 6, 2012). At vascular dysfunction, NADPH oxidase seems to have an essential role in several situations such as hypertension, atherosclerosis and endothelial dysfunction. **Objectives:** The objective of this study was to compare the effects of diapocynin (DIAPO) and apocynin (APO) on vascular reactivity of aorta from control rats and in aorta under oxidative stress induced by mercury chloride ($HgCl_2$) at 6nM. **Method:** 12 three-month-old male Wistar rats (+/- 350g) were anesthetized and the thoracic aorta isolated, segmented and mounted on organ bath system according to Nielsen & Owman (1971). Vasoconstrictor response to phenylephrine (PHE) in control and $HgCl_2$-exposed aortas were obtained in presence and absence of apocynin (0.03mM) and diapocynin (0.03mM), after 1 hour of incubation. Data are expressed as mean ± SEM and analysed by two-way ANOVA followed by post-hoc of Bonferroni ($P<0.05$). **Results:** Our results showed that diapocynin seems to be more effective than apocynin on inhibition of NADPH oxidase on vascular response to PHE in control aortic segments. However, this effect was not observed in $HgCl_2$-exposed aortas (Emax % to KCl: Untreated (n=12) 94.6±1.98, $HgCl_2$ (n=12) 111.8±2.1, APO (n=6) 75.4±3.3, DIAPO 55.3±1, APO+$HgCl_2$ (n=6) 62.1±3.2, DIAPO+$HgCl_2$ (n=6) 62.8±2.7%). **Conclusion:** Our results suggest that, in physiological conditions, diapocynin is a stronger NADPH oxidase inhibitor. However, after oxidative stress induced by mercury these difference disappear, which points to another underling mechanisms involved on vascular dysfunction after $HgCl_2$-acute exposure. This study has the approval by Ethics Committee on Animal Use Experimentation of Federal University of Pampa (CEUA/UNIPAMPA - 037/2014).

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