06 Cardiovascular and Renal Pharmacology

06.001 Biomonitoring the cardiorenal effects of *Luehea divaricata* **Mart.: An ethnoguided approach**. Tirloni CAS[1], Palozi RAC[1], Schaedler MI[1], Marques AAM[1], Guarnier LP[1], Silva AO[1], Gasparotto Junior A[1] [1]UFGD – Ciências da Saúde

Introduction: *Luehea divaricata* Mart. (Malvaceae) is an important medicinal species widely used as a diuretic in the Brazilian Pantanal region. It has been recently shown that the ethanolic supernatant obtained from leaves of this species (ESLD) has important hypotensive and diuretic activity (Tirloni et al, 2017). Nevertheless, the secondary metabolites responsible for this activity, as well as the molecular mechanisms responsible for their pharmacological effects remain unknown.
Aim: To carry out a biomonitoring study to identify possible active metabolites present in different ESLD fractions and investigate their effects on the renal and peripheral arteriolar tone, showing their interrelation with sustained diuretic and hypotensive activities. **Materials and Methods:** First, ESLD was obtained from *L. divaricata* leaves and a liquid-liquid fractionation was performed. The resulting fractions were analyzed by liquid chromatography-mass spectrometry. Then, ethyl acetate (AceFr), *n*-butanolic (ButFr) and aqueous (AqueFr) fractions were orally administered in a single dose or for seven days, to male Wistar rats. The doses were previously defined from the yield obtained in each fraction. Hydrochlorothiazide was used as a positive control. Then, blood pressure, heart rate, urinary volume, pH, density, urinary sodium, potassium, chloride, and calcium concentrations were measured. In addition, serum levels of nitrite, thiobarbituric acid reactive species, nitrotyrosine, aldosterone, vasopressin and plasma angiotensin converting enzyme activity were also measured. Finally, the direct effects of ButFr on renal and mesenteric arteriolar tone, as well as the role of nitric oxide and prostaglandins on the renal and hemodynamic effects were also investigated. ***Results:*** Of all fractions tested, only ButFr showed significant diuretic and saluretic effect. AceFr and ButFr fractions also showed acute hypotensive effect, but only ButFr maintained its response after 7 days of treatment. Prolonged treatment with ButFr was able to increase serum nitrite levels and significantly reduce oxidative and nitrosative stress markers. In addition, ButFr induced an important vasodilatory response in the renal and mesenteric arteriolar beds through the release of nitric oxide and prostaglandins. Finally, the diuretic and hypotensive effects induced by ButFr were completely blocked by previous administration of L-NAME or indomethacin, showing the direct involvement of nitric oxide and prostaglandins in these effects. **Conclusion:** ButFr obtained from *Luehea divaricata* has important and sustained diuretic and hypotensive effects. Apparently, these effects are due to the release of nitric oxide and prostaglandins, which reduces renal and peripheral arteriolar tone, leading to an increase in the glomerular filtration rate and reduction of the global peripheral resistance. This study presents ButFr as a potential complementary therapy in several situations where diuretic and hypotensive effect is required. **References:** Tirloni, C.A.S., Palozzi, R.A., Tomazetto, T.A., Vasconcellos, P.S.P., Souza, R.I.C., Santos, A.C., Almeida, V.P, Budel, J.M., Souza, L.M., Gasparotto Junior, A., 2017. Ethnopharmacological approaches to kidney disease-prospecting an indigenous species from Brazilian Pantanal. J. Ethnopharmacol. 211, 47-57. **License number of ethics committee**: 16/2015 **Financial support**: CNPq e FUNDECT

06.002 *Celosia argentea* **L. a vasodilator species from the Brazilian cerrado – An Ethnopharmacological Report**. Tolouei SEL[1], Marques AAM[2], Tirloni CAS[2], Palozi RAC[2], Schaedler MI[2], Guarnier LP[2], Silva AO[2], Passoni MT[1], Curi TZ[1], Da Silva GN[1], Grechi N[1], Dalsenter PR[1], Gasparotto-Júnior A[2] [1]UFPR – Farmacologia, [2]UFGD – Ciências da Saúde

Introduction: *Celosia argentea* L. (Amaranthaceae), popularly known as "crista de galo", is an ornamental plant grown in home gardens with beautiful red flowers with velvety texture and dark green leaves. In folk medicine, its leaves infusion is used by local healers as diuretic and hypotensive agents. However, there are no reports in the literature regarding its pharmacological effects on cardiovascular system as well as no data proving the safety of this species. Therefore, we aimed to perform a detailed ethnopharmacological investigation of the ethanol soluble fraction from *Celosia argentea* (ESCA) in male and female Wistar rats. **Methods:** First, a morpho-anatomical study was performed in order to properly characterize and provide quality control parameters for the identification of the species under investigation. Then, the extract was obtained, chemically characterized and its oral acute toxicity (ESCA 30, 300 and 2000 mg/kg) evaluated. Finally, the possible diuretic and hypotensive effects of three different doses of ESCA (30, 100 and 300 mg/kg) were investigated in rats. Besides, the mechanisms of action of this extract and its involvement with nitric oxide/cGMP and prostaglandin/cAMP pathways as well as potassium channels were evaluated. **Results:** According to our data, ESCA caused no deaths, no toxic effects in female rats nor increased urinary excretion in male rats after acute exposure. However, intermediary dose (100 mg/kg) of ESCA was able to promote a significant acute hypotension and bradycardia. Furthermore, its cardiovascular effects appear to be involved with the voltage-dependent K+ channels activation. **Conclusion:** This study has brought new scientific evidence of preclinical efficacy of *C. argentea* as a hypotensive agent in normotensive rats. These effects seem to be involved with the activation of the voltage-sensitive K+ channels contributing to the reduction of peripheral vascular resistance and cardiac output. **License number of ethics committee**: UFPR 05/2017; UFGD 21/2017
Financial support: This work was supported by FUNDECT, CNPq and CAPES.

06.003 GRK2/NO pathway increases kidney alpha1 adrenergic receptor density during sepsis. Rosales TO, Stachewski R, Assreuy J UFSC – Farmacologia

Introduction: Sepsis is a serious medical condition caused by a dysregulated immune response to infection. The onset of kidney failure in sepsis worsens the prognosis and increases the mortality. Distinct from the generalized systemic vasodilation and impaired vascular reactivity to vasoconstrictors observed in sepsis, the kidney exhibits a marked vasoconstriction. G protein-coupled receptor kinases (GRKs) are OFF elements in signal transduction as they phosphorylate several receptors, including adrenergic receptors, resulting in their labeling for internalization. Previous data of our laboratory show that sepsis activates GRK2 in aorta and heart, leading to the decrease in α_1 and β_1 adrenergic receptor density which, in turn, is associated with the decrease of vascular and cardiac reactivity. In addition, sepsis-induced nitric oxide (NO) production has a relevant role in this cardiovascular dysfunction. Considering the opposite vascular status between the kidney and the systemic circulation during sepsis, the present study aimed to evaluate the α_1 adrenergic receptor density and the putative role of NOS-2-derived NO on GRK2 levels in septic kidney. **Methods:** *F*emale Swiss and Black C57BL/6 NOS-2-KO mice were submitted to sepsis by cecal ligation and puncture (CLP). Swiss mice were treated with a NOS-2 inhibitor (1400W; 1 mg/kg), 30 min before and 6 and 12 hours after sepsis induction or treated with a NO donor (SNAP, 10 mg/Kg). Kidney were removed for α_1 adrenergic-receptor fluorescent binding assay and Western blot analysis (GRK2 and NOS-2), 24 hours after CLP or 4 hours after SNAP treatment. **Results:** Our results show that i) sepsis induced an increase (75%) in α_1 adrenergic receptor density and reduced GRK2 levels in kidney to almost none; ii) NOS-2 protein expression was increased in septic kidney; iii) decrease of GRK2 levels in kidney was prevented in NOS-2-KO mice or with 1400W treatment of septic animals; iv) treatment with NO donor halved GRK2 content in kidney. **Conclusions:** Our findings show that sepsis induced a decrease in GRK2 levels together with an increased density of α_1 adrenergic receptors in kidney. Whether this increased density is due to upregulation of the receptor or to a decreased internalization remains to be seen. The results with NOS-2-KO mice and with 1400W treatment show that sepsis-reduced GRK2 levels were prevented by the reduction of NOS-2-derived NO in the kidney of septic animals. On the other hand, treatment with a NO donor decrease GRK2 content in the kidney. These data indicated that levels of GRK2 in the kidney are strictly dependent of NO production. Why sepsis decreases renal GRK2 content and by what mechanism NO is involved demands further studies. In any event, these findings may help explain why the kidney does not display the characteristic vasodilation observed in other organs. Considering that in sepsis, the sympathetic and angiotensin tonus are increased along with a renal higher α_1 adrenergic receptor density leading to vasoconstriction, our findings may point to a new mechanism to explain sepsis-induced kidney failure. **License number of ethics committee**: 8443190617 **Financial support**: CNPq, CAPES, FINEP and FAPESC.

06.004 Hyporesponsiveness to angiotensin II in conductance but not in resistance arteries from sepsis-surviving rats. Matsubara NK, Corrêa T, da Silva-Santos JE
UFSC – Farmacologia

Introduction: Sepsis survivors have a higher risk to present long-term cardiovascular diseases, but few experimental studies have addressed long-lasting effects of this disease. We have previously described that the cecal ligation and puncture (CLP) model, with resulting mortality rate around 30%, was associated with long-lasting and time-dependent effects on the vascular reactivity to angiotensin II (AII). In this study, we hypothesized that the time-course of the changes in vascular reactivity after septic shock depends on the severity of the septic insult and also includes resistance arteries. Therefore, this study aimed to evaluate the responsiveness to vasoactive drugs in the aorta and branches from the mesenteric artery from animals that survived sepsis induced by CLP with higher mortality rates (~60%). **Methods:** Male Wistar rats were anesthetized and subjected to the CLP surgery (three non-transfixing punctures with a 14G needle), which generated a mortality rate of 60-70% in 72 h. The survivors, named S30 and S60 groups, were used in experiments at 30 and 60 days after sepsis, respectively. Sham-operated animals (SO group) were used as the control group. The animals were euthanized with anesthetic overdose, and both thoracic aorta and the entire intestine with the vascular bed was removed. Aortic rings and segments from first or second order branches of superior mesenteric artery were removed and mounted in organ baths containing a Krebs-modified buffer in a myograph for isometric force recording. Small mesenteric arteries were subjected to increasing cumulative concentrations (1 nM-100 μM) of phenylephrine (PE) and vasopressin (AVP), for construction of concentration-response curves (CRCs), and to AII (1 μM). Aortic rings were exposed to CRCs of PE, AII or AVP. The results were expressed as the mean ± standard error of the mean of force developed. For statistical analysis, Student t-test or two-way ANOVA followed by Bonferroni test were used when applicable. **Results:** Aortic rings with functional endothelium from the S30 group did not show differences in its reactivity to PE and AVP, but displayed hyporeactivity to AII (the Emax was 0.5 ± 0.04 and 0.75 ± 0.04 in S30 and SO groups, respectively; n = 4-6; p = 0.0082). However, resistance arteries had no differences for any of the drugs tested, compared with the SO group. Likewise, aortic rings of the S60 group showed unaltered reactivity to PE and AVP but presented reduced responses to AII (Emax = 0.51 ± 0.07 and 0.74 ± 0.11 g in S60 and SO groups, respectively; n = 4-7; p = 0.0415). Resistance arteries of the S60 group showed no difference to any of the drugs tested, compared with vessels from SO animals. **Conclusion:** Together with previous studies, these results suggest that the time-course of septic shock-induced long-lasting effects on the vascular responsiveness depends on the severity of the septic insult. Moreover, the profile of changes on the vascular reactivity between the conductance artery aorta and resistance mesenteric arteries are quite different, reinforcing the relevance of additional studies to explore the behavior of the cardiovascular system in those who survive to septic shock. **License number of ethics committee**: pp00566 **Financial support**: CAPES

06.005 Bradykinin increases blood pressure in endotoxemic rats: **functional and biochemical evidence of bradykinin B2-angiotensin II type 1 receptor heterodimerization** Anton EL, Fernandes D, Assreuy J, Da Silva-Santos JE UFSC – Farmacologia

Introduction: The biological effects and potential interactions of bradykinin (BK) and angiotensin II (AII) in cardiovascular diseases remain poorly understood. This study hypothesized that the vascular effects of BK would be impaired in endotoxemia, an experimental model used to study sepsis, as a consequence of the interaction between BK B2 receptor (B2R) and AII AT1 receptor (AT1R). **Methods:** Female Wistar rats (200-280 g) received intraperitoneal (i.p.) injections of sterile saline (1 ml/kg), or lipopolysaccharide (LPS; 1 mg/kg) and were anesthetized after 24 h for direct measurement of the systemic arterial pressure, which was conducted under general anesthesia. The effects of BK (6, 20 and 60 nmol/kg, i.v.) and/or AII (6, 20 and 60 pmol/kg, i.v.) were assessed before and after treatment with Hoe-140 (20 nmol/kg s.c.), [Leu8]des-Arg9-BK (100 nmol/kg, i.v.), prazosin (0.5 mg/kg i.v.), losartan (15 mg/kg, i.v.), Y-27632 (0.1 mg/kg, i.v.), or indomethacin (10 mg/kg s.c.). The effects of BK and AII were also assessed in endothelium-intact small mesenteric arteries with or without incubation of 10 µM losartan or 1 µM Hoe-140. Homogenates of resistance mesenteric arteries and aorta were used for detection of B2R, and AT1R levels, RhoA-ROCK pathway components, and B2R-AT1R interaction throughout Western blotting and co-immunoprecipitation approaches. **Results:** The hypotension induced by BK (6, 20, and 60 nmol/kg) remained unchanged, but it was followed by an increase of 30 ± 3, 54 ± 4, and 65 ± 4 mm Hg in the systolic pressure of endotoxemic rats, against 10 ± 2, 18 ± 3, and 26 ± 2 mm Hg in control animals. The pressor response to BK was not reduced by the antagonist of the alpha-1 adrenergic receptor prazosin, nor by the antagonist of the BK B1 receptor [Leu8]des-Arg9-BK, or the cyclooxygenase inhibitor indomethacin, but it was vanished by Hoe-140, a BK B2R antagonist, losartan, an AII AT1R antagonist, and Y-27632, an inhibitor of Rho-kinase. LPS-treated rats also displayed enhanced pressor responses to AII (i.e., 46 ± 7 and 77 ± 5 mm Hg in control and LPS-treated rats, respectively, for 60 pmol/kg AII), which was prevented by Hoe-140. Resistance mesenteric arteries (but not aorta, a conductance artery) from endotoxemic rats showed augmented levels of B2R and AT1R, as well as of the phosphorylated MYPT-1 subunit of myosin phosphatase, as disclosed by Western blot analyses. *In vitro* experiments using organ baths revealed that small mesenteric arteries from LPS-treated, but not from control animals, presented a contractile response to BK (3 ± 0.6 mN), which was fully avoided by losartan. Moreover, the AII-induced contraction was enhanced in these arteries, a process prevented by Hoe-140. Immunoprecipitates isolated from homogenates of resistance mesenteric arteries using anti-B2R antibody followed by immunoblotting with an anti-AT1R antibody (and vice versa) revealed higher levels of the B2R-AT1R complex in samples from LPS rats. **Conclusion:** Besides hypotension, BK can produce systemic pressor effects in endotoxemic rats, a phenomenon that appears to be mediated by direct interaction of B2R-AT1R in resistance arteries, includes activation of ROCK, and contributes to enhance the vasoconstrictor effects of AII. **License number of ethics committee**: CEUA/UFSC PP00566 **Financial support**: CNPq (448738/2014-7)

06.007 Amelioration of sepsis by inhibition of Phosphodiesterase 3. Oliveira JG[1], Sordi R[1], Oliveira MRP[2], Alves G F[1], Fernandes D[1] [1]UFSC – Farmacologia, [2]UEPG – Biologia Estrutural, Molecular e Genética

Introduction: Sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated immune response to infection, with a high incidence and mortality rate in critically ill patients. The cAMP and cGMP play a central role in signal transduction cascades, regulating many critical physiological processes that are affected during sepsis including cardiac function, inflammation, platelets aggregation and endothelial stabilization. The cellular levels of the second messengers, cAMP and cGMP, are regulated by cyclic nucleotide phosphodiesterases (PDEs) enzymes. The PDE3 possess dual specificity, acting on both cAMP and cGMP hydrolysis. To study the role of cGMP and cAMP on organ protection in sepsis, we have used cilostazol, a PDE3 inhibitor that is approved for the management of peripheral vascular diseases. **Methods:** Sepsis was induced by cecal ligation and puncture (CLP) procedure in male Wistar rats (n=32). Cilostazol (15 mg/kg, vo, n=16) or vehicle (peanut oil, n=16) was administered 6 h after the surgery and 24 h later, blood pressure, heart rate, renal blood flow, organ bath (thoracic aorta ring), urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate, nitrite/nitrate (NOx) levels, hematological analyzes, lung and kidney myeloperoxidase (MPO) and lung histopathology were performed. **Results:** The CLP procedure was associated with hypotension, hyporesponsiveness to vasoconstrictors (Phe and AII) *in vivo* and ex vivo (aorta) (Phe and KCl), reduction of renal blood flow and platelets number, and increase of heart rate, urea, AST, ALT, lactate and NOx serum levels. The treatment of CLP animals with cilostazol restored renal blood flow (Sham 496.8 ± 29.92; CLP 251.9 ± 42.0; CLP + Cilostazol 379.9 ± 36.6, perfusion units; $P<0.05$), improved the vasoconstrictive response to AII *in vivo*, and aorta responsiveness to Phe and KCl. When compared to CLP vehicle rats, cilostazol treatment reduced lactate level (CLP 47.4 ± 8.9; CLP + Cilostazol 30.1 ± 5.1, mg/dL; $P<0.05$), hematocrit (CLP 50.4 ± 2.5; CLP + Cilostazol 41.2 ± 0.8, %; $P<0.01$), lung MPO (CLP 4.0 ± 1.6; CLP + Cilostazol 0.5 ± 0.1, O.D. 650 nm/mg protein; $P<0.05$) and lung histopathological score ($P<0.05$). However, the hypotension, thrombocytopenia, and increased levels of urea, AST, ALT, NOx and kidney MPO associated with CLP procedure were not changed by cilostazol treatment ($P>0.05$). **Conclusion:** The PDE3 inhibition by cilostazol improved tissue blood perfusion and reduced organ injury, probably through cAMP and cGMP levels increasing. Thus, PDE3 inhibition may be an interesting strategy in the treatment of sepsis. Further experiments, measuring the cyclic nucleotides levels and evaluating PDE-3 expression during sepsis are needed and are ongoing in our laboratory. **License number of ethics committee**: The procedures were approved by the University Institutional Ethics Committee (Protocol number 006/2017). **Financial support**: This work was supported by CAPES Foundation (Ministry of Education of Brazil).

06.008 Chronic treatment with estrone improves endothelial dysfunction though antioxidant properties in ovariectomized Wistar rats. Oliveira TS[1], Costa RM[2], Campos HM[1], Neri HFS[1], Costa EA[1], Santos FCA[3], Tostes RC[4], Filgueira FP[2], Ghedini PC[1] [1]UFG – Farmacologia, [2]UFG – Ciências da Saúde, [3]UFG – Ciências Morfológicas e Fisiológicas, [4]FMRP-USP – Farmacologia e Fisiologia

Introduction: Conjugated equine estrogens (CEE) have been widely used by women who seek to relieve symptoms of menopause. Despite evidences describing protective effects of naturally occurring estrogens against cardiovascular risk factors, little is known about the vascular effects of estrone (E1) treatment, the main component of CEE. We have previously demonstrated that E1 have an endothelium-dependent vasorelaxant effect *in vitro*, which is mediated by activation of estrogen receptors, resulting in stimulation of NO/cGMP pathway, via Ca2+/CaM dependent activation of PI3K. The present study tested the hypothesis that *in vivo* E1 treatment improves vascular function in an estrogen-deficient animal model. **Methods:** Female 12-week-old Wistar rats were ovariectomized (OVX) or sham (Sham) operated. 60 days after surgery, the OVX group were treated with vehicle, E1 (825 µg/kg/day) or 17β-estradiol (E2) (15 µg/kg/day) for 30 days. Body mass, heart weight and retroperitoneal fat pad were determined. Arterial blood pressure was measured by an indirect tail-cuff method. Concentration-effect curves to phenylephrine (Phe, 0.1nM-30µM), a α1-adrenoreceptor agonist, to acetylcholine (ACh, 0.01nM-10µM), an endothelium-dependent vasodilator, and to sodium nitroprusside (SNP, 1pM-30µM), a nitric oxide donor, were performed in aortic rings with intact endothelium. The involvement of reactive oxygen species (ROS) on ACh-induced vasodilation was evaluated in aortic rings from Sham, OVX, E1 and E2 after preincubation (30min) with apocynin (APO), a NADPH oxidase inhibitor (10mM), and the antioxidant enzymes SOD (150U/mL) or catalase (CAT, 100U/mL). The protein levels of CAT, SOD1, SOD2, NOX1, NOX2 and NOX4, were assessed by Western blotting. Data are presented as mean±SEM of 4-7 experiments and analyzed by one-way ANOVA. A value of $P < 0.05$ was considered significant. **Results:** Phe-induced contraction was increased, and ACh-induced relaxation was reduced in endothelium-intact arteries from OVX, while no differences were observed in endothelium denuded aortic rings. There was no change in the relaxation response to SNP. E1 treatment corrected both the increased contraction and the impaired relaxation in OVX rats. Incubation with CAT, SOD or APO restored the impaired ACh-induced relaxation in aortic rings from OVX and made the responses become similar to those observed in E1-treated group. The increased protein expression of NOX4, CAT and SOD1 observed in the aorta of OVX rats were restored by E1 treatment. The protein levels of SOD2, NOX1 and NOX2 were not affected by ovariectomy. **Conclusion:** The present study indicates potential benefits of E1 treatment correcting the endothelial dysfunction in OVX rats, thought inhibition of NOX4 expression. **Financial Support:** CAPES, FAPEG, CNPq (462306/2014-3). Research approved by the Animal Research Ethical Committee from Federal University of Goias (process number 20/2013). **License number of ethics committee**: Research approved by the Animal Research Ethical Committee from Federal University of Goias (process number 20/2013) **Financial support**: Support: CAPES, FAPEG, CNPq (462306/2014-3)

06.009 Sepsis-induced long-term cardiac dysfunction in rats. Correa T, da Silva-Santos JE UFSC – Farmacologia

Introduction: A particular occurrence in sepsis is the development of cardiovascular dysfunction. Notably, cardiovascular diseases have been described as an important risk factor among those who survived sepsis. We hypothesized that depressed cardiac function is one of the long-lasting effects of sepsis. **Methods:** We have evaluated the cardiac function of male Wistar rats subjected to the cecal ligation puncture (CLP) model at 6 h, 24 h, 72 h, 15 days and 30 days after the surgery (n = 5-6/group). Age-matched sham-operated animals were used for control. The animals were anesthetized, and a pressure-volume catheter was inserted into the left ventricle chamber. The basal and dobutamine-stimulated cardiac output (CO), heart rate (HR), stroke volume (SV), end diastolic pressure (EDP), stroke work (SW), relaxation time constant (TAU), and ejection fraction (EF) were measured *in situ*. The development of force, the cardiac cycle, and the coronary perfusion pressure were also evaluated using hearts from surviving rats (30 days after CLP) in a Langendorff apparatus. Heart samples were used to measure either activity or levels of superoxide dismutase (SOD), glutathione S-transferase (GST), glutathione (GSH), lipid hydroperoxides (LOOH), myeloperoxidase (MPO), and reactive oxygen species (n = 5-6/group). **Results:** At 24 h after the CLP surgery, the CO, EF, and SW were reduced from 30.4 ± 1.9 mL/min, 43.6 ± 2.2%, and 6097 ± 469 mmHg/µl in sham animals, to 12.4 ± 1.2 mL/min, 24.4 ± 3.1%, and 2561 ± 597 mmHg/µl in CLP subjected rats. Similar differences were found at 6 h after the CLP. Importantly, all cardiac parameters were back to control levels at 72 h and remained unchanged at 15 days after the CLP. Nevertheless, when evaluated at 30 days after the CLP, sepsis-surviving animals presented decreased CO (20.85 ± 5.01 mL), EF (41.3 ± 4.8%) and SW (6481 ± 1252 mmHg/µl), compared with control animals (38.4 ± 4.1 mL, 57.8 ± 2.3%, and 10551 ± 725 mmHg/µl, respectively). On the other hand, both EDP and Tau were increased from 2.8 ± 1.8 mmHg and 9.2 ± 2.3 G (ms) in sham animals to 14.5 ± 3.3 mmHg and 14.0 ± 0.6 G (ms) in sepsis-surviving rats. Besides, dobutamine had its effects on the intraventricular pressure, ejection fraction and stroke work reduced by 58%, 20% and 50% in sepsis-surviving rats. Similarly, the development of force and dobutamine-increased contractility were also decreased by 40-50% in perfused hearts taken from sepsis-surviving rats, which also displayed reduced coronary perfusion pressure and prolonged diastolic time. Regarding the biological markers of oxidative stress, both SOD and GST activities were significantly reduced (~ 64% and 23%), GSH was increased (~ 54%), and LOOH unchanged at 30 days after CLP. Moreover, MPO was augmented by ~ 400%, and enhanced DHE fluorescence was found in hearts from sepsis-surviving rats, always compared with samples from age-matched sham-operated animals. **Conclusion:** Despite the lack of functional abnormalities at 15 days after the CLP, sepsis-surviving animals developed both systolic and diastolic dysfunction associated with signs of redox unbalance and inflammation at later periods (30 days after the CLP). A better understanding regarding the mechanisms undergoing the long-term effects of sepsis on the cardiac function may allow the development of preventive strategies to reduce the risk of cardiovascular diseases among those who survive sepsis. **License number of ethics committee**: PP00566 **Financial support**: FAPESC, SC, Brazil (TR2012000367)

06.010 Effects of adjuvant-induced arthritis (AIA) on local mechanisms that control the tonus of veins. Montenote MC[1], Pita LM[2], Oliveira PB[2], Chagas EFB[2], Spadella MA[3], Chies AB[2] - [1]Unesp-Botucatu – Farmacologia e Biotecnologia, [2]FAMEMA – Farmacologia, [3]FAMEMA – Farmacologia e Fisiologia

Introduction: In arthritis, cytokines released from the injured joints may act in extra-articular structures, thereby promoting inflammation and oxidative stress. The action of such joint-derived cytokines upon blood vessels may induce endothelial dysfunction and, as a consequence, may alter the vascular responses of substances involved in the control of vascular tonus. The emergence of these arthritis-related effects on blood vessels may vary, throughout the time course of the disease, depending on the vascular bed. In fact, endothelial dysfunction in microvascular beds precedes that occurring in the macrovascular bed. These vascular repercussions of arthritis are well described in the arterial bed, but precariously characterized in veins. Thus, the present study aimed to identify the influence of adjuvant-induced arthritis (AIA) on the noradrenaline (NOR) and angiotensin II (Ang II) responses in rat large veins. **Methods:** Twelve weeks old Male Wistar rats were distributed in Control (non-immunized) and AIA groups [immunized with Mycobacterium tuberculosis/oil (50mg/mL), injected into the right hind paw]. The effectiveness of AIA was demonstrated by the measurement of left hind paw volume, body mass and C-reactive protein titration (PCR). Animals that did not showed joint inflammation signals and or PCR positivity until the 20th day after immunization were excluded from the study. Rings of cava, femoral, jugular, mesenteric and portal veins, obtained from these animals, were mounted in organ baths and challenged by cumulative concentrations of NOR and ANG II. Isometric contractions were registered to obtain the maximum responses (Rmax) and the concentration-response curves (that permitted the calculation of pD_2). These parameters (expressed in mean ± standard error of the mean; sample size = 10-11) were compared between the groups by the Student's t test (significance when $P < 0.05$). Study approved by CEUA / FAMEMA nº 092/17. **Results:** AIA increased the left hind paw volumes (from 1.71 ± 0.01 to 2.29 ± 0.12, $p < 0.05$) and reduced body mass (from 395.6 ± 15.7 for 312.0 ± 8.30, $p < 0.0001$). In addition, all AIA animals presented positive PCR. AIA increased the pD_2 to NOR in the portal vein (from 5.88 ± 0.11 to 6.46 ± 0.22, $p=0.02$), without modifying this parameter in the other studied veins. In contrast, no significant modification of the Rmax to NOR induced by AIA was observed in any of these studied veins. AIA also did not promote significant modifications of pD2 or Rmax to Ang II in any of the studied veins. Conclusions: The obtained data show that the employed immunization was effective in promoting AIA. These data also show that the changes of NOR responses induced by AIA in veins are bed-specific, since they only occurred in the portal vein. In this vein, AIA probably impaired the endothelial function, thereby increasing its responsiveness to NOR. AIA-induced modifications of response also appear to be agonist-specific because no difference of Ang II responses was observed in any of the studied vessels. SUPPORT: CAPES and FAPESP (proc. nº 2016/08450-3). **License number of ethics committee**: 092/17 **Financial support**: CAPES and FAPESP (proc. nº 2016/08450-3).

06.011 New ROCK inhibitor reduced vascular dysfunction and cardiac hypertrophy induced by pulmonary hypertension. Montagnoli TL, Rocha BS, da Silva JS, Silva MMC, Alencar AKN, Silva GF, Oliveira RG, Sudo RT, Barreiro EJ, Fraga CAM, Zapata-Sudo G UFRJ – Desenvolvimento de Fármacos

Introduction: Pulmonary hypertension (PH) is a chronic and progressive disease characterized by increased tone in pulmonary vascular bed and muscularization of vascular walls. Rho-associated protein kinases (ROCK) are important serine/threonine kinases involved in processes of contractility and proliferation of smooth muscle cells which inhibition represents a therapeutic strategy for PH. Since a novel sulfonylhydrazone derivative named LASSBio-2020 produced 50% inhibition of ROCK-1 at 13 μM, it was tested on the cardiac and vascular dysfunction induced by PH in rats. **Methods:** PH was induced in male Wistar rats (250 ± 20 g) after 3 weeks exposure to normobaric hypoxia (10% O_2) combined with intraperitoneal (i.p.) administration of SU5416. Transthoracic echocardiography was used to confirm PH. Control group consisted of animals kept in normoxia during all protocol (n= 5). LASSBio-2020 at 20 mg/kg/day or vehicle (DMSO) was injected i.p. during 2 weeks after hypoxia period. At the endpoint, pulmonary artery acceleration time (PAT) and right ventricle (RV) wall thickness were evaluated through echocardiography. Vascular reactivity was investigated using isometric tension recording in pulmonary artery rings from all experimental groups. Vasodilator activity of LASSBio-2020 and fasudil (drug reference) was observed after exposure to increased concentrations in pre-contracted denuded vessels. **Results:** LASSBio-2020 and fasudil produced vasodilation of pulmonary artery in normoxic rats with half inhibitory concentration (IC_{50}) of 8.04 and 1.49 μM, respectively. Mean arterial pressure was significantly reduced by LASSBio-2020 from 91.0 ± 6.8 to 79.8 ± 1.0 mmHg ($p< 0.05$). In contrast, echocardiographic analysis demonstrated that PAT (23.3 ± 3.8 vs. 21.0 ± 3.7 in vehicle group), RV pressure and wall thickness were not significantly improved by treatment of LASSBio-2020. LASSBio-2020 partially reverted RV hypertrophy. Pulmonary arteries from PH rats showed impaired response to acetylcholine with reduction of maximal relaxation from 84.4 ± 5.2 to 56.9 ± 9.8% ($p<0.05$) which was reverted after treatment with LASSBio-2020 recovering the vasodilator response to control values of 78.5 ± 5.7%. **Conclusion:** LASSBio-2020 is a novel ROCK inhibitor with vasodilator activity that reverted endothelial dysfunction and cardiac hypertrophy in animals with PH. **License number of ethics committee**: CEUA/UFRJ nº 106/16 **Financial support**: INCT, CAPES, CNPq, FAPERJ

06.012 Influence of physical exercise on treatment with captopril in SHR rats. Castro QJT[1], Silva SSC[1], Watai PY[1], Guimarães HN[2], Grabe-Guimarães A[1] [1]UFOP – Farmácia, [2]UFMG – Engenharia Elétrica

Introduction: Regular physical exercise is important to prevent and to help arterial hypertension (AH) treatment (SBC, 2016). The effectiveness of AH pharmacological treatment is better when non-pharmacological treatment is adopted (RONDON and BRUM, 2003), although there are few evidences confirming dose reduction. Considering ECG intervals alteration as predictors of cardiac arrhythmias also associated with AH, we conducted an experimental study to evaluate on spontaneous hypertensive rats (SHR) the effectiveness of regular physical exercise associated to three doses of captopril. **Methods:** All the procedures were approved by CEUA/UFOP (2015/05). Physical exercise trained and sedentary male adults SHR received captopril 12.5, 25 or 50 mg/kg by oral route or vehicle (8 groups, n=5 a 8 each). For physical exercise it was used a treadmill, 18 m/min during 60 minutes per day, five days a week during eight weeks. After that, lead II ECG signal was recorded anaesthetized rats. **Results:** PR and QRS intervals of ECG were similar for all groups. Sedentary SHR presented QT (70.5 ± 1.61 ms) and QTc (127.3 ± 3.90 ms) intervals prolongation and physical exercise alone was not able to reduce it. It was observed 12.6% and 12.2% of QT (64.5 ± 1.50 ms) and QTc (102.3 ± 3.36 ms) intervals reduction in trained group treated with 50 mg/kg captopril compared to trained group treated with vehicle, and 9.0% and 9.5% related to sedentary group treated with the same dose, respectively. For sedentary groups it was observed 17.1% reduction of QTc interval when tread with captopril 12.5 mg/kg compared to vehicle treatment. No significant response on QT and QTc intervals was observed for the other groups, trained or sedentary. **Conclusion:** Regular physical exercise can contribute to QT and QTc intervals reduction and can increase pharmacological treatment effectiveness as demonstrated in male adults SHR, at least when higher dose is used. Further studies are necessary to encourage dose reduction prescription to patients that exercise regularly. **Referências:** RONDON, M. U. P. B.; BRUM, P. C. Exercício físico como tratamento não-farmacológico da hipertensão arterial. **Revista Brasileira de Hipertensão**, v. 10, n. 2, p. 134–139, 2003. SOCIEDADE BRASILEIRA DE CARDIOLOGIA. 7ª Diretriz Brasileira De Hipertensão Arterial. Rio de Janeiro: **Arquivos Brasileiros de Cardiologia**. v. 107, n. 3, p. 1-103, 2016. **Funding agencies and Acknowledgments:** FAPEMIG, CAPES, CNPq and UFOP. **License number of ethics committee**: CEUA/UFOP 2015/05 **Financial support**: FAPEMIG, CAPES, CNPq and UFOP

06.013 Apocynin ameliorates endothelial modulation on angiotensin II vasoconstrictor response in mesenteric arteries of SHR. Graton ME[1], Potje SR[2], Vale GT[3], Troiano JA[1], Benevides PS[1], Tirapelli CR[3], Nakamune ACMS[1], Bendhack LM[2], Antoniali C[1] [1]Unesp-Araçatuba – Ciências Básicas, [2]FCFRP-USP – Física e Química, [3]EERP-USP – Enfermagem Psiquiátrica e Ciências Humanas

Introduction: Angiotensin (Ang) II can regulate expression and activity of NAD(P)H oxidase (NOX), the major source of reactive oxygen species (ROS) in cardiovascular system. Previously, we observed that apocynin, a NOX inhibitor, prevented endotelial dysfunction and reduced blood pressure by increasing nitric oxide (NO) and reducing ROS concentrations in endothelial cells. We evaluated the effect of apocynin on the contractile responses to Ang II in resistance arteries of SHR and the mechanisms involved on these effects. **Methods:** SHR were treated from the 4th to the 10th week of life with apocynin (30 mg/Kg). Wistar rats were used as normotensive control. Using mesenteric arteries, we performed concentration-response curves to Ang II and determined eNOS and NOX isoforms and subunits expressions, lucigenin chemiluminescence and nitrate/nitrite levels. Data were expressed as mean ± SEM. **Results:** Apocynin increased endothelium modulation and/or NOS activity on Ang II vasoconstrictor responses in mesenteric arteries of SHR. Treatment with apocynin did not alter NO synthase activity, and eNOS, NOX1, NOXO1, and NOX4 expressions, however, it decreased NOX2 and p47phox expressions in mesenteric beds of SHR treated. Moreover, treatment apocynin was able to decrease ROS production in these vessels. **Conclusion:** All these results demonstrated that *in vivo* treatment with apocynin induces important alterations of several mechanisms that lead to the reduction of the pressor and vasoconstrictor effects of Ang II in SHR. Apocynin effect involves further mechanisms besides the modulation of vascular ROS, which improve NO availability in vascular cells of SHR. All these results demonstrated that *in vivo* treatment with apocynin induces important alterations of several mechanisms that lead to the reduction of the pressor and vasoconstrictor effects of Ang II in SHR. Apocynin effect involves further mechanisms besides the modulation of vascular ROS, which improve NO availability in vascular cells of SHR. **License number of ethics committee**: CEUA FOA 450/2015 **Financial support**: CAPES and FAPESP (2016/22180-9)

06.014 Inflammatory profile of mesenteric perivascular adipose tissue in obese mice: **a possible protective role of spleen**. Silva RNO[1], DIAS CC[2], Rodrigues SFP[1], Fock RA[2], Carvalho MHC[1], Akamine EH[1] [1]ICB-USP – Farmacologia, [2]FCF-USP – Análises Clínicas e Toxicológicas

Introduction: In obesity there is adipocyte hypertrophy and inflammation in the visceral deposits. A major deposit is the perivascular adipose tissue (PVAT), as it exerts paracrine action on the vasculature, modulating the tonus and inflammation. The spleen is the largest lymphoid organ and it is an important regulator of the immune system. The aim of the study was to evaluate the influence of the spleen on the inflammatory profile of mesenteric PVAT in obese mice. **Methods:** CEUA/ICB/USP 90/2013. C57BL/6 (4 weeks old) male mice were either splenectomized (SPX) or sham operated (SHAM) and received either control(CT) or high fat diet (HFD, OB) for 16 weeks. Spleen and mesenteric PVAT were used to analyse: protein content of cytokines (spleen: Western blot, arbitrary units, n=6; PVAT: ELISA, pg/ml normalized by mg total protein, n=5), leukocyte total number (spleen: 10^6/ml, n=10; PVAT: 10^5/ml, n=10) and immunophenotyping (flow cytometry, n=10) and spleen cell chemotaxis for PVAT (*in vitro* transmigration, 10^4/ml/mg PVAT, n=8). Statistical analysis: values are mean ± SEM; two-way ANOVA or t-test; significance level $p < 0.05$. **Results:** The content of IL-10 was reduced (CT: 93.4 ± 8.3; OB: 66.7 ± 6.9) and TNF-α was increased (CT: 63.7 ± 11.4; OB: 174.7 ± 46.1) in the spleen of OB animals. The number of total leukocytes was reduced (CT: 9.0 ± 1.2, OB: 5.3 ± 0.6), but the relative population of T cells (CD4+ and CD8+), B cells, NK cells (NK1.1+ CD49b+), monocytes (Mac-1+ F4/80+) and granulocytes (Mac-1+ Gr.1+) in spleen were similar between the OB and CT groups. There was no difference in the content of pro-inflammatory cytokines in PVAT of OB-SHAM, but IL-6 and IL-1β were increased in the PVAT of OB-SPX in comparison to CT (IL-6 - CT: 17.7 ± 2.8; OB-SHAM: 5.9 ± 0.8; OB-SPX: 14.0 ± 4.0) (IL-1β - CT: 47; OB-SHAM: 17.1 ± 1.8; OB-SPX: 28.9 ± 7.2). The content of the anti-inflammatory cytokine IL-10 was increased in PVAT of OB-SHAM and OB-SPX group (CT: 51.3±10.6; OB-SHAM: 139.0±16.7; OB-SPX: 120.0±12.8). The number of total leukocytes in PVAT was increased in OB-SPX in comparison to CT group, but there was no difference for the OB-SHAM (CT: 2.1 ± 0.6; OB-SHAM: 2.8± 0.3; OB-SPX: 3.7±0.2). The relative population of $CD4^+$ T cells in PVAT was similar in all groups. $CD8^+$ T cells in PVAT of OB-SHAM were similar to CT but were decreased in OB-SPX (CT: 52.1± 7.5; OB-SHAM: 48.0 ± 4.2; OB-SPX: 26.0 ± 2.6). B cells in PVAT of OB-SHAM, but not in OB-SPX, were decreased in comparison to CT (CT: 30.2± 4.4; OB-SHAM: 14.8 ± 2.0; OB-SPX: 20.4 ± 1.5). M1 ($CD11c^+$) and M2 (CD206) macrophages were, respectively, increased and decreased in PVAT OB-SPX, whereas there was no difference for OB-SHAM (M1 - CT: 4.5± 0.6; OB-SHAM: 5.5 ± 0.9; OB-SPX: 10.1 ± 0.9) (M2 - CT: 18.1± 3.5; OB-SHAM: 8.2 ± 2.4; OB-SPX: 3.4 ± 1.4). Spleen leukocyte migration to PVAT was reduced in both OB-SHAM and OB-SPX groups (CT: 228.4 ± 50.0; OB-SHAM: 90.0 ± 16.5; OB-SPX: 74.8 ± 9.1). **Conclusion:** Feeding with HFD for 16 weeks was not able to promote inflammation in the mesenteric PVAT and spleen may play a protective role. **License number of ethics committee**: CEUA/ICB/USP 90/2013 **Financial support**: CNPq, FAPESP e CAPES.

06.015 Aging affects the expression of Ca^{2+} handling proteins related to cardiac dysfunction programmed by neonatal leptin treatment. Marques EB, Souza KP, Scaramello CBV - UFF – Fisiologia e Farmacologia

Introduction: Previous data have shown that neonatal leptin treatment programs cardiac dysfunction in 30, 150 and 365 days-old male rats in an age different way (Marques et al., Int J Cardiol. 181C: 141, 2015; Marques et al., SBFTE2017, panel 06.021). According to literature, changes in Ca^{2+}-handling proteins in the heart may underlie cardiac dysfunction (Mora et al., PLoS One, 12(11): e0187739, 2017), including along aging process (Feridooni et al., J Mol Cell Cardiol. 83: 62, 2015). The aim of this work was to evaluate if aging affects Ca^{2+}-regulatory proteins expression related to cardiac dysfunction programmed by neonatal leptin treatment. **Methods:** Male newborn Wistar rats were divided into two groups: Leptin group (L), treated with daily leptin (8µg/100g sc), and Control group (C), treated with saline, for the first 10 days of lactation. At postnatal days 30 (C30xL30), 150 (C150xL150) and 365 (C365xL365) rats were euthanized and the hearts (n=4-6 for group) were excised to obtain cardiac homogenates (Bambrick et al., J Pharmacol Meth, 20: 313, 1988). The samples were used in western blot assays to evaluate protein levels of sarcoplasmic reticulum and plasma membrane Ca^{2+}-ATPase (SERCA and PMCA), phosphorylated and non-phosphorylated phospholamban (p-PLB and PLB), Na^{+}/Ca^{2+} exchanger (NCX), Na^{+}/K^{+}ATPase (NKA) and FKBP12 (Laemmli. Nature, 227: 680, 1970). Data were presented as mean (±SEM), analyzed by Student t test and considered statistically different if $P<0.05$(*) compared to respective control. **Results:** Western blot analysis showed significant changes on SERCA (C30: 1.52±0.19xL30: 2.49±0.39*; C150: 1.51±0.17xL150: 2.61±0.35*; C365: 0.82±0.09xL365: 0.51±0.07*), p-PLB/PLB ratio (C30: 2.11±0.62xL30: 5.88±1.56*; C150: 1.34±0.38xL150: 3.02±0.44*; C365: 0.33±0.01xL365: 0.19±0.04*), NCX (C150: 2.37±0.32xL150: 1.54±0.16*; C365: 1.13±0.16xL365: 1.76±0.14*) and NKA (C150: 4.33±0.60xL150: 1.82±0.32*; C365: 0.77±0.06xL365: 1.76±0.19*) protein levels in an age-dependent manner. PMCA and FKBP12 expression were unchanged. **Conclusion:** Differently from postnatal days 30 and 150, p-PLB/PLB ratio is lower and SERCA is downregulated, while NCX and NKA are upregulated in 365 days-old rats submitted to neonatal leptin treatment compared to respective control. SERCA-mediated Ca^{2+} uptake seems to decline while cytosolic Ca^{2+} removal by NCX increases, an alteration often seen in failing hearts (Wasserstrom, J Physiol, 588(Pt 7): 1027, 2010; Coppini et al., Glob Cardiol Sci Pract, 2013(3): 222, 2013). NKA upregulation should be a consequence of the secondary active transporter overexpression because of intracellular Na^{+} concentration increase (Shimizu et al., J Physiol Sci. 59(1): 63, 2009). These findings suggest that aging affects the molecular mechanism related to the programmed cardiac injury abolishing compensatory changes that are important to the preservation of diastolic function. **Financial Support:** FAPERJ, CNPq, CAPES, PROPPI/UFF. **Ethics Committee Approval Number**: CEUA/UFF812-16.

06.016 O-GlcNAcylation and the enzymes involved in this post-translational modification in blood vessels of normotensive and hypertensive pregnant rats.
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Introduction: O-glycosylation by N-acetyl-glucosamine (O-GlcNAc) decreases nitric oxide synthase (eNOS) phosphorylation leading to a lower nitric oxide (NO) bioavailability. We hypothesized that vascular proteins O-GlcNAc are decreased in blood vessels of pregnant Wistar and spontaneously hypertensive rats (SHR) and contribute to reduce NO bioavailability. We evaluated the expression of O-GlcNac-proteins, O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA) expressions and the effects of PugNAc (OGA inhibitor) on reactivity to PE in aorta and mesenteric artery of pregnant Wistar and SHR. **Methods:** Ethics committee FOA-UNESP proc. 00730/2015. We performed the analysis of O-GlcNAc, OGT and OGA expression by Western Blot and OGA activity by biochemical analysis in aortic and mesenteric bed homogenates from non-pregnant (NP) and pregnant (P) Wistar and SHR with 12 weeks old (n= 6). Aorta rings or mesenteric artery rings from different group (n= 5-7) were incubated in DMEM in the absence or presence of PugNAc (100 µM) for 24 hours. Rings, with and without endothelium, or with endothelium in the presence of L-NAME (100 µM, 30 min), were stimulated with cumulative concentrations of phenylephrine- PE (1 nM to 10 µM). The results were compared among groups (Student`s t test or ANOVA, $p < 0.05$). **Results:** Pregnancy reduced O-GlcNAc proteins expression in aorta in mesenteric artery from Wistar and SHR. The OGA expression was not altered in these vessels and OGT expression was decreased only in mesenteric bed of P SHR. OGA activity was increased in aorta and bed mesenteric of P Wistar and in mesenteric bed of P SHR, but it was reduced in aorta from P SHR. PE vascular reactivity was reduced in blood vessels from P rats than in NP rats from both groups. PugNAc did not alter PE reactivity in aortas from NP Wistar (Emax: 26.16±0.43 mN) and NP SHR (Emax: 18.71±1.7 mN). Aortas from P Wistar incubated with PugNAc showed greater maximum contractile effect to PE (Emax: 32.75±1.17 mN) than aortas from P Wistar (Emax: 23.40±1.18 mN) non-incubated with PugNAc. However, PugNAc did not alter the contractions stimulated by PE in aortas from P SHR (Emax: 13.46 ±1.49 mN). Mesenteric artery rings from P Wistar incubated with PugNAc (Emax: 23.39±1.8 mN) showed increased reactivity to PE when compared to the control (Emax: 17.32±0.8 mN). PugNAc reduced endothelium blunted effect in blood vessels and reversed low reactivity to PE in aorta and in mesenteric artery from P Wistar. **Conclusion:** The reduced vascular proteins O-GlcNAc expression in aorta and mesenteric artery from Wistar observed on late pregnancy seems to be due to the increased OGA activity and it contribute to reduced reactivity to PE in different blood vessels from P Wistar rats, while in pregnant SHR OGT seems to be involved with reduced O-GlcNAc. **Financial support**: FAPESP 2015/09373-0; 2016/22180-9 and CAPES.
License number of ethics committee: FOA-UNESP proc. 00730/2015
Financial support: FAPESP 2015/09373-0; 2016/22180-9 and CAPES

06.017 Hydrogen Sulfide (H_2S) production is altered in mesenteric arteries from rats with mild periodontitis. Jesus FN, Teixeira SA, Costa SKP, Muscará MN ICB-USP – Farmacologia

Introduction: H_2S has important roles in the vascular system and during the inflammatory response. The activation of ATP-dependent potassium (K_{ATP}) channels is one of the well-defined mechanisms of H_2S actions; however other mechanisms have been shown, such as those involving the modulation of NO actions. In this way, we decided to evaluate the participation of H_2S in the *in vitro* response of mesenteric artery from rats with periodontitis, a disease condition characterized by dysfunction of the vascular NO-soluble guanylate cyclase (sGC)-cyclic guanosine monophosphate (cGMP) pathway. **Methods:** The experimental protocol was approved by the local Ethics Committee for Animal Experimentation (CEUA/ICB 170, 2/113, 2011). Male Wistar rats (10-14 wk-old) were anesthetized by the i.p. administration of ketamine (80 mg/kg) and xylazine (20 mg/kg). Periodontitis was induced by placing a cotton ligature around both the left and right lower first molars (group P); sham rats (S) had the ligature immediately removed. Seven days later, the rats were killed by anesthesia overdosing and the mesenteric bed was dissected. Third-branch artery segments were mounted on a wire myograph in order to evaluate the *in vitro* responses to the increasing concentrations of acetylcholine (ACh) and sodium nitroprusside (SNP), as well as in the presence of the selective K_{ATP} channel blocker glibenclamide (Gli, 10 µmol/l) or the non-selective K^+ channel blocker tetraethylammonium (TEA). In another set of experiments, the *in vitro* perfusion pressure of the whole mesenteric bed was analysed in response to increasing bolus doses of ACh, SNP and the spontaneous H_2S donor sodium hydrosulfide (NaHS), as well as in the presence of 100 µmol/l aminooxyacetic acid (AOAA), a non-selective cystathionine-γ-lyase (CSE) and cystathionine-β-synthase (CBS) inhibitor. From the response curves, potency (pA_2) and maximal response (E_{max}) values were calculated. In addition, mesenteric artery samples from both experimental groups were subjected to Western blot analysis in order to study the expression of the H_2S producing enzymes CSE, CBS and 3-mercaptopyruvate sulfurtransferase (3-MST), as well as the *in vitro* production of H_2S. Differences between the groups were analyzed by unpaired Student *t*-test. **Results:** The relaxation to SNP was significantly lower in the P group in comparison with S animals. When analyzed as tension of isolated mesenteric artery segments (pA_2: 8.0±0.3 vs. 7.1±0.1, $P<0.05$), these differences were abolished in the presence of either Gli or TEA. The difference in perfusion pressure of the mesenteric beds (E_{max}: 86.0±12.1% vs. 49.9±4.6, $P<0.05$) was abolished in the presence of AOAA. No differences were observed in the response to ACh or NaHS, either alone or in the presence of Gli, TEA or AOAA. No differences between the groups were observed in terms of CSE, CBS or 3-MST expression, although mesenteric arteries obtained from group P produced higher amounts of H_2S than the vessels obtained from group S (1,092±86 vs. 646±78 nmol/min/mg prot; $P<0.01$). **Conclusions:** Based on the results above, we conclude that the presence of periodontitis in rats results in a reduced response of the mesenteric vessels to exogenous NO, which involves K^+ channel-mediated hyperpolarization and H_2S production. Regarding the effective H_2S source and the molecular mechanisms underlying its involvement, additional experiments are in progress in order to properly address these issues. **Financial Support:** FAPESP, CNPq, CAPES. **License number of ethics committee**: CEUA/ICB 170, 2/113, 2011 **Financial support**: FAPESP, CNPq, CAPES.

06.018 Agonist-mediated differential modulation of nitric oxide production and intracellular calcium effectiveness by vascular alpha-adrenergic receptors in early and late periods of experimental sepsis. Bernardelli AK, Da Silva-Santos JE UFSC – Farmacologia

Introduction: We have shown that each vascular bed responds quite differently to adrenergic receptor (α1-ARs) agonists in sepsis. Thus, we investigated whether the stimulation of α1-ARs by different ligands in sepsis results in a distinct rate of nitric oxide stimulation or intracellular Ca^{2+} mobilization. **Methods:** Male Wistar rats (3-4 months old) were used in this study. Sepsis was induced by the cecal ligation and puncture (CLP) model, and the animals were euthanized 6 h (CLP 6 h group), or 18 h (CLP 18 h group) later for removal of the thoracic aorta. Vessels from naïve rats were used as control. Only endothelium-intact preparations were used in this study. The effects of cumulative concentrations (1 nM–300 □M) of phenylephrine (PE) and norepinephrine (NE) were evaluated using regular physiological saline solution (PSS), with or without the previous incubation with the nitric oxide synthase (NOS) inhibitors L-NAME (100 μM), 1400W (10 μM), or S-Methyl-L-thiocitrulline (10 μM). Additionally, aortic rings of the control, CLP 6 h and CLP 18 h groups were maintained for 15 minutes in Ca^{2+}-free PSS before being stimulated with 1 μM PE or NE. Besides, after contraction with 1 μM PE or NE, dose-response curves for $CaCl_2$ (10 μM–100 mM) were recorded. In another protocol, the rings were maintained 30 min in Ca^{2+}-free depolarizing PSS, and curves for $CaCl_2$ were obtained. **Results:** Using regular PSS, PE-induced contraction was reduced from 2.5 ± 0.08 g to 1.7 ± 0.1 and 1.01 ± 0.08 at 6 and 18 h after the CLP surgery. There was no hyporeactivity to NE in arteries from the CLP 6 h group, but the response to NE was decreased from 2.7 ± 0.12 to 1.7 ± 0.11 in the CLP 18 h group (n=6/group). The non-selective NOS inhibitor L-NAME was able to increase contraction in all experimental groups. Interestingly the selective inhibitors of the inducible (iNOS) and neuronal (nNOS) isoforms of NOS, 1400W and S-methyl-L-thiocitrulline, respectively, increased the responses to PE, but not to NE in the CLP 6 h group. However, at 18 h after the CLP, the inhibition of either iNOS or nNOS was able to increase the contraction for both vasoconstrictors. $CaCl_2$-induced contraction in Ca^{2+}-free depolarizing solution (as % of responses to 120 mM KCl) was not impaired at 6 h after the CLP, but it was reduced from 120.7 ± 4.9% in control to 57.7 ± 10.0 % in aortic rings from the CLP 18 h group (n =4-5, $p < 0.05$). When evaluated in arteries maintained in the non-depolarizing Ca^{2+}-free medium previously incubated with PE, the contractile response to $CaCl_2$ was reduced in both CLP 6 and 18 h groups (n =4-6). However, the $CaCl_2$-induced contraction was decreased in the CLP 18 h, but not in the CLP 6 h group in vessels stimulated by NE (n = 4-6). Despite the unaltered responses to NE in Ca^{2+}-free solution, the PE-induced phasic contraction was reduced from 31.1 ± 3.8% in control to 13.8 ± 2.3% of 120 mM KCl-induced contraction ($p < 0.05$) in aortic rings from the CLP 18 h group (n = 5). **Discussion:** Contractility mediated by two different agonists of α1-ARs, PE and NE, differs in early stages of sepsis, despite the putative sharing of the same signaling pathway. Our results indicate that such difference is dependent on nitric oxide modulation. On the other hand, advanced stages of sepsis are associated with an impaired functionality of Ca^{2+}-dependent contractile machinery, rendering the aortic rings less responsive regardless the ligand used. **License number of ethics committee**: CEUA/UFSC (PP00566). **Financial support**: CNPq (448738/2014-7)

06.019 Hypotensive effect of a hydroalcoholic extract obtained from the fruit peels of *Plinia peruviana*. Ascenso R, Ribeiro-do-Vale RM, Maraschin M, Da Silva-Santos JE
UFSC – Farmacologia

Introduction: It was demonstrated vasoactive effects *in vitro* from the peels of *Plinia peruviana* fruits, popularly known in Brazil as "jabuticaba". We hypothesized that a crude hydroalcoholic extract of the fruit peels of *Plinia peruviana* (EPP) might produce hypotension in rats, and we have evaluated its effects *in vivo* on the blood pressure (BP) of *Rattus norvegicus*. **Methods:** Both male and female Wistar rats were used in this study. For assessment of diuresis, female rats were treated with 30, 100, or 300 mg/kg (p.o.) of EPP and were placed in metabolic cages for 8 h. Control groups received vehicle (water, 1 mL), or furosemide (5 mg/kg, s.c.). To measure BP, the animals were anesthetized with ketamine/xylazine (100/20 mg/kg, i.m.), and had their femoral veins and the carotid artery cannulated for drug administration and pressure recording. After stabilization of the BP, a continuous infusion of phenylephrine (PE, 4 µg/kg/min) was initiated and maintained during the entire period of evaluation. Under the pressor effect of PE, different groups of female rats received the EPP (30, or 100, or 300 mg/kg) directly into the duodenum (i.d.). Time-matched control experiments were conducted in animals with continuous infusion of PBS (10 µL/min), or PE infusion plus vehicle or 50 mg/kg captopril (both i.d.). For comparison, the effect of EPP (300 mg/kg, i.d.) was evaluated in male rats. The latency and maximal effect of EPP on the BP and heart rate were continuously evaluated during 30 min. In additional experiments, the hypotensive effect of EPP (100 mg/kg, i.d.) was evaluated in animals infused with L-NAME (7 mg/kg/min), or previously treated with ODQ (2 mg/kg, i.v.) or TEA (20 mg/kg, i.v.), and subjected to PE infusion. Additionally, the effect of a nanoemulsion prepared with EPP (30 mg/kg, i.v.) was evaluated in PE infused female rats (n = 6-7 in all experiments). The results are expressed as the mean ± standard deviation. **Results:** Oral administration of EPP did not increase diuresis. Intraduodenal administration of 300 mg/kg EPP decreased the diastolic pressure (DP) by 25 ± 4 mmHg. Infusion of PE increased the DP from 76 ± 9 to 135 ± 9 mmHg, and resulted in enhanced and dose-dependent hypotensive effects of EPP, without significant effects in the heart rate. For instance, the DP was reduced by 28 ± 9, 39 ± 10, and 57 ± 17 mmHg after 30, 100, and 300 mg/kg of EPP, respectively; for comparison, captopril decreased the DP by 32 ± 8 mmHg. A similar pattern of reduction of the BP was found in male rats. Continuous infusion of L-NAME increased the DP to 143 ± 7 mmHg. Although administration of EPP (100 mg/kg) had reduced the DP of L-NAME infused animals by 30 ± 8 mmHg, the latency for the beginning of the hypotensive effect was delayed from an average of 2 min in PE-infused to 7 min in L-NAME infused animals. The previous treatment with ODQ or TEA did not reduce the hypotensive effect of EPP (100 mg/kg). The hypotensive effect was enhanced for the nanoemulsion formulation of EPP, which quickly decreased the DP of PE infused rats by 89 ± 4 mmHg. **Conclusion:** Preparations obtained from the fruit peels of *Plinia peruviana* did not present any diuretic effect, but were able to dose-dependently reduce the systemic arterial pressure of anesthetized rats. Our results suggest that this hypotensive effect is, at least in part, dependent on nitric oxide production, but it was not reduced by guanylate cyclase inhibition or blockade of TEA-sensitive potassium channels. **License number of ethics committee**: 5371190815 **Financial support**: FAPESC (TR2012000367) and CAPES, with an M.Sc. fellowship to Ascenso, R.

06.021 AT_1 receptors mediate ethanol withdrawal-induced cardiac oxidative stress. Assis VO[1], Gonzaga NA[1,2], Pereira LC[1], Brigagão C[1], Tirapelli CR[1] [1]EERP-USP – Farmacologia, [2]FMRP-USP – Farmacologia

Introduction: The abrupt interruption of ethanol consumption in heavy drinkers lead to cardiovascular, physiological and behavioral changes that are collectively known as Ethanol Withdrawal Syndrome. We have shown that ethanol withdrawal (EW) induces activation of the rennin-angiotensin system (RAS) with further increase in blood pressure and vascular oxidative stress[1]. These responses were prevented by losartan, a selective antagonist of AT_1 receptors. The molecular mechanisms underlying the cardiac effects of EW are not well defined. Understanding that ethanol withdrawal increases the circulating levels of angiotensin II and that this peptide may increase cardiac oxidative stress under distinctive circumstances, we hypothesized that EW would induce deleterious effects in the heart via angiotensin II/AT_1 receptors. Here, we investigated a possible role for angiotensin II/AT_1 receptors in the cardiac effects induced by EW. **Methods:** Male Wistar rats (250g) were divided into 6 groups: Control: animals received water ad libitum for 21 days and daily gavage (DG) of vehicle (CV) or losartan (LST-10mg/kg/day - CL); Ethanol: animals were treated with ethanol 9% (v/v) for 21 days and DG of vehicle (EV) or LST (EL); Ethanol Withdrawal: animals were treated in the same way that ethanol group for 21 days and after that ethanol was removed and the animals received water ad libitum until the 23rd day. During EW period (48h) rats received DG of vehicle (EWV) or LST (EWL). At the end of the treatment, blood and cardiac tissue (left ventricle) were collected for biochemical analysis. The activity of creatine kinase-MB (CK-MB) was measured by colorimetric assay, while the levels of superoxide anion (O2-) were detected by chemiluminescence of lucigenin. The expression of the following proteins was detected by Western Immunoblotting: eNOS, AT_1 and AT_2 receptors, Nox2 and Nox4. Two-way ANOVA followed by Bonferroni was used to compare the results. The protocols were approved by the Ethics Committee (CEUA#11.1.1432.53.5). **Results:** EW did not affect serum activity of CK-MB. On the other hand, EW increased O2- generation in the left ventricle (RLU/mg protein; n=8) (CV=77±4; CL=66±4; EV=78±4; EL=71±5; EWV=108±5*; EWL=69±5) and losartan prevented this response. Neither ethanol nor losartan affected the expression of eNOS, Nox2, Nox4, AT_1 and AT_2 receptors. **Conclusion:** EW increases cardiac oxidative stress via AT_1 receptor activation. [1]Gonzaga NA, Vale GT, Parente JM, Yokota R, De Martinis BS, Casarini DE, Castro MM, Tirapelli CR. Ethanol withdrawal increases blood pressure and vascular oxidative stress: a role for angiotensin type 1 receptors. Ethanol. 2018 April; (in press) **License number of ethics committee**: CEUA#11.1.1432.53.5 **Financial support**: CNPq and FAPESP

06.022 Vasodilator effect of leaf and stem hydroalcoholic extracts of *Kielmeyera membranacea* (**Calophyllaceae**). Reis NF[1], Alves NS[2], Melo CM[1], Araújo MH[2], Barth T[2], Muzitano MF[2], Raimundo JM[1] [1]UFRJ – Farmacologia de Produtos Bioativos, [2]UFRJ – Produtos Bioativos

Introduction: Cardiovascular diseases (CVDs) are the number one cause of death worldwide, with an estimative of 17.7 million deaths in 2015[1]. Among the most important risk factors for CVDs, hypertension is often associated with functional and structural changes in heart and vessels. The use of vasodilators allows the control of hypertension by promoting the relaxation of vascular smooth muscle, whose tone is regulated by endothelium dependent and independent mechanisms. Plants are a major source of bioactive compounds, and different groups of secondary metabolites show vasodilatory activity[2]. There are still no reports in the literature on the cardiovascular activity of *Kielmeyera membranacea* (Calophyllaceae), an endemic species of Brazil. Thus, the aim of this work was to investigate and compare the vasodilator effect of leaf (KMLE) and stem (KMSE) hydroalcoholic extracts of K. membranacea. Also, it was investigated the vasodilator effect of the biflavonoid podocarpusflavone A, which was identified in both extracts. **Methods:** The vasodilatory activity were assessed in Wistar rat aortas (200-260 g) prepared for isometric tension recording. Aortic rings were placed in vertical chambers filled with Krebs-Henseilet solution, continuously oxygenated with carbogenic mixture, at 37 ºC. After the equilibration period of the preparation, the contractile response to phenylephrine (10 µM) was measured before and after exposure to increasing concentrations of the extracts or podocarpusflavone A (0.1 to 100 µg/mL). In order to investigate the involvement of endothelium factors in the vasodilator effect, experiments were performed in aorta with and without endothelium. All the experimental protocols were approved by the Ethics Committee on Use of Animals of Campus UFRJ-Macaé (License MAC019). **Results:** KMLE and KMSE induced vasodilation in a concentration-dependent manner in aortas with endothelium. The relaxant effects were 88.3 ± 3.8 % and 78.7 ± 2.5 %, at the concentration of 30 µg/mL of HMLE and KMSE, respectively ($P<0.05$, n=6). In aortas without endothelium, HMLE and KMSE did not alter the vascular tone, indicating that the vasodilatory activity of both extracts is dependent on endothelial factors. Podocarpusflavone A, 30 µg/mL, produced vascular relaxation of 68.3 ± 5.0 % in aorta with endothelium. Chemical profile analysis through HPLC-DAD showed that the extracts presented different chromatographic profiles and that HMLE (6.6 µg/mg) presented a higher concentration of podocarpusflavone A than KMSE (1.4 µg/mg). **Conclusion:** The leaf and stem hydroalcoholic extracts of K. membranacea produce endothelium-dependent vasodilation of rat aorta and this species could be a source of vasodilator compounds. The biflavonoid podocarpusflavone A seem to be one of the main secondary metabolites responsible for the vasodilator effect of K. membranacea. **References:** [1]WHO Fact Sheet Cardiovascular Diseases, 2017; [2]Luna-Vázquez et al. Molecules 18: 5814, 2013. **License number of ethics committee**: MAC019 **Financial support**: CNPq, FAPERJ, PIBIC

06.023 Cardiac remodeling in acute myocardial infarction decreases with new agonist adenosine receptor (**LASSBIo-1027**). Beltrame F[1], Da Silva JS[2], Montagnoli TL[2], Melo L[2], Cunha VMN[2], Maia RC[2], Fraga CAM[2], Barreiro EJ[2], Sudo RT[2], Zapata-Sudo G[2] [1]UFRJ, [2]UFRJ – Desenvolvimento de Fármacos

Introduction: Acute myocardial infarction (MI) deflagrate an intense inflammatory response which results in cardiac dysfunction, cell death, and ventricular remodeling. Cardiac remodeling is a crucial factor for the development of heart failure (HF), therefore there is requirement to develop additional treatment strategies, such as the detection of new targets, to prevent heart failure after MI. **Objective:** The aim was to investigate a new agonist of the adenosine A_{2A} and A_3 receptors, named LASSBio-1027 on the experimental MI in rats due to its vasodilatory and anti-inflammatory profile. **Methods:** Protocols were approved by Animal Care and Use Committee at Universidade Federal do Rio de Janeiro. The experimental MI was induced in male Wistar rats (180-200 g) by left coronary descending artery occlusion under isoflurane anesthesia (2%) and animals with no motility of the anterior wall were included in MI group. The experimental groups consisted of sham-operated (sham) and infarcted (MI) treated orally with either vehicle or LASSBio-1027 (30 and 70 µmol/kg) during 7 days. All animals underwent to an echocardiography: diastolic function using the determination of mitral flow (ratio between early transmitral filling velocity and tissue doppler, E/e') and systolic function which was determined by ejection fraction. Histological analysis was used to evaluate cardiac fibrosis in the infarct border area. Inflammation and apoptosis were evaluated in heart tissues using western blot to determine expression of TNF-□ and pERK1/2. Immunohistochemistry was used to determine □-SMA and iNOS in infarct border. **Results:** Diastolic dysfunction was observed one week after MI with an increase of E/e' from 22.9 ± 1.6 (sham) to 37.0 ± 3.7 ($P<0.01$) which recovered to 23.9 ± 5.3 ($P<0.05$) after treatment with 70 µmol/kg of LASSBio-1027. Reduced ejection fraction of 36.6 ± 2.0% was observed in MI group, however, this parameter returned to normal value (47.0 ± 7.4%) when infarcted animals were treated with LASSBio-1027 (70 µmol/kg), indicating the improvement of systolic function. Deposition of collagen was detected in the border area of infarction with 31.5 ± 4.2% ($P<0.01$) when compared to sham (3.6 ± 0.9%) and treatment with 30 and 70 µmol/kg of LASSBio-1027 reduced to 23.3 ± 2.2 and 19.7 ± 3.2% ($P<0.05$), respectively. Additionally, in infarct border LASSBio-1027 (70 µmol/kg) reduced cell infiltrate of 227.5 ± 31.1 (MI) to 137.4 ± 2.6 interstitial cell/□^{2} ($P<0.05$). LASSBio-1027 (30 µmol/kg) reduced the myofibroblast activation because the increased □-SMA expression induced by MI was altered from 36.8 ± 2.9% to 0.7 ± 0.4% ($P<0.01$) which was similar to sham group (0.4 ± 0.1%). Similarly, it was also observed that the increased iNOS expression of 25.3 ± 1.9% (sham = 7.3 ± 4.5%) was reduced in MI-LASSBio-1027 group with 6.1 ± 1.8% ($P<0.05$). MI increased the expression of TNF-□ and p-ERK1-2/ERK1-2 in comparison to sham and LASSBio-1027 recovered the expression of them to control values. **Conclusion:** Since LASSBio-1027 could alter the elevated TNF-α and p-ERK1-2/ERK1-2 which contributes to HF after MI through a local inflammatory response, increased matrix metalloproteinase-2 activity and reduced cardiomyocyte apoptosis, this compound could interfere with the cardiac remodeling after MI. **License number of ethics committee**: 103/17

06.024 Antihypertensive effect of novel *N*-acylhydrazone derivates. Rocha BS, da Silva JS, Gelves LGV, Fraga CAM, Barreiro EJ, Sudo RT, Zapata-Sudo G UFRJ – Desenvolvimento de Fármacos

Introduction: Systemic arterial hypertension is a multifactorial condition and considered a risk factor for cardiac, encephalic, renal dysfunction and metabolic complication. Two new compounds named LASSBio-1791 and LASSBio-1792 were designed to activate the adenosine A_{2A} receptor and their pharmacological profile was investigated after administration in spontaneously hypertensive rats (SHR). **Methods:** Protocols were approved by Animal Care and Use Committee at Universidade Federal do Rio de Janeiro under protocol number 103/17. Vascular reactivity was evaluated using isometric tension recording of pre-contracted thoracic aorta from male Wistar rats (200-220 g) after exposure to increasing concentrations of either LASSBio-1791 or LASSBio-1792 (0.1 – 100 µM) (n=4 each). Hemodynamic parameters such as systolic (SBP), diastolic (DBP) and mean (MBP) blood pressure and heart rate (HR) were determined after intravenous administration of 10 and 30 µmol/kg of both compounds in SHR (12-14 weeks old) to investigate their antihypertensive effect. **Results:** LASSBio-1791 and LASSBio-1792 promoted vasodilation in aortic rings because maximal relaxation of 36.6 ± 8.2 and 72.7 ± 8.0% was observed after exposure to 100 mM, respectively. The concentration to promote 50% of relaxation for LASSBio-1792 was 56.4 ± 9.6 µM. Systolic and diastolic pressure were reduced in SHR by LASSBio-1791 from 178.4 ± 8.7 to 140.5 ± 3.7 mmHg and from 121.0 ± 6.5 to 70.0 ± 15.4 after intravenous administration of 10 µmol/kg. Additionally, HR was reduced in a dose dependent manner from 258.3 ± 10.5 to 160.4 ± 31.9 and from 270.2 ± 14.9 to 130.7 ± 24.6 bpm with 10 and 30 µmol/kg of LASSBio-1791, respectively. Antihypertensive effect and reduced HR were also observed with the administration of LASSBio-1792 in SHR. Injection of 30 µmol/kg reduced the SBP from 214.5 to 151.3 mmHg, DBP from 149.8 to 63.9 mmHg and MBP from 178.1 to 102.7 mmHg. HR was decreased from 330.6 to 208.5 bpm. **Conclusions:** Novel N-acylhydrazone compounds produced vasodilator effect and reduced blood pressure in SHR. **Financial support**: CNPq, CAPES, INCT-INOFAR, FAPERJ. **License number of ethics committee**: 103/17

06.025 Vasodilator effect of the N-acylhydrazone derivative LCSO13, **a potent myeloperoxidase inhibitor**. Gomes PVCL[1], Quimas JVF[1], Santos DC[2], Souza ALF[2], Silva LL[1], Raimundo JM[1] [1]UFRJ-Macaé – Farmácia, [2]UFRJ-Macaé – Química

Introduction: Endothelial dysfunction is a common feature of cardiovascular diseases and involves oxidative stress and reduced endothelium-dependent vasodilation. Myeloperoxidase (MPO) has emerged as a key therapeutic target in vascular diseases and mediates endothelium dysfunction by producing reactive oxidant species and nitric oxide inactivation[1]. Thus, substances that reduce oxidative stress and restore vascular tone could be of pharmacological interest for the treatment of cardiovascular diseases. N-acylhydrazone (NAH) derivatives have shown different pharmacological activities, including vasodilator, antiplatelet, anti-inflammatory and MPO inhibition[2,3]. Our group has been studying the pharmacological profile of NAH derivatives, which has shown MPO inhibitory and antioxidant activities. The aim of this work was to investigate the vasodilator effect of the NAH derivative N'-((1H-Indol-3-yl)methylene)isonicotinohydrazide (LCSO13). **Methods:** The vasodilator effect was assessed in aorta isolated from Wistar rats (200-260 g). Aortic rings were placed in vertical chambers filled with Krebs-Henseilet solution, continuously oxygenated with carbogen gas at 37 ºC. After equilibration under 1 g resting tension for 90 min, the integrity of endothelium was verified. It was used aortic rings with and without endothelium. Then, the contractile response to phenylephrine (10 µM) was measured before and after exposure to increasing concentrations of LCSO13 (1 to100 µM; n= 4-6). All experimental protocols were approved by the Animal Care and Use Committee of Campus UFRJ-Macaé (License MAC019). **Results:** Among a group of 15 NAH derivatives, LCSO13 was selected for vascular studies since it was the most potent in inhibiting MPO activity (EC_{50} 0.5 ± 0.2 µM). LCSO13 induced a concentration-dependent vasodilation of aortic rings with endothelium, with maximal effect of 87.9 ± 3.2 % and EC_{50} of 13.4 ± 1.8 µM. In endothelium-denuded rings, maximal effect was reduced to 42.2 ± 9.2 % ($P<0.001$). In order to verify the influence of conformational restriction in the vasodilator activity of LCSO13, an analogue with C=N double bound reduction, LCSO13R, was tested. This compound presented a similar maximal relaxant effect (87.9 ± 1.5 %; $P>0.05$) in aortas with endothelium, although it was less potent (EC_{50} 38.4 ± 1.2 µM; $P<0.01$). Removal of endothelium abolished LCSO13R-induced vasodilation. The mechanisms involved in the vasodilator effect are still being investigated. **Conclusion:** LCSO13 induced intense vasodilation, which was partially dependent on endothelium. The C=N double bound in LCSO13 seem to be crucial for its endothelium-independent vasodilation. The vasodilator effect of LCSO13, associated with the MPO inhibitory activity previously observed, indicates that this compound could be useful for reducing vascular dysfunction associated with cardiovascular diseases. **References:** [1]Maiocchi et al. Free Rad Biol Med 86, S28, 2015. [2]Rollas et al. Molecules 12, 1910, 2007. [3]Soubhye et al. ACS Med Chem Lett 8, 206, 2017. **License number of ethics committee**: MAC019 **Financial support**: PIBIC, FAPERJ, CNPq

06.026 Endogenous production of nitric oxide via eNOS does not contribute to the vasodilator effect of sodium nitroprusside in aortas from SHR. Da Silva MH, Potje SR, Vercesi JA, Grando MD, Bendhack LM FCFRP-USP – Física e Química

Introduction: Nitric oxide (NO) is produced in the endothelial cells by the NO-synthase (eNOS). It is described that eNOS can be uncoupled in the vessels of spontaneously hypertensive rats (SHR). Sodium nitroprusside (SNP) is a classical NO donor that releases NO inducing vasodilatation. This study aimed to investigate the contribution of NO endogenously produced by eNOS to the SNP-induced vasodilatation. **Methods:** Endothelium-intact (E+) or denuded (E-) aortic rings were mounted in an organ bath system in order to record the isometric tension. The relaxation induced by SNP was studied in phenylephrine- induced contraction in E+ and E- aortas. In order to verify the role of eNOS activation on the relaxation induced by SNP, E+ aortic rings were stimulated with increasing concentrations of SNP in the absence (Control) and after incubation for 30 min with L-NAME (eNOS inhibitor), L-Arg (eNOS substrate), BH_4 (eNOS cofactor). The maximum effect (ME) and pD_2 (-log of the concentration of SNP that produced half-maximal relaxation) were analyzed, and the responses obtained were compared to the given control. **Results:** SNP induced relaxation in a similar way in E+ (ME: 108.1 ± 3.7%, pD_2: 7.92 ± 0.13; n=7) and E- (ME: 119.1 ± 7.1%, pD_2: 8.14 ± 0.16; n=3). After eNOS inhibition with L-NAME, SNP-induced relaxation was not altered (ME: 110.4 ± 2.5%, pD_2: 7.91 ± 0.20; n=6). In a similar way, the relaxation was not changed by L-Arg (ME: 108.3 ± 4.6%, pD_2: 7.76 ± 0.01; n=4) and by BH_4 (ME: 122.2 ± 4.9%, pD_2: 7.79 ± 0.14; n=6). **Conclusions:** These results indicate that in SHR aortas, endogenous NO produced via eNOS does not contribute to the SNP-induced vasodilatation, because the eNOS inhibitor and the eNOS activators had no effect. **License number of ethics committee**: Ethics Committee: University of São Paulo - USP 18.1.151.60.1 **Financial support**: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP 2017/14797-9) and CNPq (304137/2014-6).

06.027 Physiopathology mechanisms of cardiovascular disease programmed by perinatal undernutrition in male and female Wistar rats. Farias RS, Araújo GA, Marques EB, Rocha NN, Scaramello CBV UFF – Fisiologia e Farmacologia

Introduction: Previous data from our research group using Wistar rats have shown that the increase of litter size and the consequent decrease of milk supply leads to low perinatal weight (Farias et al. SBFTE 2017). According to Barker Theory, adult diseases may have origin on fetal and infant nutritional status (Barker, BMJ. 301 (6761): 1111,1990). The aim of the present work was to verify if perinatal undernutrition programs CV disease and investigate its physiopathology mechanisms in post pubertal male and female rats originated from enlarged litters. **Methods:** Rat pup litters were randomly adjusted to 8 or 12 male (M) and female (F) animals per mother (in a proportion of 1: 1) on day 1 of life, originating 2 groups – Control (C) and Undernutrition (U). All animals were assessed at postnatal day 150 being submitted to hemodynamic evaluation, echocardiography and maximal effort ergometric test as described by Marques et al. (Int J Cardiol.181C: 141,2015). Serum lipids and glucose levels were also determined using commercially available Labtest Brasil kit. Data were presented as mean±standard error of the mean, analyzed by Student t test and considered statistically different if $P<0.05$(*) compared to respective control. **Results:** The levels of triglycerides (TG: CM = 28.52±6.33xUM=83.57±4.32*; CF = 46.70±6.52XUF=69.40±5.15*), VLDL (CM = 5.70±1.26xUM = 16.71±0.86*; CF = 9.34±1.30XUF=13.87±1.03*) and atherogenic index described by TG/HDL ratio (CM=0.62±0.11xUM=2.08.71±0.18*; CF=2.58±0.56XUF=5.28±0.78*) were higher in male and female rats submitted to undernourishment during lactation compared to respective controls. These animals presented enlargement of intraventricular septum thickness (CM=0.137±0.008xUM=0.186±0.014*; CF=0.114±0,003XUF=0.172±0,0012*), left ventricle posterior wall thickness (CM=0.135±0.010xUM=0.187±0.014*; CF=0.115±0.003XUF=0.177±0.012*) and relative wall thickness (CM=0.387±0.002xUM=0.589±0.040*;CF=0.454±0.061XUF=0.771±0.084*). Different from males, female rats from enlarged litters presented higher systolic blood pressure (CF=138.85±7.8XUF=162.89±2.29mmHg*) and lower maximum speed developed on maximal effort ergometer test (CF=2.02±0.07XUF=1.00±0.10km/h*). **Conclusion:** Perinatal undernutrition raises the risk of coronary artery disease development and promotes a concentric remodeling of left ventricle in both male and female rats. However, females from enlarged litters, but not males, present high blood pressure and lower cardiorespiratory capacity. CV disease programmed by undernourishment during lactation and its physiopathology mechanism seem to be sex-dependent. **License number of ethics committee**: CEUA/UFF812-16 **Financial support**: FAPERJ, CNPq, CAPES, PROPPI/UFF

06.028 Determination of the antioxidant potential of medicines used on the treatment of chronic kidney disease. Oliveira BM[1], Michelin AP[1], Semeão LO[1], Matsumoto AK[1], Casagrande R[2], Barbosa DS[1] [1]UEL – Análises Clínicas e Toxicológicas, [2]UEL – Ciências Farmacêuticas

Introduction: The imbalance between oxidant and antioxidant agents is called oxidative stress (OS). This process increases the lipids, proteins, carbohydrates and DNA oxidation, resulting in toxic effects on cells and tissues. As in many chronic diseases, the OS presents a relevant part of the processes involved in Chronic Kidney Disease (CKD), impairing renal function and contributing to disease evolution. Besides the dialytic treatment in advanced patients, some medications are used in the disease's control, encompassing all CKD's phases. The aim of this study was to evaluate if the drugs commonly used in the treatment of these pathologies present antioxidant effect to identify any other action mechanism beyond those already known. **Methods:** The antioxidant potential *in vitro* was evaluated for the drugs simvastatin, hydrochlorothiazide, furosemide and enalapril by colorimetric techniques 2,2-diphenyl-1-picrylhydrazyl (DPPH•) and 2,2'azino-bis (3-ethylbenzthiazoline-6-sulphonic acid – ABTS+). The reduction of the DPPH radical was based on its capability of reacting with hydrogen donating substances. In the presence of antioxidants, it receives H^+, therefore being reduced. The ABTS+ technique monitors the decay of the cation-radical $ABTS^{\cdot+}$ by its removal when a sample containing substances with antioxidant properties is added. All the methodologies were applied in triplicate and under protection from light. **Results:** This study demonstrated that the simvastatin in the DPPH• test presented an IC50 of 27.56 µg/mL, maximum activity of 61.66% in the concentration of 46.36 µg/mL. Enalapril, hydrochlorothiazide, furosemide and acetylsalicylic acid didn't show any results in DPPH• colorimetric methodology. Regarding the methodology of the ABTS+, the enalapril presented an IC50 of 0.622 µg/mL, maximum activity of 63.03% in the concentration of 0,909 µg/mL, simvastatin presented an IC50 of 25.90 µg/mL, maximum activity of 87,15% in the concentration of 132.53 µg/mL, furosemide presented an IC50 of 231.55 µg/mL, maximum activity of 91.52% in the concentration of 1132.02 µg/mL and hydrochlorothiazide presented an IC50 of 140.97 µg/mL, maximum activity of 80,25% in the concentration of 872,09 µg/mL. **Conclusion:** By the study results, we are able to conclude that in ABTS+ assay, the best *in vitro* antioxidant activity between the tested drugs was from enalapril, followed by simvastatin. This last one, besides confirm the electrons donate capacity in ABTS+ assay, also confirmed the hydrogen donate capacity by the DPPH• test. However, more studies should be done due to the lack of results in relation to these drugs antioxidant activity in the scientific literature, aiming at an improvement in the patient's condition, considering that oxidative stress is closely linked with CKD. Special thanks to CNPq for the financial support Process number: 2.491.476
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06.029 Treatment with Olanzapine does not alter vascular function of normotensive rats. Campos HM, Neri HFS, Oliveira TS, Brito RB, Ghedini PC UFG – Farmacologia

Introduction: Patients with schizophrenia have a higher incidence of cardiovascular mortality associated with the use of antipsychotic drugs like olanzapine (OLZ). Rats treated with a single intraperitoneal dose of OLZ had reduced blood pressure, venous tone and cardiac contractile function within 60 min of administration, which was associated with orthostatic hypotension commonly found in patients during the start of antipsychotic therapy (Vascul Pharmacol. 2014;62(3): 143-9). Chronic treatment with OLZ has produced variable cardiovascular results and studies are necessary for more light on the OLZ long-term cardiovascular changes. **Objective:** To evaluate the effects of OLZ treatment for 30 days on the vascular function of normotensive rats. **Methods:** Normotensive male Wistar rats (12 weeks old) were randomized into four groups (n = 6): Vehicle (V); olanzapine 0.3 mg/kg (OLZ 1); olanzapine 3,0 mg/kg (OLZ 2); olanzapine 15 mg/kg (OLZ 3). All treatments were administered daily by gavage at volume of 5 mL/kg during 4 weeks. At the end of the treatment, all animals were anesthetized with ketamine (0.7 mL/Kg, i.p.) and xylazine (0.1 mL/Kg, i.p.) and analysis of vascular reactivity was carried out in thoracic aortic segments evaluated by concentration-response curves to phenylephrine (Phe) and acetylcholine (Ach). Vasorelaxant effect of Ach (10^{-11} ? 10^{-5} M) was evaluated in the endothelium-intact vessels pre-contracted with Phe (10^{-6} M). Vasoconstriction by Phe (10^{-10} ? 10^{-4} M) was evaluated in endothelium -intact and -denuded vessels. For each concentration?response curve, the maximal effect (E_{max}) and the concentration of agonist that produced 50% of the maximal response (log EC_{50}) were calculated using nonlinear regression analysis. A value of $p < 0.05$ was considered. All protocols were approved by the Animal Research Ethical Committee from Federal University of Goiás (process number 20/2013) **Results:** The data of E_{max} obtained with Ach were similar among the groups (V = 88.52 ± 9.08 %; OLZ 1= 87.24 ± 14.62%; OLZ 2= 82.54 ± 9.33% and OLZ 3= 93.90 ± 7.47%). The E_{max} promoted by Phe in both endothelium ?intact (V= 1.67 ± 0.31g; OLZ 1= 1.89 ± 0.45g; OLZ 2 = 2.06 ± 0.67g and OLZ 3= 1.90 ± 0.59g) and -denuded vessels (V = 2.65 ± 0.57g; OLZ 1= 2.73 ± 0.81g ; OLZ 2 = 2.49 ± 0.63g and OLZ 3= 2.77 ± 0.21g) were not different among the groups. Similarly, the EC_{50} values for ACh vasorelaxant effect (V= 6.84 ± 0.8; OLZ 1= 7.11 ± 0.10 ; OLZ 2= 7.09 ± 0.06 and OLZ 3= 7.10 ± 0.08) and for Phe vasoconstriction effect in endothelium -denuded (V= 6.63 ± 0.08; OLZ 1= 6.84 ± 0.11; OLZ 2= 6.71 ± 0.14 and OLZ 3= 6.72 ± 0.16) and -intact vessels (V= 7.31 ± 0.09; OLZ 1= 7.52 ± 0.13; OLZ 2= 7.39 ± 0.13 and OLZ 3= 7.03 ± 0.09) did not differ among the groups ($P > 0.05$). **Conclusion:** The results here obtained suggest the treatment with OLZ for 30 days does not alter the vascular function of the normotensive rat thoracic aorta. **License number of ethics committee**: 20/2013 **Financial support**: FAPEG

06.030 Impact of ethanol consumption on the nephrotoxicity induced by cyclophosphamide. Sousa AH, Tirapelli CR, do Vale GT EERP-USP – Enfermagem Psiquiátrica e Ciências Humanas

Introduction: Long term ethanol consumption may cause damages to distinctive organs including the kidneys. The tissue damage induced by ethanol involves the increase of oxidative stress, which is characterized by the increased generation of reactive oxygen species (ROS). Cyclophosphamide is an antineoplastic drug widely used in the treatment of some types of cancer and autoimmune diseases. This drug induces several adverse effects such as nephrotoxicity. We aimed to evaluate whether ethanol consumption would aggravate the renal damage caused by cyclophosphamide. **Methods:** Male C57BL/6J mice were divided into four groups: 1) Control (C): mice received water ad libtum; 2) Ethanol (E): mice were treated with ethanol 20% (vol./vol.); 3) Cyclophosphamide (CC): water ad libtum and intraperitoneal injection of cyclophosphamide (300 mg/kg) 24h before euthanasia; 4) Ethanol + Cyclophosphamide (EC): ethanol 20% and intraperitoneal injection of cyclophosphamide (300 mg/kg) 24h before euthanasia. The study was approved by the local ethics committee (#2017.5.93.22.5). Results were analyzed using Two-way ANOVA followed by Bonferroni's test ($p<0.05$). **Results:** Animals of E and EC groups showed a lower intake of mice chow (g/week) and liquid (ml/week) compared to C and CC groups (Chow: C: 27.7±0.3, n=30; E: 20.7±0.3, n=29*; CC: 26.4±0.2, n=30; EC: 18.9±0.5, n=30* / Liquid: C: 39.7±2.4, n=30; E: 20.3±0.7, n=29*; CC: 38.8±1.3, n=30; EC: 20.2±0.8, n=30*). Animals of the E group showed lower weight gain compared to the C group. Similarly, animals of the CC and EC groups showed lower weight gain compared to their respective control groups (C: 29.1±0.1, n=30; E: 27.2±0.1, n=29*; CC: 27.0±0.4, n=30$^{\#}$; EC: 24.9±0.3, n=30$^{\#}$). No differences were observed in serum Na^{+} and K^{+} levels among groups. However, animals of the EC group showed higher serum concentration of urea (mg/dL) (C: 62.1±1.6, n=9; E: 64.9±1.4, n=9; CC: 64.8±1.1, n=8; EC: 71.1±1.6, n=8*). The mice treated with ethanol (groups E and EC) showed higher concentration of creatinine (mg/dL), compared to C group (C: 0.20±0.02, n=12; E: 0.28±0.03, n=10*; CC: 0.24±0.02, n=12; EC: 0.32±0.02, n=7*). **Conclusions:** Cyclophosphamide induces renal dysfunction and ethanol consumption do not aggravates this response. **License number of ethics committee**: 2017.5.93.22.5

06.031 Perivascular adipose tissue aggravates sepsis-induced vasoplegia. Awata WMC[1], Gonzaga NA[1], Borges VF[1], Cunha FQ[1], Tirapelli CR[2] [1]FMUSP – Farmacologia, [2]EERP-USP – Farmacologia

Introduction: Sepsis is an organic dysfunction caused by an unregulated host response to life-threatening infection. When treatment of sepsis is ineffective, the condition may progress to severe hypotension that is drug-irresponsive. Perivascular adipose tissue (PVAT) is recognized as a regulatory element in vascular biology that is implicated in the pathophysiology of cardiovascular diseases. However, little is known about the effects of sepsis in the modulatory action of PVAT. **Objectives:** Evaluate the effect of experimental (lethal) sepsis in the modulatory action that PVAT exerts on vascular tone and the possible mechanisms underlying this response. **Methods:** Male Wistar rats (250-300 g) were randomized into 2 groups: 1) Sham: the cecum was exteriorized without ligation and puncture; 2) CLP: lethal sepsis was induced using the cecal ligation and puncture (CLP) model. The thoracic aorta with or without PVAT (PVAT + and PVAT-, respectively) was isolated 6 h after sepsis for functional and biochemical assays. Concentration-response curves for phenylephrine were obtained in rings with (E+) and without endothelium (E^-). In another set of experiments the curves for phenylephrine were obtained after incubation (30min) with one of the following drugs: L-NAME (non-selective inhibitor of NOS), 1400W (selective inhibitor of iNOS), 7-nitroindazole (7-NI, selective nNOS inhibitor), tiron (superoxide anion scavenger), catalase (enzyme that decomposes H_2O_2), A779 (*Mas* receptor antagonist), 4-aminopyridine (voltage-sensitive K^+ channel inhibitor), glibenclamide (ATP-sensitive K^+ channel inhibitor) or indomethacin (non-selective COX inhibitor) [CEUA #2017.5.86.22.9]. Two-way ANOVA followed by Bonferroni test ($p < 0.05$) was used to compare the results. **Results:** In PVAT- aortas sepsis decreased the contraction (in mN) induced by phenylephrine, when compared to sham. In PVAT+ arteries CLP induced a more pronounced reduction of phenylephrine-induced contraction (PVAT-: Sham: 10.6 ± 0.1, n=10; CLP: 7.8 ± 0.4*, n=9; PVAT+: Sham: 7.6 ± 0.3, n=10; CLP: 3.8±0.5*, n=12). The increased anti-contractile effect of PVAT in the septic condition was not found in arteries after incubation with L-NAME (14.9 ± 1.1, n=7), 7-NI (8.1 ± 1.1, n=7), 1400W (10.8 ± 0.6, n=6), A779 (7.2 ± 0.7, n=12) or indomethacin (6.6 ± 0.6, n=13). Tiron, catalase, 4-aminopyridine and glibenclamide did not alter phenylephrine-induced contraction in the CLP group. Increased generation of O_2^- (RLU/mg protein) was detected in PVAT from CLP rats (672.1 ± 65*, n=6), when compared to PVAT of the Sham group (447.3 ± 41, n=7). Conversely, CLP did not affect the concentration of H_2O_2 in PVAT. Increased prostaglandin (PG) I_2 levels were detected in PVAT from CLP rats (27.7 ± 7.2*, n=7), when compared to PVAT of the Sham group (11.4 ± 3.8, n=6), but no alteration in PGE_2 levels was found. **Conclusion:** Sepsis increases the anti-contractile action of PVAT by a mechanism that involves the production of nitric oxide (NO) by iNOS and nNOS. Angiotensin (1-7) and PGI_2 also contribute to the increased anti-contractile effect displayed by PVAT during sepsis. **Financial support**: CAPES **References:** Buras, J.A.; Nat. Rev. Drug Discov; 4: 854, 2005. Gao YJ.; Curr Pharm Des; 13(21): 2185, 2007. Singer, M; JAMA.;315(8): 801, 2016. **License number of ethics committee**: 2017.5.86.22.9

06.032 Effects of PDE-4 inhibition on cardiovascular and inflammatory changes induced by sepsis. Alves GF, Oliveira JG, Nakashima MA, Assreuy J, Da Silva-Santos JE, Fernandes D UFSC – Farmacologia

Introduction: Sepsis and septic shock are associated with high mortality rates and are considered one of the major public health problems worldwide. Sepsis is characterized by an intense inflammatory process, myocardial dysfunction, increased vascular permeability and platelet aggregation which together leads to cardiovascular collapse. During sepsis there is impairment in the cAMP pathway. cAMP is one of the most potent signaling molecules to stabilize the endothelial barrier, increase cardiac contractility and inhibits platelet aggregation. Phosphodiesterase-4 (PDE-4) is an important enzyme that has been found in several tissues that suffer damage during sepsis, such as kidney, lung, and heart, and is responsible for cAMP hydrolysis, regulating its intracellular levels. Therefore, we evaluated the effect of roflumilast (RFM), a clinically approved PDE-4 inhibitor on cardiovascular collapse and organ damage during experimental sepsis. **Methods:** Sepsis was induced by cecal ligation and puncture (CLP) procedure in male rats (250-300 g). Six hours after the CLP or Sham procedure the animals were randomly assigned to receive RFM (0.3 mg/kg, s.c) or vehicle. Twenty-four hours after surgery, mean arterial pressure and heart rate were recorded. Thereafter, blood samples were collected for measurement of nitrite/nitrate (NOx), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate levels. Heart tissues were also harvested to myeloperoxidase (MPO) assay. **Results:** The CLP induced hypotension (Sham: 112.8±3.4 n=4; CLP: 82.1±2.4 n=4, in mmHg) and tachycardia (Sham: 238.5±14.2 n=4; CLP: 352.2±26.3 n=4, in bpm). RFM treatment reduced the heart rate in septic rats (CLP+RFM: 277.1±15.7 n=4; $P<0.05$). CLP also induced thrombocytopenia (Sham: 736.6±60.1 n=8; CLP: 451.4±47.5 n=8, in 10^3 cells/mm^3) and lymphopenia (Sham: 5.17±0.68 n=8; CLP: 2.47±0.38 n=8, in 10^3 cells/mm^3). There was an increase in the relative number of monocytes (Sham: 7.6±1.1 n=8; CLP: 9.9±1.4 n=8, in %) and granulocytes (Sham: 39.1±6.5 n=8; CLP: 51.5±2.8 n=8, in %), but the absolute number did not change. None of these parameters were changed by RFM. There was also an increase in MPO activity in the heart of CLP animals (Sham: 0.179±0.01 n=8; CLP: 0.250±0.01 n=8, in OD/mg protein) that was reduced by RFM (CLP+RFM: 0.171±0.007 n=8; $P<0.05$). Compared to sham, septic animals exhibited an increase in levels of plasma NOx (Sham: 38.1±4.8 n=7; CLP: 98.8±14.8 n=8, in µM), AST (Sham: 86.6±3.5 n=8; CLP: 140.1±7.3 n=8, in U/mL), ALT (Sham: 39.4±1.6 n=8; CLP: 59.3±4.8 n=8, in U/mL) and lactate (Sham: 8.5±1.3 n=8; CLP: 51.6±8.4 n=8, in mg/dL). RFM treatment reduced the levels of CLP-induced ALT (CLP+RFM: 43.9±3.9 n=8; $P<0.05$) and lactate (CLP+RFM: 19.3±6.5 n=8; $P<0.05$). The levels of NOx and AST were not changed by the RFM treatment. **Conclusion:** In summary, we have demonstrated that PDE-4 inhibition reduces inflammation in heart tissue, reduces the hepatic damage and improved tissue perfusion during sepsis. These results suggest that increasing cAMP through PDE-4 inhibition has protective effect during sepsis. Further studies, including cAMP measuring and PDE-4 expression are needed to clarify the role of cAMP during sepsis development. **License number of ethics committee**: CEUA-UFSC 1667100417
Financial support: CAPES

06.033 Effects of prolonged use of a combined hormonal injectable contraceptive on aorta and plasma oxidative status in female Wistar rats. Nery LCES[1], Braz L[2], Silva LL[2], Blanc HNH[2], Raimundo JM[1] - [1]UFRJ-Macaé – Produtos Bioativos e Biociências, [2]UFRJ-Macaé – Farmácia

Introduction: Combined hormonal oral contraceptive (COC) is the most used hormonal contraceptive method, despite it is associated with an increased cardiovascular risk[1]. Combined hormonal injectable contraceptive (CIC), which contains different estrogen-progestin combinations, is an alternative to the use of COC. However, the cardiovascular effects related to the prolonged use of CIC are still not well defined. Therefore, the aim of this work was to evaluate the morphological and functional vascular effects of the prolonged use of a CIC containing norethisterone enanthate (NE) and estradiol valerate (EV) in female Wistar rats. Effects on oxidant status of plasma were also evaluated. **Methods:** All experimental protocols were approved by the Ethics Committee on Use of Animals of Campus UFRJ-Macaé (MAC037). A group of 30 female Wistar rats (8 to 10 weeks of age) was randomly divided into 2 groups: CIC – each rat received an intramuscular injection, once-weekly, of the contraceptive (1 mg NE + 0,1 mg EV in 0,02 mL) for 8 weeks; control (CTL) – each rat received the same volume of vehicle (castor oil) by the same route and for the same period. Indirect evaluation of hormonal status was assessed through vaginal cytology and uterus weight. Body weight, food intake and non-invasive blood pressure (tail-cuff method) were measured once a week during the treatment period. Concentration-response curves for phenylephrine (10^{-9} to 10^{-5} M) and acetylcholine (10^{-9} to 10^{-5} M) were performed in aortic rings prepared for isometric tension recording. Also, aortas were submitted to histological process for morphological and histomorphometric analyses. Plasma oxidative stress was evaluated through the measurement of total oxidant status (TOS) and thiobarbituric acid reactive substances (TBARS). **Results:** The CIC group presented reductions in the weekly food consumption (17.39 ± 0.67 g; $P<0.05$) and total body weight gain (AUC 104.10 ± 9.13; $P<0.05$) during the experimental period, in comparison with the CTL rats (19.37 ± 0.36 g and AUC 167.80 ± 16.71, respectively). The uterine weight of CIC rats was increased (CTL = 0.31 ± 0,02 g; AIC = 0.57 ± 0,04 g; $P<0.001$) and vaginal cytology showed characteristics of proestrus and metestrus phases, as expected due to the hormonal action. There was no change in blood pressure during the treatment and vascular reactivity (maximal effect and potency) to phenylephrine and acetylcholine was not altered by CIC treatment. TOS (CTL: 4.60 ± 0.71 □mol H_2O_2 eq/mg ptn; CIC: 10.78 ± 2.87 □mol H_2O_2 eq/mg ptn; $P<0.05$) and TBARS (CTL: 16.78 ± 1.99 □mol MDA/mg ptn; CIC: 11.99 ± 0.86 □mol MDA/mg ptn; $P<0.05$) values on plasma were significantly reduced in the CIC group. There was no difference in total aorta wall thickness, as well as in intima/media and tunica adventitia thickness, between groups. **Conclusion:** Our results indicate that the long-term use of CIC did not produce alterations in blood pressure and in morphology and vascular reactivity of aorta. On the other hand, it was observed an improvement in the oxidant status of plasma and a significant loss of weight. **Reference:** [1]LIZARELLI, P.M. Contraception, 79: 35, 2009. **License number of ethics committee**: MAC037 **Financial support**: FAPERJ, CAPES, PIBIC

06.034 Time course of the articular and extra-articular manifestations of Adjuvant-Induced Arthritis (**AIA**). Pita LM[1], Montenote MC[2], Oliveira PB[1], Spadella MA[3], Chies AB[1] [1]FAMEMA – Farmacologia e Fisiologia, [2]IBB-Unesp – Farmacologia e Biotecnologia, [3]FAMEMA – Fisiologia

Introduction: Rheumatoid arthritis (RA) is a disease classically characterized by an inflammatory response in the joints. However, several extra-articular manifestations related to RA have also been described. Such manifestations of RA may occur early or in a later stages of the disease. In addition, although extra-articular manifestations are already well-characterized, there are still doubts regarding their time course. Thus, the present study proposes to characterize, in rats, the time course of the manifestations of adjuvant-induced arthritis (AIA) in joints, heart, kidneys, skeletal muscle and adipose tissue. **Methods:** Twelve weeks old Male Wistar rats were studied either in the control condition (without immunization) or 4, 15 and 40 days after AIA induction. AIA was induced by Mycobacterium tuberculosis/mineral oil (50 mg/mL), injected into the right hind paw. The effectiveness of AIA was confirmed by flogistic signs in the left paw and by the positivity of reactive C protein (PCR). Animals that did not showed joint inflammation signals and/or PCR positivity until the 20th day after immunization were excluded from the study. The left hind paw diameter, feed intake, body mass (g), masses of the heart and left kidney (both normalized by body mass), skeletal muscle mass (sum of masses of extensor *digiorum longus*, soleus and gastrocnemius muscles, normalized by length of the tibia) and adipose tissue mass (sum of masses of retroperitoneal and periepididimal fat, normalized by length of the tibia) were analyzed. The data (10-20 animals/group) were expressed as mean ± SEM and compared by one-way analysis of variance (ANOVA) followed by Bonferroni's post test; significance if $p<0,05$. Study was approved by the CEUA of Faculdade de Medicina de Marília (nº 158/17). **Results:** The left hind paw diameters were increased by AIA already on day 15 (from 5,27 cm (control) to 7,91 cm; $p=0,0017$) and reached 9.66 cm on the 40th day ($p<0,001$ regarding the control group). Only on the 40th day, there was a significant reduction in food intake (Control: 58,65±1,560g – 40th: 35,35±1,115g; $p<0,0001$). The body mass was already reduced in 15th day (from 400,5±18,94g to 343,5±20,55g, $p<0,001$) and remained lower in comparison to control until the 40th day (389,7±10,17g, $P=0,0027$). The skeletal muscle mass was reduced already in the 15th day [from 1,243 ± 0,02g (control) to 0,917±0,06g, $p <0.001$), reaching 0,673±0,05g on the 40th day ($p<0,001$ regarding the Control group). No significant reductions in adipose tissue mass were observed. Increase in left kidney mass was observed only on the 40th day (from 3,830±0,08g (control) to 4,786±0,13g, $p<0,0001$), with no change in heart mass. **Conclusion:** Changes in body mass and skeletal muscle occur in the early stage of AIA, in parallel to paw edema. Changes in food intake and kidney mass occur later. Mass of the heart and adipose tissue do not appear to change significantly by AIA. **Financial Support:** Fapesp (processos 2016/08450-3 e 2017/00746-3). **License number of ethics committee**: 158/17

06.035 Increased placental nitric oxide and restored endothelium-dependent vasodilation underlie pravastatin effects against fetal growth restriction and hypertension in pregnant rats. Chimini JS, Possomato-Vieira JS, Santos-Silva ML, Dias-Junior CA IBB-Unesp – Farmacologia

Introduction: Hypertensive disorders of gestation are associated with greater maternal and fetal complications marked by pathophysiological changes that lead to endothelial dysfunction. Increases in vascular resistance and decreases in nitric oxide (NO) bioavailability due to endothelial dysfunction may be involved hypertension-in-pregnancy and compromise placental transport capacity to the fetus. Statins upregulate the expression of endothelial NO synthase (eNOS) and reestablish NO levels (Lecaripentie, Drugs, 773.788, 2012). Therefore, we aimed to examine pravastatin effects on placental NO bioavailability, vascular and endothelial functions, maternal hypertension and fetal growth restriction in pregnant rats. **Methods:** Pregnant Wistar rats were randomly distributed into four groups: normal pregnant (Norm-Preg); pregnant+Pravastatin (Preg-Pravastatin); hypertensive pregnant (HTN-Preg) and hypertensive pregnant+Pravastatin (HTN-Preg+Pravastatin). Hypertension was induced by desoxycorticosterone acetate (DOCA) and high salt intake. Animals were injected intraperitonially with DOCA 12.5 mg/Kg/ml on gestational day one, followed by weekly injections of DOCA 6.25 mg/Kg/ml and drinking water was replaced by 0.9% saline solution. Pravastatin was administered on gestational days 10-19 (10 mg/Kg/daily - gavage). Systolic blood pressure (SBP) was recorded by tail cuff plethysmography on gestational days 9, 12, 14, 16, and 19. After euthanasia, fetal and placental weights were recorded. Placental NO levels were measured by Griess assay. Vascular reactivity experiments were performed in thoracic aorta rings with intact endothelium which were stimulated with increasing concentrations of phenylephrine (PHE, 10^{-10} to 10^{-4} M). To investigate endothelial function, rings were pre-contracted with PHE (10^{-6} M) and then increasing concentrations of acetylcholine (ACh, 10^{-9} to 10^{-4} M) were added. **Results:** Treatment with pravastatin blunted increases in SBP (HTN-Preg+pravastatin; 136±2 mmHg) versus HTN-Preg group (160±2 mmHg). Both fetal and placental weights were significantly decreased in HTN-Preg group (1.75±0.02 and 0.25±0.0 g, respectively) versus Norm-Preg group (2.84±0.1 and 0.33±0.01 g, respectively) and HTN-Preg+Pravastatin group (2.40±0.04 and 0.46±0.01 g, respectively). Placental NO levels were increased in HTN-Preg+Pravastatin (23±2 µg/100 mg tissue) versus HTN-Preg, Preg+Pravastatin and Norm-Preg groups (15±2; 17±3 and 14±1 µg/100 mg tissue, respectively). pEC50 value to PHE significantly increased in HTN-Preg group and the E_{max} value to ACh significantly decreased versus other experimental groups. Interestingly, E_{max} and pEC50 to PHE and ACh of HTN-Preg+Pravastatin group was similar to Norm-Preg and Preg-Pravastatin groups and significantly different to HTN-Preg group. **Conclusions:** Treatment with pravastatin increases placental NO bioavailability, blunts the increase in SBP, attenuates fetal growth restriction and restore vascular relaxation in hypertensive pregnancy by improvement of endothelial function. **Financial support**: CAPES **License number of ethics committee**: 960/2017

06.036 Loss of anti-contractile effect mediated by aortic perivascular adipose tissue-derived hydrogen sulfide in hypertensive pregnancy. Polonio LCC, Possomato-Vieira JS, Chimini JS, Dias-Junior CA IBB-Unesp – Farmacologia

Introduction: Preeclampsia is a pregnancy related hypertensive disorders characterized by increased blood pressure, often proteinuria and other clinical symptoms that affect 8-12% pregnant women after the 20thgestational week (ACOG, 2013). Perivascular adipose tissue (PVAT), may participate in the control of vascular tone through the release of vasoactive substances, including hydrogen sulfide (H_2S) (Beltowski,J; Can J Physiol Pharmacol, v93, p. 889, 2015). H_2S is a gaseous molecule, enzymatically produced by cystathionine gamma lyase (CSE) that has been shown to exert vascular tonus regulation, predominantly vasodilation (Aydinoglu, F; Nitric Oxide, v 70, p.51, 2017). Moreover, it has been shown that H_2S is reduced in preeclampsia (Wang, K, Circulation, v. 127, p. 2514, 2013). Therefore, we aimed to evaluate the involvement of PVAT-derived H_2S in the vascular modulation of hypertensive pregnancy. **Methods:** Female Wistar rats (220-300g) were mated and then allocated in individual cages. Animals were divided into two different groups, as follow: Normal Pregnant (Norm-Preg) and Hypertensive Pregnant (HTN-Preg). Animals from HTN-Preg group received desoxycorticosterone acetate (DOCA) 12.5 mg on gestational day one, followed by weekly injections of DOCA 6.25mg and water was replaced by 0.9% saline solution. Systolic blood pressure (SBP) was measured by tail cuff plethysmography throughout gestation, totalizing eight scattered days during pregnancy. On pregnancy day 20, animals were killed under isoflurane overdose. Thoracic aorta was removed and divided into four rings as follow: +PVAT + endothelium (E), +PVAT –E, -PVAT +E, -PVAT –E. Preparations were pre-contracted with phenylephrine (PHE) (10^{-6}M) followed by ACh (10^{-4}M) to test endothelial integrity. After tissue equilibration, aortic rings were challenged with cumulative concentrations of (Phe - 10^{-12}–10^{-4}M) in absence or in presence of DL-Propargylglycine (PAG, 10^{-3}M), an inhibitor of CSE. Tissue was also challenged with cumulative concentrations of KCl. In another set of experiments, tissue was pre-contracted with PHE (10^{-6}M) and challenged with L-cysteine (L-Cys – 10^{-2}M). **Results:** Injections of DOCA increased Systolic blood pressure during pregnancy. No differences were found in KCL-induced contraction in Norm-Preg rats. However, in HTN-Preg, presence of PVAT induced an anti-contractile effect to KCl. PVAT induced an anti-contractile effect to PHE in Norm-Preg rats. In contrast, we observed loss of PVAT anti-contractile effect to PHE in HTN-Preg rats. Moreover, in presence of PAG we observed a loss of anti-contractile effect in +PVAT-E ring from Norm-Preg rats, which was not observed in HTN-Preg. No differences in response to L-Cys were noted in Norm-Preg group. However, in HTN-Preg rats L-Cys induced a contraction in –PVAT+E whereas a relaxation was observed in +PVAT+E, +PVAT-E and –PVAT-E rings. **Conclusions:** In HTN-Preg animals, PVAT may exerts anti-contractile effects involving modulation of ion channels-mediated contraction. Moreover, our data suggest that H_2S may participate in the regulation of PVAT anti-contractile effect during pregnancy and that this protective mechanism is compromised in hypertensive pregnancy. **License number of ethics committee**: 1083/2018 **Financial support**: CAPES

06.037 Sildenafil Reduces Hypertension in Seven-day Lead-treated Rats Souza-Paula E, Chimini JS, Nascimento RA, Dias-Junior CAC IBB-Unesp – Farmacologia

Introduction: Lead is a common environmental pollutant and it is capable of causing cardiovascular disorders such as arterial hypertension. This phenomenon may be explained by the increased formation of reactive oxygen species (ROS), reduced bioavailability of nitric oxide (NO) or increased vascular reactivity to constrictor agents. Sildenafil is a phosphodiesterase inhibitor currently used for the treatment of erectile dysfunction. Also, it has innovated the treatments of hypertensive crises, pulmonary hypertension and myocardial ischemia due to inhibition of phosphodiesterase 5 (PDE5). This inhibition promotes cyclic guanosine monophosphate (cGMP) accumulation. The cGMP production results from the activation of soluble guanylate cyclase by the NO produced in endothelial cells. These mechanisms induce relaxation of the smooth muscle as a result of decreases in intracellular levels of calcium (Korkmaz-Icöz, S. Br J Pharmacol. 175, 223, 2018). **Methods:** Male Wistar rats (250-400g) were distributed into three groups, as follow: sildenafil+lead (Pb+Sil), saline+lead (Pb) and saline+sodium acetate (Sham). The animals from the Pb+Sil and Pb groups received intraperitoneally (i.p) lead acetate 8µg/100g/daily on the first day of the protocol. The intoxication was maintained with lead acetate 0.1µg/100g/daily for seven subsequent days. The group Pb+sil received sildenafil 15mg/kg/day by gavage. Sham group received sodium acetate i.p. in the same concentrations of lead acetate compared to the other groups. Sham Group also received saline by gavage 0.5 mL/Kg/day. Systolic blood pressure (SBP) was measured by tail cuff plethysmography every day until the eighth day when the animals were killed. Vascular reactivity experiments were performed in thoracic aorta rings with intact and mechanically removed endothelium. Aortic rings were stimulated with increasing concentrations of phenylephrine (Phe, 10^{-10} to 10^{-4}M). To investigate endothelial function, rings were pre-contracted with PHE (10^{-6} M), followed by increasing concentrations of acetylcholine (ACh, 10^{-9} to 10^{-5} M) in absence or presence of N(G)-Nitro-L-arginine methyl ester (L-NAME - 10^{-4}M). **Results:** Sildenafil reversed the increase in SBP on 6th and 7th day of protocol (120±4.4; 124±6.0 mmHg, respectively, in Pb+sil group) versus Pb group (153±4.4; 162±1.9 mmHg). Non-lead exposed group (Sham) did not evince alterations in SBP throughout the experimental period. No significant differences were observed among the experimental groups in concentration-response curves to Phe, KCl and ACh. **Conclusion:** Treatment with sildenafil reestablished the normal biological levels of SBP in lead-induced hypertension. However, this may not be related with improvements in aortic vascular reactivity. Ethics Committee IBB/UNESP (Protocol#1081/2018)

06.038 Structure-Activity/Kinetics relationship of cardenolides and Bufadienolides for inhibition of pig kidney Na^+K^+-ATPase. Azalim PN, Quintas LEM, Noël F UFRJ – Farmacologia Bioquímica e Molecular

Introduction: The Na^+,K^+-ATPase (NKA) is an integral membrane protein present in all eukaryotic cells, a member of the P-type ATPases and is responsible for maintaining the cell's electrochemical gradient. The main inhibitors of NKA are cardiotonic steroids (CTS), characterized by a steroidal nucleus in a Cis-Trans-Cis configuration, a lactone ring of five (cardenolides) or six (bufadienolides) members at C17 and, for some of them, an osidic portion at C3. The objective of present work was to evaluate 13 CTS, among cardenolides and bufadienolides, to establish a structure-activity relationship (SAR) for the potency and kinetics of their inhibitory effect on porcine kidney NKA. **Methods:** The K^+-dependent *pNPPase* reaction was used in order to assess both the potency and kinetics of the inhibitory effect of CTS on NKA, through colorimetric measurement of the p-nitrophenol liberated during the reaction performed at 37°C in a medium containing (in mM): KCl 50, $MgCl_2$ 3, p-nitrophenylphoshate (p-NPP) 3, EGTA 1 and Maleate-Tris 20 (pH 7.4), in the absence or presence of different concentrations of the CTS. Absorbance (430 nm) was measured in a 96-well plates reader every 15 min for determination of IC_{50} values, until equilibrium (4 h). **Results:** ***1.*** *Osidic portion (n=3)*: The presence of an osidic portion at C3 increased the potency of CTS as observed by comparing the equilibrium IC_{50}´s between: 1.ouabain (3.96 µM) and its genin (25.7 µM); 2.digoxin (2.34 µM) and its genin (3.87 µM); 3. digitoxin (0.74 µM) and its genin (1.84 µM). In the three cases studied, the presence of the osidic portion highly increased the time needed for equilibrium (when IC_{50} value is minimum), since it is already attained after 15 min incubation (ie. $T_{1/2} < 15$ min) with the 3 genins contrarily to what occurred with ouabain ($T_{1/2}$: 17.6 min), digoxin ($T_{1/2}$: 44.1 min) and digitoxin ($T_{1/2}$: 65.7 min). **2.** *Ciclization at C14-15 (n=3):* Cyclization at C14-15 (epoxy instead of hidroxil) decreased the potency of bufadienolides as observed by comparing the equilibrium IC_{50}´s between bufalin (0.57 µM) and resibufogin (2.38µM). Cyclization also decreased the time to reach equilibrium since $T_{1/2}$ is 31.5 min for bufalin but less than 10 min for resibufogin. Addition of a carbozymethyl group at the C16 of resibufogin (becoming cinobufagin) increased the potency (IC_{50} decreased 12 times) and time to reach the equilibrium ($T_{1/2}$ 34.8 minutes). **Conclusion:** Loss of the osidic portion at C3 (cardenolides) and ciclization at C14-15 (bufadienolides) decreased the potency and time to reach equilibrium of the inhibitory effect on p-NPPase activity of pig kidney NAK. These results also show that long times of incubation are necessary to ensure correct values of IC_{50}s for inhibition of NKA by CTS. **Animal Research Ethical Committee:** no needed (kidneys obtained at slaughterhouse). **Financial support**: FAPERJ, CNPq, CAPES.

06.039 High ethanol consumption in females from spontaneously hypertensive rat strain are reversed by Losartan. Boeder AM, Ramborger P, Marchette RCN, Fadanni GP, Linder AE, Izídio GS UFSC – Farmacologia

Introduction: Currently, there is a high incidence of women involved in the excessive ethanol consumption among 2 billion people that consume some alcoholic beverage. It is suggested that women are more sensitive to the deleterious effects of ethanol abuse in comparison to men. Despite this evidence, male animals are mostly used in preclinical and basic research. Therefore, in the present study, we propose, in female rats, a new genetic model for the biological basis of ethanol consumption. For that, herein we evaluated spontaneous ethanol consumption using females of SHR (Spontaneously Hypertensive Rat) and SLA16 (SHR.LEW.*Anxrr16*) strains and related this consumption with the blood pressure. **Methods**. In experiment 1, SHR and SLA16 females (8-weeks old) were submitted to spontaneous ethanol consumption during ten days. Basically, animals were isolated and had access to filtered water and 10% ethanol solution[1]. In experiment 2, the blood pressure (BP) measurement was performed in naïve rats by a noninvasive method (tail plethysmography) and direct measurement (anesthetized rats).[2] In experiment 3, to elucidate a possible relationship between ethanol consumption and blood pressure, the SHR and SLA16 females were treated with the antihypertensive drug (antagonist selective for AT1 receptors) that blocks the effects of Angiotensin II. For this, Losartan 15 mg/kg was administered orally for 12 days[3], mixed in hazelnut cream[4], and the ethanol consumption and BP were evaluated by the same methods already mentioned. All experimental procedures were performed between 1 p.m. and 6 p.m. Student's t-test (strain) or repeated measures analysis of variance (ANOVA) (strain and time) was performed in experiments 1 and 2. Two-way repeated measures ANOVA (strain, treatment and time) was performed in experiment 3. $p \leq 0.05$ was considered significant. **Results.** In experiment 1, a significant effect of strain was observed in total ethanol consumption ($p=0.0002$), where SHR consumed more ethanol than the SLA16 females. In experiment 2, there were significant differences in mean arterial pressure between the strains ($p=0.012$). SHR females presented higher blood pressure when compared to SLA16 females. In experiment 3, a significant effect of treatment was observed ($p=0.0001$), where both strains presented a reduction in blood pressure after losartan. Moreover, a suggestive interaction between strain and treatment ($p=0.055$) was observed in the total ethanol consumption (g/kg). The losartan was effective in reducing the ethanol consumption only in SHR strain ($p=0.039$). **Conclusion.** The females from SHR strain presented high levels of spontaneous ethanol consumption, which could be related to the renin-angiotensin system. SHR and SLA16 are genetically similar differing only by a *locus* on chromosome 4. This *locus* seems to be enough to produce differences in ethanol consumption and blood pressure. Then these strains could be valuable in the research of genes involved with ethanol consumption. **References:** 1. SPANAGEL, R. Psychopharmacology. v. 122, p. 369, 1995. 2. ZHAO, X. Current Protocol in Mouse Biology. v. 1, p. 105, 2011. 3. RODRIGUES, S. Life Science. v. 78, p. 2280, 2006. 4. DIOGO, L. N. J Am Assoc Lab Anim Sci. v. 54, p. 549, 2015. **License number of ethics committee**: PP00903 **Financial support**: CAPES and CNPQ

06.040 Anticontractile function of aortic Perivascular Adipose Tissue (**PVAT**) **in Senescence-Accelerated Mouse Prone** (**SAMP**). Barros PR, Miyoshi VP, Carvalho MH, Akamine EH ICB-USP – Farmacologia

Introduction: Aging per se is considered a risk factor for the development of cardiovascular diseases by promoting structural and functional vascular changes, such as endothelial dysfunction. Perivascular Adipose Tissue (PVAT) is able to secrete factors that modulate vascular function in a paracrine way, exerting an anticontractile action. Influence of aging on PVAT function is still poorly understood. The senescence-accelerated mouse prone (SAMP8) is a model of age-related cognitive decline and could be useful to study the age-related vascular alterations. We have previously demonstrated that 3-month old SAMP8 already display reduced endothelium-dependent relaxation and increased noradrenaline-induced contraction in comparison to SAMR1 (mice with normal aging). In the present study, we aimed to evaluate the anti-contractile function of PVAT, and the involved mechanisms, in aorta of SAMP8. **Methods:** Concentration-response curves for noradrenaline were evaluated in endothelium-intact (E+) thoracic aorta rings with (PVAT+) and without (PVAT-) PVAT from SAMR1 and SAMP8. PVAT- rings were obtained by removing all surrounding fat and connective tissue from the vessel with forceps and scissors. For further investigation of the mechanisms involved in anticontractile action of PVAT in SAMP8, curves to noradrenaline were performed in aortic rings with endothelium removed (E-) or in E+ rings in the presence of: nitric oxide synthase inhibitor (LNAME), non-selective cyclooxygenase inhibitor – indomethacin (INDO), superoxide dismutase (SOD) and catalase (CAT). Results are shown as mean ± standard error mean. **Results:** The maximum response (maxR) to noradrenaline in SAMR1 was not modified by the presence of PVAT, but it was reduced in SAMP8 (PVAT-: 1.6 ± 0.2 g; PVAT+: 1.1 ± 0.1 g), in comparison to the respective PVAT- rings, showing absence of anticontractile action of PVAT in SAMR1. In SAMP8, neither removal of the endothelium nor incubation of E+ rings with L-NAME modified the maxR to noradrenaline in PVAT- rings, but both increased the maxR in PVAT+ rings (E-: 1.6 ± 0.2 g; E+/LNAME: 1.7 ± 0.1 g) in comparison to the respective E+ rings. Incubation with INDO and CAT, but not with SOD, reduced the maxR to noradrenaline in PVAT- rings of SAMP8 (INDO: 1.0 ± 0.03; CAT: 0.7 ± 0.2). On the other hand, incubation with SOD, but not with INDO and CAT, reduced the maxR to noradrenaline in PVAT+ rings of SAMP8 (0.4 ± 0.01 g). **Conclusion:** The present results show that, different from SAMR1, PVAT from SAMP8 exert an anticontractile action, suggesting a compensatory mechanism to endothelial dysfunction. Moreover, cyclooxygenase-derived constrictor products and hydrogen peroxide mediate the contraction induced by noradrenaline in SAMP8. The anticontractile action of PVAT in SAMP8 could be dependent of endothelium and involves nitric oxide. Reduction of superoxide anion and/or accumulation of hydrogen peroxide may potentiate the anticontractile action of PVAT in SAMP8. **License number of ethics committee**: 82-2017 **Financial support**: CAPES, FAPESP

06.041 Activation of nitric oxide/guanylate-cyclase pathway accounts for the vasodilatory effect of a gastrointestinal digested whey protein hydrolysate. Pereira NR[1], Ozorio L[2], Mellinger-Silva C[3], da Silva-Santos JE[1] [1]UFSC – Farmacologia, [2]UFRJ – Química, [3]Embrapa Agroindústria de Alimentos - Bioquímica

Introduction: Whey-derived hydrolysates are putatively associated with biological activities, such as vasodilation, mainly attributed to inhibition of the angiotensin-converting enzyme. This study aims to investigate the mechanisms underlying vascular activity of a low-cost commercial whey protein hydrolysate, and the influence of gastrointestinal digestion on its effects. **Methods:** Thoracic aortas from anesthetized male Wistar rats (90-120 days) were removed and placed into organ bath chambers for isometric tension recording. Cumulative concentrations of spray-dried whey protein hydrolysate (PC3 spray; 1, 3, 5, and 10 mg/mL), and PC3 spray subjected to *in vitro* digestion (DWPH; 3, 10, 30, 50 and 100 µg/mL) were added into the chamber after a previous contraction with phenylephrine (PE; 1 µM). The experiments were carried out in endothelium-denuded (E-) and endothelium-intact (E+) aortic rings. In E+ preparations, the vasodilation promoted by DWPH was also evaluated during incubation with L-NAME (100 µM), ODQ (10 µM), c-PTIO (300 µM), tetraethylammonium (10 mM), 4-aminopyridine (1 mM), and glibenclamide (10 µM). **Results:** After contraction with PE, maximal relaxation (Rmax) induced by cumulative concentrations of PC3 spray was 81 ± 5.8% [EC_{50} = 4.5 (3.5-5.6) mg/mL], and 90.9 ± 4.8% [EC_{50} = 5.1 (3.6-7.2 mg/mL)], in E+ and E- vessels, respectively. On the other hand, DWPH-induced relaxation (3-100 µg/mL) reached a Rmax of 94.7 ± 3.1% [EC_{50} = 26.6 (20.8-33.4) □g/mL] and 10.7 ± 3.8% (EC_{50} not measurable), in E+ and E- aortic rings (n = 6/group). Previous incubation with the non-selective nitric oxide synthase inhibitor L-NAME, or the nitric oxide scavenger c-PTIO, reduced the maximal relaxation induced by DWPH to 12.1 ± 5.1% and 27.5 ± 2.3%, respectively (*n* = 4-5/group). A similar pattern of inhibition was found in arteries subjected to the soluble guanylate cyclase inhibitor ODQ (*n* = 5). The vasodilatory effect of DWPH was not susceptible to inhibition by glibenclamide and 4-aminopyridine, selective inhibitors of Kir6.1 ATP-sensitive and voltage-gated potassium channels, respectively. Nonetheless, the non-selective K_{Ca} potassium channel blocker tetraethylammonium reduced the maximal relaxation induced by DWPH to 14 ± 1.6% (*n* =4). **Conclusion:** The results obtained in this set of experiments support increasing evidence that whey-derived protein hydrolysates possess vasodilatory effects, and suggest that gastrointestinal digestion results in the generation of peptides with enhanced potency. Moreover, our data disclosed that the vasodilation induced by peptides obtained from whey protein hydrolysates after *in vitro* digestion, at least in the range of concentrations used, are also dependent on nitric oxide production, activation of guanylate cyclase, and requires the opening of calcium-activated potassium channels. **License number of ethics committee**: 5371190815 **Financial support**: CNPq with an M.Sc. fellowship to Pereira, N.R.

06.042 Impairment of anti-contractile effect of thoracic aorta PVAT of high fed diet mice involves AT2 but it is independent of Mas Receptor. Marques BVD, Silva RNO, Akamine EH ICB-USP – Farmacologia

Introduction: Obesity is a global health problem and it predisposes the obese individual to vascular dysfunction. Perivascular adipose tissue (PVAT) is located around most blood vessels and is known to produce diverse vasoactive substances that act both in the smooth muscle and endothelium, participating in the control of vascular tonus. In physiological conditions, PVAT has an anti-contractile effect in the vascular tonus. However, in obesity the PVAT anti-contractile effect is lost. Several components of the renin angiotensin aldosterone system (RAAS) are expressed in PVAT, such as angiotensin II and angiotensin 1-7, and these can participate in the control of vascular tonus. The objective of this study was to assess the participation of the vasodilator axes of RAAS in the loss of the anti-contractile effect in obese mice. **Methods:** The protocols were approved by ICB-USP Animal Research Ethical Committee (# 53/2017). Four-week-old C57Bl6/J male mice were submitted to a control diet (CTL) or a high fat diet (OB) for 16 weeks. Mice were anesthetized with sodium thiopental (50 mg/kg, i.p.). Thoracic aorta vascular reactivity was performed in 2 mm rings with (PVAT+) or without (PVAT-) PVAT. Concentration-response curves to noradrenaline were constructed; some of them were constructed in the presence of AT2 receptor antagonist PD123,319 (1 mM), Mas receptor antagonist A779 (1 mM) and nitric oxide synthase (NOS) inhibitor L-NAME (100 mM). The maximal response was calculated (maxR). The difference between the areas under the curves in the absence and presence of antagonists/inhibitor (ΔAUC) was evaluated. Results are shown as mean ± standard error of the mean. (n) represents the number of mice/group. Statistical analysis: two-way ANOVA followed by Tukey's test; significance level was $p < 0.05$. **Results:** PVAT decreased maxR in CTL [CTL PVAT-: 2.11 ± 0.14 mN/mm (16) vs CTL PVAT+: 1.48 ± 0.12 mN/mm (17)], but not in OB rings [OB PVAT-: 0.82 ± 0.07 mN/mm (15) vs OB PVAT+: 0.69 ± 0.09 mN/mm (16)]. Incubation of aortic rings with PD123,319 increased the noradrenaline contraction in CTL and OB. In CTL rings, the increase caused by PD123,319 was higher in presence of PVAT [ΔAUC; CTL PVAT-: 1.68 ± 0.47 (7) vs CTL PVAT+: 4.56 ± 0.45 (4)], but in OB rings the PD123,319 effect was similar in PVAT- and PVAT+ [ΔAUC; OB PVAT-: 0.99 ± 0.26 (7) vs OB PVAT+: 1.54 ± 0.23 (8)]. The noradrenaline contraction was also increased in the presence of A779 in CTL and OB rings. The effect of A779 was similar in PVAT- and PVAT+ in CTL [ΔAUC; CTL PVAT-: 2.44 ± 0.44 (7) vs CTL PVAT+: 2.79 ± 0.42 (8)] and in OB rings [ΔAUC; OB PVAT-: 1.86 ± 0.23 (7) vs OB PVAT+: 2.17 ± 0.38 (9)]. L-NAME increased the noradrenaline contraction in both groups and the increase was not different between PVAT- and PVAT+ rings in CTL [ΔAUC; CTL PVAT-: 4.83 ± 1.08 (4) vs CTL PVAT+: 5.98 ± 1.01 (4)] and in OB [ΔAUC; OB PVAT-: 4.24 ± 0.83 (4) vs OB PVAT+: 2.48 ± 0.82 (5)]. L-NAME effect appeared lower in OB PVAT+ compared to CTL PVAT+, but there was no statistical difference ($p=0.07$). **Conclusion:** Until the moment our results suggest that the anti-contractile action of the thoracic aorta PVAT is impaired in OB mice and that this impairment involves alteration of the angiotensin II AT2 receptor, but not the angiotensin 1-7 Mas receptor. **License number of ethics committee**: ICB-USP 53/2017 **Financial support**: Capes, CNPq and FAPESP.

06.043 Methylglyoxal potentiates thrombin-, collagen- and U46619- induced human platelet aggregation Salles CA[1], Freitas CF[2,1], Lescano CH[1], Naime AC[1], Santos I[3], Antunes E[1] [1]FCM-Unicamp – Farmacologia, [2]PUC-Minas – Ciências Biológicas, [3]FCM-Unicamp – Ciências Médicas

Objectives: Methylglyoxal (MGO), a metabolite of the glycolysis pathway, is a precursor of advanced glycation end products (AGE). Levels of MGO are elevated in pre-diabetic and diabetic patients (Dornadula 2015 et al., 2015). Because increased AGE formation has been proposed as one mechanism involved in vascular complications and platelet activation of diabetes (Hadas K et al., 2013), this study aimed to evaluate the effects of MGO on the human washed platelet aggregation induced by thrombin, collagen and U46619 (TXA2 analogue). **Methods:** Platelets were isolated from peripheral blood of human healthy volunteers (7 men and 10 women, aged 27.8±1.9 y.o.). Platelet samples (200 µL) were incubated with MGO (0.08-0.64 mM), and subsequently activated with thrombin (0.01 to 1.0 U/ml), collagen (0.2 to 20 µg/ml) or U46619 (0.25 to 2,0 µM). Platelet aggregation was performed with an optical aggregometer (PAP-8E Platelet Aggregation Profiler, PA, USA). **Results:** Thrombin produced concentration-dependent platelet aggregation, achieving a maximum response at 0.3 and 1 U/ml (0.8±0.5, 15.7±4.7, 27.8±5.5, 59.6±5.4 and 63.9±3.3% for 0.01, 0.03, 0.1, 0.3 and 1 U/ml, respectively). Pre-incubation with MGO (0.08 mM) markedly increased ($p<0.001$) the thrombin-induced platelet aggregation (14.0±13.5, 60.6±4.7, 72.4±4.2, 79.4±4.4 and 77.2±2.7%, respectively). Similar data were observed using higher concentrations of MGO (0.16 and 0.64 mM). In separate samples, addition of collagen induced concentration-dependent platelet aggregation (0.2±0.2, 8.6±3.7, 14.4±4.4, 41.4±4.9 and 53.2±5.3% for 0.2, 1.0, 2.0, 10.0 and 20.0 µg, respectively). Pre-incubation with MGO (0.08 mM) significantly increased ($p<0.001$) the collagen-induced aggregation (0.5±0.3, 38.7±11.5, 38.3±6.8, 56.8±4.9 and 57.1±4.9%, respectively). Higher concentrations of MGO (0.16 and 0.64 mM) also significantly potentiated collagen-induced aggregation. U46619 induced concentration-dependent platelet aggregation (4.2±1.9, 17.7±5.5, 42.1±4.6 and 44.1±4.8% for 0.25, 0.5, 1.0 and 2.0 µM, respectively) Pre-incubation with MGO (0.64 mM) increased ($p<0.05$) U46619-induced aggregation (14.3±9.5, 44.8±14.7, 58.0±5.2 and 64.8±3.1%, respectively). Lower MGO concentrations (0.08 and 0.16 mM) had no effect on U46619-induced aggregation. Next, we evaluated if the potentiation of thrombin-, collagen- and U46619-induced platelet aggregation by MGO was due to inactivation of nitric oxide (NO) – cyclic GMP signaling. Incubation with the NO donor sodium nitroprusside (10 µM) inhibited by 60.2%, 37.7% and 23.3% the thrombin-, collagen- and U46919-induced aggregation, respectively ($p<0.05$). However, MGO (0.08 a 0.64 mM) had no effect on the inhibitory effect of SNP on platelet aggregation. **Conclusion:** MGO enhances human platelet aggregation induced by thrombin, collagen and U46619 by mechanisms independent of inactivation of NO-cGMP signaling. **License number of ethics committee**: (Protocol No. 66299817.4.0000.5137) **Financial support**: FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo- Brasil)

06.044 Effects of ambient pollution on the development of nonalcoholic fatty liver disease and cardiovascular risk in fat diet-fed APOE $^{-/-}$ mice. Marques CL[1], Soares AG[1], Araujo LCC[2], Carvalho CR[2], Teixeira SA[1], Antunes V[2], Muscará MN[1], Costa SKP[1] - [1]ICB-USP – Farmacologia, [2]ICB-USP – Biofísica e Fisiologia

Introduction: We have shown that neonatal exposure of male mice to the electrophilic ambient pollutant 1,2-naphthoquinone (1,2-NQ), commonly found in diesel exhaust particles (DEP), affected cardiovascular function and exacerbated allergic airway inflammation at adulthood [1,2]. Interestingly, an important correlation between DEP exposure and enhancement of non-alcoholic fatty liver disease (NAFLD) has been shown [3], as well as compelling evidence that has substantiated a significant link between NAFLD and cardiovascular disease [4]. Thus, a close follow up on the impact of early exposure to 1,2-NQ for NAFLD development and cardiovascular risk stratification in diet-fed APOE $^{-/-}$ mice from parental preconceptional diet-fed APOE -/- mice is required. **Material and Methods:** Apolipoprotein deficient male mice (ApoE$^{-/-}$, 20g) were used and fed with either a regular chow or high fat diet (HFD) for 3 weeks. Over the neonatal period (days 6, 8 and 10), the animals inhaled for 15 min 1,2NQ (100 nM) or its vehicle, and groups divided as follows: 1) Apoe $^{-/-}$ + vehicle fed with regular chow, 2) Apoe $^{-/-}$ mice + 1,2NQ fed with a regular chow, 3) Apoe $^{-/-}$ + vehicle fed with HFD, and 4) 1,2NQ group fed a HFD. Mice were submitted to daily weighing and ECG analysis at 42 days of age. They were euthanized on day 43. The right atria were isolated and mounted on a wire myograph and subjected to pharmacological tests (e.g. adrenergic or cholinergic stimuli), while liver and blood samples were obtained and prepared for biochemical and histological analysis. **Results:** Changes related to body weight were not detected among groups, but retroperitoneal fat deposition was associated with HFD in mice exposed to both vehicle and 1,2-NQ. In the HFD group prior exposed to 1,2-NQ there were changes in some metabolic parameters, such as increased serum total cholesterol and high-density lipoprotein (HDL), AST and ALT levels. The 1,2-NQ exposure and HFD did not significantly increase liver weight compared to mice exposed to 1,2-NQ. In contrast, mice exposed to 1,2-NQ and fed with standard diet exhibited high maximum response to norepinephrine in the right atria reactivity (206 bpm) compared to control group (175 bpm). In the presence of HFD there was a potentiation of the maximum response in mice exposed to 1,2-NQ. No alterations were observed in the carbachol-induced concentration response curve when compared to all groups. **Conclusion:** In spite of the preliminary data in a small population, these findings suggest that early exposure to 1,2-NQ in the HFD group led to a exacerbation of positive chronotropic effect in the right atria. The association between preconceptional overnutrition and the offspring´s overnutrition status and early exposure to 1,2-NQ suggest an acquired increased risk for atrial defects and steatosis development. **License number of ethics committee**: 48/2016 **Financial support**: CAPES, CNPq and FAPESP

06.046 Increases in relaxation mediated by Perivascular Adipose Tissue (PVAT) may counterbalance the loss of anti-contractile effect of PVAT in normal pregnancy. Possomato-Vieira JS, Tozzato GPZ, Chimini JS, Dias-Junior CA IBB-Unesp – Farmacologia

Introduction: Several vascular adaptations occur during healthy pregnancy in order to maintain proper conditions to the growing fetus. In this context, it has been shown that the perivascular adipose tissue (PVAT) may exert a role in the control of vascular tone (Zaborska, KE; Br J Pharmacol, v.174, 3388, 2017). We therefore investigated the vascular effect of PVAT surrounding the thoracic aorta of virgin and normal pregnant (Norm-Preg) rats. **Methods:** Female Wistar rats (220-250g) were mated and then allocated in individual cages. Animals were divided into two groups: virgin rats and normal pregnant (Norm-Preg) rats. Systolic blood pressure (SBP) was evaluated by tail-cuff plethysmography on gestational days 19-20. After SBP measurements animals were killed under isoflurane overdose and thoracic aorta was removed. Thoracic aorta was divided into four rings as follow: +PVAT + endothelium (E), +PVAT –E, -PVAT +E, -PVAT –E. Rings were placed in an organ bath containing Krebs-Henseleit solution at 37^{o}C, pH 7.4, gassed with 95% O_2 and 5% CO_2, under basal tension of 1.5g. Some preparations had their endothelium mechanically removed. Preparations were challenged with cumulative concentrations of phenylephrine (Phe – 10^{-9}–10^{-4} M) in absence or in presence of cocktail of inhibitors containing desipramine (0.1 µM), corticosterone (10 µM), yohimbine (0.1 µM) and propranolol (0.1 µM) to block the neuronal and extraneuronal monoamine uptake, α2- and β-adrenoceptors, respectively. Concentration-response curves to KCl (10-120 mM) were also constructed. Vascular tissue was pre-contracted with Phe (10^{-6}M) and concentration-response curves to acetylcholine (ACh – 10^{-9}–10^{-4}M) and to sodium nitroprusside (SNP – 10^{-12}–10^{-4}M) were constructed. Vascular tone modifications were recorded by isometric force transducers and expressed as concentration-response curves. Log of EC_{50} and maximal response E_{max} (n= 5-6) were compared by two-way ANOVA/Tukey (significance when $P < 0.05$). **Results:** Both groups presented physiological values of SBP. PVAT promoted a decrease in E_{max} and pEC_{50} values to Phe in virgin rats. In Norm-Preg rats, PVAT also induced a decrease in Phe E_{max} values, which was lower than those observed in virgin rats. Presence of cocktail of inhibitors did not alter responses to Phe in both groups. PVAT induced increases in SNP pEC_{50} and decreases in E_{max} in virgin rats. Importantly, these differences disappeared in Norm-Preg rats. No differences were observed in the contraction induced by KCl and in ACh responses in both groups. **Conclusion:** PVAT compromised the non-endothelial-derived NO relaxation in virgin rats. Interestingly, pregnancy restored relaxation responses. Also, we observed an anti-contractile response to Phe induced by PVAT in both groups. Nevertheless, anti-contractile responses were lower in Norm-Preg versus virgin rats. Taken together, our data indicate that an increase in relaxation pathways may counterbalance the loss of anti-contractile effects exerted by PVAT during normal pregnancy in rings without endothelium, which suggest that in case of endothelial dysfunction, such as preeclampsia, PVAT may assume a protective role in vasculature. **License number of ethics committee**: IBB/UNESP (Protocol# 619/2014) **Financial support**: CAPES and FAPESP

06.047 Melatonin reduces weight gain and restores anti-contractile effect of aortic perivascular adipose tissue (PVAT) in obese rats. Gonzaga NA[1], Tirapelli CR[2]
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Introduction: perivascular adipose tissue (PVAT) is the adipose tissue adjacent to most of the arteries, which produces and releases numerous vasoactive factors, cytokines, and adipokines, influencing vascular function and the pathogeneses of vascular disease. Pathophysiological conditions such as obesity cause PVAT disfunction, leading to vascular disease. **Objective:** evaluate whether melatonin would be able to restore the anti-contractile function of aortas from obese rats. **Methodology:** male Wistar rats (280-300g) were divided into 4 groups: Control: animals were fed with a normal pelleted chow (12.6kJ/g - Nuvilab®, Nuvital, Brazil) for 10 weeks; Obese: animals were fed with a high sugar diet (13.35 kJ/g - HSD) which consisted of 33% chow, 33% Nestlé condensed milk, 7% sucrose, and 27% water, for 10 weeks. Melatonin (Mel): animals were fed with a normal diet and received melatonin (5mg/kg/day – p.o.) for the last 2 weeks; Obese-Melatonin (Obese-mel): animals were fed with a high sugar diet and received melatonin (5mg/kg/day – p.o.) for the last 2 weeks. All animals were weighed weekly throughout and at the end of 10 weeks aortas with ($PVAT^+$) or without PVAT ($PVAT^-$) were collected for vascular reactivity and biochemical assays. Cumulative concentration-response curves for phenylephrine were obtained in aortas with intact (E+) or denuded endothelium (E-) and the Emax (mN) and pD_2 values were analyzed. Aortic and PVAT superoxide anion (O_2^- - RUL/mg protein) levels were measured using chemiluminescence of lucigenin. All protocols were approved by the Ethics Committee (CEUA: 2017.5.91.22.2). **Results:** rats fed a high sugar diet for 10 weeks showed a significant increasein weight gain (556.5±6g) when compared to control group (695.0±10g). Melatonin treatment at the last 2 weeks decrease weight gain in approximately 25% (596.0±15g) in obese rats. Thoracic aortic rings with PVAT presented a significant decrease in phenylephrine-induced contraction when compared to rings without PVAT. However, rings with PVAT from obese rats showed a significant increase in phenylephrine-induced contraction when compared to control animals and melatonin treatment restore the anti-contractile effect of PVAT (Control: 8.0±0.1; Obese: 10.8±0.7*; Mel: 7.9±0.7; Obese-Mel: 7.8±0.3; n=5 per group). Removal of PVAT increased contractions to phenylephrinein control and obese animals that were not significantly different between the groups (Control: 12.2±0.2; Obese: 11.2±0.8; Mel: 11.0±0.7; Obese-Mel: 11.0±0.7; n=5 per group). Thoracic aortas with or without PVAT from obese animals showed a significant increase in O_2^- production when compared to control animals and melatonin treatment reestablish the physiological levels of O_2^- ($PVAT^-$: Control: 39.9±3; Obese: 95.7±10*; Mel: 38.7±6; Obese-Mel: 47.6±11; and $PVAT^+$: Control: 71.1±15; Obese: 110.7±10*; Mel: 82.4±6; Obese-Mel: 90.4±20; n=5 per group). **Conclusions:** Treatment with melatonin for 2 weeks restores the loss of the anti-contractile effect of PVAT induced by obesity. Additionally, we found that melatonin decreased obesity-induced O_2^- generation in the PVAT. **Funding:** CAPEs **Keywords:** Melatonin, Oxidative stress, PVAT, Obesity **License number of ethics committee**: CEUA: 2017.5.91.22.2 **Financial support**: CAPEs

06.048 Molecular mechanisms underlying cardiac dysfunction programmed by early weaning in male Wistar rats. Alvim-Silva T[1], Barros RBM[1], Oliveira DF[2], Pinto LMO[2], Nascimento JHM[2], Scaramello CBV[1] [1]UFF – Fisiologia e Farmacologia, [2]UFRJ – Biofísica

Introduction: Previous data from our research group have shown cardiometabolic risk increase, diastolic dysfunction and cardiorespiratory capacity decrease in male Wistar rats submitted to early weaning (Barros et al. SBFTE 2014; Barros et al. FESBe 2015; Alvim-Silva et al. SBFTE 2016; Alvim-Silva et al. SBFTE 2017). The aim of the present work was to investigate molecular mechanisms underlying cardiac dysfunction programmed by early weaning in 365 days-old male Wistar rats. **Methods:** Male Wistar rats were randomly divided into control (C) and early weaning (EW) groups, being physically separated from their mothers at postnatal day 21 and 18 respectively. At postnatal day 365, all animals were submitted to electrocardiography (EKG) and then they were euthanized. The hearts were excised to perform Langendorff assays or to obtain cardiac homogenates (Bambrick et al., J Pharmacol Meth, 20: 313, 1988). The experiments with isolated hearts were performed in the presence/absence of 0.3-300nM isoprenaline (Marques et al., Int J Cardiol. 195: 48, 2015). The homogenates were used in western blot assays to evaluate protein levels of sarcoplasmic reticulum and plasma membrane Ca^{2+}-ATPase (SERCA;PMCA), phospholamban (PLB), Na^{+}/Ca^{2+} exchanger (NCX) and Na^{+}/K^{+}ATPase (NKA) (Laemmli. Nature, 227: 680, 1970). Data were presented as mean±standard error of the mean, analyzed by Student t test and considered statistically different if $P<0.05$(*) versus control. **Results:** No differences about EKG parameters were noticed. Despite left ventricular developed pressure (LVDP) and cardiac performance indexes (± dp/dt) have been increased by isoprenaline in a concentration-dependent manner in both groups, these parameters were statistically lower in EW compared to C at 300nM of the β-agonist (LVDP: 317.68%±53.35% x 163.49%±11.55%*; (+)dp/dt: 399.22±65.96% x 196.89±30.35%*; (-)dp/dt: 416.38±70.96% x 159.24±11.97%*). Western blot analysis showed significant changes on SERCA (8.93±2.18 x 13.28±0.45*) and NCX (1.02±0.25 x 1.88±0.21*) protein levels but not about PMCA, PLB nor NKA expression. **Conclusions:** Isolated heart assays have demonstrated that β-adrenergic inotropic and lusitropic effect are impaired in 365 days-old male rats submitted to early weaning. The observed downregulation of SERCA and overexpression of NCX suggest that Ca^{2+} uptake to sarcoplasmic reticulum decline while cytosolic Ca^{2+} removal for extracellular medium increases, an alteration often seen in failing hearts (Wasserstrom, J Physiol, 588(Pt 7): 1027, 2010; Coppini et al., Glob Cardiol Sci Pract, 2013(3): 222, 2013). This molecular mechanism leads to the impairment of β-adrenergic signaling that is important in the beginning of heart failure for systolic and diastolic function preservation (Bernstein *et al*., Prog Pediatr Cardiol.; 31(1): 35, 2011; Rodriguez *et al*., PLoS One; 9 (4): e96400, 2014). **License number of ethics committee**: CEUA/UFF812-16 **Financial support**: FAPERJ, CNPq, CAPES, PROPPI/UFF

06.049 Effect of drugs with action on the renin-angiotensin system on the physical performance of hypertensive rats. Santos DC[1], Veríssimo LF[1], Raquel HA[1], Volpini VL[1], Marques LAC[1], Gomes MV[2], Fernandes KP[3], Michelini LC[4], Pelosi GG[1] [1]UEL – Ciências Fisiológicas, [2]UNOPAR – Ciências da Saúde, [3]UNOPAR – Ciências da Reabilitação, [4]USP – Ciências Biomédicas, Fisiologia e Biofísica

Introduction: Studies have shown that main mediator of the renin angiotensin system (RAS) angiotensin II (Ang II) is a key peptide in regulation muscle function and is related to beneficial or deleterious effects in these cells. Thus, pharmacological approaches capable RAS modulating can represent potential benefits in muscle structure and function. The aim this study was to evaluate the effects of pharmacological treatment with the angiotensin converting enzyme inhibitor enalapril or the angiotensin receptor antagonist losartan on physical, cardiovascular, molecular and epigenetic parameters in spontaneously hypertensive rats (SHR), submitted to the treadmill progressive stress test. **Methods:** Male adults SHR were divided into 3 groups: control (CTL: water, n=11), enalapril (ENA: 10mg/kg/day, n=13), losartan (LOS: 10mg/kg/day, n=15), such administration being performed for 28 consecutive days by gavage. Mean arterial pressure (MAP), heart rate (HR) and distance traveled (meters) of these animals were evaluated by treadmill progressive test, every 7 days during drug administration period. After 28 days of treatment, a group of animals (CTL: 5, ENA: 5, LOS: 6) remained into the home cage for 7 days without receiving any treatment, to evaluate the late effect to the treatment. Gastrocnemius muscle, soleus and plasma were collected to the oxidative and antioxidative capacity and the overall DNA methylation analyses. **Results:** Administration of Enalapril modulates body weight animals, preventing weight gain during treatment, mainly after 14th day of treatment ($p = 0,0222$). Significant MAP reduction of animals treated with enalapril was observed after fourth week of treatment ($p = 0,0146$). Enalapril did not improve the running capacity distance by the animals, but it avoided its decline when compared to the other groups ($p = 0,1163$). There was no difference in the running capacity distance by the animals, when compared treatment effect and late effect, one week after the end of the treatment ($p = 0,0516$). Running capacity rats were reduced in the losartan group after 21th day of treatment ($p=0,004$). Enalapril or losartan did not change the total oxidative and antioxidative capacity of the plasma and muscles evaluated by the FRAP assay (gastrocnemius $p=0,4702$, soleus $p=0,0843$, plasma, $p=0,3510$), ABTS (gastrocnemius $p=0,4212$, soleus: $p=0,1488$, plasma: $p=0,5280$), NBT (gastrocnemius $p=0,8812$, soleus $p=0,5300$, plasma $p=0,0653$) and MDA (gastrocnemius $p=0,8354$, soleus $p=0,9972$, plasma $p= 0,4096$) of the animals submitted to the treadmill maximum stress test. Enalapril but not losartan increased the overall methylation of the skeletal muscle cell DNA of the gastrocnemius muscle ($p=0,0239$) from animals to the maximal treadmill stress test. **Conclusions:** These findings confirm the protective effect of enalapril on the musculoskeletal system and its effect on body weight modulation, as well as demonstrate for the first time that the pharmacological intervention focused on the modulation of RAS using ACE inhibitors enalapril can promote epigenetics changes in the muscle skeletal muscle submitted to the maximum stress test. **License number of ethics committee**: CEUA/UEL n. 30987.2014.73 **Financial support**: Scholarship and funding of the project by CAPES

06.050 Effects of b-blockers with intrinsic sympathomimetic activities on myocardial dysfunction and immune response in septic mice. Silva KP[1], Silva-Junior ED[2], Baker JG[3], Cunha FQ[4], Pupo AS[1] - [1]IBB-Unesp – Farmacologia, [2]UFRN – Biofísica e Farmacologia, [3]University of Nottingham – Queen's Medical Centre, [4]FMUSP – Farmacologia

Introduction: Myocardial dysfunction is a common feature in septic patients contributing to high mortality rates. Recent findings showed that the cardiac depression in sepsis is related to □-adrenoceptor internalization (Dal Secco, D., Am J Physiol Heart Circ Physi., 313: H149, 2017). This study investigates whether □-blockers with intrinsic sympathomimetic activities (efficacies) ranging from "zero" (propranolol) to "moderate" (carvedilol<pindolol<alprenolol) ameliorate the cardiac function and inflammatory response in sepsis by preventing □-adrenoceptor internalization, but still providing some degree of cardiac stimulation. <pindolol**Methods:** Sepsis was induced in C57BL/6 male mice (6-8 weeks olds) by cecal ligation and puncture followed by treatment with propranolol, carvedilol, pindolol or alprenolol at doses chosen based on the K_D and half-life of each drug. Drugs were given after surgery during the hyperdynamic phase of sepsis. After 24h of sepsis induction, mice were used in the following experiments: (a) [^{3}H]CGP-12177 binding assay to estimate □-adrenoceptors density in the heart; (b) isolated heart in Langendorff system to evaluate cardiac function; (c) ELISA to quantify serum and cardiac IL-1□ and TNF levels; (d) bacteremia. **Results:** □-adrenoceptor density at the cell surface was reduced by ≈50% in the heart of septic mice (Bmax in fmol.mg^{-1} protein=4.7±0.7, n=5 *vs* 8.8±1, n=5 in naïve mice). The reduction in □-adrenoceptor density induced by sepsis was prevented by treatments with propranolol (Bmax=13.8±2.9, n=5), alprenolol (7.7±0.6, n=5) and pindolol (8.4±0.8, n=5), but not by carvedilol (5.8±1.7, n=5). The performance of the isoprenaline-stimulated heart from septic mice was severely compromised (Heart rate= 322±59 bpm; dP/dT_{max}=343±61 mmHg/s; dP/dT_{min}= -287±51 mmHg/s, n=5) when compared to hearts from naïve mice (Heart rate= 556±23; dP/dT_{max}= 894±122; dP/dT_{min}= -1245±210; n=5). Treatment of mice with propranolol and alprenolol restored the heart rate ($Emax_{propranolol}$= 468±15bpm; $Emax_{alprenolol}$= 528±32; n=5), dP/dTmax ($Emax_{propranolol}$= 1162±190 mmHg/s; $Emax_{alprenolol}$= 1090±95; n=5) and dP/dTmin ($Emax_{propranolol}$= -1141±167 mmHg/s; $Emax_{alprenolol}$=-759±211; n=5); however, treatment with carvedilol and pindolol was ineffective in preventing the cardiac depression induced by sepsis. High levels of IL-1□ (932±473 pg/mL, n=4) and TNF (785±26 pg/mL, n=4) were found in serum from septic mice. Treatment with □-blockers diminished serum IL-1□□(in pg/mL: propranolol= 33□±17; carvedilol= 68±16; pindolol= 259±236; alprenolol= 459±292; n=3-5) and TNF levels (in pg/mL: propranolol= 43±18; carvedilol= 86±29; pindolol= 95±76; alprenolol= 72±73 pg/mL; n=3-5). □-blocker treatment diminished IL-1□ levels in the heart (in pg.mg^{-1} protein: propranolol= 2±0,4; carvedilol= 2±0.2; pindolol= 2±0.3; alprenolol= 2±0.2; n=5-8 vs untreated septic animals 4.7±1.4; n=5);however, only propranolol treatment reduced bacteremia (Log UFC/ml= 1,2; 95%CI =0.6-2.1 vs untreated septic animals Log UFC/ml= 3,5; 95%CI= 2.8-4.5; n=5). **Conclusion:** Treatment with □-blockers with moderate intrinsic sympathomimetic activity (e.g. alprenolol) may be a novel approach to protect cardiac function and reduce inflammatory response during sepsis. (CEUA #40/2017).</pindolol<pindolol</pindolol **License number of ethics committee**: CEUA #40/2017

06.051 Pharmacological evaluation of new drug prototypes in smooth muscle. Teixeira RGS[1], Santos NN[2], Resende GO[2], Ponte CG[3], Santos WC[1] [1]UFF – Farmacologia, [2]IFRJ – Química, [3]IFRJ – Ciências Biomédicas

Introduction: Treatment of some chronic diseases can be significantly impaired by the adherence to therapy. Systemic Arterial Hypertension (SAH), despite the vast therapeutic arsenal available, continues to grow on the world stage. Hyperactive Bladder Syndrome (HBS) also has its treatment compromised by the adherence to therapy. Molecules that can alter the contractile profile of aorta or bladder smooth muscle *in vitro* are potential candidates for new clinical approaches. quinazoline derivatives are promising molecules and also are complexes derived from Salan. This study aimed to investigate the effect of Quinazolines derived compounds and Salan complex compounds in rat aorta and bladder as new templates for possible application as new therapeutic approaches in the treatment of SAH and HBS. **Methods:** The tested substances were added at cumulative concentrations to specific agonist pre-stimulated tissues (aorta and bladder) and the data were recorded by a system of vertical isometric voltage recording system. The concentrations used were 1μM, 3μM, 10μM and 30μM on the plateau of pre-contractions induced by Phenylephrine (Phe) in the aorta and by Carbacol in the bladder. **Results:** In the aorta, substances Quin02 (IC_{50} = 1,18 x 10^{-6}M), Quin04 (IC_{50} = 2,82 x 10^{-6}M), Quin05 (IC_{50} = 5,98 x 10^{-6}M), Quin07 (IC_{50} = 2,91 x 10^{-5}M), Quin08 (IC_{50} = 2,82 x 10^{-6}M) and Quin09 (IC_{50} = 1,34 x 10^{-5M}) of the "Quin" series presented a vasorelaxation effect. While only FC18 (EC_{50} = 8,24 x 10^{-5}M) of the substances belonging to FC substances class showed a vasoconstriction effect in the pre-contracted aorta with Phe. On the other hand, the follow two substance (FC15 – IC_{50} = 2,81 x 10^{-4}M; Quin07 – IC_{50} = 9,75 x 10^{-5}M), one of each series, showed significant effect in bladder relaxation but only at the highest concentration employed. **Conclusion:** These results may spoil the possible use as potential prototypes for new drugs. In the sequence of the study, we intent to continue evaluation of the observed effects of Quin02, Quin04 e Quin08 that show highest pharmacological potential and to investigate the physiological mechanisms involved in the actions on the contractile activity to the aorta. In addition, we also will analyze their cytotoxicity, as well as use molecular modeling techniques to guide the synthesis of new analogues for greater efficiency and potency. **References:** ALAGARSAMY, V. Eur. J. Med. Chem. v. 151, p. 628, 2018. DRAGOUN, M. J Cancer Res Clin Oncol. v. 144, p. 685, 2018. HAMEED, A. Expert Opin. Ther. Pat. v. 28, p. 281, 2018. PETER, J. M. Revista Femina. v. 37, p. 505, 2013. PESCH, T. BMC Cancer. v. 16, p. 469, 2016. **License number of ethics committee**: CEUA-UFF 765/2017 **Financial support**: Capes, Faperj e CNPq

06.053 Troponin I proteolysis by Matrix Metaloproteinase (MMP)-2 may be associated with chronic cardiac remodeling and dysfunction in renovascular hypertension. Parente JM[1], Omoto ACM[2], Fazan R[2], Castro MM[1] [1]FMRP-USP – Farmacologia, [2]FMRP-USP – Fisiologia

Introduction: MMP-2 is present within the cardiomyocytes and degrades troponin I, which may contribute to cardiac dysfunction. Inhibition of MMP-2 activity with doxycycline improved cardiac ventricular dysfunction associated with ischemia and reperfusion injury by reducing troponin I proteolysis. The objective of this study is to verify whether increased MMP-2 activity contributes to hypertension-induced chronic morphological and functional changes in the heart by degrading troponin I. **Methods:** Male Wistar rats were sham or two kidney-one clip (2K1C) operated and treated with water or doxycycline (15 mg/kg/day) by gavage for six weeks, starting from the tenth week of hypertension. Tail-cuff plethysmography and echocardiogram were performed during the hypertension development. Rats were euthanized with sixteen weeks of hypertension and hearts were used for: morphological analysis by H&E staining; gel and *in situ* zymography; troponin I levels by western blotting and DHE fluorescence. Statistical analysis was performed by two-way ANOVA. All protocols were approved by CEUA (023/2015-1). **Results:** Systolic blood pressure (SBP) of 2K1C rats was higher than control group during ten weeks of hypertension and doxycycline did not decrease it in 2K1C. At sixteen weeks of hypertension, 30% of 2K1C rats had left ventricular eccentric hypertrophic remodeling (LVEHR) while the rest of it remained at left ventricular concentric hypertrophic remodeling (LVCHR). Interestingly, only 12.5% of 2K1C rats treated with doxycycline had LVEHR (1of 8 rats). Histological analysis and echocardiogram were used to separate 2K1C rats in LVCHR (2K1C-H) and LVEHR (2K1C-D). The left ventricular chamber area (LVCA) was higher in 2K1C-D rats when compared to 2K1C-H and Sham and this change was prevented by doxycycline. 2K1C rats had an increase in the left ventricle wall thickness (LVWT), heart weight (HW) and intraventricular septum thickness (IST). However, there was no difference in these parameters between 2K1C-D and 2K1C-H. Doxycycline prevented only the increased IST in 2K1C-D. At ten weeks of hypertension, 2K1C rats show no changes in the ejection (EF) or shortening fraction (SF), but at the sixteenth week, 2K1C-D rats had reduced EF and SF when compared to 2K1C-H, thus suggesting cardiac dysfunction. Doxycycline prevented these changes and preserved cardiac function without reducing SBP. Analysis of MMP-2 activity by gel zymography showed a tendency to increase the activity of 72 kDa MMP-2 in 2K1C-H and 2K1C-D and treatment with doxycycline apparently decreased it. *In situ* zymography showed an increase in the gelatinolytic activity in the 2K1C-D when compared to 2K1C-H and this was followed by a tendency to appear oxidative stress in these hearts as shown by DHE fluorescence. Treatment with doxycycline tended to reverse these changes. Furthermore, increased levels of degradation products of troponin I were observed in 2K1C-D when compared to 2K1C-H and doxycycline tended to decrease it. **Conclusion:** Increased activity of MMP-2 may contribute to troponin I proteolysis in the heart of hypertensive rats and this effect may contribute to cardiac remodeling and dysfunction. **Financial support**: CAPES, CNPq and FAPESP. **License number of ethics committee**: 023/2015-1

06.054 Is programming by neonatal leptin treatment different between male and female Wistar rats? Souza KP, Marques EB, Rocha NN, Scaramello CBV UFF – Fisiologia e Farmacologia

Introduction: Previous report showed that neonatal leptin treatment programs an increased cardiovascular risk (CV) and an age-dependent cardiac dysfunction in male Wistar rats (Marques et al., Int J Cardiol. 181C: 141, 2015). Epidemiological data show substantial differences between males and females about the development and the prognosis of CV diseases (Sandberg et al., Biol Sex Differ. 3: 7, 2012). Preliminary data from our research group indicated that the programed cardiac injury in males involves changes about Ca^{2+} handling proteins level that had an opposite pattern of expression in females also submitted to neonatal leptin treatment (Souza et al., SBFTE2017, panel 06.033). Therefore, the aim of this work was to verify if the programming of CV risk and cardiac function by hyperleptinemia neonatal is also different between male and female Wistar rats in prepubertal phase. **Methods:** Twenty-four male and female newborn Wistar rats were divided into four equal groups: Male (ML) and Female (FL) Leptin groups, receiving daily leptin (8µg/100g sc), and Male (MC) and Female (FC) Control groups, receiving saline, for the first 10 days of lactation. Body weight and food consumption were monitored and besides anthropometric and nutritional evaluation, all animals were submitted to hemodynamic evaluation, echocardiography and maximal effort ergometric test at postnatal day 30. Data were presented as mean±standard error of the mean, analyzed by Student t test or two-way ANOVA and considered statistically different if $P<0.05$(*) compared to respective control. **Results:** Independently of the sex, pups submitted to leptin treatment presented greater body weight between postnatal days 18 and 21 compared to pups that received saline. This profile remained for 30 days-old females (FC=83.28±0.71xFL=90.17±1.65*g), despite no differences have been seen about nutritional parameters as the greater food consumption observed for males (MC=91.11±0.59xML=96.08±1.47*g) between postnatal days 21 and 30. Hemodynamic parameters, such as systolic, diastolic and mean blood pressure, as well as cardiorrespiratory capacity evaluated by ergometer test, were not affect by leptin neonatal treatment neither in males nor females. However, some changes have been already noticed about echocardiography parameters (cm) in females but not in males. Leptin treatment have determined greater left ventricle internal diameter in sístole (cm) (FC=0.150±0.009xFL=0.260±0.020*) and diminished intraventricular septum thickness in diastole (cm) (FC=0.120±0.005xFL=0.110±0.004*), left ventricle posterior wall thickness in diástole (cm) (FC=0.130±0.003xFL=0.110±0.004*) and relative wall thickness (FC=0.560±0.040xFL=0.420± 0.040*). **Conclusion:** These data suggest that leptin neonatal treatment promotes hyperphagia in males and increases CV risk in females by favouring a sustained overweight. Ecocardiography data suggest that maybe dilated cardiomyopathy development is in course. Programming seems to be sex-dependent and older animals will be evaluated to verify if cardiac dysfunction is better characterized with aging as seen in a prior study performed just with males. **License number of ethics committee**: CEUA/UFF812-16. **Financial support**: FAPERJ, CNPq, CAPES, PROPPI/UFF.

06.055 Influence of physical exercise on ECG of SHR rats treated with losartan. Watai PY[1], Castro QJT[1], Silva SSC[1], Becker LK[2], Guimarães AG[1] [1]UFOP – Cipharma, [2]UFOP – Cedufop

Introduction: Hypertension treatment can be non-pharmacological alone or associated with drug treatment. The effectiveness of pharmacological treatment of hypertension seems to be better when non-pharmacological treatment is present. Considering ECG intervals alteration as predictors of cardiac arrhythmias also associated with hypertension, this study aimed to evaluate the effectiveness of regular physical exercise in response to losartan, a selective antagonist of AT1 receptors. **Methods:** All the procedures were approved by CEUA/UFOP (2015/05). Sedentary and trained (8 groups, n=6 each) male spontaneous hypertensive rats (SHR) received losartan (2.5, 5 or 10 mg/kg) or vehicle by oral route. Trained groups were submitted to physical exercise on a treadmill 18 m/min, during 60 min, five days a week for eight weeks. After the last day of training, lead II ECG signal was recorded. **Results:** PR and QRS intervals were similar for all groups. Sedentary SHR vehicle treated presented QT and QTc intervals prolongation and physical exercise alone was not able to reduce it. QT and QTc intervals were similar between SHR treated with losartan compared to vehicle. QT interval of sedentary losartan treated groups was 67.8 ± 2.03 ms, 63.5 ± 1.00 ms, 64.7 ± 0.74 ms and for trained losartan treated groups was 65.3 ± 1.04 ms, 64.6 ± 0.74 ms and 64.5 ± 0.87 ms, respectively for 2.5, 5 and 10 mg/kg and compared to vehicle of sedentary (70.5 ± 1.61 ms) and trained (69.2 ± 1.64 ms) groups. QTc interval of sedentary losartan treated groups was 122.9 ± 4.38 ms, 122.2 ± 6.77 ms, 115.4 ± 4.46 ms and for trained losartan treated groups was 118.9 ± 3.01 ms, 118.4 ± 3.51 ms and 116.5 ± 2.82 ms, respectively for 2.5, 5 and 10 mg/kg and compared to vehicle of sedentary (127.3 ± 3.90 ms) and trained (116.5 ± 3.54 ms) groups. **Conclusion:** Treatment with losartan associated or not with physical exercise was not able to reduce to QT and QTc intervals prolongation observed in SHR. **Acknowledgments:** FAPEMIG CNPq; UFOP; CAPES.
License number of ethics committee: CEUA/UFOP (2015/05) **Financial support**: PIBIC/FAPEMIG

06.056 Mechanisms of cardiovascular disease programed by perinatal overnourishment in male and female wistar rats. Araújo GA, Farias RS, Marques EB, Rocha NN, Scaramello CBV UFF – Farmacologia e Fisiologia

Introduction: Previous data from our research group have shown that the decrease of litter size leads to perinatal overweight due to the increase of milk supply. In addition, male Wistar rats presented a higher abdominal/thoracic circumference ratio in adulthood which is associated to an increased cardiovascular disease (CVD) risk (Amaral et al. SBFTE 2017). As epidemiological data points to sexual dimorphism in CVD development (Sandberg & Ji, Biol Sex Differ.3: 7,2012), the aim of this work was to investigate mechanisms of CVD programed by perinatal overnourishment in male and female Wistar rats. **Methods:** Rat pup litters were randomly adjusted to 8 or 4 male (M) and female (F) animals per mother (in a proportion of 1: 1) on day 1 of life, originating Control (C) and Overweight (O) groups. The animals were assessed at postnatal day 90 (CMxOM;CFxOF) being submitted to hemodynamic evaluation, echocardiography and maximal effort ergometer test (Marques et al. Int J Cardiol.181C: 141,2015). Determination of serum lipids and glucose levels were performed using commercially available Labtest Brasil kit. Data were presented as mean±SEM, analyzed by Student *t* test and considered statistically different if $p<0.05$(*). **Results:** Changes were noticed between overnourished and control animals about systolic (CM=107.8±1.5 x OM=122.3±3.1*;CF=113.4±1.7 x OF=107.7±1.9*) and mean blood pressure (CM=71.3±0.7 x OM=77.7±1.0*). Although females from small litters have presented higher serum LDL-c (CF=27.4±1.4x OF=40.6±4.8*), only males have shown an enlarged intraventricular septum thickness (CM=0.156±0.006cm x OM=0.175±0.006cm*) and left ventricle posterior wall thickness (CM=0.157±0.006cm x OM=0.176 ±0.005cm*) in diastole compared to respective controls. None differences about maximum speed developed, time spent and distance travelled were observed on maximal effort ergometer test. **Conclusions:** Hypertension is a condition of chronic pressure overload that may alter ventricular geometry, leading to concentric remodeling (Koren et al. Ann Intern Med. 114(5): 345, 1991), as only seen for male rats submitted to overnourishment during lactation in this work. Here female rats in pubertal fase seemed to be cardioprotected by estrogen against hypertension and its consequences (Ashraf, Vongpatanasin. Curr Hypertens Rep. 8(5): 368, 2006), despite of the higher level of LDL seen on those from small litters. So the programming o CVD by perinatal overnourishment seems to be sex-dependent. **Financial Support:** FAPERJ, CNPq, CAPES. **License number of ethics committee**: CEUA-UFF812/2016 **Financial support**: FAPERJ, CNPq, CAPES

06.057 Pentoxifylline attenuation of vascular hypertrophy is not associated with TNF-α pathway in renovascular hypertension. Pereira MP[1], Bonacio GF[2], Garcia VT[2], Alvez CAC[2], Dellalibera-JovillianoR[1], Franca SC[2], Tanus-Santos JE[3], Rizzi E[2] [1]UNAERP – Medicina, [2]UNAERP – Biotecnologia, [3]FMRP-USP – Farmacologia

Introduction: Angiotensin II activates matrix metalloproteinases (MMP), which contributes to vascular remodeling during hypertension. Angiotensin II and tumoral necrose factor (TNF)-α are involved in reactive oxygen species (ROS) formation which is responsible for *in vitro* MMP-2 activation in endothelial cells. Mounting evidences demonstrate that pentoxifylline (PTX), a non-selective inhibitor of phosphodiesterases clinically used for chronic occlusive arterial diseases, may inhibit proinflammatory cytokines such as TNF-α. In addition, the beneficial effects of PTX treatment have been associated with antioxidants properties. Thus, the aim of the present study was to evaluate whether inhibition of TNF-α reverses vascular hypertrophy in 2-kidney and 1-Clip (2K1C) rats through inhibition of MMP-2 activity. Etanercept (ETN), a known inhibitor of TNF-α, was used as the positive control in this study. **Methods:** 2K1C and Sham rats were treated 2 weeks after surgery with Vehicle, PTX (50 or 100 mg/Kg/day) daily or with ETN 3X/week (0.3 mg/Kg/day). Systolic blood pressure (SBP) was monitored weekly by tail plethysmography. Histological changes in aortas were examined in sections of 4 µm stained with hematoxylin/eosin. The expression of MMP-2 in aorta was determined using gelatin zymography assays. TNF-α levels were analyzed using the enzyme-linked immunosorbent assay (ELISA). Procedures were approved by the UNAERP Animal Research Ethics Committee (protocol number: 15/2016). **Results:** SBP of 2K1C rats treated only with vehicle reached 191±4 mmHg after 6 weeks of hypertension. Higher dose of PTX decreased the SBP to 171±5 mmHg ($P<0.05$), but the lower dose of ETN treatment did not affect the SBP in 2K1C rats (in mmHg: 181±6 and 181±9; $P>0.05$). 2K1C untreated animals showed a significant increase in the media/lumen ratio when compared to Sham group ratio (in %: from 12.0±0.6 to 15.0±1.1; $P<0.05$), which was attenuated only by the treatment with higher dose of PTX (12.6±0.6%; $P<0.05$ vs 2K1C vehicle), while low dose of PTX or ETN did not affect the vascular hypertrophy ($P>0.05$). 2K1C hypertension induced significant increases in total MMP-2 levels (in Arbitrary units (A.U.): 1.3±0.08 to 2.3±0.3; $P<0.05$) compared to sham group (?). Although thisMMP-2 upregulation was not significantly reduced by the treatments, the highest dose of the PTX presented a great tendency to attenuate MMP-2 levels in 2K1C rats ($P>0.05$). TNF-α levels were increased in 2K1C rats (70±2 pg/ml) when compared with the Sham group (53±1 pg/ml; $P<0,05$). The treatments with ETN and PTX100 decreased the TNF-α levels (in pg/ml: 59±3 and 43±2; respectively) and the treatment with lower dose of PTX did not change this cytokine levels (66±1 pg/ml). **Conclusion:** The highest dose of PTX exerts protective effects on blood pressure, vascular remodeling and MMP-2 in 2K1C rats by mechanisms independent of the TNF-α inhibition. Although chronic treatments with ETN reduced plasmatic levels of TNF-α, we did not observe improvement in vascular hypertrophy during renovascular hypertension. **License number of ethics committee**: 15/2016 **Financial support**: FAPESP

06.058 Resveratrol decreases MMP-2 activity *in vitro* **and attenuates renovascular hypertension-induced PVAT remodeling**. Fumagalli GB[1], Rosalin MG[2], Camassola GB[2], Carmo MFA[2], Basso FG[3], Restini CBA[4], Sanchez ER[1] [1]UNAERP – Biotecnologia, [2]UNAERP – Medicina, [3]UNAERP – Odontologia, [4]Michigan State University – Pharmacology & Toxicology, MI - USA

Introduction: The protective antioxidant effects of resveratrol (RESV) have been enlightened in endothelial cells, particularly during hypertension. However, is still uncertain whether RESV could protect hypertension-induced PVAT alteration. Increased reactive oxygen species (ROS) formation activates matrix metalloproteinases (MMP) is a possible mechanism by which hypertension induces vascular remodeling and dysfunction. Thus, RESV could decrease ROS formation and inhibiting MMP, which contribute to attenuate PVAT remodeling during hypertension. In this regard, a molecular docking study showed that RESV can inhibit MMP directly, independent of antioxidant effects. The present study evaluated if RESV inhibits MMP-2 *in vitro* and whether RESV treatment ameliorating PVAT remodeling in 2-kidney and 1-Clip (2K1C) hypertensive rats. **Methods:** Firstly, the *in vitro* effects of RESV on MMP-2 were evaluated by gelatin zymography. Plasma from rats were diluted 1: 1 with sample buffer and subjected to electrophoresis on 12% SDS-polyacrylamide gel electrophoresis co-polymerized with gelatin (0.1%) as the substrate. After electrophoresis was completed, the gel was divided in three parts and each one was incubated with activator buffer containing: (1) ethanol (vehicle), (2) RESV (0.1 mM), and (3) RESV (1 mM) for 16h. The gels were stained and destained. Gelatinolytic activities were detected as unstained bands against the background of Coomassie blue-stained gelatin. MMP-2 activity was assayed by densitometry using ImageJ program. To evaluate the effects of RESV on PVAT remodeling, 2K1C and Sham rats were treated from the fourth weeks after surgery with vehicle, RESV (40 mg/Kg/day) 3Xweek during 2 weeks. Systolic blood pressure (SBP) was monitored weekly by tail cuff plethysmography. Histological changes in PVAT from aortas were examined in sections of 4 µm stained with hematoxylin/eosin. The area of each cell individually and the number of the cells per area (500X500 pixels) was measured in the microphotograph (400X). Procedures were approved by the UNAERP Animal Research Ethics Committee (no.06/2016). Results: Highest concentration of RESV inhibited MMP-2 activity *in vitro* (from 100±4% to 72±6%). Previous data reported by our group showed that 2K1C rats had their SBP reduced if treated with RESV during four weeks. In the present study only two weeks of treatment did not change the SBP (mmHg: vehicle= 178±12; RESV= 166±8), but the protective mechanism is been started since then. Indeed, hypertension decreased the area of each cell from PVAT (47±11%) resulting in the increased number of the cells per area (195±30%) when compared with to the Sham group (118±30% and 100±14%, respectively). RESV treatment decreased these alterations in 2K1C rats (113±27% and 122±23%, respectively). **Conclusions:** RESV directly reduced MMP-2 activation *in vitro*, suggesting a possible role of this flavonoid on cardiovascular remodeling, but this mechanism needs to be evaluated. However, in line with this suggestion, RESV reversed the PVAT remodeling in hypertensive rats. Thus, the protective effects of RESV on the cardiovascular alterations could also be attributed to its effects on PVAT. **Financial support**: UNAERP and CNPq. **License number of ethics committee**: no.06/2016

06.059 Effect of caspofungin on heart rate variability in mice. Mourão RS[1], de Paula DCC[1], Araújo CM[1], Leite EA[2], Guimarães HN[3], Guimarães AG[1] [1]UFOP – Farmácia, [2]UFMG – Produtos Farmacêuticos, [3]UFMG – Engenharia Elétrica

Introduction: Cardiotoxicity was reported when caspofungin was evaluated ex vivo in rat heart and in isolated ventricular myocytes. Considering the endocarditis induced by Candida species and the use of caspofungin to treat this condition, we evaluated caspofungin *in vivo* cardiac effects on heart rate variability (HRV) using the efficacious dose against Candida albicans. HRV parameters are currently used to describe drug general effects on autonomic nervous system, mainly sympathetic and parasympathetic division. **Methods:** Female Swiss mice received by intraperitoneal route (IP) during 5 days, vehicle or caspofungin 10 mg/kg (dose previously determined *in vivo* against C. albicans). Electrocardiogram (ECG) lead II signal was obtained by telemetry on freely moving mice to measure HRV parameters, before and after (1, 6, 12 and 24 hours after the first and the fifth dose) caspofungin administration. The analyzed parameters were RR: mean of RR interval; SDNN: standard deviation of normal to normal intervals: RMSSD: root mean square of successive RR interval differences; TINN: baseline width of the triangular interpolation of the RR interval histogram; HRV Triangular Index: integral of the interval histogram divided by the histogram maximum. **Results:** No significant alteration was observed for HRV parameters on conscious mice after caspofungin. All parameters were similar after caspofungin treatment when compared to baseline values of the same group and compared to vehicle treatment at the same time. One hour after the first dose, RR (ms) was 160.6 ± 30.03 ms and 152.2 ± 14.27, SDNN (ms) was 68.4 ± 19.18 and 58.0 ± 11.61, RMSSD (ms) was 96.0 ± 27.30 and 83.8 ± 17.30, TINN (ms) was 112.6 ± 78.97 and 123.7 ± 49.24, HRV Triangular Index was 8.5 ± 4.12 and 9.5 ± 3.24, all respectively for vehicle and caspofungin treatment. Twenty-four hours after the last dose, RR (ms) was 141.8 ± 6.79 ms and 143.6 ± 12.09, SDNN (ms) was 57.0 ± 5.89 and 65.2 ± 5.98, RMSSD (ms) was 81.4 ± 8.28 and 90.4 ± 8.43, TINN (ms) was 68.1 ± 10.54 and 130.5 ± 44.85, HRV Triangular Index was 7.0 ± 1.14 and 9.5 ± 2.63, all respectively for vehicle and caspofungin treatment. For the other time evaluated and comparison with baseline there was also no significant difference. **Conclusion:** Five days mice treatment with caspofungin showed *in vivo* cardiac safety based on HRV parameters. **License number of ethics committee**: 2014/37 **Financial support**: FAPEMIG (Rede NANOBIOMG), CNPq, CiPharma (PROAP)/UFOP, PROPP/UFOP.

06.060 Role of the transcription factor NRF2 on aldosterone-induced vascular ROS generation. Rodrigues D[1], Costa RM[2], Tostes RC[1] - [1]FMRP-USP – Farmacologia, [2]UFG – Farmacologia

Aldosterone (Aldo) is a hormone synthesized in the glomeruli zone of the suprarenal cortex and is a mineralocorticoid (MR) receptor agonist. Aldo mainly controls hydroelectrolyte balance and its secretion is stimulated under low blood pressure conditions and by activation of the Renin-Angiotensin-Aldosterone System. However, chronic increases in aldosterone levels (hyperaldosteronisn) increases blood pressure and induces hypertension. In the cardiovascular system, Aldo stimulates reactive oxygen species (ROS) generation through the activation of NAD(P)H oxidase enzymes. ROS contribute to vascular dysfunction since it reduces the bioavailability of nitric oxide in endothelial cells, thereby increasing vascular smooth muscle contractile tone. Several cell types have developed adaptive programs to counteract oxidative stress. Nuclear factor erythroid 2–related factor 2 (NRF2), for example, recruits the transcriptional machinery to promote the expression of various antioxidant proteins, being one of the main factors in the adaptive response to oxidative stress. **Hypothesis:** We hypothesized that Aldo negatively regulates the antioxidant system NRF2, favoring ROS accumulation and subsequent vascular dysfunction **Methods:** In a preliminary study, endothelial cells (EA.hy926) were used to evaluate ROS production by lucigenin chemiluminescence and NRF2 activity by a nuclear translocation assay. Cells were treated with Aldo in different ranges of time and concentration, with or without eplerenone (10^{-6} M, 30 min, MR antagonist). **Results:** Aldo for 30 min stimulated the generation of ROS only in the concentration 10^{-7} M [Lucigenin (RLU) – Basal: 53.6±6.5; 10^{-9} M: 60.4±10.4; 10^{-8} M: 63.6±15.3; 10^{-7} M: 108.7±12.5]. Aldo at the concentration of 10^{-7} M increased ROS generation within 30 minutes and 1 hour. However, Aldo-induced ROS generation became even more evident after 3, 6 and 12 hours [Lucigenin (RLU) – Basal: 55.96±3.2; 30 min: 103.8±7.9; 1 h: 107.2±7.3; 3 h: 164.6±10.4; 6 h: 157.7±12.8; 12 h: 169.1±9.7]. Eplerenone prevented Aldo-induced (10^{-7} M for 30 min) increase in the generation of ROS [Lucigenin (RLU) – Basal: 53.6±6.5; Aldo: 111.2±13.1; Aldo+eplerenone: 54.3±16.6]. Stimulation of endothelial cells with Aldo increased NFR2 translocation at 30 min, 1 and 3 hours. Aldo-induced highest levels of ROS generation (3, 6 and 12 hours) was associated with decreased NRF2 activity. **Conclusion:** These data indicate that Aldo increases ROS in endothelial cells and this increase is associated with MR activation and decreased NRF2 activity. We are currently evaluating whether NRF2 activation with L-sulforaphane prevents ROS generation at later time points. **Financial support:** FAPESP, CAPES, CNPq. This study was approved by the Ethics Committee on Animal Experimentation of the Ribeirao Preto Medical School (030/2018).

06.061 Cardiovascular evaluation of the effects of quercetin and phosphodiesterase-5 inhibitor (lodenafil carbonate) on monocrotaline-induced pulmonary arterial hypertension in rats. Baptista EF[1], Menengat TA[2], Oliveira APF[1], Barbosa RAQ[1], Seara FAC[1], Alves AS[1], Oliveira DF[1], Maciel L[1], Miranda BT[1], Ponte CG[2], Nascimento JHM[1] [1]UFRJ – Biofísica, [2]IFRJ – Ciências Biomédicas Aplicadas

Introduction: Pulmonary arterial hypertension (PAH) is a disease characterized by increased pulmonary vascular resistance, which may be experimentally induced by the administration of monocrotaline. Quercetin and phosphodiesterase-5 inhibitor (IPDE-5) appear as new alternatives for the treatment of PAH because they have a vasodilatory, immunomodulatory, antiprofilerative, apoptotic and antioxidant effect. This study aimed to test the therapeutic potential of quercetin and IPDE-5, alone or in combination, in the prevention of cardiovascular changes associated with the development of monocrotaline-induced pulmonary arterial hypertension in rats. **Methods:** The study was divided into 5 experimental groups: CTRL (Control); MCT (monocrotaline); MCT+QUERC (MCT+quercetin); MCT+IPDE-5 (MCT + phosphodiesterase-5 inhibitor); and MCT+QUERC+IPDE-5. Adult male Wistar rats received a single injection of monocrotaline (60 mg/kg, i.p) and after 14 days the treatments were started: Quercetin (100 mg/kg/day, gavage) and IPDE-5 (25 mg/kg/day gavage) were injected for 14 consecutive days. The following variables were analyzed: electrocardiogram (ECG), echocardiogram (ECO), right ventricular systolic and diastolic pressure (RVSP and RVDP), physical effort test, cardiac weight, cardiac and pulmonar arterial remodeling, pulmonary protein expression of TNF-α and IL-10, and cardiac gene expression of SOD1 and 2, GPX1 and 3, Catalase, BNP, α and β-MHC. **Results:** Hypertensive animals (MCT) presented increased RVSP, RVDP and right ventricular hypertrophy, increased QTc interval, reduced vagal tone, increased heart weight corrected by body weight, decreased exercise tolerance, reduced pulmonary flow, increased TNF-α levels and increased β/α-MHC ratio. Only the MCT+QUERC group prevented increased cardiac electrical remodeling (QTc interval reduction). Both therapies (quercetin or IPDE-5) alone or in combination were able to reduce RV and pulmonary arterial remodeling evaluated by histology. In cardiac hemodynamics, only the isolated therapies reduced RVSP, however, the 3 treated groups reduced the RVDP. In ECO, only the group treated with IPDE-5 showed to be effective in increasing pulmonar vascular flow by increasing PAT (pulmonary flow acceleration time) and PAT/ET (PAT/pulmonary flow ejection time) indexes. Regarding the physical effort test, the 3 different treated groups presented better exercise tolerance. In the protein analysis of pulmonary cytokines, only the IPDE-5 group reduced levels of TNF-α and in the gene expression of antioxidant enzymes, the MCT+QUERC group only increased GPX3 levels. **Conclusion:** The combination therapy of quercetin and IPDE-5 did not potentiate the therapeutic effects of each therapy in isolation. In general, the therapeutic use of quercetin and IPDE-5 alone was more effective than when associated, being possible that some form of drug interaction happened that was unfavorable to the proposed objective. **License number of ethics committee**: CEUA CCS/UFRJ nº 087/15 **Financial support**: CNPq, Faperj, IFRJ e Capes.

06.062 Increased Matrix Metalloproteinase (MMP)-2 activity and focal adhesion kinase expression may contribute to arterial hypertrophic remodeling in renovascular hypertension. Silva PHL, Mendes AS, Castro MM, Belem-Filho IJA, Correa FMA FMRP-USP – Farmacologia

Introduction: Vascular remodeling is an active process that involves cell growth, migration and degradation of extracellular matrix in response to hemodynamic changes. Increased MMP-2 activity cleaves extracellular substrates such as collagen thus leading to the exposure of its critical structural sites. This effect may activate cell surface integrins, which triggers FAK phosphorylation and proliferative and migratory stimulus that may contribute to hypertension-induced arterial hypertrophy. Thus, the hypothesis here is to analyze whether increased MMP-2 activity contributes to hypertension-induced hypertrophic arterial remodeling by activating collagen turnover and FAK pathway. **Methods:** Rats were submitted to 2-kidneys-1Clip (2K-1C) surgery or laparotomy, without implantation of the silver clip. Systolic blood pressure was weekly monitored by tail-cuff plethysmography. Mean arterial pressure was measured by an invasive technique. Rats were treated for 8 weeks with doxycycline (30 mg/kg/day, gavage). Aortas were analyzed by histomorphometry with H&E and picrosirius, gelatin zymography and western blot for FAK and MMP-2. **Results:** There was a significant increase in the systolic blood pressure in the 2K-1C rats when compared to controls ($p<0.05$). By plethysmography, doxycycline decreased systolic blood pressure in 2K-1C rats only in the seventh week; however, by the invasive technique, treatment with doxycycline was completely able to reduce the increased mean arterial pressure in hypertension ($p<0.05$). We also investigated whether doxycycline reduces hypertrophic vascular remodeling in hypertension. Although doxycycline did not completely reduce the increased M/L in hypertensive rats, it tended to reduce cell hyperplasia and collagen deposition in aortas. The long-term use of doxycycline may be beneficial in prevent hypertension-induced development of other cardiovascular diseases. Although we did not observe a significant reduction in the aortic thickening in hypertensive rats, we still believe that this drug may reduce mechanistic parameters of cell proliferation and migration before causing any effect in aortic hypertrophy. By using gelatin zymography, we observed increased activity of MMP-2 in the aortas of hypertensive animals when compared to controls ($p<0.05$). Treatment with doxycycline did not reduce the increased activity of MMP-2 in 2K-1C rats. We also determined the protein expression of MMP-2 by western blot and doxycycline tended to reduce the increased levels of MMP-2 in 2K-1C. By contributing to the turnover of arterial collagen, MMP-2 may mediate hypertrophic signaling via FAK in the vascular smooth muscle cells. Western blotting for FAK was performed in the aortas and it was observed that doxycycline could reverse increased FAK expression in 2K-1C rats ($p<0.05$). **Conclusion:** Increased activity of MMP-2 may contribute to arterial hypertrophic remodeling in hypertension by contributing to FAK signaling and collagen turnover. **License number of ethics committee**: 119/2017 **Financial support**: CNPq and FAPESP

06.063 Pentoxifylline exerts anti-inflammatory effects, **inhibits MMP-2 activity and attenuates renovascular hypertension-induced cardiac hypertrophy**. Vitorino TR[1], Bonacio GF[1], Dellalibera-Jovilliano R[2], Franca SC[1], Prado CM[3], Rizzi E[1] [1]UNAERP – Biotechnology, [2]UNAERP –Medicine, [3]FZEA-USP – Veterinary Medicine

Introduction: Pentoxifylline (PTX) is a non-selective inhibitor of phosphodiesterases, which also inhibits cytokines. Matrix metalloproteinases (MMP) activation contributes to hypertension-induced heart remodeling and its activity may be increased by tumor necrosis factor (TNF)-α and interleukin (IL)-1β *in vitro* studies. In this regard, we hypothesized that immunomodulatory properties of PTX downregulate MMP-2 and attenuate cardiac hypertrophy induced by renovascular hypertension. **Methods:** 2-kidney and 1-Clip (2K1C) rats and Sham rats were treated 2 weeks after surgery with Vehicle or with PTX (50 or 100 mg/Kg/day) daily by four additional weeks. Systolic blood pressure (SBP) was monitored weekly by tail plethysmography. Body weight and heart weight were evaluated and used to assess cardiac index of hypertrophy. Heart expression of MMP-2 was determined by gelatin zymography assays. Plasma levels of TNF-α and IL-1β were analyzed by the enzyme-linked immunosorbent assay (ELISA). Data were considered significant when $p<0.05$. Procedures were approved by the UNAERP Animal Research Ethics Committee (protocol number: 15/2016). **Results:** Systolic blood pressure of 2K1C rats reached 196±4 mmHg after 6 weeks of hypertension, which was attenuated by the treatment with the higher dose of PTX (169±6 mmHg, $p<0.05$). The lower dose of PTX did not affect the SBP in 2K1C rats (182±8 mmHg; $p>0.05$). No difference was observed in the body weight among all the experimental groups ($p>0.05$). However, vehicle hypertensive rats showed increased heart weight when compared to the Sham group (1.16±0.03g and 0.94±0.04g, $p<0.05$). The higher dose of PTX reduced heart weight in 2K1C rats (0.99±0.03g; $p<0.05$) reducing the cardiac hypertrophy while the lower dose of PTX did not affect the heart weight in 2K1C rats ($p>0.05$). The treatment with the higher dose of PTX significantly attenuated the MMP-2 levels when compared to 2K1C (620±21 and 489±21 arbitrary unity, $p<0.05$). The lower dose of PTX did not affect the cardiac MMP-2 levels in 2K1C rats. TNF-α and IL-1β levels were increased in 2K1C rats (71±3 pg/ml and 36±1 pg/ml, respectively) when compared with the Sham group (50±1 pg/ml and 26±1 pg/ml; $p<0.05$). Besides the treatment with lower dose of PTX did not change the cytokines levels in 2K1C rats, the higher dose normalizes the TNF-α and IL-1β levels in hypertension (44±2 pg/ml and 21±1 pg/ml, respectively; $p<0.05$). **Conclusion:** The beneficial effects of PTX on cardiac hypertrophy are dose-dependent and seem to involve the reduction of blood pressure, immunomodulatory properties and cardiac MMP-2 reduction. **License number of ethics committee**: 15/2016 **Financial support**: FAPESP

06.064 Inhibition of myeloperoxidase and vascular peroxidase 1 by substituted chalcones for reduction of vascular dysfunction. de Moura GR[1], Silva MVT[2], de Souza ROMA[3], Leal ICR[4], Muzitano MF[1], Silva LL[2], Raimundo JM[1] [1]UFRJ-Macaé – Produtos Bioativos e Biociências, [2]UFRJ-Macaé – Farmácia, [3]UFRJ – Química, [4]UFRJ – Farmácia

Introduction: Oxidative stress plays a crucial role in the pathophysiology of vascular dysfunction associated with cardiovascular diseases and risk factors such as diabetes mellitus. Different studies show that enzymes such as myeloperoxidase (MPO) and vascular peroxidase 1 (VPO1) participate in vascular dysfunction by catalyzing the formation of the potent oxidant hypochlorous acid (HOCl), contributing to endothelial dysfunction, inflammation and tissue remodeling [1-2]. The aim of this work was to investigate the pharmacological potential of a series of substituted chalcones (ChC), that presents anti-inflammatory activity [3], for inhibition of MPO and VPO1 activity and reduction of high glucose-induced endothelial dysfunction. **Methods:** The effects of the ChC on peroxidative activity of MPO and VPO1 were determined by measuring the oxidation of tetramethylbenzidine (TMB), after reacting with H_2O_2, which was detected spectrophotometrically at 450 nm. The effects on the clorination cycle of MPO was determined based on the reaction of HOCl with taurine to form taurine chloramine, which oxides the chromophore (5′-thio-2-nitrobenzoic acid) TNB (412 nm) to a colorless product. The mean inhibitory concentration (IC_{50}) of ChC was determined by obtaining concentration-response curves. Vascular effects were evaluated in aortas isolated from Wistar rats prepared for isometric tension recording. Concentration-response curves for acetylcholine (ACh; 10^{-9} to 10^{-5} M) were obtained before and after incubation with control glucose (11 mM) or high glucose (44 mM) for 3 h. In parallel trials, a selected ChC (30 µM) was co-incubated for 3 h. All experimental protocols were approved by the Ethics Committee on Use of Animals of Campus UFRJ-Macaé (License MAC019). **Results:** From a group of 26 ChC, 5 were able to inhibit MPO peroxidase activity by more than 80%: ChC4, ChC19, ChC27, ChC31 and ChC32 (IC_{50}: 2.8 ± 1.7; 3.4 ± 0.1; 1.2 ± 0.1; 18.3 ± 6.2 and 38.4 ± 13.7 µM, respectively). On the MPO chlorinating activity, only 3 were statistically active, ChC4, ChC19 and ChC27. The inhibition of the peroxidase pathway of VPO1 was higher than 60% for ChC4, ChC19 and ChC27 (IC_{50}: 50.3 ± 0.1; 3.2 ± 0.1; 2.3 ± 0.1 µM, respectively). Incubation of aortic rings for 3 h with 44 mM glucose reduced the maximal relaxation induced by Ach (control: 78.3 ± 3.3%; 44 mM glucose: 58.7 ± 2.0%; $P<0.05$), without alteration of pEC_{50} (control: 6.5 □ 0.1; 44 mM; glucose: 6.8 □ 0.1; $P>0.05$). Co-incubation with ChC27 reversed the endothelial dysfunction induced by high glucose, with maximal relaxation to Ach of 85.4 ± 2.3% and pEC_{50} of 7.2 ± 0.1. **Conclusion:** ChC27 was a potent inhibitor of both MPO and VPO1 activities, which seem to be related to the substituents dimethylamine on A ring and methoxyl on B ring. ChC27 restored high glucose-induced endothelium dysfunction, indicating its pharmacological potential for vascular dysfunction associated with diabetes mellitus. **References:** [1] Tian et al., Biochem Biophys Res Commun, 484, 572, 2017. [2] Yang et al., Biochem Biophys Res Commun, 439, 511, 2013; [3] Ventura et al., Molecules, 20, 8072, 2015. **License number of ethics committee**: MAC019 **Financial support**: CNPq, CAPES

06.065 IL-1β knockout receptor prevents vascular dysfunction and remodeling in angiotensin II-induced hypertension. Fedoce-Garcia A, Pereira CA, Carneiro FS
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Introduction: Hypertension is associated with chronic low-grade inflammation, and the interleukin (IL)-1β plays a causal role in the pathogenesis development. Vascular and circulating levels of IL-1β are elevated in hypertension. Inflammasome or IL-1β inhibition results in decreased blood pressure, renal damage, and inflammation in several models of hypertension. Furthermore, IL-1β has direct effects on the vasculature upon short-term incubation, including endothelial dysfunction, increased vascular smooth muscle migration and proliferation, and augmented contractile response in denuded mesenteric resistance arteries (MRA) from hypertensive rats. While it is well established that vascular dysfunction and remodeling are critical features in arterial hypertension, it is still unclear whether IL-1β contributes to those processes. Therefore, we hypothesized that IL-1β promotes vascular dysfunction and remodeling in Angiotensin II- (ANGII)-induced hypertension. **Methods:** Subcutaneous infusion of ANGII (1µg/kg/min, for 14 days) via osmotic mini-pump was used to induce arterial hypertension in mice aged 12 weeks. C57BL/6 control mice (WT) and IL-1β receptor knockout mice (IL-1R KO) were divided into four groups: 1) WT; 2) WT + ANGII; 3) IL-1R KO; 4) IL-1R KO + ANGII. Blood pressure (BP) was measured by tail-cuff plethysmography and by intra-arterial catheter method. Vascular function and structure were accessed by isometric tension and pressure myograph, respectively, using second-order MRA. IL-1β serum levels were detected using ELISA. **Results:** IL-R1 KO and WT mice had similar systolic blood pressure levels (IL-R1 KO: 114.8 ± 2.0 vs WT: 124.1 ± 5.0; mmHg; n=6), which was measured by tail-cuff method before ANGII-induced hypertension. BP increase was not prevented in IL-1R KO one week after ANGII infusion (IL-1R KO 155.5 ± 5.5 vs WT: 166.8 ± 8.5; mmHg; n=6). Invasive BP measurement demonstrated an elevation in mean arterial pressure in WT mice after ANGII infusion (WT: 106.0 ± 4.4 vs WT + ANGII: 160.8 ± 2.3; mmHg; n=3-6). ANGII-induced hypertension reduced acetylcholine-induced vascular relaxation in WT mice, and IL-1R KO prevented this vascular dysfunction (WT + ANGII: 67.6 ± 5 vs IL-1R KO + ANGII: 119.3 ± 9.2; % of relaxation; n=5-6). MRA cross-sectional area (CSA) was increased in WT + ANGII, but IL-1R KO + ANGII mice were protected. Nevertheless, normotensive IL-1R KO displayed increased CSA when compared to WT. ANGII-induced hypertension caused a four-fold increase in IL-1β serum levels in WT mice (WT: 92 ± 21 vs WT + ANGII: 461 ± 5; pg/ml, n=3-5), but not in IL-1R KO mice (IL-1R KO + ANGII: 104.1 ± 24; pg/ml, n=4). Surprisingly, IL-1β serum levels were higher in IL-1R KO when compared to WT mice. **Conclusion:** These results indicate that IL-1β receptor absence prevents vascular injury and remodeling but not BP elevation in ANGII-infused animals. IL-1β pharmacological inhibition may represent a new therapeutic approach to treat hypertension-associated vascular dysfunction. **License number of ethics committee**: 179/2017 **Financial support**: CNPQ, CAPES, FAPESP

06.066 Verapamil decreases MMP-2 during cardiac hypertrophy in hypertension by reducing Calpain-1 activity and expression. Mendes AS[1], Omoto AC[2], Neto-Neves EM[1], Fazan R[2], Tanus-Santos JE[1], Castro MM[1] [1]FMRP-USP – Farmacologia, [2]FMRP-USP – Fisiologia

Introduction: Arterial hypertension-induced cardiac hypertrophy (CH) is characterized by thickening of the left ventricle walls and diminution of the left chamber diameter. The increased activity and expression of calpain-1 and matrix metalloproteinase (MMP)-2 were shown in different models of CH and possess relationship with the pathophysiologic changes in CH. The objective here is to analyze whether calpain-1 contributes to increase the protein levels of MMP-2 in the heart and whether this mechanism results in CH-induced cardiac changes. **Methods:** Two kidney-one clip (2K1C) hypertensive male Wistar rats (180-200g) and their respective controls (Sham) were orally treated with verapamil (VRP, 8mg/kg/bid) or vehicle during 8 weeks. Systolic blood pressure (SBP) was monitored in the rats during 10 weeks of hypertension. Left ventricle (LV) was analyzed by histology to evaluate its thickening. Calpain-1 activity was analyzed by casein zymography. Calpain-1 and MMP-2 expression were analyzed by Western Blot. Hearts were submitted to morphological and functional evaluation by echocardiography. All the protocols were approved by Ethical Committee in Animal Research of Ribeirao Preto Medical School (43/2017). **Results:** After 10 weeks, the SBP had sustained increase and treatment with VRP was not able to decrease it in any time of hypertension (Sham: 120.80±5.43; Sham+VRP: 116.35±3.29; 2K1C: 208.68±7.37; 2K1C+VRP: 211.01±6.77, n=9-10, $p<0.05$ 2K1C vs Sham). Analysis of ventricle mass and thickening showed that VRP reverted CH-induced pressure overload (Sham: 2.33±0.14; Sham+VRP: 2.54±0.16, 2K1C: 2.94±0.24; 2K1C+VRP: 2.25±0.16, n=4-8, $p<0.05$ 2K1C vs all groups). It was observed increased calpain-1 activity and expression in 2K1C rats when compared to sham groups and VRP decreased it (Sham: 750.80 ± 48.10; Sham + VRP: 679.00 ± 39.40; 2R-1C: 1470.00 ± 112.20; 2R-1C + VRP: 921.80 ± 154.50; n = 4-7, $p<0.05$ 2K1C vs all groups). It was also observed increased MMP-2 expression in hypertrophied hearts and VRP decreased it. Data obtained by echocardiography corroborated with the cardiac morphology showed by histology in hypertensive animals and VRP decreased all the hypertrophied changes. **Conclusion:** Increased calpain-1 and MMP-2 expression seem to be involved with renovascular hypertension-induced CH. VRP administration suggests that calpain-1 activity and expression are regulated by calcium and may alter MMP-2 expression in these hearts. **Financial support**: CAPES, CNPq, FAPESP. **License number of ethics committee**: 43/2017

06.067 *Talinum paniculatum* (**Jacq**.) **Gaertn**. **as a potent diuretic agent**. Romão PVM[1], Tolouei SEL[2], Tirloni CAS[1], Marques AAM[1], Guarnier LP[1], Schaedler MI[1], Passoni MT[2], Curi TZ[2], Silva GN[2], Grechi N[2], Dalsenter PR[2], Gasparotto Junior A[1] [1]UFGD – Farmacologia, [2]UFPR – Farmacologia

Introduction: *Talinum paniculatum* (Jacq.) Gaertn. (Talinaceae), popularly known as "major gomes", is a weedy species widely used as food source. In Mato Grosso do Sul State (Brazil), leaf infusion of this species is prescribed as diuretics by local healers. However, there are no reports in the literature proving its cardiorenal effects. For this reason, we aimed to evaluate the diuretic potential of *T. paniculatum* after prolonged (7 days) exposure. **Methods:** First, *T. paniculatum* leaves were dried in an air circulation oven for 5 days and then grounded into powder form. The infusion was prepared by adding 1 liter of boiling water to each 100 g of powder. The infusion was treated with 3 volumes of EtOH, which gave rise to a precipitate and an ethanol soluble fraction (ESTP). Then, thirty fasted (12 hours) male rats (n = 6), received oral doses of saline solution (0.9% NaCl; 5 mL/100 g) before treatments in order to impose body uniformity of salt and water. Following salinization, animals received daily doses of ESTP (30, 100 or 300 mg/kg), hydrochlorothiazide (HCTZ, 25 mg/kg) or vehicle (1 mL/100 g) by gavage. Immediately after the first treatment, animals were individually placed in metabolic cages and urine was collected every 24 hour on days 1, 3 and 7. Density, pH, volume and electrolyte concentrations (Na^+, K^+ and Cl^-) were determined. At the end of the experiments, animals were euthanized by isoflurane anesthesia (inhalation) followed by exsanguination. All procedures involving animals were previously approved by the Ethics Committee in Animal Experimentation from the Federal University of Paraná (protocol: 05/2017) and Federal University of Grande Dourados (protocol: 21/2017). **Results:** All doses tested (30, 100 and 300 mg/kg) increased diuresis on day 1 (10.22 ± 0.94, 10.10 ± 0.72, 9.15 ± 0.60 ml/100g/8h, respectively), 3 (17.49 ± 1.588, 19.89 ± 1.63, 17.37 ± 0.88 ml/100g/8h, respectively) and 7 (25.73 ± 2.22, 30.17 ± 2.54, 25.89 ± 1.08 ml/100g/8h, respectively) after treatments and were statistically different from the control group (day 1: 4.11 ± 0.30 ml/100g/8h; day 3: 7.38 ± 0.40 ml/100g/8h and day 7: 14.10 ± 0.98 ml/100g/8h). ESTP 30 and 100 mg/kg were able to increase Na^+ excretion on day 3 (1.85 ± 0.35 and 2.37 ± 0.07 mmol/L/8h) and 7 (4.08 ± 0.37 and 4.66 ± 0.10 mmol/L/8h) after administration. ESTP 100 and 300 mg/kg increased K^+ (4.49 ± 0.17 and 4.49 ± 0.32 mmol/L/8h) and Cl^- (5.14 ± 0.20 and 5.06 ± 0.26 mmol/L/8h) excretion on day 3 and all doses were able to increase K^+ (7.56 ± 0.73, 8.66 ± 0.31 and 9.44 ± 0.80 mmol/L/8h) and Cl^- (8.52 ± 1.02, 9.62 ± 0.24 and 10.24 ± 0.72 mmol/L/8h) excretion on day 7. In addition, ESTP 30 and 100 mg/kg significantly decreased pH values on day 7 (11.1 ± 0.41 and 10.85 ± 0.66, respectively). As expected, HCTZ was able to significantly increase diuresis (15.50 ± 1.62 and 33.16 ± 3.31 ml/100g/8h) and electrolyte contents such as Na^+ (3.2 ± 0.24 and 7.6 ± 0.45 mmol/L/8h), K^+ (4.3 ± 0.5 and 11 ± 0.79 mmol/L/8h) and Cl^- (4.9 ± 0.3 and 13.9 ± 0.75 mmol/L/8h) in urine samples on days 3 and 7. No changes were observed in urine density. **Conclusion:** Data presented show important information about the ethnomedicinal properties of *T. paniculatum*. In addition, the study presents the ESTP as a possible herbal medicine, especially when a sustained diuretic effect is required. **License number of ethics committee**: 05/2017 and 21/2017 **Financial support**: This work was supported by FUNDECT, CNPq and CAPES.

06.068 Contribution of the NLRP3 Inflammasome to vascular dysfunction induced by supraphysiological levels of testosterone. Alves JV[1], Costa RM[2], Pereira CA[1], Souza NL[3], Tostes RC[1] [1]FMRP-USP – Farmacologia, [2]UFG – Farmacologia, [3]UFG – Fisiologia

Introduction: Testosterone modulates vascular tone and cardiac performance. Both supraphysiological and subphysiological testosterone levels are associated with increased cardiovascular risk. Athletes who use testosterone, usually at supraphysiological doses, exhibit increased blood pressure, left ventricular hypertrophy, increased inflammatory markers and vascular dysfunction. Activation of NLRP3 inflammasome, a component of the innate immune system, contributes to the production of proinflammatory cytokines, leading, among other effects, to vascular dysfunction. In this context, we hypothesized that supraphysiological levels of testosterone promote generation of mitochondrial reactive oxygen species (mROS), NLRP3 inflammasome activation and vascular dysfunction. **Methods:** Male, 12 week-old C57Bl/6J (WT) mice were used. Vessels were incubated with testosterone (10^{-6} M, 2 hours). In addition, mice were treated with testosterone propionate [TP (10 mg/kg)] or vehicle (Veh) for 30 days. Vascular function was evaluated in thoracic aortic rings, using a myograph system. Concentration-response curves for phenylephrine (PE) and acetylcholine (ACh) were performed in vessels with endothelium. To assess whether NLRP3 contributes to testosterone effects, NLRP3 knockout (NLRP3-/-) mice were used. **Results:** Vessels from WT mice incubated with testosterone exhibited increased aortic contractile responses to PE [(mN) Vehicle: 5.7 ± 0.1 n=10 *vs*. Testosterone: 9.0 ± 0.2 n=8] and this effect was prevented in NLRP3-/- mice [(mN) 6.2±0.2 n=3]. Furthermore, testosterone reduced ACh-induced vasodilation [Values are expressed relatively to the contraction triggered by PE 2 µM (% Relaxation) – Vehicle: 92.3 ± 2.7 n=10 *vs*. Testosterone: 52.0 ± 2.6 n=8] and this effect was almost completely prevented in NLRP3-/- mice (87.2 ± 1.2 n=4). Treatment of WT mice with TP increased aortic contractile responses to PE [(mN) Vehicle: 6.4 ± 0.3 n=10 *vs*. TP: 10.6 ± 0.3 n=8] and this effect was prevented in NLRP3-/- mice [(mN) 6.8 ± 0.2 n=5]. TP also decreased ACh-induced dilation in arteries from control mice [(% Relaxation) – Vehicle: 89.5 ± 1.5 n=10 *vs*. TP: 70.3 ± 2.2 n=8], but not in arteries from NLRP3-/- mice (86.5 ± 2.6 n=6). Testosterone *in vitro* (arteries incubated with testosterone) and *in vivo* (treatment of mice with TP) increased vascular mROS, determined by lucigenin and DHE fluorescence. mROS generation was prevented by carbonyl cyanide m-chlorophenyl hydrazone (CCCP, 10^{-7} M 30 min), a mitochondrial uncoupler. Furthermore, aortas incubated with testosterone and mice treated with TP showed increased expression of Caspase-1, as determined by western blot, and this effect was prevented by CCCP. **Conclusion:** These data indicate that supraphysiological levels of testosterone induce vascular dysfunction via mROS generation and NLRP3 inflammasome activation. **License number of ethics committee**: 032/2018 **Financial support**: FAPESP, CAPES, CNPq.

06.069 Disruption of the enterosalivary circuit of nitric oxide inhibit antihypertensive efects of L-Arginine in 2K1C model. Batista RIM[1], Nogueira RC[1], Ferreira GC[1], Oliveira-Paula GH[2], Damacena de Angelis C[1], Pinheiro LC[1], Tella SOC[1]
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Introduction: Nitric oxide (NO) is produced by nitric oxide synthases (NOS), having as the only substrate for this production the amino acid L-arginine. In addition to this pathway, the bioavailability of NO can be increased by the enterosalivary cycle of nitrite in which dietary nitrate is converted to nitrite by the action of oral bacteria with nitrate reductase activity and this nitrite is swallowed and converted to NO in the acidic environment of the stomach. This NO can be oxidized to nitrite and this to nitrate, restarting the cycle. Thus, the hypothesis is that mouthwash (MW) would attenuate the beneficial effects of L-arginine in hypertension**. Methods:** Hannover rats were divided into 4 groups SHAM and 4 groups 2k1C (2 kidney 1 Clip). Treatment with L-arginine (in the drinking water 10mg / mL) and / or MW (applied to the oral mucosa with the aid of a swab) was done during four weeks. Rats from the groups **2K1C+Vehicle** and **SHAM+Vehicle** received only water. All procedures were approved by the CEUA of the USP campus of Ribeirão Preto (Protocol nº 142/2017). **Results:** The nitrate reductase activity of oral bacteria was decreased by the use of MW (t test, p=0.0273). Systolic blood pressure increased significantly in the 2K1C rats compared to the Sham group in the first week of hypertension ($p<0.05$). Treatment with L-arginine was able to lower blood pressure in all treatment weeks ($p<0.05$). The treatment with MW was not able to cause changes in the pressure in both the Sham and 2K1C rats, but it significantly reversed the effect of L-arginine in all weeks of treatment ($p<0,05$). Regarding aortic reactivity, hypertension was able to decrease relaxation to acetylcholine, shifting the curve to the right, causing impairs in both pD2 (negative log of the concentration that produces half of the maximum effect) and in Emax (maximum effect) (pD2=7,9210±0,0564 e Emax=97,960±1,593 para o Sham+Vehicle e pD2=7,3960±0,1506 e Emax=74,760±3,888 for the group 2K1C+Vehicle, $p<0,05$). L-arginine was able to improve the vascular function of hypertensive rats, making the acetylcholine relaxation response similar to that of Sham groups (pD2=7.525±0.158 and Emax=91.090±4.459, $p<0.05$ relative to the 2K1C+Vehicle group) and concomitant administration with MW reversed this improvement (pD2=0.0790±0.0874 and Emax=79.210±2.582, $p<0.05$ relative to the 2R1C+L-arginine group). Regards to plasma nitrite and nitrosothiois concentrations, treatment with L-arginine in hypertensive rats increased nitrite concentrations $F_{(1.21)}=4.466$, p=0.0467 compared to the Sham+L-arginine group. The concentration of nitrosothiois showed a tendency to increase in hypertensive rats treated with L-arginine in relation to the other study groups, but this trend was not statistically significant. **Conclusion:** In our study, L-arginine was able to reduce blood pressure and improve vascular function in hypertensive rats and the MW was able to reverse these effects. Therefore, these results suggest that maintenance of the enterosalivary cycle is important for the antihypertensive effect of L-arginine. **License number of ethics committee**: 142/2017 **Financial support**: Cnpq Capes Fapesp

06.070 Alterations in the local renin-angiotensin system of rat major salivary glands after losartan administration. Cano IP[1], Dionísio TJ[2], Cestari TM[2], Santos CF[2]
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Introduction: Patients taking anti-hypertensive drugs usually claim of hyposalivation as a side effect. This research aimed to characterize the local renin-angiotensin system (RAS) in rat major salivary glands in regular conditions and in comparison, with a group of animals taking a recommended daily dose of losartan for 7 days. **Methods:** After ethics committee approval, 14 rats were studied (2 groups, n=7 in each). After euthanasia, parotid, sublingual and submandibular glands were dissected and used for quantitative polymerase chain reaction (qPCR) and immunohistochemical (IHC) preparation and analyses. Renin (REN), Angiotensin Converting Enzyme (ACE), ACE2, Angiotensin-II Receptor (AT)-1, AT2 and MAS Receptor (MASr) were the targets for both analyses. ANOVA was performed and the differences between groups were identified by Bonferroni's post-test at a significance level of $p<0.05$. **Results:** Losartan treatment caused a statistically relevant decrease in parotid MASr as well as submandibular AT1, AT2 and ACE. **Conclusion:** losartan evoked significant alterations in the local RAS of rat major salivary glands, which could be involved in local control of blood pressure and water balance modifications. Since saliva production also depends on these vascular mechanisms, future studies should evaluate whether parotid and submandibular local RAS components contribute to saliva control. **License number of ethics committee**: CEEPA FOB USP 015/2013 **Financial support**: FAPESP 2014/12975-9, FAPESP 2015/03965-2 and FAPESP 2017/17747-2

06.072 Serum from preeclamptic patients who ingested acute grape juice increased nitric oxide and decreased heme oxigenase-1 production in endothelial cells. Caldeira-Dias M[1], Machado JSR[2], Cavalli R[2], Sandrim V[1] [1]IBB-Unesp – Farmacologia, [2]FMRP-USP – Ginecologia e Obstetrícia

Introduction: Preeclampsia is characterized by hypertension and proteinuria at ≥ 20 weeks of gestation and it is the leading cause of fetal-maternal morbidity and mortality worldwide (Report of the National High Blood Pressure Education Program Working Group. Am J Obstet Gynecol, v.183, p.1, 2000; WHO. Am J Obstet Gynecol, v.158, p.80, 1988). The pathophysiology of this syndrome is complex and involves several processes. One of these, widely validated in the literature is the oxidative stress, which is characterized by the prevalence of free radical production and/or reduction of antioxidant activity (Williamson, RD. Pregnancy Hypertens, v.8, p.1, 2017). Recently, grape juice has been studied for its antioxidant potential, in addition to its anti-inflammatory and hypolipidemic properties (Castilla, P. Am J Clin Nutr, v.84, p.252, 2006). Grape juice is rich in polyphenols, such as resveratrol, which has been shown to present a protective effect in preeclampsia by regulating nitric oxide (NO) and heme oxygenase-1 (HO-1) production (Ungvari, Z. Am J Physiol Heart Circ Physiol, v.299, p.18, 2010; Hannan, NJ. Sci Rep,v.7, p.1819, 2017). However, there are no studies on the role of grape juice in preeclampsia. We examined the effect of serum from preeclamptic patients before and after one hour of grape juice intake on cell viability, NO, ROS and HO-1 productionin human umbilical vein endothelial cells (HUVECs). **Methods:** HUVECs were incubated with 10% (v/v) serum collected from preeclamptic patients before (0h group; n=4) and after one hour (1h group; n=4) of whole red grape juice intake (200 mL) for 24h. Prior grape juice intake, women were in a fast period of approximately 12 hours.Cell viability and ROS production were measured in the cells by PrestoBlue and DCFH intracellular fluorescence dyerespectively. Nitrite (NO primary metabolite) and HO-1 concentrationswere measured in cell supernatants by Griess assay and ELISA respectively. **Results:** Cell viability and ROS production were not significantly different between HUVECs incubated with serum from 0h and 1h groups (96.5±0.6 vs. 84,4±9.9 % viability, p=0.375, respectively; 3357±326 vs. 3663±144 fluorescence intensity, p=0.375, respectively). Nitrite production was increased in 1h compared to 0h (0.938±0.039 vs. 0.852±0.023 □M, p=0.017, respectively), whereas HO-1 concentration was decreased (229.3±4.3 vs. 276.0±12.6 pg/mL, p=0.0004, respectively). **Conclusion:** After one hour of acute grape juice intake, serum from preeclamptic patients was able to increase NO production in endothelial cells, suggesting a beneficial effect of the grape juice, especially regarding endothelial dysfunction known to be involved in preeclampsia. The decrease in HO-1 concentration may be because its induction occurs as a result of an oxidative status, which may be lower after one hour of grape juice intake. **License number of ethics committee**: process number 2.602.100/2018 - Hospital das Clínicas de Ribeirao Preto **Financial support**: São Paulo Research Foundation (FAPESP) #2015/20461-8, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and The Brazilian National Council for Scientific and Technological Development (CNPq) #2014-5/305587

06.073 Activation of the cold-sensing TRPM8 channel triggers relaxation of internal pudendal artery demonstrating increased sensitivity in the spontaneously hypertensive rats: **Is it a new target to erectile dysfunction**? Silva DF[1], Wenceslau CF[2], McCarthy CG[2], Szasz T[2], Ogbi S[2], Webb RC[2] [1]UFBA – Fisiologia e Farmacologia, [2]UFBA – Fisiologia

Erectile dysfunction (ED) is frequently encountered in patients with arterial hypertension. Due to low pharmacological response to phosphodiesterase type 5 (PDE-5) inhibitors in patients with vascular endothelial damage, the search for new drugs and therapeutic targets is of paramount importance to ED treatment. The aim of this study was to investigate the expression and function of cold-sensing TRPM8 channel in internal pudendal artery (IPA) as well as to clarify the mechanism of action involved on the observed responses in both normotensive and hypertensive rats. For this, we performed experiments integrating physiological, pharmacological, biochemical and cellular techniques, to better understand the effects of TRPM8 activation on the penile function in hypertensive rats. All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH) and were reviewed and approved by the Institutional Animal Care and Use Committee of Augusta University. Our results demonstrated the expression of TRPM8 channels in the IPA by western blotting and immunofluorescence technique. Furthermore, we observed that these channels are also expressed in vascular smooth muscle cells from IPA. In addition, TRPM8 activation, by both a cooling compound icilin (10^{-8} – 10^{-4} M) and cold temperature (thermal stimulus), induced vasorelaxation in IPA pre-contracted by phenylephrine (10^{-6}M). All these effects were reduced by BCTC (2x10-6 M), a TRPM8 blocker. Furthermore, the results showed that the concentration-response curve to icilin was shifted to the right in different conditions, such as: the absence of the vascular endothelium, in the presence of L-NAME (10^{-4} M), or indomethacin (10^{-5} M) or by a combination of charybdotoxin (10^{-7} M) and apamin ($5x10^{-6}$ M). These data indicate that endothelium-derived relaxing factors (EDRFs) are important for the vasodilator effect induced by TRPM8 activation. However, no changes were observed in the icilin-induced effect in the presence of iberiotoxin (10^{-7} M) and TEA (3 mM), both BKCa channels blockers, suggesting that, at least in IPA, this signaling pathway is not involved after subsequent activation of TRPM8. Interestingly, icilin-induced vasodilation was significantly higher in IPA from spontaneously hypertensive (SHR) compared to normotensive wistar rats. However, we could not observe significant difference of the TRPM8 expression in IPA from SHR and wistar, suggesting that probably the sensitivity of TRPM8 channels seems to be higher in pudendal arteries from hypertensive compared to normotensive rats. In conclusion, the data demonstrates, for the first time, the expression and function of TRPM8 channels in the pudendal artery, emphasizing the importance of these channels in the penile vasculature, the mechanism of action underlying their activation and increased sensitivity of these channels in IPA from hypertensive rats, and all together, demonstrating the potential of this channel as a new target for the treatment of hypertension associated-ED. **Financial support**: NIH, CNPq and CAPES. **License number of ethics committee**: Não apresenta numeração. Aprovação americana realizada pela Augusta University **Financial support**: NIH, CNPq and Capes.

06.074 *O*-GlcNAcylation of endothelial nitric oxide synthase compromises the anti-contractile effect of perivascular adipose tissue in metabolic syndrome Costa RM[1], Silva JF[2], Alves JV[2], Dias TB[2], Rassi DM[2], Garcia LV[3], Lobato NS[1], Tostes RC[2] [1]UFJ – Ciências da Saúde, [2]FMRP-USP – Farmacologia, [3]FMRP-USP – Biomecânica, Medicina e Reabilitação do Aparelho Locomotor

Introduction: Under physiological conditions, the perivascular adipose tissue (PVAT) negatively modulates vascular contractility. This property is lost in experimental and human obesity and in the metabolic syndrome, indicating that changes in PVAT function may contribute to vascular dysfunction associated with increased body weight and hyperglycemia. The *O*-linked β-N-acetylglucosamine (*O*-GlcNAc) modification of proteins (*O*-GlcNAcylation) is a unique posttranslational process that integrates glucose metabolism with intracellular protein activity. Increased flux of glucose through the hexosamine biosynthetic pathway and the consequent increase in tissue-specific *O*-GlcNAc modification of proteins have been linked to multiple facets of vascular dysfunction in diabetes and other pathological conditions. **Hypothesis:** We hypothesized that chronic consumption of glucose, a condition that progresses to metabolic syndrome, leads to increased *O*-GlcNAc modification of proteins in the PVAT, decreasing its anti-contractile effects. Therefore, the current study was devised to determine whether a high-sugar diet increases *O*-GlcNAcylation in the PVAT and how increased *O*-GlcNAc interferes with PVAT vasorelaxant function. **Methods:** To assess molecular mechanisms by which *O*-GlcNAc contributes to PVAT dysfunction, thoracic aortas surrounded by PVAT were isolated from Wistar rats fed either a control or high sugar diet, for 10 and 12 weeks. **Results:** Rats chronically fed a high sugar diet exhibited metabolic syndrome features, increased *O*-GlcNAcylated-proteins in the PVAT and loss of PVAT anti-contractile effect. PVAT from high sugar diet-fed rats for 12 weeks exhibited decreased NO formation, reduced expression of endothelial nitric oxide synthase (eNOS) and increased *O*-GlcNAcylation of eNOS. High sugar diet also decreased OGA activity and increased superoxide anion generation in the PVAT. Visceral adipose tissue samples from hyperglycemic patients showed increased levels of *O*-GlcNAc-modified proteins, increased ROS generation and decreased OGA activity. **Conclusion:** These data indicate that *O*-GlcNAcylation contributes to metabolic syndrome-induced PVAT dysfunction and that *O*-GlcNAcylation of eNOS may be targeted in the development of novel therapies for vascular dysfunction in conditions associated with hyperglycemia. **License number of ethics committee**: 206/2016 **Financial support**: FAPESP-CRID 2013/08216-2

06.075 HFD-induced kidney alterations in rats with metabolic syndrome: **Decreased anti-oxidant defence and endothelial dysfunction**. Flores EEI[1], Pereira ENGDS[1], Silvares RR[1], Rodrigues KL[1], Albuquerque CFG[2], Albuquerque CFG[2], Neto HCDCF[2], Daliry A[1] [1]Fiocruz – Investigação Cardiovascular, [2]Fiocruz – Imunofarmacologia

Introduction: The excess of caloric intake characteristic of western diets is linked to obesity and the metabolic syndrome (MS) worldwide. MS is a risk factor for cardiovascular disease, type 2 diabetes, cirrhosis and chronic kidney disease (CKD). Excess accumulation of lipids in non-adipose tissues may lead to a lipotoxicity with increase of advanced glycation end products (AGEs) deposition, which in turn can contribute to progressive decrease of renal function. This study aims to investigate the presence of renal endothelial dysfunction in rats with MS induced by high fat diet (HFD) intake. **Methodology:** In Wistar rats, the MS animal model was induced by 20 weeks of HFD feeding and biochemical markers, oxidative stress, inflammation and microcirculatory parameters were analyzed at the end of the protocol. Tissue perfusion in renal microcirculation was examined by laser speckle contrast imaging (LSCI). Oxidative stress was analyzed by TBARs and the inflammatory markers by ELISA (TNF-α and IL-1β). RT-PCR was used to analyze gene expression of eNOs, IL-6, VCAM, NADPH oxidase, catalase and receptor for AGE (RAGE) gene expression. **Results:** The group of rats with MS showed decrease in catalase mRNA transcripts (CTL 1.0 ± 0.1 *vs* HFD 0.7 ± 0.1) and catalase enzyme activity (CTL 3.9 ± 0.8 *vs* HFD 1.6 ± 0.4) compared to CTL animals. There were no significant changes in NADPH gene expression or MDA content in kidneys of MS-induced rats. Concerning inflammatory markers in the kidney, there was a significant increase of IL-1B in MS-rats compared to CTL animals ($P<0.05$) (CTL 6.5 ± 1.7 *vs* HFD 9.1 ± 3.4), while there were no significant changes in TNF-alfa and IL-6 mRNA transcript levels in kidneys of MS group of rats. Concerning the endothelial dysfunction, while kidneys of CTL rats showed an increase of 12% in microvascular blood flow in response to acetylcholine (Ach), HFD-induced MS rats showed a significant decrease in microvascular blood flow after Ach administration, with a reduction of 26% in microvascular blood flow. Both eNOS and VCAM gene expression were not altered in kidneys of MS-rats. The participation of AGE-RAGE axis was evaluated in Kidneys of HFD-induced MS rats, and we observed that kidney AGE content was less abundant in MS rats compared with CTL animals (CTL 0.8 ± 0.03 *vs* HFD 0.7 ± 0.3). The RAGE gene expression was not altered among the studied animal groups. Biochemical analyses did not shown evidences of microalbuminuria. **Conclusions:** Decreased anti-oxidant defense and endothelial dysfunction are involved in HDF-induced renal damage. Further experiments are needed to elucidate the role of AGE-RAGE axis in renal alterations induced by HFD-feeding. **License number of ethics committee**: CEUA license L-034/2016 **Financial support**: CNPQ, FAPERJ e PAPES/FIOCRUZ