

## 06. Cardiovascular and Renal Pharmacology

**06.001 Electrical field stimulation induces endothelium-dependent contraction of human umbilical cord vessels.** Britto Júnior J<sup>1</sup>, Jacintho FF<sup>1</sup>, Murari GF<sup>1</sup>, Campos R<sup>1</sup>, Moreno RA<sup>2</sup>, Antunes E<sup>1</sup>, Mónica FZ<sup>1</sup>, De Nucci GD <sup>1</sup>Unicamp, <sup>2</sup>UECE

Electrical field stimulation (EFS) has been used for decades in classical pharmacological preparations in order to characterize the mediators released by neural endings involved in smooth muscle contraction or relaxation. Since most part of the human umbilical cord has no innervation, EFS has never been used in this preparation. The aim of this study was to investigate the effect of EFS in human umbilical cord vessels. Segments of the human umbilical cord were obtained from normotensive parturient and the human umbilical artery (HUA) and the human umbilical vein (HUV) were isolated and mounted in organ bath chambers. Electrical field stimulation-induced contractions in both HUA ( $2.35 \pm 1.31$  mN and  $3.77 \pm 2.31$  mN for 8 Hz and 16 Hz respectively, n=24) and HUV ( $3.81 \pm 2.54$  mN and  $6.26 \pm 4.51$  mN for 8 Hz and 16 Hz respectively, n=25). The addition of tetrodotoxin did not alter the EFS-induced contractions in both tissues (n=4). The endothelium removal almost abolished the EFS-induced contractions in both vessels (n=5). In sandwich preparation, *donor* tissue (with endothelium) released a factor(s) that promoted contraction of the *recipient* tissue (endothelium removal) in both HUA and HUV (n=3, respectively). The nature of the factor(s) released by endothelium remains to be characterized. **Keywords:** Tetrodotoxin; ATP; L-NAME; Serotonin; Smooth muscle.

**06.002 Causes of cardiac decompensation and its influence on the mortality of hospitalized patients with heart failure in General Hospitals in Maceió, AL.** Silva RZ, Rivera IR, Mendonça MA, Galdino EBT, Oliveira-Filho AD, Neves SJF, Costa FA UFAL

**Introduction:** Heart failure (HF) is a complex clinical syndrome in which the heart is unable to eject enough blood to meet the metabolic needs of tissues or to do so only at high filling pressures. The identification of the etiology and causes of decompensation of HF can have a decisive impact on the treatment and prognosis of the disease (Rohde LE, et al). **Objective:** To identify the causes of decompensation that led to the hospitalization of adult patients with HF in general public and private hospitals that provide services to the Sistema Único de Saúde (SUS), as well as to try to indicate their influence on the main outcome - death - of such patients. **Methods:** A prospective, cross-sectional study was carried out at Hospital Geral do Estado de Alagoas (HGE-AL), Hospital Universitário da UFAL, and Hospital Veredas, Maceió - AL, all providers of SUS services. Patients were divided into two groups: 1) death group and 2) surviving group. The main factors of decompensation of HF were identified and comparisons were made between groups. Categorical variables were compared by chi-square or Fisher's exact tests, with significant correlations being considered at  $p < 0.05$ . **Results:** Between August 2014 and July 2015, 237 patients with NYHA functional class IV had the following characteristics: mean age =  $63 \pm 15$  years, men (52.7%), hypertensive patients (74.3%), diabetics (35.4%); (36.4%), hypertensive (31.3%), idiopathic (14.5%), chagasic (10.5%) and valvular (7.3%) etiologies; mean ejection fraction =  $38.0\% \pm 14.0\%$ . Dyspnea was the main symptom reported (97.5%) and previous hospitalizations reported 89.9% of the patients. Medications in use (non-exclusive): angiotensin receptor blocker (65.8%), diuretics (59.7%), beta-blockers (57.3%), spironolactone (46.3%), angiotensin converting enzyme inhibitors (45.1%), digital (25.6%) and nitrates (9.7%). Comparing the main decompensation factors of HF between group 1 (death,  $n = 49$  or 20.7% of the sample) and group 2 (survivor,  $n = 188$  or 79.3% of the sample) were the following results found. Infections: 31 X 64,  $p < 0.001$ ; renal injury (creatinine  $> 1.4$  mg/dl): 15 X 31,  $p = 0.01$ ; acute coronary syndromes: 12 X 56,  $p = 0.23$ ; pulmonary edema: 3 X 6,  $p = 0.18$ ; tachyarrhythmias: 10 X 39,  $p = 0.48$ ; bradyarrhythmias: 2 X 4,  $p = 0.36$ . **Conclusion:** Hospital mortality was very high (20.7%) when compared to that reported in international registries (4.0%). Among the decompensation factors, only infections and renal injury presented a statistically significant relation regarding the influence on the mortality of the study population. **Reference:** Rohde LE, Montera MW, Bocchi EA, Clausell NO, Albuquerque DC, Rassi S, et al. Diretriz Brasileira de Insuficiência Cardíaca Crônica e Aguda. Arq Bras Cardiol. 2018; 111(3): 436-539. Ethics Committee: Approved under opinion 779.353/2014.

**06.003 AAL 195, a phosphodiesterase-4 inhibitor, induces hypotensive and vasorelaxant effects in SHR.** Silva JCG, Bernardino AC, Paulino ET, Oliveira KRV, Machado MLDP, Rodrigues AKBF, Vieira SP, Araújo Júnior JX, Schmitt M, Ribeiro EAN UFAL

**Introduction:** Hypertension is one of the main risk factors for the development of cardiovascular diseases (SBC, 2016), and even many antihypertensive drugs available, many patients do not reach ideal blood pressure levels (CONSOLIM-COLOMBO, 2011). Therefore, phosphodiesterase (PDE) inhibitors may appear as a new option for treating hypertension. PDEs are enzymes that selectively hydrolyze cAMP/cGMP. Studies show that PDE inhibitors may be useful in the therapy of diseases of multifactorial origin, such as hypertension (LUGNIER, 2006). Therefore, the objective of this work is to evaluate the actions of a PDE4 inhibitor, AAL 195, on the cardiovascular system of SHR rats.

**Methods:** Approval by the ethics committee for animal experimentation UFAL: n° 15/2018. Male spontaneously hypertensive rats were used for all experiments. For measurement blood pressure (BP) and heart rate (HR) catheters were inserted in the abdominal aorta and inferior vena cava, and after 24 hours, the experiments were performed. For evaluation of vascular reactivity, superior mesenteric arteries rings were maintained in organ bath solution until use. The results were expressed as mean  $\pm$  s.e.m. All analysis was performed using GraphPad™ Prism 5.0®. **Results:** In non-anesthetized rats, AAL 195 (0.1; 0.5; 1 and 5 mg/kg, i.v.) promoted a dose-independent reduction of systolic ( $-20.7 \pm 2.2$ ;  $-24.8 \pm 2.0$ ;  $-23.0 \pm 3.9$ ;  $-34.6 \pm 3.8$  %, respectively), diastolic ( $-31.0 \pm 1.2$ ;  $-34.4 \pm 2.8$ ;  $-38.4 \pm 6.0$ ;  $-51.3 \pm 4.2$  %, respectively) and mean ( $-24.6 \pm 1.7$ ;  $-28.5 \pm 2.1$ ;  $-28.9 \pm 4.6$ ;  $-40.9 \pm 3.8$  %, respectively) arterial pressure. However, it promoted an increase in heart rate ( $7.8 \pm 2.5$ ;  $13.4 \pm 3.7$ ;  $10.9 \pm 3.3$ ;  $12.1 \pm 3.8$  %, respectively), probably a reflex tachycardia. The vascular effect was investigated, AAL 195 ( $10^{-9}$ - $3 \times 10^{-5}$  M) induced vasorelaxation ( $E_{max}$ :  $114.63 \pm 6.6$  % and  $pD_2$ :  $-6.43 \pm 0.10$  M). Removal of the endothelium attenuated the effect promoted by AAL 195 ( $E_{max}$ :  $95.50 \pm 1.54$  %,  $p < 0.05$ ), without displacement of the curve to the right ( $pD_2$ :  $-6.25 \pm 0.05$  M). To assess the specificity of the vasorelaxant effect, a concentration-response curve to the AAL 195 was obtained in the presence of 80 mM KCl. Under these conditions, AAL was able to promote vasorelaxant effect ( $E_{max}$ :  $100.62 \pm 1.19$  %), but significantly reduced pharmacological potency ( $pD_2$ :  $-6.25 \pm 0.05$  M). Therefore, AAL 195 promotes vasorelaxative effect in an unspecific way and probably there is the partial involvement of  $Ca^{2+}$  channels in this effect. These results corroborate the vasorelaxant effect found in wistar rats, where AAL 195 promoted a decrease in intracellular  $Ca^{2+}$  influx (SILVA, 2017). **Conclusion:** AAL 195 promoted a hypotensive effect mediated in part by vasorelaxation endothelium-dependent and partial involvement of  $Ca^{2+}$  channels. **Key words:** Hypotension. Phosphodiesterase-4 Inhibitors. Vasodilation. **Financial support:** FAPEAL, CAPES, CNPq and UFAL **References:** CONSOLIM-COLOMBO, F. M. et al. Rev Bras Hipertens. v. 18, P. 149-52, 2011. LUGNIER, C. Pharmacol Ther. v. 109, p. 366-398, 2006. SBC. 7ª diretriz brasileira de hipertensão arterial. v. 107, 104 p., 2016. SILVA, J. C. G. Dissertação (Mestrado em Ciências da Saúde). UFAL, 2017.

**06.004 Matrix Metalloproteinase (MMP)-2 contributes to decrease dystrophin and troponin i in hypertension-induced cardiac remodeling and dysfunction.**

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**Introduction:** Increased blood pressure contributes to the transition from cardiac hypertrophy to heart failure. MMP-2 is a protease involved in cardiac remodeling mainly due extracellular matrix proteolysis. However, MMP-2 is also activated within cardiac myocytes, which contributes to cardiac remodeling and dysfunction. In fact, inhibition of MMP-2 activity with doxycycline improved cardiac ventricular dysfunction associated with ischemia and reperfusion injury. MMP inhibitors contributed to prevent MMP-2-induced troponin I and dystrophin proteolysis during acute ischemia and reperfusion injury to the heart. So, the hypothesis is that increased MMP-2 activity contributes to hypertension-induced chronic cardiac morphological and functional changes by degrading troponin I and dystrophin. **Methods:** Male Wistar rats were submitted to two kidney-one clip (2K1C) or sham surgery and were treated with doxycycline (15 mg/kg/day) or its vehicle (water) by gavage from tenth to sixteenth weeks post-surgery. Systolic blood pressure (SBP) was weekly assessed by tail-cuff plethysmography, while echocardiogram was performed on the tenth, thirteenth and sixteenth weeks of hypertension to analyze the morphological and functional parameters of the left ventricles. *In situ* zymography analyzed gelatinolytic activity and troponin I and dystrophin levels were investigated by western blotting and immunofluorescence, respectively. Co-localization of MMP-2 and dystrophin was analyzed by double immunofluorescence followed by confocal microscopy, and picosirius staining evaluated the collagen levels. Results were analyzed by Two-way ANOVA. The Ethics Committee in Animal Research of Ribeirao Preto Medical School approved all protocols (023/2015-1). **Results:** SBP was increased in 2K1C rats compared to Sham groups at sixteen weeks ( $p < 0.05$ ) and it was not decreased by treatment with doxycycline. In this time of renovascular hypertension, 25% (6 of 24) of 2K1C rats had left ventricular eccentric hypertrophic remodeling (LVEHR (2K1C-D)) while others remained at left ventricular concentric hypertrophic remodeling (LVCHR (2K1C-H)). On the other hand, doxycycline was able to prevent this transition, since in 2K1C group treated with doxycycline only 17.6% (3 of 17) had LVEHR while others 2K1C had LVCHR. Furthermore, ejection and shortening fractions were reduced in 2K1C-D rats when compared to 2K1C-H and treatment with doxycycline preserved both parameters. MMP-2 activity was increased in 2K1C-H and 2K1C-D rats and doxycycline decreased it ( $p < 0.05$ ). 2K1C-D rats also presented higher amounts of degradation products of troponin I when compared to 2K1C-H and Sham groups and doxycycline prevented it ( $p < 0.05$ ). Dystrophin levels were decreased in the left ventricles of both 2K1C groups ( $p < 0.05$ ) and doxycycline also reversed them. Moreover, collagen deposition increased in the 2K1C-D rats and treatment with doxycycline prevented it ( $p < 0.05$ ). **Conclusion:** Increased MMP-2 activity contributes to decrease dystrophin and troponin I levels in hypertensive rats, thus leading to chronic cardiac remodeling and dysfunction. **Financial support:** CAPES, CNPq and FAPESP.

**06.005 Vasodilator potential of ruthenium complexes containing imidazole derivatives in preparations of rat aorta artery.** Barbosa FWX, Silveira JAM, Rocha DG, Gouveia FS, Uchôa BO, Silva FAO, Marinho AD, Jorge RJB, Lopes LGF, Siqueira RJB, Monteiro HSA UFC

**Introduction:** The ruthenium is a metal that allows high affinity to the NO due to its chemical structure, forming nitrosyl-ruthenium complexes (RuNO). Complexes based on ruthenium may be metallopharmaceuticals with potential medical applications, especially for the treatment of cardiovascular diseases. The objective of this study was to perform a pharmacological screening to evaluate the vasodilator potential in conductance vessels of ruthenium complexes cis-[Ru(bpy)<sub>2</sub>(2-MIM)Cl]<sup>+</sup> (FOR011A), cis-[Ru(bpy)<sub>2</sub>(2-MIM)<sub>2</sub>]<sup>2+</sup> (FOR011AA), cis-[Ru(bpy)<sub>2</sub>(2-MIM)(NO<sub>2</sub>)]<sup>+</sup> (FOR711A) and cis-[Ru(bpy)<sub>2</sub>(2-MIM)(NO)]<sup>3+</sup> (FOR811A). **Methods:** Aortic rings of Wistar rats were precontracted with phenylephrine (1 µmol/L) or KCl (60 mmol/L) for subsequent creation of a concentration-effect curve (0.01 to 30 µmol/L) with the compounds and registration in a data system. The effects of the metallocompounds studied were compared to the precursor molecules cis-[Ru(bpy)<sub>2</sub>(Cl)<sub>2</sub>] (FOR000) and 2-methylimidazole (L11A) (negative controls), sodium nitroprusside (SNP) and BAY 41-2272 (positive controls). **Results:** According to the results, in the preparations pre-contracted with PHE, the four compounds were able to induce concentration-dependent relaxation, reversing 100% of the contraction triggered with discrete differences between their efficacies. However, there was a large difference between their potencies (demonstrated by the concentrations required to reach 50% of the maximum response, CE<sub>50</sub>) when compared FOR011A and FOR811A with FOR011AA and FOR711A. The compounds produced a vasodilator effect with variable potencies (FOR011A: CE<sub>50</sub> = 0.19 [0.14-0.26] µmol/L and maximum efficacy (E<sub>MAX</sub>) = 101.32 ± 1.84%; FOR011AA: CE<sub>50</sub> = 0.62 [0.45-0.87] µmol/L and E<sub>MAX</sub> = 105.27 ± 2.45%; FOR711A: CE<sub>50</sub> = 0.47 [0.39-0.57] µmol/L and E<sub>MAX</sub> = 112.06 ± 1.90%; FOR811A: CE<sub>50</sub> = 0.20 [0.16-0.26] µmol/L and E<sub>MAX</sub> = 113.40 ± 1.78%). When analyzing the performance of the ruthenium complexes submitted to electromechanical coupling by K<sup>+</sup>, a great difference was observed between CE<sub>50</sub> showed by FOR011A, when compared to the ones presented by FOR011AA, FOR711A and FOR811A. The complexes FOR711A and FOR811A, which had the nitrite and nitrosyl radicals respectively, were able to induce concentration-dependent relaxation, reversing about 90% to 100% of the K<sup>+</sup> induced-contraction, with a slightly difference on the efficacy. The compounds (FOR011A and FOR011AA) lacking these radicals showed a very low efficacy (E<sub>MAX</sub><20%). Thus, the potencies of the metallocompounds studied were smaller than the potency exhibited by SNP and higher than the one from FOR000 and L11A. It was also similar to BAY, on FOR811A and FOR011A, and smaller on FOR011AA and FOR711A. **Conclusion:** Therefore, it is possible to conclude that the ruthenium complexes studied, especially FOR811A and FOR011A, have a potential vasodilator effect and that these substances should also be better investigated through different methods of research, like a study of cell viability, toxicity, hemodynamic parameters, among others, in order to obtain more data on these compounds, so we can verify and elucidate their physiological mechanisms in the organism. **License number of ethics committee:** CEUA-UFC 03/2016 **Financial support:** CAPES and CNPQ.

**06.006 Evaluation of the pharmacological potential of the hydroethanolic extract of the peels from *Passiflora edulis* fo. *flavicarpa degener* in treatment of hypertension in rats.** Cabral B<sup>1</sup>, Gonçalves TAF<sup>2</sup>, Medeiro IA<sup>2</sup>, Rezende AA<sup>1</sup>, Zucolotto SM<sup>1</sup> <sup>1</sup>UFRN, <sup>2</sup>UFPB

**Introduction:** *P. edulis* (Passifloraceae), known as yellow passion fruit is an important medicinal plant. Different preparations of *P. edulis* have been popularly used to treat high blood pressure (Jamir et al., 1999). However, the authenticity of these effects is not known. Thus, the objective of this study was to evaluate the antihypertensive effect of the hydroethanolic extract obtained from peels of *P. edulis* and the pharmacological mechanisms involved. **Methods:** For the study of antihypertensive activity, spontaneously hypertensive rats (SHR) and Wistar Kyoto rats (WKY) were used. The animals were distributed in normotensive control (WKY); hypertensive control (SHR) and hypertensive treated with hydroethanolic extract of the peels (AFM) at doses of 200 and 400 mg/kg, orally. After 28 days of treatment, direct blood pressure was measured. After euthanasia, the mesenteric artery was removed and constructed concentration-response curves with acetylcholine (ACh) in the presence and absence of inhibitors L-NAME and indomethacin. Maximum relaxation corresponded to maximum response (MR) for the highest concentration used. **Results:** The SHR control animals had systolic, diastolic and mean pressure (mmHg) of  $233.2 \pm 3.9$ ;  $166.0 \pm 2.7$ ;  $186.79 \pm 2.7$ , respectively. Treatment with AFM at the dose of 200 mg/kg reduced to  $205.2 \pm 4.5$ ;  $144.02 \pm 4.2$ ;  $160.40 \pm 3.5$ , respectively and at the dose of 400 mg/kg reduced to  $202.8 \pm 4.5$ ;  $147.6 \pm 5.5$ ;  $166.02 \pm 4.8$ , respectively. In addition, the treatment was able to reverse endothelial dysfunction in hypertensive rats, this can be verified by increasing the vasorelaxant response to ACh in AFM-treated rats at doses of 200 and 400 mg/kg (MR =  $84.7 \pm 5.9$  %;  $98.0 \pm 3.8$  %, respectively), when compared to SHR-control (MR =  $75.3 \pm 4.0$  %). To evaluate the probable mechanism responsible for the reduction of endothelial dysfunction, the arteries were incubated with indomethacin for the evaluation of the cyclooxygenase pathway and with L-name for the evaluation of the nitric oxide pathway. In the presence of indomethacin (10  $\mu$ M), the ACh-promoted vasodilatory effect was not attenuated in SHR groups treated with AFM at doses of 200 and 400 mg/kg ( $86.5 \pm 9.6$  %;  $97.4 \pm 12.5$  %,  $p < 0.05$ , respectively), when compared to SHR-control ( $84.7 \pm 5.3$  %). In rats incubated with L-name (100  $\mu$ M), there was a decrease in the maximum response induced by acetylcholine in the group that received AFM at the dose of 400 mg/kg (MR =  $1.7 \pm 3.6$  %,  $p < 0.05$ ), when compared to SHR-control (MR =  $8.8 \pm 4.7$  %). Thus, it is suggested that the AFM extract causes reduction of endothelial dysfunction, probably by stimulating nitric oxide synthase (eNOS). **Conclusion:** AFM has an important hypotensive effect. Apparently, these effects are due to a greater availability of nitric oxide that reduces the vascular tonus leading to a reduction of the global peripheral resistance, a phase II clinical study is being conducted to confirm this effect. Thus, the use of *P. edulis* peel extract to develop a herbal medicine can be an innovative product with high clinical potential to treat hypertension. **References:** Jamir, T. T. et al. Folklore medicinal plants of Nagaland, India. *Fitoterapia*, 70,4, 395-401, 1999. **Financial support:** CNPq, CAPES. **CEUA:** (UFPB/015/2017).

**06.007 Chlorhexidine mouthwash attenuates the antihypertensive effects and vascular MMP-2 downregulation induced by L-arginine in two kidney, one clip hypertensive rats.** Batista RIM<sup>1</sup>, Nogueira RC<sup>1</sup>, Ferreira GCF<sup>1</sup>, Paula GHO<sup>1</sup>, Angelis CD<sup>1</sup>, Pinheiro LC<sup>2</sup>, Santos JET<sup>1</sup> <sup>1</sup>FMRP-USP, <sup>2</sup>EERP-USP

**Introduction:** Antihypertensive effects of L-arginine are associated with stimulated nitric oxide (NO) synthesis by NO synthases and increased nitrate concentrations. Nitrate may enter the enterosalivary cycle of nitrate, which generates NO from nitrate and depends on oral bacteria, which have nitrate reductase activity. This study examined the hypothesis that chlorhexidine mouthwash attenuates the beneficial effects of L-arginine in hypertension by interfering with oral bacteria. **Methods:** Male Wistar Hannover rats were divided into 4 Sham-operated groups and 4 hypertensive groups (2 kidney 1 clip hypertension) treated with vehicle (water), Chlorhexidine, L-arginine (10 g/L in drink water) or L-arginine+Chlorhexidine. Systolic pressure was monitored weekly for 4 weeks of treatment. Saliva nitrate reductase activity, aortic reactivity and measurement of NO metabolites concentrations were performed. Vascular reactive oxygen species (ROS) formation was determined by DHE (dihydroethidium). Assessment of L-arginine effects on aortic MMP-2 expression and activity was performed by western blot and gel and in situ zymography. To examine the effects of L-arginine treatment on vascular remodeling, morphometric analyses were carried out in aortas. CEUA FMRP: Protocol n<sup>o</sup> 142/2017.

**Results:** The nitrate reductase activity of oral bacteria decreased with the use of Chlorhexidine. Treatment with L-arginine lowered blood pressure in relation to non-treated hypertensive rats and Chlorhexidine significantly reversed this effect. Hypertension was able to decrease aortic relaxation in response to acetylcholine shifting the concentration-response curve to acetylcholine to the right. L-arginine improved the vascular function of hypertensive rats, whereas chlorhexidine prevented the improvement of vascular function caused by L-arginine. L-arginine increased nitrate concentrations in the aorta from hypertensive rats and Chlorhexidine reversed this effect. Also, mouthwash tended to decrease plasma concentrations of nitrite, nitrosylated species and nitrosothiols. L-arginine treatment reversed the increase in vascular ROS levels caused by hypertension and the use of Chlorhexidine tended to block this effect. Hypertension increased the vascular expression and activity of MMP-2 and L-arginine treatment blocked this effect. Chlorhexidine prevented the effects of L-arginine on vascular MMP-2. Both cross-sectional area (CSA) and media to lumen (M/L) ratio increased in hypertensive animals and L-arginine did not modify these parameters. **Conclusion:** Our results show that L-arginine reduced the blood pressure, improved the vascular function, and diminished ROS production and MMP-2 activity in hypertensive rats. These effects were blunted by chlorhexidine mouthwash. Our findings suggest that maintenance of the enterosalivary cycle is important for the antihypertensive and anti-MMP-2 effects of L-arginine.

**06.008 Analyses of tubular transporters in visceral leishmaniasis patients before and during treatment with liposomal Amphotericin B.** Bezerra GF<sup>1</sup>, Lima DB<sup>1</sup>, Meneses GC<sup>1</sup>, Magalhães EP<sup>1</sup>, Azevedo IEP<sup>1</sup>, Silva Júnior GB<sup>2</sup>, Daher EF<sup>1</sup>, Martins AMC<sup>2</sup> <sup>1</sup>UFC, <sup>2</sup>Unifor

**Introduction:** The treatment with amphotericin is related to tubular renal damage. The use of liposomal amphotericin B (L-AMB) is effective for the treatment of visceral leishmaniasis (VL), although it is a less nephrotoxic formulation than deoxycholate AMB (D-AMB), it may be related to the development of acute kidney injury (AKI) in VL patients. Urinary exosomes originate from various cell types such as podocytes, cells of proximal, distal convoluted tubule and epithelial cells transitional. The results in urine samples have been shown as an initial step in the discovery of new biomarkers. The aim of this work was to evaluate the tubular renal transporters in urinary exosomes of VL patients treated with L-AMB who developed AKI. **Methods:** The study was realized with VL patients (n=17) hospitalized at the São José Hospital of Infectious Diseases, Fortaleza, Ceará. The work was approved by the ethics committee CAAE: 61488016.8.3001.5044. AKI was measured according to the KDIGO. Urine samples were collected before the treatment and 120 hours after starting treatment with L-AMB. The samples were centrifuged at 17,000g to remove whole cells, large membrane fragments, and other debris. The supernatant was centrifuged at 200,000g to obtain low-density membrane pellets (exosomes). The pellets were suspended in isolation solution with protease inhibitors. Urinary protein levels were determined by western blot of isolated exosomes of VL patients, which developed AKI, and healthy volunteers. We evaluated two tubular transporters: aquaporin 2 (AQP2) and Na/H exchanger (NHE3). Bands corresponding to protein expression of AQP2 and NHE3 were quantified by densitometric analysis using Image Lab and they are expressed as percentages of control. The expression of protein was corrected by a constitutive protein of exosome and compared to healthy controls. **Results:** Seven patients (41%) evolved to AKI according KDIGO criteria during hospital stay. Two of these AKI patients had urinary exosomes analyzed. The samples without treatment compared with healthy controls, the expression of NHE3 increased in 11 and 81 times and the AQP2 expression increased in 9 and 1,7 times. During the treatment, these expressions decreased, but still were increased compared to healthy control. They showed a reduction of expression of NHE3 in 71,10% and 31,19% during the treatment. The AQP2 also reduced in 36,30% and 58,65% in the same period. **Conclusion:** The alterations observed on NHE3 and AQP2 transporters suggest tubular alterations caused by the disease and the decreased expression of these transporters during the treatment with L-AMB could be related with the AKI evolution. **Acknowledgments:** Financial support for this study was provided by the Brazilian Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

**06.009 Negative inotropic effects and subcellular disorganization induced by *Crotalus durissus cascavella* venom in cardiac tissue.** Silva LB<sup>1</sup>, Simões LO<sup>1</sup>, Alves QL<sup>1</sup>, Araújo FA<sup>1</sup>, Hora VRS<sup>1</sup>, Jesus RLC<sup>1</sup>, Soares MBPS<sup>2</sup>, Meira CS<sup>2</sup>, Aguiar MC<sup>1</sup>, Couto RD<sup>1</sup>, Cruz JS<sup>3</sup>, Santos MAV<sup>2</sup>, Silva LLC<sup>1</sup>, Vasconcelos DFSA<sup>1</sup> <sup>1</sup>UFBA, <sup>2</sup>CPqGM-Fiocruz, <sup>3</sup>UFMG

**Introduction:** The snake *Crotalus durissus cascavella* (CDC) is found mainly in areas of the caatinga of the Northeast region of Brazil and has demonstrated a broad network of biological functions for therapeutic purposes including action on the cardiovascular system. Previous studies by our research group have demonstrated, experimental evidence that CDC was negative inotropic effect, but it was not demonstrated whether this effect would be related to a therapeutic or cardiotoxic activity. **Aim:** To investigate the impact of CDC venom on cardiac tissue, assessing the therapeutic and/or cardiotoxicity effects of venom. **Methods:** Changes on the contractility of cardiomyocytes were evaluated by means of the cardiomyocyte length alteration technique, using a border detection system. A series of tests were conducted to investigate whether the negative inotropic effect induced by CDC was related to cardiac damage, such as: Cytotoxicity using the resazurin assay; determination of creatine kinase activity (Total-CK and CK-MB); morphological and ultrastructural analysis and evaluation of cardiac electrical activity. Evaluation of the effects of CDC on anesthetized rats ECG was performed in the set of *in vivo* experiments. This study was approved by CEUA (CEUA-ICS / UFBA n ° 072/2014). Statistical analyzes were performed using Student's t-test or one-way ANOVA followed by Bonferroni post-test, when appropriate using. **Results:** CDC induced a reduction of contractility on isolated cardiomyocytes. The addition of CDC crude venom (7.5, 15 and 30 µg/mL) did not induce significant alterations in cell proliferation, nor in the enzymatic activity of total-CK and CK-MB. Data from light microscopy showed that the incubation of rat atria with CDC (30 µg/mL) for 20 min, did not induce any significant morphological changes in cardiac tissue when compared to control. Ultrastructural evaluation demonstrated that cardiac cells from control, isoproterenol and CDC groups revealed swollen and displaced intermyofibrillar mitochondria with disorganization of cristae, however this effect was mostly observed in the CDC group. Additionally, perfusion of isolated rat hearts with CDC venom did not substantially alter cardiac electrical activity. In anesthetized animals CDC induced bradycardia observed on ECG. Our results suggest that the negative inotropic effect induced by CDC venom is unrelated to cardiac toxicity but may be related to subcellular disorganization and/or mitochondrial dysfunction. Financial support: Fundação de Amparo à Pesquisa do Estado da Bahia -FAPESB.

**06.010 Lethal sepsis increases the anti-contractile action of PVAT by a mechanism that involves NO and PGI<sub>2</sub>.** Awata WMC<sup>1</sup>, Gonzaga NA<sup>1</sup>, Borges VF<sup>1</sup>, Carnio EC<sup>2</sup>, Cunha FQ<sup>1</sup>, Tirapelli C<sup>2</sup> <sup>1</sup>FMRP-USP, <sup>2</sup>EERP-USP

**Introduction:** Sepsis induces vascular hyporesponsiveness to vasoconstrictive agents. Perivascular adipose tissue (PVAT) displays anti-contractile action in various blood vessels. 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 the vascular tone and the possible mechanisms underlying such response. **Methods:** Male Wistar rats (250-300 g) were randomized in 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 CLP surgery for functional and biochemical assays. Concentration-response curves for phenylephrine were obtained. In the absence or after incubation (30min) with one of the following drugs: L-NAME (non-selective inhibitor of NOS), carboxy-PTIO (NO scavenger), 1400W (selective inhibitor of iNOS), 7-nitroindazole (7-NI, selective nNOS inhibitor), ODQ (guanylyl cyclase inhibitor), tiron (superoxide anion scavenger), catalase (enzyme that decomposes H<sub>2</sub>O<sub>2</sub>), apamin (low conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channel inhibitor), charybdotoxin (high conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channel inhibitor), 4-aminopyridine (voltage-sensitive K<sup>+</sup> channel inhibitor), glibenclamide (ATP-sensitive K<sup>+</sup> channel inhibitor), indomethacin (non-selective COX inhibitor) or RO1138452 (selective prostacyclin IP receptor antagonist). [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 aortas 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 involves the participation of NO since this response was not found in arteries after incubation with L-NAME (14.9±1.1, n=7), carboxy-PTIO (15.6±1.1, n=14), 7-NI (8.1±1.1, n=7) and 1400W (10.8±0.6, n=6). Similar results were found in the presence of ODQ (9.9±1.0, n=9) and apamin (6.2±0.6, n=5). Besides that indomethacin (6.7±0.7, n=13) and Ro1138452 (6.3±0.3, n=5) reversed the hypocontractility mediated by PVAT in aortas from CLP rats. Tiron, catalase, 4-aminopyridine, charybdotoxin and glibenclamide did not alter phenylephrine-induced contraction in the CLP group. Increased generation of O<sub>2</sub><sup>-</sup> (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<sub>2</sub>O<sub>2</sub> in PVAT. Increased prostaglandin (PG)<sub>12</sub> 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<sub>2</sub> levels was found. In situ quantification of O<sub>2</sub><sup>-</sup> and nitric oxide using fluorescent dyes revealed that sepsis increased the levels of both in PVAT. **Conclusion:** Lethal sepsis increases the anti-contractile action of PVAT by a mechanism that involves the activation of the NO-cGMP pathway and the opening of Ca<sup>2+</sup>-dependent K<sup>+</sup> channel of low conductance. PGI<sub>2</sub> also contribute to the increased anti-contractile effect displayed by PVAT during sepsis. Financial Support: CAPES

**06.011 Acute and prolonged diuretic effect of 1,3,5,6-Tetrahydroxyxanthone in normotensive and hypertensive rats.** Mariano LNB<sup>1</sup>, Boeing T<sup>1</sup>, Silva RCMVAF<sup>1</sup>, Cechinel-Filho V<sup>1</sup>, Niero R<sup>1</sup>, Silva LM<sup>2</sup>, Andrade SF<sup>1</sup>, Souza P<sup>1</sup> <sup>1</sup>Univali, <sup>2</sup>UFPR

**Introduction:** This study aimed to evaluate the acute and prolonged diuretic effect of 1,3,5,6-tetrahydroxyxanthone (THX), a natural xanthone isolated from *Garcinia achachairu* Rusby in rats. **Methods:** Female Wistar normotensive (NTR) and spontaneously hypertensive rats (SHR) received an oral treatment with THX, hydrochlorothiazide (HCTZ) or vehicle (VEH). Cumulative urine volume and urinary parameters were evaluated at the end of 8-h (acute analysis) or 7 days (dose-repeated treatment analysis) experiment. The effects of THX in combination with diuretics of clinical use (HCTZ, furosemide and amiloride), as well as with L-NAME (a nitric oxide synthase inhibitor), atropine (a muscarinic receptor blocker) and indomethacin (an inhibitor of the cyclooxygenase) were also explored. **Results:** THX was able to stimulate 8-h diuresis of both NTR and SHR, as well as urinary Na<sup>+</sup> and K<sup>+</sup> excretion, at a dose of 0.1 mg/kg, p.o. The combination with HCTZ or furosemide, but not with amiloride, significantly enhanced THX-induced diuresis. The diuretic effect with HCTZ plus THX treatment was accompanied by an increase of the urinary Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup> excretion. On the other hand, when given THX in combination with amiloride, there was a significant increase of Na<sup>+</sup> and a decrease of K<sup>+</sup> excretion, an effect characteristic of this class of diuretics. Moreover, the diuretic effect of THX was heightened after pretreatment with L-NAME, and its ability to induce diuresis was not prevented neither in the presence of indomethacin nor in the presence of atropine. The urinary volume of both NTR and SHR after 7 days were significantly augmented with the THX treatment, with final values reaching an increase of 54% and 63%, respectively, when compared with the VEH-treated group. This effect was associated with increased levels of urinary excretion of Na<sup>+</sup> and K<sup>+</sup>, and a decrease of Ca<sup>2+</sup> excretion. None of the treatments modified urinary pH values and did not cause any change in body weight, or food and water consumption. Importantly, although significant modifications in urinary electrolyte excretion were found, plasma content of Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup> and Ca<sup>2+</sup> was not affected by any of the treatments. Regarding the renal analyzes, when compared with the VEH-treated NTR group, the activity of superoxide dismutase (SOD), glutathione S-transferase (GST) and reduced glutathione (GSH) levels in kidney homogenates of the SHR group were decreased, while the generation of lipid hydroperoxides (LOOH), nitrite levels and catalase enzyme (CAT) activity were significantly increased. The daily treatment with THX was able to reduce the LOOH in kidney homogenates obtained from SHR. Finally, THX augmented the levels of nitrite, a marker of nitric oxide production, in the plasma obtained from SHR group when compared with VEH-treated group. **Conclusion:** This study showed both the acute and prolonged diuretic effect of THX and its protective renal properties in hypertensive rats, by inducing diuresis and saluresis, as well as by its ability to restore oxidative balance in kidney tissue. **Research support:** CNPq, CAPES, FAPESC and UNIVALI. Authorization from CEUA/UNIVALI: 028/17p.

**06.012 Atorvastatin and sildenafil improve seven-day lead-exposed rats' hypertension.** Paula ES, Polonio LCC, Tozzato GPZ, Dias Júnior CAC Unesp-Botucatu

**Introduction:** Exposure to low lead (Pb) levels has been associated with cardiovascular disorders, including hypertension. This phenomenon may be explained by increased reactive oxygen species (ROS) with concomitant reduced nitric oxide (NO) bioavailability, which result in vascular hyperresponsiveness to constrictor agents (Varize ND; Indian J Med Res, 128(4), 426-35, 2008). In search of pharmacological agents to attenuate Pb-induced hypertension, drugs that activate NO pathway may have this therapeutic potential, for example: atorvastatin and sildenafil. Atorvastatin upregulates NO synthesis and sildenafil inhibit cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase 5 (PDE5), while both drugs have showed to reduce oxidative stress (Mason RP, J Physiol Pharmacol 66(1): 65-72, 2015; Gonçalves-Rizzi VH, Eur J Pharmacol 5,822: 119-127, 2018). Thus, these drugs may simultaneously protect against endothelial dysfunction caused by exposure to Pb. Therefore, we aimed to investigate whether isolated and associated treatment with atorvastatin and sildenafil may prevent endothelial dysfunction after seven-day lead-exposed rats. **Methods:** Male Wistar rats (250-400g) were distributed into four groups: Pb+atorvastatin (Pb+Atorva), Pb+sildenafil (Pb+Sild), Pb+saline (Pb) and saline+sodium acetate (Sham). Pb+Atorva, Pb+Sild and Pb rats received Pb acetate (8µg/100g of body weight) on the first day of the protocol. Pb levels were maintained with Pb acetate (0.1µg/100g/day) for consecutive seven days. Pb (or sodium) acetate was intraperitoneally (i.p) administrated. Atorvastatin (20mg/kg/day) and sildenafil (15mg/kg/day) or saline (0.5 mL/Kg/day) were administrated by gavage once daily. 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. Aorta rings were submitted to cumulative concentration-response curves to potassium chloride (KCl, 10-120 mM) and phenylephrine (Phe, 10<sup>-10</sup>-10<sup>-4</sup>M). To examine endothelial function, aorta rings were pre-contracted with Phe (10<sup>-6</sup> M) followed by cumulative concentration-response curves to acetylcholine (ACh, 10<sup>-9</sup>-10<sup>-5</sup> M) in absence (or presence) of N(G)- Nitro-L-arginine methyl ester (L-NAME, 10<sup>-4</sup>M). All procedures were approved by institutional ethics committee (CEUA: 1081/2018). **Results:** Atorvastatin and sildenafil prevented increases in SBP on day 6 and 7 compared to Pb group. Sham group showed no change in SBP throughout the experimental period. Moreover, no significant differences were found in KCl and Phe-induced contractions from all groups. However, impaired relaxation induced by ACh was found in aorta rings from Pb and Pb+Atorva groups, while sildenafil prevented this effect. **Conclusion:** Atorvastatin and sildenafil treatments protect against Pb-induced hypertension, while sildenafil but not atorvastatin prevents endothelial dysfunction caused by Pb. Financial support: CAPES and FAPESP.

**06.013 Hydralazine reduces mortality in sepsis animal model.** Santos DM<sup>1</sup>, Silva EAP<sup>1</sup>, Pereira EWM<sup>1</sup>, Marinho YYM<sup>1</sup>, Heimfarth L<sup>1</sup>, Quintans-Júnior LJ<sup>1</sup>, Menezes IAC<sup>2</sup>, Santos MRV<sup>1</sup>, Quintans JSS<sup>1</sup> <sup>1</sup>UFS, <sup>2</sup>UFPR

**Introduction:** Sepsis is characterized by an amplified inflammatory response resulting from a microorganism infection. This response causes changes in the cardiovascular system and presents mortality around 45.8%. Among treatments for sepsis, the vasopressors are traditionally used. However, currently it has been demonstrated the use of vasodilators in the treatment of sepsis. Thus, the aim of this study was to evaluate if hydralazine, a recognized vasodilator, is able to reduce mortality in sepsis animal model. **Methods:** Healthy male Wistar rats (CEPA #20/17) were divided into 03 groups: Sham (n = 5); Sepsis (GS; n = 8); Sepsis + Hydralazine (GSH; n = 8). Each animal was anesthetized and cannulated in the abdominal aorta for hemodynamic recordings. Sepsis was induced 24 h later by cecal ligation and puncture (CLP). Afterwards, all animals were monitored by 48 h and clinical sepsis score (CSS), blood lactate, body temperature, mean arterial pressure (MAP), heart rate (HR), and mortality were evaluated. Statistical analysis was performed using Kaplan–Meier survival analysis and log rank test to mortality and two-way ANOVA with Bonferroni post-test, considering  $p < 0.05$ . **Results:** Sepsis increased CSS (from  $1.5 \pm 0.5$  to  $10.5 \pm 0.6$  u.a.;  $p < 0.05$ ), lactate (from  $15.6 \pm 0.6$  to  $36.8 \pm 1.9$  mg/dL;  $p < 0.01$ ) and mortality (from 0 to 50%). Hydralazine treatment reduced the CSS (from  $10.5 \pm 0.6$  to  $6.0 \pm 0.4$  u.a.;  $p < 0.05$ ), lactate (from  $36.8 \pm 1.9$  to  $18.5 \pm 1.2$  mg/dL;  $p < 0.01$ ), and without death. Sepsis decreased MAP from  $115.9 \pm 5.6$  to  $103.3 \pm 3.5$  mmHg ( $*p < 0.05$ ), which was reduced further by hydralazine treatment (from  $103.3 \pm 3.5$  to  $89.5 \pm 2.3$  mmHg;  $*p < 0.05$ ). On the other hand, HR was unchanged in all groups. Sepsis induced a dropdown on body temperature, which was prevented by hydralazine. **Conclusion:** Treatment with hydralazine reduces mortality in animals with sepsis probably by improving clinical and cardiovascular parameters, blood lactate, and temperature. Furthermore, it is possible to infer that hydralazine can be improving perfusion of organs. This contributes to the absence of mortality in the group. This work was supported by CNPq (304634/2015-8), and FAPITEC/SE (01844/2011), Brazil.

**06.014 Phytochemical screening and evaluation of the vascular effects of the aqueous extract from *Morus Nigra* L. in rats.** Silva WFP<sup>1</sup>, Silva SB<sup>1</sup>, Alves SML<sup>2</sup>, Machado JCB<sup>1</sup>, Ferreira MRA<sup>1</sup>, Mendes RFV<sup>1</sup>, Soares LAL<sup>1</sup>, Ximenes RM<sup>1</sup>, Araújo AV<sup>1</sup>  
<sup>1</sup>UFPE, <sup>2</sup>UPE

**Introduction:** *Morus nigra* L. is a species of the genus *Morus*, family Moraceae. Popularly, the tea from its leaves is popularly used for the treatment of diabetes, high cholesterol, cardiovascular diseases and obesity. Species of this genus have a large number of secondary metabolites, such as alkaloids, coumarins, flavonoids, triterpenes and steroids, which may be associated to the prevention of endothelial dysfunction, reduced blood coagulation and cardioprotective effects. Therefore, we have identified the main chemical constituents present in the aqueous extract of the leaves of *Morus nigra* L. (AEMn), as well as its vascular effects in rat aorta. **Methods:** Thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC) analysis of the AEMn were used for phytochemical screening. Aortic rings of male Wistar rats (2-3 months) were mounted in an organ bath. The responses were recorded in an acquisition system (AVS Project, Brazil). Cumulative concentration-effect curves were performed for the AEMn in aortic rings precontracted with phenylephrine, in the absence or in the presence of the endothelium. The phytochemical screening was expressed in g% (grams of metabolite per 100 g of the extract). For the statistical analysis, the Prisma program (Graph Pad Software, version 5.0) was used. To access the difference among the groups, Student's t-test for paired samples was used and the significance level was set at  $p < 0.05$ . All protocols were approved by the Ethics Committee on the Use of Animals (CEUA) of the Federal University of Pernambuco under No. 0037/2018. **Results:** Phytochemical screening of the extract showed that the main compounds found were: Ellagic acid:  $0.24 \pm 0.0024$  (0.96) g%, Rutin:  $0.33 \pm 0.0021$  (0.62) g%, Derivatives flavonoids:  $0.39 \pm 0.0006$  (0.43) g% and ellagic acid derivatives:  $0.18 \pm 0.0001$  (0.05) g%. The extract was able to induce relaxation of the aortic rings with endothelium ( $ME = 33,143 \pm 6,976g$ ,  $pD_2 = 8,739 \pm 0,259g$ ,  $n = 6$ ), but not in endothelium-denuded aortic rings ( $ME = 4,000 \pm 6,280g$ ,  $n = 5$ ). **Conclusion:** The extract induced relaxation of the aorta artery of rats in an endothelium - dependent manner, an effect that may be associated with the flavonoid derivatives present in it. However, further studies will be needed a better understanding of the mechanisms of action of the aqueous extract of *Morus nigra* L (AEMn). **Acknowledgment:** This study was supported by research grants from the National Council for Scientific and Technological Development (CNPq). **References:** ERCISLI, S.; ORHAN, E. Chemical composition of white (*Morus alba*), red (*Morus rubra*) and black (*Morus nigra*) mulberry fruits. Food Chemistry, v. 103, n. 4, p. 1380-1384, 2007. ALMEIDA, M. G. J. D. et al. Validation of an alternative analytical method for the quantification of antioxidant activity in plant extracts. Latin American Journal of Pharmacy, v. 32, n. 1, p. 90-95, 2013.

**06.015 Translational effects of peptide Kef-1 from probiotic Kefir: Anti-hypertensive and antioxidant properties.** Aires R<sup>1</sup>, Amorim FG<sup>2</sup>, Côco LZ<sup>2</sup>, Pimenta AB<sup>2</sup>, Leal MA<sup>1</sup>, Vasquez EC<sup>1</sup>, Campagnaro BP<sup>2</sup>, Meyrelles SDS<sup>1</sup> <sup>1</sup>UFES e <sup>2</sup>UVV-ES

Studies have shown that in addition to the benefits on the intestinal microbiota, the probiotic Kefir also has a great therapeutic potential on several diseases, such as hypertension. However, little is known about the bioactive components and mechanisms of action involved in the antihypertensive activity observed by Kefir consumption. In a previous proteomics study by our research group, were identified 35 peptides inhibitors of angiotensin converting enzyme (ACE) in Kefir. Therefore, this study aimed to evaluate the effects of an ACE inhibitor peptide (ACEi), named Kef-1, derived from Kefir on blood pressure (BP) and its antioxidant activity in an experimental model of arterial hypertension. Kef-1 was synthesized (Aminotech) and its inhibitory activity on ACE was confirmed *in vitro* (~72% compared to captopril). For *in vivo* protocols were performed with mice (C57Bl/6, male, ~23g) submitted to clipping of the left renal artery to produce renovascular hypertension (2K1C). After 2 weeks, the BP measurement was performed with plethysmography and the animals were divided into 2 groups: 2K1C vehicle (water) and 2K1C treated (10 mg/kg/day, via gavage, for 7 days). In the Sham animals, the same surgical procedures were performed except for the renal artery clip placement and they were used as control. At the end of the treatment, the animals were euthanized, and the aorta was extracted for histological analysis and isolation of smooth muscle cells (SMC). The thickness of the aortic midlayer was evaluated after staining with hematoxylin and eosin. The cytoplasmic levels of reactive oxygen species, viability and cellular apoptosis were evaluated through flow cytometry. Animal Ethics Committee-UJV (#489-2018). Data are reported as mean±SEM and one-way ANOVA and Tukey's test were used. Level of significance was fixed in  $p < 0.05$ . Systolic blood pressure (SBP) and mean blood pressure (MBP) were reduced in the group treated with Kef-1 (SBP:  $\Delta 17.5 \pm 6.3$  mmHg; MBP:  $\Delta 20 \pm 3.9$  mmHg) when compared to the control groups (Sham and 2K1C vehicle). The thickness of the aortic midlayer was also reduced in the 2K1C Kef-1 group when compared to the 2K1C vehicle group (Sham:  $1.41 \pm 0.06$ ; 2K1C vehicle:  $2.15 \pm 0.14$ ; 2K1C Kef-1:  $1.98 \pm 0.05$ ). In the aorta SMC the treatment was able to reduce the levels of hydrogen peroxide (Sham:  $1410 \pm 58$ ; 2K1C vehicle:  $1822 \pm 113$ ; 2K1C Kef-1:  $1478 \pm 59$  u.a.,  $p < 0,05$ ). The 2K1C Kef-1 group showed a reduction of the cellular apoptosis ( $8.6 \pm 2.5\%$ ) in comparison to the 2K1C vehicle group ( $21 \pm 3.5\%$ ; Sham:  $8 \pm 1.2\%$ ), thus presenting increase of viable cells after treatment (2K1C Kef-1:  $89 \pm 2.5\%$  vs 2K1C vehicle:  $77 \pm 3.4\%$ ; Sham:  $87 \pm 1.1\%$ ). These results suggest that Kef-1 peptide from a probiotic food has potential prospectation as drug for anti-hypertensive treatment. Financial support: CNPq; FAPES; CAPES (Finance Code 001).

**06.016 Angiotensin II- induced contraction is due to reactive oxygen species production in renal hypertensive rat aortas.** Fahning BM, Bendhack L FCFRP-USP

The renovascular experimental model of hypertension (2K-1C) is characterized by the activation of the Renin-Angiotensin-Aldosterone System (RAAS), in which AngII is the main mediator that activates AT<sub>1</sub> receptors promoting vascular smooth muscle cells (VSMC). This study aimed to evaluate the effects of AngII on endothelial and VSMC and to verify the modulation between the enzymes NO- Synthase (eNOS), cyclooxygenase (COX) and reactive oxygen species (ROS) production by Nox. Concentration-response curves for AngII were constructed in 2K-1C rat aortas with intact endothelium (E+), in the absence and after incubation for 30 min with the enzymes inhibitors: Nox (apocynin), eNOS (L-NAME), COX (Indomethacin) or the AT<sub>2</sub> antagonist (PD123319). Also, concentration-response curves for AngII were constructed in denuded aortas (E-). The maximum effect (Emax) and pD<sub>2</sub> values were compared to control. AngII-induced contraction in 2K-1C rat aortas was not changed by L-NAME (pD<sub>2</sub> 8.50 ± 0.15 vs 8.36 ± 0.14; Emax 0.46 ± 0.13 g vs 0.22 ± 0.08, n=7-10) and Indomethacin (pD<sub>2</sub> 7.98 ± 0.21 vs 8.36 ± 0.14; Emax 0.26 ± 0.08 g vs 0.23 ± 0.08 g, n=5-7). PD123319 did not alter AngII-induced contraction in 2K-1C rat aortas. The endothelium removal increased the Emax of AngII-induced contraction in the 2K-1C aortas (0.59 ± 0.10 g vs 0.22 ± 0.08 g, n=7-9, p= 0.016). Apocynin abolished the contraction stimulated with AngII in 2K-1C rat aortas (Emax 0.04 ± 0.02 g vs 0.23 ± 0.08 g, n=7-8). Therefore, our results demonstrated that AngII activates AT<sub>1</sub> receptors that increase ROS production via Nox. However, the endothelium presented an anti-contractile effect that is not due to eNOS or COX activation. Moreover, AT<sub>2</sub> receptors do not play an important role on these mechanisms. **Ethical Committee:** 17.1.617.60.0 CEP/FCFRP Financial support: Fapesp process: 2016/20322-0

**06.017 Supraphysiological levels of testosterone induces cardiac dysfunction via NLRP3 inflammasome activation.** Alves JV<sup>1</sup>, Costa RM<sup>2</sup>, Omoto ACM<sup>2</sup>, Silva J<sup>2</sup>, Tostes RCA<sup>1</sup> <sup>1</sup>FMRP-USP, <sup>2</sup>UFG

**Introduction:** Testosterone modulates vascular tone and cardiac performance. Both supraphysiological and subphysiological testosterone levels are associated with increased cardiovascular risk. Athletes who use testosterone at supraphysiological doses exhibit increased blood pressure, higher inflammatory markers levels, vascular dysfunction, and cardiac hypertrophy. NLRP3 inflammasome activation as part of the innate immune system response contributes to proinflammatory cytokines production, which leads to cardiac hypertrophy as one of its effects activations. Hypothesis: We hypothesized that supraphysiological levels of testosterone promote cardiac dysfunction by NLRP3 inflammasome activation. **Methods:** Male, 12 week-old C57Bl/6J (WT) and NLRP3 knockout (NLRP3<sup>-/-</sup>) mice were used. Mice were treated with testosterone propionate [TP (10 mg/kg)] or vehicle for 30 days. Cardiac function was evaluated using echocardiography. After in vivo experiments, histological and molecular assays were performed. Results: Echocardiography showed severe cardiac dysfunction in WT mice treated with TP, as reduction of the ejection fraction, shortening fraction, cardiac output and systolic volume. In addition, there was an increase in interventricular septum, left ventricle posterior wall and left ventricle internal diameter. All these effects were prevented in NLRP3<sup>-/-</sup>. In cardiac histomorphometric parameters, WT mice treated with TP showed increased in septal thickness [WT\_Vehicle: 878.6 ± 39.5 (mm) n=5 vs. WT\_TP: 1097.0 ± 36.9 (mm) n=8] and free wall thickness [WT\_Vehicle: 945.1 ± 30.6 (mm) n=5 vs. WT\_TP: 1196.0 ± 48.6 (mm) n=8]. Furthermore, were determined the expression of the components of NLRP3 inflammasome by western blot and ELISA. WT mice treated with TP showed increased expression of NLRP3 receptor [WT\_Vehicle: 0.007075 ± 0.001353 (AU) n=3 vs. WT\_TP: 0.01778 ± 0.002799 (AU) n=5] and Caspase-1 [WT\_Vehicle: 52.63 ± 11.29 (AU) n=5 vs. WT\_TP: 138.03 ± 11.55 (AU) n=4]. The increase of Caspase-1 was prevented in NLRP3<sup>-/-</sup> [NLRP3<sup>-/-</sup>\_Vehicle: 112.59 ± 11.99 (AU) n=3 vs. NLRP3<sup>-/-</sup>\_TP: 112.03 ± 0.82 (AU) n=3]. The cardiac IL-1β expression was increased in WT mice treated with TP [WT\_Vehicle: 15.26 ± 3.52 (pg/mg) n=5 vs. WT\_TP: 35.94 ± 4.86 (pg/mg) n=3], but this effect was prevented in NLRP3<sup>-/-</sup> [NLRP3<sup>-/-</sup>\_Vehicle: 18.82 ± 3.25 (pg/mg) n=3 vs. NLRP3<sup>-/-</sup>\_TP: 11.89 ± 7.73 (pg/mg) n=3]. **Conclusion:** These data indicate that supraphysiological levels of testosterone induce cardiac dysfunction via NLRP3 inflammasome activation. Financial support: FAPESP, CAPES, CNPq. This study was approved by the Ethics Committee on Animal Experimentation of the Ribeirao Preto Medical School (032/2018).

**06.019 Ascorbate decreases nitrosylation and increases blood pressure in septic shock.** Pinheiro LC<sup>1</sup>, Persona IS<sup>1</sup>, Tirapelli C<sup>1</sup>, Cunha FQ<sup>2</sup>, Santos JET<sup>2</sup>, Lacchini R<sup>1</sup>  
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**Introduction:** Septic shock treatment is complex, generally expensive and ineffective. During sepsis there is a significant increase in the production of NO. The systemic increase in NO results in a myriad of effects. NO derived from several cells is essential for the control of infection, but also exerts vascular effects, such as vasodilatation. Treatment for reversion of septic shock usually requires the infusion of vasoconstrictors, often with low efficacy. In this context, NO may form S-nitrosothiols that would result in long-term protein modifications, which in general result in inhibition thereof. We believe that S-nitrosylation during sepsis plays a critical role in the response to vasoconstrictors in shock. This study aimed at investigating the increase in total protein nitrosylation in septic shock in heart and aorta and to quantify nitrosylation of protein kinase C (PKC) protein kinase A (PKA) and GAPDH. We also investigated the impact of reduction of nitrosylation on blood pressure. **Methods:** The C57 mice had their arterial vein and femoral vein cannulated. Invasive blood pressure was measured in free-moving animals three days after surgery. Sepsis was then induced by cecal ligation puncture (CLP). After 10 h, invasive systolic blood pressure (SBP) was recorded and the animals were treated with saline or ascorbate (200 mg/kg i.v.). SBP was recorded for 2 hours and the animals were euthanized. Aorta, heart and plasma were collected. Concentrations of plasma nitrite and nitrosylated species were measured by chemiluminescence. The nitrosylated protein was measured by SNO-RAC. The results were analyzed by ANOVA and data are showed as mean  $\pm$  S.E.M. **Results:** Severe hypotension was observed 10 h after the induction of sepsis by CLP. (SBP 119  $\pm$ 9 mmHg to 46 $\pm$ 6 mmHg, P<0.05). Treatment with saline solution did not alter blood pressure. Treatment with ascorbate increased blood pressure (SBP 39  $\pm$ 6 mmHg to 53 $\pm$ 7 mmHg, P<0.05). Septic shock increased plasma levels of nitrite and nitrosylated species in 12 hours. On the other hand, treatment with ascorbate decreased nitrite levels and nitrosylated species. Total protein nitrosylation increased twofold in the aorta, heart and liver 12 hours after sepsis. Further, septic shock increased nitrosylation of PKC in the aorta (2.1  $\pm$ 0.2% to 9.2 $\pm$ 6 %, P<0.05), heart (3.8  $\pm$ 0.3 % to 7.6 $\pm$ 2%, P<0.05) and liver. It was found increased nitrosylation in PKA and GAPDH in the heart and liver. Animals treated with ascorbate showed a significant decrease of nitrosylation in PKC and GAPDH in all tissues analyzed. **Conclusion:** Septic shock was associated with increased nitrite and nitrosylated plasma species and increased nitrosylation of total protein in the aorta and heart. Nitrosylation of the central proteins of vascular responses, such as protein kinase C protein kinase A, may be associated with septic shock and may be a target in hypotension. Treatment with ascorbate reduced total nitrosylation and PKC and GAPDH and increased blood pressure in animals with shock. These data suggest a relevant participation of nitrosylation in hypotension and suggest target proteins. **Financial Support.** FAPESP 2017-07570-8

**06.020 Alpha-1A adrenoceptor signaling impairment during experimental sepsis.** Borges VF<sup>1</sup>, Silva Júnior ED<sup>2</sup>, Abrão EP<sup>3</sup>, Silva KPD<sup>4</sup>, Cunha TM<sup>3</sup>, Alves-Filho JCF<sup>3</sup>, Carneiro FS<sup>3</sup>, Tostes RC<sup>3</sup>, Baker J<sup>5</sup>, Pupo AS<sup>4</sup>, Cunha FQ<sup>3</sup> <sup>1</sup>USP, <sup>2</sup>UFRN, <sup>3</sup>FMRP-USP, <sup>4</sup>Unesp-Botucatu, <sup>5</sup>University of Nottingham

**Introduction:** infection control by innate immunity depends of the microbial products recognition by resident cells. This interaction leads to the intracellular pathways activation, resulting in the inflammatory mediators production. In response to these mediators, there are modifications in the local microvasculature and in the circulating neutrophils, that allow the passage of these cells to the infectious focus. Sepsis is a life threatening organ dysfunction associated with the dysregulated hosts response to infection. Neutrophils recruitment to the infectious focus is impaired and the cardiovascular responses are uncontrolled, culminating in infection control failure, bacteremia, multiple organ dysfunctions and death. The impact of cardiovascular complications during sepsis is high because the mortality among patients presenting them is significantly higher. There is hyporesponsiveness to vasoconstricting agents and, consequently, failure to reestablish vascular tonus. Our research group has identified reduction of adrenergic receptors on the surface of cells by exacerbation of the pathways associated with the internalization of GPCRs in sepsis. **Objective:** the objective of this study is to identify biased agonists of the Gq protein pathway as potential candidates for the recovery of vascular tonus in septic patients. **Methods:** wildtype C57/BL6 mice were submitted to cecal ligation and puncture (CLP) sepsis model. Besides the survival curves, other sepsis parameters were obtained, such as cell migration, bacteremia, mean arterial pressure and heart rate. To determinate the  $\alpha$ -1 adrenoceptors quantity in the tissues, we performed the 3H-prazosin binding assay. Cumulative concentration-effect curves for the selected ligands in mesenteric arteries preparations of septic or control mice without endothelial cells were also performed. **Results:** after the sepsis model standardization through survival analysis, neutrophil migration failure and bacteremia, we observed that the model of sepsis major severity occurs with persistent decrease in blood pressure and heart rate. We evaluated the amount of adrenergic receptors in tissues such as the aorta, mesenteric arteries and heart of these animals and observed that there is reduction of these receptors during sepsis. When evaluating the contractile response of first order mesenteric arteries without endothelium obtained from septic mice, we can observe that these animals lose the response to selective agonists for the  $\alpha$ -1A adrenoceptors, indicating the loss of this kind of receptor during the disease. **Conclusion:** our results indicate that  $\alpha$ 1-receptors, mainly the  $\alpha$ 1A-subtype, are reduced in septic tissues and can be related to the sepsis vasoplegia. Financial support: FAPESP, CAPES, CNPq.

**06.021 Consequences of different nutritional insults during lactation in early life of male and female wistar rats.** Vieira CB, Fernandes I, Castro-Pinheiro C, Alvim-Silva T, Souza KP, Rocha NN UFF

**Introduction:** Malnutrition during critical periods of life, such as lactation, can cause metabolic and functional changes in adulthood (Langley-Evans SCProc Nutr Soc. 72(3): 317-25, 2013; Saunders and Smith Clin Med (Lond) 10(6): 624-7, 2010). Literature reports that time course and temporal association of the consequent disease remain unsettled (Rodriguez et al. PLoS ONE 9(4), 1-10, 2014). In addition, incorporating concepts of sex-specific analysis in basic research is largely neglected (Ventura-Clapier et al. Cardiovasc Res 113, 711-24, 2017). Therefore, the aim of this work was to evaluate body, nutritional and cardiovascular parameters of young male and female Wistar rats submitted to different nutritional insults during lactation as early weaning and mother's food restriction. **Methods:** This work is approved by local ethics' committee (812/2016). Pups physically separated from their mothers at postnatal day 18 or nursed by females submitted to 30% food restriction during lactation were compared to respective control about body mass index, food consumption, blood pressure as well as cardiac structure and function at postnatal day 30. Data were presented as mean and standard error and analyzed by Student's t-test, being considered statistically significant if  $p < 0.05$ . **Results:** Body mass index ( $0.47 \pm 0.01 \text{g/cm}^2$ ), food consumption ( $133.9 \pm 2.5 \text{g}$ ) and left ventricle diameter in diastole ( $0.426 \pm 0.174 \text{cm}$ ), but not feed efficiency ( $0.280 \pm 0.007$ ), of males from restricted mothers were lower than respective control ( $0.40 \pm 0.01 \text{g/cm}^2$ ;  $115.5 \pm 2.3 \text{g}$ ;  $0.373 \pm 0.016 \text{cm}$ ;  $0.310 \pm 0.01$ ). Different from males, females nursed by restricted mothers have presented only lower blood pressure ( $167.7 \pm 3.3 \text{mmHg}$ ) compared to control ( $148.5 \pm 7.6 \text{mmHg}$ ). Food intake was lower in early-weaned females ( $140 \pm 0.01 \text{g}$ ) compared to respective control ( $121 \pm 0.01 \text{g}$ ). However, as they have also presented inferior body mass ( $102.0 \pm 3.7 \times 86.8 \pm 1.3 \text{g}$ ) and naso-anal length ( $14.70 \pm 0.19 \times 14.02 \pm 0.20 \text{cm}$ ), no difference was observed about body mass index, distinctly from males in which this parameter was reduced ( $0.43 \pm 0.01 \times 0.39 \pm 0.01 \text{g/cm}^2$ ). **Conclusion:** In general, malnutrition during lactation promoted differences about alimentary behavior and blood pressure as well as about body and echocardiographic parameters. However, data suggest that consequences of malnutrition during lactation in young life depends on the type of the insult as well as on sex. **Financial Support:** CAPES, FAPERJ.

**06.022 The supplementation with *Dipteryx alata* Vog.'s almond oil has antithrombotic and vasomodulate effects in rats.** Oliveira JCPL, Veras RC, Silva-Luis CC, Azevedo FLAA, Arruda AV, Alves RMFR, Araújo IGA, Medeiros IAUFPPB

**Introduction:** It is described in the literature that intake of vitamin E could decrease the risk of platelet aggregation and thrombus formation in blood vessels. *Dipteryx alata* is a Brazilian tree that its almond oil (DaAO) has significant concentrations of vitamin E, becoming its oil a nutraceutical candidate. Thus, this study aimed evaluate the effect of DaAO as antithrombotic and the capacity to modulate vessel reactivity. **Methods:** It was used 4 groups of Wistar rats treated throughout 10 days with saline solution (control group), or 0.42 mg of vitamin E, or DaAO (treated with 0.21 mg/Kg or 0.42 mg/Kg) and in the 11<sup>o</sup> day the experiments were made. The thrombus formation was stimulated by ferric chloride (50% w/v) in the carotid artery and the blood flow was monitored with an ultrasonic flow probe, measuring the time of total occlusion of artery (Radomski, Br J Pharmacol, v. 146, p. 882, 2005). To evaluate platelet aggregation the blood was collected, centrifugated at 120 g for 10 minutes to obtain platelet-rich plasma (PRP) and at 1,000g for 15 minutes to the platelet-poor plasma (PPP). 300 µl of PRP was incubated for in an aggregometer and the platelet aggregation was stimulated with 16.7 µM of ADP or 100 µM of phorbol-12-myristate 13-acetate (PMA) (Yoneda, Br J Pharmacol, v. 142, p. 551, 2004). Finally, thoracic aorta was removed, rings (1-2 mm) were set up in organ bath to the construction of concentration-response with cumulative concentrations of Phe (0.009 µM – 10.0 µM), Ach (0.009 µM – 10.0 µM) and Sodium nitroprusside – NPS – (0.001 µM – 10.0 µM). The results were expressed as mean ± standard error of mean (SEM) and statistical analyses were made with ANOVA followed by Tukey's post-test. Ethics Committee on Animal Use (CEUA/UFPB): 128/2016. **Results:** The treatment with 0.42 mg DaAO (1,127.5 ± 32.02 s) was capable to increase de time of occlusion (p<0.05) of carotid arterial for the thrombus when compared with the control (472,5 ± 32,02 s) and corroborating with this data the weight of the thrombus in the rats treated with 0.42 DaAO (wet: 1.500 ± 0.230 mg; dry, after 24h: 0.733 ± 0.189 mg) was less (p<0.05) than the control group (wet: 2.383 ± 0.294 mg; dry, after 24h: 1.917 ± 0.296 mg) and the platelet aggregation ADP-stimulated was decreased (p<0.05) in the group of rats treated with 0.42 DaAO (53.25 ± 4.87%) compared with the control group (77,14 ± 2,55%). Both doses of DaAO (0.21 mg: E<sub>max</sub>=54.33 ± 8.94%, pD<sub>2</sub>=6.96 ± 0.16; 0.42 mg: E<sub>max</sub>=31.62 ± 4.62%, pD<sub>2</sub>=7.6 ± 0.16) was capable to decrease the aorta response to Phe when compared with control group (E<sub>max</sub>=100 ± 6.08%, pD<sub>2</sub>=6.27 ± 0.13) and finally the dose of 0.42mg DaAO (E<sub>max</sub>=100.64 ± 1.38%, pD<sub>2</sub>=11.00 ± 0.04) increase de vasorelaxation potency of nitric oxide when compared with all other groups. **Conclusions:** Thus, DaAO has a dose-dependent effect as antithrombotic and antiplatelet agent and the modulation of the ADP pathway to these is suggestive. Also, the treatment with the oil decreased the effect of sympathomimetic agonist, while positively modulated NO-mediated relaxation. Financial support: CNPq/IDEP/UFPB.

**06.023 Contribution of the aryl hydrocarbon receptor (AhR) to vascular dysfunction in mice fed with a hyperlipidic diet.** Bolsoni JA, Silva JF, Tostes R FMRP-USP

**Introduction:** Obesity is a major risk factor for cardiovascular diseases. In obesity, there is an imbalance in the production of vasoactive molecules, compromising the vascular function (Xia N. et al, 2016; Roberts et al, 2000). In addition, obese individuals have inadequate suppression of the Renin Angiotensin System (RAS), leading to endothelial dysfunction and hypertension (Asferg et al, 2013). In addition, knockout animals for the Aryl Hydrocarbon Receptor (AhR) present lower blood pressure and lower contractile responses to Angiotensin II, which is associated with a lower activation of the RAS. In this context, AhR may influence vascular function and vasomotor response (Agbor et al, 2011). Thus, our study investigates whether obesity-associated endothelial dysfunction in mice is related to the activation of RAS driven by AhR. Our hypothesis is that the absence of AhR in obese knockout animals protects aortic function due to decreased activation of the RAS. **Methods:** 15 weeks-old C57BL/6J (WT) mice and knockout mice for AhR (AhR<sup>-/-</sup>) were divided in four groups: WT fed a control diet (C), WT fed a Western type hyperlipidic diet (W), AhR<sup>-/-</sup> fed a control diet, and AhR<sup>-/-</sup> fed a hyperlipidic diet. Mice were fed the diets for 10 weeks. Metabolic profile (gain of body mass and serum levels of total cholesterol, HDL, LDL, triglycerides and glucose) and vascular reactivity [thoracic aortic rings submitted to cumulative concentration-effect curves to Phenylephrine (Phe) and Acetylcholine (ACh)] were determined. All experiments with animals were approved by the Comissão de Ética no Uso de Animais (CEUA) of the Faculty of Medicine of Ribeirão Preto (FMRP) of the University of São Paulo (USP), on the protocol 056/2018. **Results: Metabolic Profile:** The hyperlipidic diet induced gain of body mass (in grams) in WT (C= 32,1±0,6 vs. W=37,5±1,6) and AhR<sup>-/-</sup> (C= 31,3±0,9 vs. W= 35,5±1,6) mice. Regarding total cholesterol and HDL, AhR<sup>-/-</sup> mice fed the hyperlipidic diet did not show a significant increase of serum levels of total cholesterol [(in mg/dL) C= 73,0±18,4 vs. W= 78,0±9,5], as observed in WT mice (C= 96,9±10 vs. W= 156,9±28); or in the levels of HDL [(in mg/dL) C= 46,6±19,7 vs. W= 43,2±9,6] compared to the WT mice (C= 56,6±7,7 vs. W= 100,2±19,4). The results obtained so far, showed no differences in the serum concentrations of triglycerides, LDL or glucose among the experimental groups. **Vascular Reactivity:** There was no difference in the maximum contractile response to Phe among the WT groups and AhR<sup>-/-</sup> mice fed the Western diet: WT [(in mN) C= 6,89±0,7 vs. W= 7,9±0,9] e AhR<sup>-/-</sup> (W= 8,8±1,3). Contractile responses to Phe were decreased in AhR<sup>-/-</sup> mice fed the control diet (4,9±0,2). Regarding vascular relaxation responses to ACh (in %), AhR<sup>-/-</sup> groups exhibited similar responses (C= 82,9±6,4 vs. W= 86,5±3,9), whereas in the WT groups, the relaxation responses to ACh were decreased in the group fed the hyperlipid diet (C= 83,8±2,9 vs. W= 67,9±2,6). So, WT mice fed the hyperlipidic diet presented vascular dysfunction, characterized by decreased responses to ACh, an effect not observed in AhR<sup>-/-</sup> mice fed the hyperlipidic diet. **Conclusion:** The results obtained so far indicate that the absence of AhR attenuates dyslipidemia and vascular dysfunction in mice fed a hyperlipidic diet. Therefore, AhR represents an important therapeutic target for prevention of obesity-associated metabolic and vascular dysfunction. **Financial Support:** FAPESP

**06.024 Cardioprotective effects of *Plinia cauliflora* (Mart.) Kausel in a rabbit model of doxorubicin-induced congestive heart failure.** Tirloni CAS, Romão PVM, Palozzi RAC, Guarnier LP, Gasparotto Júnior A UFGD

Breast cancer is a public health issue and the most common type of cancer that affects women. Among the chemotherapeutic drugs that are used to treat breast cancer is doxorubicin. Its use, however, is limited because of dose-dependent oxidative stress and cardiotoxicity. In Brazil, the fruit of a native species that is popularly known as “jabuticaba” (*Plinia cauliflora* Mart. Kausel) is widely used to treat asthma, throat inflammation, and gastrointestinal and cardiovascular disturbances. However, there are no reports in the literature proving its cardioprotective effects. To evaluate the possible cardioprotective effects of a hydroethanolic extract of *Plinia cauliflora* (EEPC) in female rabbits in a model of doxorubicin-induced congestive heart failure to simulate the female population who is undergoing breast cancer treatment. Thirty female New Zealand rabbits were subjected to 6 weeks of doxorubicin administration to induce heart failure (CEUA N°: 11/2018). EEPC was orally administered at doses of 75 and 150 mg/kg daily for 42 days. Enalapril (5 mg/kg) was used as a reference cardioprotective drug. At the end of the experimental period, blood pressure and heart rate were recorded. Serum parameters, including lipid profile, troponin, creatinine, and brain natriuretic peptide were measured. The electrocardiographic profile and renal vascular reactivity were evaluated. Cardiac morphometry was performed, and the tissue enzymatic antioxidant system was investigated. **Results:** From EEPC, thirty-seven compounds were detected, including organic acids, phenolic acid derivatives, flavonoids, anthocyanins and hydrolysable tannins (gallotannins and ellagitannins). EEPC treatment induced a cardiorenal protective response, prevented hemodynamic and functional alterations, and prevented ventricle remodeling. These effects were associated with the normalization of creatinine and brain natriuretic peptide levels and modulation of the tecidual antioxidant defense system. The present study demonstrated that EEPC may prevent doxorubicin-induced heart failure by modulating the antioxidant defense system, reducing reactive oxygen species-induced damage, preventing alterations of hemodynamic and endothelial function, and preventing damage to the cardiac structure. EEPC, especially at the highest dose tested, may be considered a cardioprotective coadjuvant to prevent doxorubicin-induced cardiotoxicity. **Acknowledgments** Fundação de Apoio ao Desenvolvimento Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT, Brazil,, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brasil).

**06.025 Involvement OF NADPH-oxidase enzyme in the nephroprotective and antioxidant effect of (-)- $\alpha$ -bisabolol.** Magalhães EP<sup>1</sup>, Sampaio TL<sup>1</sup>, Silva BP<sup>1</sup>, Menezes RRPPB<sup>1</sup>, Marinho MM<sup>1</sup>, Santos RP<sup>2</sup>, Martins AMC<sup>1</sup> <sup>1</sup>UFC, <sup>2</sup>UFC-Sobral

**Introduction:** The hypoperfusion is one of the main causes of Acute Renal Injury (AKI), leading to a reduction in oxygenation and nutrient supply, characterizing the Ischemia and Reperfusion Syndrome (I/R). The I/R can be prejudicial to renal tissue because it reduces oxygen supply after ischemia, and when re-oxygenated, it diverts the hyper-reactive oxygen to ROS production, causing mitochondrial damage, changes in the electron transport chain, preventing oxidative phosphorylation and ATP production. In this context, NADPH plays a very important role in the protection of tissues from oxidative injury, since during this process their stocks are depleted, since the enzyme NADPH-oxidase (NOX) uses them to catalyze the electron transfer reaction, producing the ROS. Therefore, inhibition of isoforms, such as renal NOX4, is proposed as an innovative pharmacological tool in preventing disorders related to oxidative stress. In this sense, many natural products, such as terpenes with antioxidant and anti-inflammatory activity have been investigated to treat and delay renal diseases, such as (-)- $\alpha$ -bisabolol (BIS), which has a nephroprotective effect already described and an antioxidant potential to be explored. **Methods:** (-)- $\alpha$ -bisabolol was obtained from Sigma-Aldrich with 99% purity. To evaluate the interaction of the BIS with the NOX4, molecular docking assay was performed and, to ratify the findings, an enzymatic activity assay was made using a commercial kit, which evaluates spectrophotometrically the conversion of NADPH in NADP<sup>+</sup>. To induce *in vitro* I/R, human proximal tubular cells (HK-2) were kept in ischemia in anaerobic chamber with medium (DMEM) deprived of the usual supplementation of glucose, pyruvate and fetal bovine serum for 24 hours, followed by the reperfusion, characterized by the replacement to an aerobic atmosphere for 3 hour and supplementation of DMEM. After the reperfusion, the treatment with BIS (250 and 62.5  $\mu$ M) was performed and cells were incubated for 24 hours. The expression of NOX4 was evaluated by Western blotting the control group, in the group exposed to I/R protocol and in the BIS-treated group. The relative expression (RE) of the enzyme was determined in relation to the  $\beta$ -actin protein. The results were expressed as mean  $\pm$  standard error of the mean, using one-way ANOVA followed by Bonferroni post-test for comparison of the groups treated with the control group ( $p < 0.05$ ), using GraphPad Prism 5.0 software. **Results:** At the studied concentrations, the treatment with BIS decreased the conversion of NADPH in NADP<sup>+</sup> in the enzymatic activity assay (CT  $-56.2 \pm 0.7$ ; BIS 250  $\mu$ M  $-65.1 \pm 0.5$ ; BIS 62.5  $\mu$ M  $-73.7 \pm 0.3$ ), possibly through inhibition of NOX4 through interaction with clusters present at the catalytic site of the enzyme, as demonstrated in the molecular docking assay. The Western blot analysis showed that the I/R protocol upregulated the expression of NOX4 (RE =  $2.99 \pm 0.04$ ) in comparison with control group (RE =  $1.00 \pm 0.03$ ). The treatment with BIS 250  $\mu$ M was able to reduce by more than 50% the expression (RE =  $1.49 \pm 0.08$ ) and the BIS 62,5  $\mu$ M reduced it by 20% (RE =  $2.48 \pm 0.07$ ). **Conclusion:** The data suggest that the effect of BIS can be due to the downregulation of the expression of the NADPH-oxidase enzyme, especially the NOX4 isoform, which is one of the main factors responsible for the generation of ROS and renal damage caused by I/R. **Acknowledgments:** We thank CNPq, CAPES and UFC for their financial and institutional support.

#### 06.026 Evaluation of antiplatelet and vascular effects of *Canna indica* L in rats.

Brazão SC, Lima GF, Machado LR, Moraes IA, Motta NAV, Brito FCF UFF

**Introduction:** The use of plants for the treatment of cardiovascular diseases is widespread in folk medicine, especially in developing countries, due to the limitations of health services. Such conduct has led to numerous researches in order to seek further clarification on the active principles as well as their pharmacokinetics and pharmacodynamics. Thus, therapeutically active substances used in contemporary medicine are developed. The present study aims to evaluate the cardiovascular activities of ethanolic extracts from different parts of *Canna indica* L. *Canna indica*, popularly known as Biri or Imbiri, is a monocotyledon belonging to the order Zingiberales, in the northwest region of Rio de Janeiro. Plants of the genus *Canna* are widely used in folk medicine and previous studies with *Canna indica* L. have shown curative effects for infections, rheumatism and hepatitis, as well as evidence of antioxidant properties (Zhang et al., 2010). Phytochemical analysis of Cl. root and rhizomes extract showed compounds such as polyphenols (flavonoids) (Kumbhar, Biochemistry and Biophysics Report., 16: 50-55, 2018). Data from literature show that polyphenols have effects reducing platelet aggregation in different extracts (Kasimu, Journal of Ethnopharmacology., 168: 116-121, 2015). The present study aims to evaluate the properties of different extracts of *Canna indica* L. in platelet aggregation and vascular reactivity in rats. **Methodology:** The animal protocols were approved by the Ethics Committee for Experimental Research of the Federal Fluminense University (CEUA/UFF 858/2016). Adult male Wistar rats (200-250g) (n= 4) were euthanized by cervical dislocation under ketamine and xylazine anesthesia. The rat blood was removed by cardiac puncture and collected into tubes containing a 3.8% trisodium citrate solution. The rat platelet-rich plasma (PRP) and the platelet-poor plasma (PPP) was prepared by centrifugation. The platelet aggregation ex vivo was monitored by the turbidimetric method described by Born and Cross (1963), using a platelet lumi-aggregometer. The PRP was incubated by five minutes with extracts (100µg, 500µg, 1mg, 2mg and 3mg/ml). Ethanol was employed as vehicle. The aggregation was induced by ADP (5 µM). Platelet aggregation was expressed as percentage of aggregation in response to ADP. Thoracic aortas were excised for vascular reactivity assay. The aorta rings were placed in chambers containing a freshly prepared Krebs-bicarbonate nutrient solution. The maximal contraction of the aorta was measured using the extracts as agonist (1mg/ml). Data were analyzed using one-way ANOVA followed by a post-hoc Bonferroni Multiple Comparison Test,  $P < 0.05$ . **Results:** In leaf extract, we observed that platelet aggregation induced by ADP 5 µM in PRP of rats was reduced at the concentration of 1mg/ml ( $35.0 \pm 5.6\%$ ), 2 mg/ml ( $26.5 \pm 6.5\%$ ) and 3mg/ml ( $23.5 \pm 4.9\%$ ) compared to vehicle group ( $57.3 \pm 2.7\%$ ). In the vascular reactivity evaluation, flowering, roots and leaves extracts showed an improvement of contractile response in aorta ( $2.24 \pm 0.02\%$ ,  $2.55 \pm 0.02\%$ ,  $2.19 \pm 0.03\%$ ), respectively, when compared to vehicle group ( $0.08 \pm 0.01\%$ ).  $*p \leq 0.05$ . **Conclusion:** This study showed the effects of *Canna indica* extracts at reducing platelet aggregation and improving contractile response in aortas. The screening is still in progress and further analysis needs to be performed. These results suggest that *Canna indica* extracts can play an important role at cardiovascular system. **Financial support:** CNPq, CAPES, PROPPI-UFF, FAPERJ.

**06.027 Effect of luteolin on endothelial superoxide anion generation.** Cruz YMC<sup>1</sup>, Assunção HCR<sup>2</sup>, Bertolino J<sup>2</sup>, Fernandes L<sup>1</sup> <sup>1</sup>Unifesp-Diadema, <sup>2</sup>Unifesp

**Introduction:** Endothelial oxidative stress affects vascular function, interfering with the whole circulatory system. One of the most important agent involved in the production of reactive oxygen species at the endothelial site is the endogenous octapeptide Angiotensin II (Ang II). By activating AT1 receptors, Ang II stimulates NAD(P)H oxidase and superoxide anion ( $O_2^-$ ) generation, resulting in the reduction of nitric oxide (NO) availability and vasoconstriction. Luteolin is a flavonoid with antioxidant effect previously reported in several tissues, but its effects on endothelial cells are poorly understood. The present work investigated the generation of  $O_2^-$  in rat endothelial cells, analyzing the possible antioxidant effect of luteolin isolated and in association with Ang II. **Methods:** Cultured endothelial cells from rat vena cava were pre-treated with the fluorescent dye DHE [10  $\mu$ M] for 30 minutes and exposed to luteolin [10, 20 and 50  $\mu$ M] for 10 minutes. Cells were fixed (PFA 4%) and coverslips were analyzed in a confocal microscope. The fluorescence intensity was quantified by densitometry (n=6). In another set of experiments,  $O_2^-$  generation was investigated by a spectrofluorometer in cells pre-treated with DHE (10  $\mu$ M, 30 minutes) and incubated with Ang II [1 $\mu$ M] in the presence or absence of luteolin [20  $\mu$ M], for 10 minutes (n=7). The protein expression of NOX-2 [a NAD(P)H oxidase isoform] was analyzed by western blot in cells exposed to Ang II [1 $\mu$ M] in the presence or absence of luteolin [20  $\mu$ M], for 24 hours (n=4). Results are represented as mean  $\pm$  sem and expressed in arbitrary units. Results: Images obtained from confocal microscopy showed a significant reduction on  $O_2^-$  generation after luteolin 10 $\mu$ M ( $5.5 \pm 0.6^*$ ), 20  $\mu$ M ( $3.0 \pm 0.1^*$ ) and 50  $\mu$ M ( $5.8 \pm 0.9^*$ ) in comparison to untreated cells ( $12.8 \pm 0.9$ ). Spectrofluorimetry assays demonstrated a marked increase in endothelial  $O_2^-$  levels after association of Ang II [1  $\mu$ M] and luteolin [20  $\mu$ M] ( $165.2 \pm 25.5^*$ ) when compared to untreated cells ( $94.6 \pm 8.4$ ). NOX 2 expression was not altered by Ang II or luteolin alone, but significantly enhanced by association ( $2.02 \pm 0.5^*$ ) when compared to untreated cells ( $0.55 \pm 0.1$ ). (\* $p < 0.05$ ) **Conclusion:** Luteolin reduces  $O_2^-$  generation by endothelial cells. This effect may account for enhancement of vascular NO bioavailability. However, in the presence of Ang II, the flavonoid increases oxidative stress, and probably contributes to endothelial NOX-2 expression and activation. These results suggest that the antioxidant effect of luteolin at the endothelial site must be further evaluated, since it depends on the presence of other agents with pro-oxidative activity. CEP UNIFESP 2689270319 Supported by CNPq and FAPESP (2017/22028-5)

**06.028 Isoflurane presents a trend in enhancing nitric oxide-dependent vasodilation response.** Souza CRR, Paula ES, Dias Júnior CAC Unesp-Botucatu

**Introduction:** Isoflurane is one of the most used volatile anesthetics for general anesthesia, once it presents low blood-gas partition coefficient (EGER, American Journal of Health-System Pharmacy, v.61, pS3, 2004), but, its security to the cardiovascular system has not been completely established. It is already known that isoflurane may influence the vasomotor response, impairing endothelium-dependent vasodilatation caused by reduction in releasing of nitric oxide (NO) in male rats (NAKAMURA, Canadian Journal of Anaesthesia, v.41, p.340, 1994). However, isoflurane effects in female rats are unclear. **Methods:** Female *Wistar* rats were randomly assigned as following: non-pregnant anesthetized group (Non-Preg+Iso) and non-pregnant rats which were not anesthetized (Non-Preg group). Non-Preg+Iso group was anesthetized for 150 minutes, blood pressure and heart rate were recorded every 15 minutes. Blood pressure and heart rate were recorded in triplicate in Non-Preg group before the experiment. After the rats were killed, vascular reactivity experiments were performed in abdominal aorta rings with intact and mechanically removed endothelium. Aorta rings were stimulated with cumulative concentrations of phenylephrine (Phe,  $10^{-9}$  to  $10^{-4}$ M). To investigate endothelial function, rings were pre-contracted with Phe ( $10^{-6}$  M) followed by cumulative concentrations of acetylcholine (ACh,  $10^{-9}$  to  $10^{-4}$  M) in absence or presence of N(G)-Nitro-L-arginine methyl ester (L-NAME  $10^{-4}$ M). Results: No significant difference was observed among the experimental groups in concentration-response curves to Phe, ACh and ACh in presence of L-NAME; however, it was observed a trend in relaxation-induced by ACh in anesthetized group. **Conclusion:** Although no significant differences were observed among concentration-response curves, we speculate that isoflurane could have activated NO synthesis. Financial Support: FAPESP Ethics committee (Protocol CEUA 1166/2019)

**06.029 Biochemical characterization in young and adult male and female Wistar rats submitted to neonatal overnourishment.** Pedro SS, Amaral GA, Souza KP, Vieira CB UFF

**Introduction:** Previous data of our research group have shown that the decrease of litter size leads to a greater body weight during lactation due to the increase of milk supply. Male, but not female, Wistar rats presented a higher abdominal/thoracic circumference ratio accompanied by alterations left ventricle geometry in adulthood (Amaral et al. SBFTE 2017/2018). Literature reports that sex may affect cardiovascular disease presentation, its diagnosis and prognosis, impacting on clinical practice (Barrett-Connor, J of Cardiovasc. Trans. Res. 2: 256-57, 2009). As literature reports that time course and temporal association of the consequent disease is also relevant (Rodriguez et al. PLoS ONE 9(4), 1-10, 2014), the aim of this work was to analyze serum biochemical profile of young and adult male and female Wistar rats submitted to overnourishment during lactation. **Methods:** At birth, the offspring were randomly adjusted to 8 or 4 animals (1 male: 1 female) per mother, originating control (C) and overnourishment (O) groups respectively, both males (CMxOM) and females (CFxOF). Blood samples were collected at postnatal days 30 and 150. Serum lipids and glucose levels (mg/dL) were measured using Labtest Brasil kit. Data were presented as mean and standard error, analyzed by Student *t* test and considered statistically different if  $p < 0.05$  (\*). **Results:** Young OM rats presented higher LDLc ( $14.85 \pm 2.72 \times 35.38 \pm 4.93^*$ ), non-HDLc ( $25.57 \pm 2.35 \times 46.11 \pm 5.18^*$ ), Castelli Index I ( $1.72 \pm 0.09 \times 2.53 \pm 0.23^*$ ), and Castelli Index II ( $25.57 \pm 2.35 \times 46.11 \pm 5.18^*$ ) compared to respective control. The same profile was observed at postnatal day 150, when OM rats have also presented higher glucose ( $127.3 \pm 16.59 \times 185.7 \pm 15.77^*$ ) and decreased HDL ( $34.13 \pm 2.02 \times 18.93 \pm 2.11^*$ ) levels. Different from males, young OF rats showed solely increased glucose ( $334.50 \pm 44.49 \times 531.90 \pm 32.66^*$ ) and decreased HDLc ( $32.80 \pm 3.22 \times 23.68 \pm 1.89^*$ ) levels compared to their controls. Glucose concentration remained higher in older OFrats but no differences were observed about HDLc. **CONCLUSIONS:** Data shown that overnourishment during lactation changes metabolism parameters in young and adult animals. Female rats seem to developed glycemic impairment while males presented importantly lipemic dysfunction which may be related to the higher fat abdominal accumulation and may explain changes in left ventricle geometry previously reported in adulthood. **Financial Support:** FAPERJ, CNPq, CAPES. **The use of animals was approved by:** CEUA-UFF812/2016.

**06.030 Pharmacological investigation of the action mechanism of a natural substance extracted from *Piper rivinoides* in vascular reactivity.** Barenco TS<sup>1</sup>, Espírito-Santo LC<sup>1</sup>, Souza PDN<sup>2</sup>, Marques AM<sup>3</sup>, Ramalho TC<sup>4</sup>, Nascimento JHM<sup>1</sup>, Ponte CG<sup>2</sup> <sup>1</sup>UFRJ, <sup>2</sup>IFRJ, <sup>3</sup>Fiocruz, <sup>4</sup>UFLA

**Introduction:** For at least 60,000 years, plants are used as a medicinal tool because of their bioactive substances, mainly with low toxicity<sup>1</sup>. The *Piper* genus includes more than 1,000 species that are widely distributed in tropical and subtropical regions, and consumed worldwide, such as black pepper. This genus has already shown a large number of physiologically active substances with pharmacological value, such as lignans and neolignans<sup>2,3</sup>. Among them, conocarpane has some important biological characteristics<sup>4</sup>, however, non is known about its effects in cardiovascular system. The aim of this study was to evaluate the effect of conocarpane in vascular reactivity from the 2nd branch of mesenteric artery, in addition to identify a possible mechanism of action. **Methods:** Male Wistar rats weighing 200-300g were used (CEUA/CCS/UFRJ protocol No. 087/15). The effect of conocarpane in vascular reactivity, as well as in presence of inhibitors/blockers - ODQ, 4-aminopyridine (4-AP), glibenclamide, iberiotoxin (IbTX), indomethacin, atropine and loratadine – were evaluated in rings from the 2nd branch of mesenteric artery, using a Mulvany's myograph. The rings were kept in Krebs-Henseleit solution at 37 °C, aerated with carbogen mixture and contracted with phenylephrine. Molecular docking procedures were performed by Molegro Virtual Docker, with the overall energy of the complex calculated as a sum of van der Waals, electrostatic, hydrogen-bonding and torsion stress terms. **Results:** In vascular reactivity, conocarpane presented vasodilator activity in tension generated by the rings of mesenteric arterioles with intact endothelium (IC<sub>50</sub> = 1.08 μM); this effect was significantly reduced in rings without endothelium (IC<sub>50</sub> = 11.9 μM). In presence of inhibitors/blockers, we observed that the vasodilator effect of conocarpane was partially inhibited by IbTX, glibenclamide, 4-AP and loratadine, totally inhibited by ODQ, minimally reduced by indomethacin and atropine had no effect. In the docking, with the observation that conocarpane could be involved in relaxation via histamine receptor, conocarpane and histamine structures were docked, within the receptor interaction site, and doxepin was re-docked. Conocarpane structures were deeply embedded in the active pocket, indicating their good complementarity with the active site and the rationality and integrity of strategies to find new pharmacological targets. Two possible poses (A and B) were obtained during the coupling: A, more stable than B, however, B has hydrogen-bonding energy greater than A. **Conclusion:** We conclude that conocarpane has a vasodilatory effect on resistance arteries, with great influence of the endothelium, and this mechanism may be related to histamine receptors antagonism. However, some studies are still needed to confirm their antagonistic effect. **Financial Support:** CAPES, CNPq, IFRJ <sup>1</sup>FABRICANT, DS. The Value of Plants Used in Traditional Medicine for Drug Discovery. Environ Health Perspect. 109: 69. 2001. <sup>2</sup>JARAMILLO, M. A.; et al. Phylogeny and patterns of floral diversity in the genus *Piper* (Piperaceae). Am J Bot. 2001. 88(4): 706-716. <sup>3</sup>RAIMUNDO, JM. The lignan eudesmin extracted from *Piper truncatum* induced vascular relaxation via activation of endothelial histamine H1 receptors. Eur J Pharmacol. 606: 150. 2009. <sup>4</sup>SARTORELLI, P. Enantioselective conversion of p-hydroxypropenylbenzene to (+)-conocarpan in *Piper regnellii*. PlantScience. 161: 1083. 2001.

**06.031 Endothelial dysfunction induced by inhibitors of gastric acid secretion.** Lopes JMS<sup>1</sup>, Nogueira RC<sup>1</sup>, Parente JM<sup>1</sup>, Paula GHO<sup>1</sup>, Pinheiro LC<sup>2</sup>, Castro MM<sup>1</sup>, Santos JET<sup>1</sup> <sup>1</sup>USP, <sup>2</sup>EERP-USP

**Introduction:** Previous findings showed that treatment with omeprazole adversely affects vascular function, impairs vascular redox biology and promotes endothelial dysfunction<sup>1,2</sup>. These findings are relevant to patients with cardiovascular diseases using PPIs, and therefore investigating whether other drugs used to treat dyspeptic diseases cause similar problems is important. In this context, the impact of ranitidine on vascular biology and function remains unknown. We examined the effects of ranitidine on blood pressure, vascular function and redox biology. **Methods:** Male Wistar rats (180-200 g) were treated once daily for four weeks with vehicle (V) or ranitidine (R) 100 mg/kg orally. Omeprazole (O) 10 mg/kg i.p. was used as a positive control. Systolic blood pressure (SBP) was assessed weekly by tail-cuff plethysmography. By the end of the fourth week of treatment, the animals were euthanized and the gastric washing pH was evaluated. We evaluated vascular reactivity to acetylcholine in aorta. Endothelial function was evaluated upon concentration response to ACh [molar] in rat thoracic aorta. To assess vascular oxidative stress, two independent biochemical assays were used: dihydroethidium (DHE) and chemiluminescent lucigenin assay. **Results:** Ranitidine or omeprazole did not affect SBP. Gastric pH increased with both treatments. Ranitidine or omeprazole decreased the maximum responses of aortic rings in response to acetylcholine (Emax: V=106.5±2.0%; O=92.2±2.1%; R=93.9±1.7%; both P<0.05). Omeprazole (P<0.05), but not ranitidine, shifted the concentration effect curve in response to acetylcholine to the right (pD2: V=7.50±0.05; O=7.20±0.06; R=7.40±0.05). Ranitidine and omeprazole increased vascular oxidative stress assessed by DHE (V=10.2±0.7; O=20.4±1.6; R=17.3±1 arbitrary units; both P<0,05) and by lucigenin chemiluminescence assay (V=66.6±9.4; O=152±9.7; R=121±11.5 RLU/mg; both P<0.05). **Conclusion:** ranitidine and omeprazole have similar pro-oxidant effects on the vasculature and cause endothelial dysfunction. Financial support: CNPq and FAPESP. This study was approved by the Institutional Animal Care and Use Committee of the Faculty of Medicine of Ribeirao Preto, University of Sao Paulo under the registration number 171-2016. 1. Pinheiro, L.C., et al., *Omeprazole impairs vascular redox biology and causes xanthine oxidoreductase-mediated endothelial dysfunction*. Redox Biol, 2016. **9**: p. 134-143. 2. Ghebremariam, Y.T., et al., *Unexpected effect of proton pump inhibitors: elevation of the cardiovascular risk factor asymmetric dimethylarginine*. Circulation, 2013. **128**(8): p. 845-53.

**06.032 Itaconimides derivatives induce vasodilation, negative inotropism and hypotension.** Moraes RA<sup>1</sup>, Alves QL<sup>1</sup>, Camargo SB<sup>1</sup>, Medeiros CFA<sup>2</sup>, Hora VRS<sup>3</sup>, Jesus ADM<sup>3</sup>, Cechinel-Filho V<sup>4</sup>, Stiz DS<sup>4</sup>, Corrêa R<sup>4</sup>, Vasconcelos DFSA<sup>3</sup> <sup>1</sup>Fiocruz, <sup>2</sup>USP, <sup>3</sup>UFBA, <sup>4</sup>Univali

**Introduction:** Hypertension is a risk factor for various cardiovascular diseases, representing a major public health challenge. Moreover, control of the hypertension for many patients with currently therapy is still insufficient, and the investigation of alternatives therapeutics is necessary. In this way, analogues from phyllanthimide, available in *Phyllanthus sellowianu*, were synthesized, N-phenyl-itaconimide (Imide-1), N-4-methyl-phenyl-itaconimide (Imide-2), N-4-methoxy-phenyl-itaconimide (Imide-3) and N-4-chloro-phenyl-itaconimide (Imide-4). The aim of this study was to investigate, for the first time, the cardiovascular activities of these itaconimides in the vascular and cardiac tissues, with propose of describing the mechanisms of action involved in the observed responses. **Methods:** Male Wistar rats (200-300g) were euthanized and the superior mesenteric artery and atria were isolated for recordings of isometric tension in an organ bath. In another set of the experiments, rats were fitted with polyethylene catheters inserted into the lower abdominal aorta and inferior vena cava through the left femoral artery and vein, respectively, in order to record blood pressure and heart rate. Imide-3 was intravenously administered at 0.1, 1 and 10 mg·kg<sup>-1</sup> randomly. (CEUA/UFBA nº120/2017). **Results:** Cumulative administration of itaconimides (3x10<sup>-8</sup> to 3x10<sup>-4</sup>M) in pre-contracted mesenteric artery rings with phenylephrine (1µM) induced endothelium-independent vasorelaxation. Additionally, Imide-3 induced vasorelaxation in rings exposed to a depolarizing-tyrode solution containing 60 mM KCl or 20 mM KCl similar to the control, suggesting the non-participation of K<sup>+</sup> channels. Imide-3 attenuated Ca<sup>2+</sup> influx in a concentration-dependent manner. As well, imide-3 reduced CaCl<sub>2</sub>-induced contraction in nominally calcium-free medium, in the presence of cyclopiazonic acid (20µM), phenylephrine (1µM) and nifedipine (1µM), indicating a reduction of Ca<sup>2+</sup> influx by receptor-operated channels (ROC) and/or store-operated channels (SOC). The presence of SKF 96365 (10<sup>-5</sup>M), SOC blocker, did not significantly alter the vasorelaxant effect induced by imide-3. Moreover, imide-3 induced a negative inotropic effect (47.0% ± 15.1, n=5) compared to control (100 ± 0) without causing a significant change in atrial rhythmicity. *In vivo* studies, in non-anesthetized normotensive rats, imide-3 (10mg/kg) lowered blood pressure (-57.3% ± 6.83mmHg) and induced bradycardia (-78.1% ± 1.76bpm, n=5). **Conclusion:** These results suggest that the itaconimides induce vasorelaxation, independent of endothelium-derived relaxing factors. In addition, imide 3 induces vasodilatation, most likely due to the inhibition of calcium influx through Ca<sub>v</sub> and ROC and it also induces negative inotropic effect. These effects probably are responsible by the decreasing of blood pressure and bradycardia observed in the *in vivo* experiments. In this way, the results observed were promising, showing the possibility of new therapeutic option for hypertensive patients, in the future. **Financial support:** CAPES, FAPESB and CNPq.

**06.033 Omeprazole promotes vascular remodeling via upregulation of xanthine oxidoreductase and Matrix Metalloproteinase-2 Activity.** Nogueira RC<sup>1</sup>, Pinheiro LC<sup>2</sup>, Lopes JMS<sup>1</sup>, Parente JM<sup>1</sup>, Conde SO<sup>3</sup>, Paula GHO<sup>1</sup>, Castro MM<sup>3</sup>, Santos JET<sup>3</sup>  
<sup>1</sup>USP, <sup>2</sup>EERP-USP, <sup>3</sup>FMRP-USP

**Introduction:** Omeprazole, a proton pump inhibitor, causes endothelial dysfunction by upregulating xanthine oxidoreductase (XOR) activity which leads to redox imbalance. Reactive oxygen species (ROS) derived from XOR activity may increase matrix metalloproteinases (MMPs) expression and activity, mainly MMP-2, a protein involved in vascular remodeling. Thus, our aim was to analyze whether omeprazole entails vascular remodeling in aorta, depending on XOR and MMP-2 activation. **Methods:** The study was approved by the Animal Research Ethical Committee (171-2016). Male rats weighting 200g (n=40) were assigned to 4 treatment groups: Control (C-treated with vehicle tween 2%- gavage); Allopurinol (A- treated with the XOR inhibitor Allopurinol- 50mg/kg/day-gavage), Omeprazole (O- treated with the PPI Omeprazole- 10 mg/kg/day-intraperitoneal) and Omeprazole+Allopurinol group (O+A), that received both drugs concomitantly. The experimental protocol lasted 4 weeks. Systolic blood pressure (SBP) was measured by tail cuff plethysmography. By the end of treatments, the animals were submitted to euthanasia and the pH of the gastric washing was measured. The thoracic aorta was collected to study vascular reactivity, biochemical analysis of ROS, morphometric analysis and MMPs activity assay. **Results:** There was no change in SBP in all treatment groups. Allopurinol did not alter any of the parameters that were analyzed in the present study, in comparison to C group. Gastric washing pH increased in O and O+A groups. Omeprazole decreased the maximum effect of acetylcholine in aortic rings, while the omeprazole+allopurinol prevented it. The O group presented increased ROS levels and MMP-2 activity in aorta, which were prevented by allopurinol. Omeprazole also induced hypertrophic remodeling, which was prevented by the association with allopurinol. **Conclusion:** Omeprazole caused aortic remodeling, vascular dysfunction, oxidative stress and MMP-2 upregulation. Allopurinol prevented the deleterious effects of omeprazole, suggesting that XOR is an essential enzyme for the deleterious effects of omeprazole on the vasculature. **Financial Support:** CNPq, FAPESP and CAPES. **Keywords:** Omeprazole, xanthine oxidoreductase, matrix metalloproteinase, allopurinol, vascular remodeling. **Reference:** 1- PINHEIRO, L. C. et al. *Redox Biol*, v. 9, p. 134, 2016. 2- VALENTIN, F. et al. *Fundam Clin Pharmacol*, v. 19, p. 661, 2005.

**06.034 Renal vascular reactivity in sepsis: A putative mechanism for sepsis-induced kidney failure.** Rosales TO, Nardi GM, Assreuy J UFSC

**Introduction:** Sepsis is defined as a life-threatening organ dysfunction caused by an overwhelming immune response to infection. Sepsis clinical findings include vasodilatation and decrease of reactivity to vasoconstrictors leading to severe and untreatable hypotension. Nitric oxide (NO) has been shown to be an important player in sepsis vascular dysfunction. If in addition to this kidney failure takes place, the prognostic is worsened. The mechanism of kidney failure in sepsis is under debate. What is known is, in the opposition to the systemic vasculature, renal vascular reactivity to catecholamines is preserved in sepsis. G protein-coupled receptor kinases (GRKs) are protein kinases that recognize and phosphorylate activated G protein-coupled receptors (GPCR), such as adrenergic receptors, labeling them for internalization. Previous data of our laboratory show that NOS-2-derived NO activates G protein-coupled receptor 2 (GRK2) in aorta and heart, leading to the decrease in  $\alpha_1$  and  $\beta_1$  adrenergic receptor density which, in turn, is associated with the decrease in vascular and cardiac reactivity in sepsis, respectively. Therefore, we aimed to study the putative relationship among kidney GRK2,  $\alpha_1$  adrenergic receptor and NO in sepsis. **Methods:** Female wild type Swiss and 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 CLP. Renal blood flow measurement, alpha 1 adrenergic-receptor fluorescent binding assay and Western blot analysis for GRK2 and NOS-2 were performed 24 hours after CLP. Normal Swiss mice were treated with a NO donor (SNAP, 10 mg/Kg) and analyses were carried out 4 hours later. Swine kidney epithelial cell line (LLC-PK1) were treated with SNAP (100  $\mu$ M) in the presence or not of an inhibitor of soluble guanylate cyclase (ODQ, 1  $\mu$ M). **Results:** Our results show that i) sepsis reduced GRK2 levels to almost nil and induced a simultaneous increase (75%) in  $\alpha_1$  adrenergic receptor density in the kidney; ii) basal renal blood flow decreased in septic animals; iii) NOS-2 protein expression increased in septic kidney; iv) the disappearance of GRK2 was prevented in NOS-2-KO mice or with 1400W treatment in Swiss mice; v) treatment with the NO donor reduced GRK2 content in kidney of normal mice and in LLC-PK1 cells; and vi) the decrease of GRK2 in LLC-PK1 cells was prevented by soluble guanylate cyclase inhibition. **Conclusions:** Sepsis leads to renal vasoconstriction. Sepsis also induced a profound reduction in kidney GRK2 content along with an increased density of  $\alpha_1$  adrenergic receptors. This reduction is highly dependent on NO pathway in both septic kidney and LLC-PK1 cells. Therefore, our data showing that the preserved response to vasoconstrictors in the septic kidney may well be due to the disappearance of GRK2 caused by NO point to a new mechanism that explain sepsis-induced kidney failure. Financial support: CNPq, CAPES and FINEP.

**06.035 Pharmacological evaluation of a quinazoline derivate in vascular reactivity.**  
Teixeira RGS<sup>1</sup>, Barenco TS<sup>2</sup>, Espírito-Santo LC<sup>3</sup>, Resende GO<sup>3</sup>, Ponte CG<sup>3</sup>, Santos WC<sup>1</sup>  
<sup>1</sup>UFF, <sup>2</sup>UFRJ, <sup>3</sup>IFRJ

**Introduction:** Quinazolines and analogs are molecules with heterocyclic ring that display several pharmacological activities such as anti-hypertensive effect<sup>1</sup>. Molecules that can modulate vascular reactivity *in vitro* are potential candidates for new clinical approaches in numerous diseases included Systemic Arterial Hypertension (SAH) and Pulmonary Arterial Hypertension (PAH)<sup>2,3</sup>. In screening experiments, we evaluated the activity of nine derivatives of quinazolines on the vascular reactivity in rat aorta, being the effects of Quin 02 the most promising. We therefore came up to investigate a possible mechanism of action for Quin 02 in rat aorta. **Methods:** Male Wistar rats weighing between 250-300 grams were used. Cumulative concentrations of Quin 02 ( $10^{-8}$ M –  $3 \times 10^{-5}$  M) were tested in endothelium-intact and endothelium-denuded aortarings contracted with Phe (phenylephrine) and high K<sup>+</sup> (60 mM). Vessels were maintained in Krebs-Henseleit solution (pH=7,4) at 37°C and aerated with a carbogen mixture. Data were recorded by a vertical isometric voltage recording system. The effect of Quin 02 ( $3 \times 10^{-6}$  M;  $1 \times 10^{-5}$  M) was also tested in endothelium-intact rings in presence of the following inhibitors: glibenclamide, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), 4-aminopyridine (4-AP), iberiotoxin (IbTX), N<sup>w</sup>-nitro-arginina-metil-ester (L-NAME), indomethacin, loratadine or atropine. **Results:** Compound Quin02 showed an endothelium-dependent vasorelaxant effect ( $IC_{50} = 1,37 \times 10^{-6}$ M) in aorta rings; this result was reduced in endothelium-denuded aortarings ( $IC_{50} = 65,35 \times 10^{-6}$ M,  $P \leq 0.001$ ). Furthermore, Quin 02 did not show any significant relaxation on contractions by high K<sup>+</sup> (60mM). The vasodilator effect was totally reversed in the presence of 10  $\mu$ M ODQ (soluble guanylyl cyclase inhibitor), significantly inhibited by 100  $\mu$ M L-NAME (nitric oxide synthase inhibitor) in both concentration of Quin 02 and partially inhibited by 10  $\mu$ M loratadine only at  $10^{-5}$  M Quin 02. The other inhibitors employed (4-AP, IbTX, indomethacin, glibenclamide and atropine) did not significantly alter the effect of Quin 02. **Conclusion:** Results suggested the involvement of nitric pathway in the vasodilator effect of Quin 02, since the presence of ODQ and L-NAME in experimental protocols blunted the vasodilatation. Besides, our results discharged the participation of potassium and calcium channels in the vasorelaxation. In order to ascertain our preliminary conclusions, experiments on resistance arteries and also on the cytotoxicity of Quin 02 will be performed in the sequence of the investigation. Financial support: CAPES, FAPERJ, CNPq Process number (CEUA-UFF): 765/2017 **References:** <sup>1</sup>KHAN, I. Eur. J. Med. Chem. v. 76, p.193 2014. <sup>2</sup>ALAGARSAMY, V. Eur. J. Med. Chem. v. 151, p.628, 2018. <sup>3</sup>PARACHA, TU. Molecules. v. 24(2), p.281, 2019.

**06.036 *In vitro* vasorelaxant activity of the mitochondria-targeted hydrogen sulfide (H<sub>2</sub>S)-donor AP39 on murine mesenteric artery rings.** Marques LAC<sup>1</sup>, Teixeira SA<sup>1</sup>, Torregrossa R<sup>2</sup>, Whiteman M<sup>2</sup>, Costa SKP<sup>1</sup>, Muscara MNP<sup>1</sup> <sup>1</sup>ICB-USP, <sup>2</sup>University of Exeter

**Introduction:** Previous studies with the mitochondria targeted H<sub>2</sub>S-donor AP39 showed important protective effects during oxidative stress, including improved cell metabolism and inhibition of mitochondrial oxidant activity in cultured endothelial cells. We thus decided to study the effects of AP39 on vascular resistance arteries. **Methods:** The experimental protocol was approved by the local Ethics Committee for Animal Experimentation (CEUA/ICB N°7759060218). C57BL/6 male mice were euthanized under anesthesia (xylazine, 20 mg. kg<sup>-1</sup> and ketamine, 80 mg.kg<sup>-1</sup>, i.p.), the mesenteric beds were excised, first-order mesenteric arteries were isolated and 2 mm length rings were prepared. The rings were mounted on a wire myograph for isometric tension registry. The responses to both AP39 and the spontaneous H<sub>2</sub>S donor NaHS were studied under phenylephrine pre-contraction in the vessels with (E<sup>+</sup>) or without endothelium (E<sup>-</sup>, after mechanical removal). These responses were also evaluated in presence of the non-selective COX inhibitor indomethacin (Indo, at 10 μM), the non-selective NOS inhibitor L-NAME (100 μM), the soluble guanylate cyclase inhibitor ODQ (10 μM), the non-selective inhibitor of the H<sub>2</sub>S-producing enzymes CBS and CSE, AOAA (100 μM), the non-selective K<sup>+</sup> channel blocker tetraethylammonium (TEA, at 3 mM) and the selective K<sub>ATP</sub> channel blocker glibenclamide (Gli, at 10 μM). E<sub>max</sub> and pA<sub>2</sub> (-logEC<sub>50</sub>) were obtained from the concentration-response curves and differences due to the different agents were analyzed by Student's t test. The expression of the main H<sub>2</sub>S-producing enzymes (CSE, CBS and 3MPST) in the arteries was analyzed by Western-blot. **Results:** The vasorelaxant responses of both AP39 and NaHS were significantly attenuated after the removal of endothelium (AP39 E<sub>max</sub> E<sup>+</sup>=72.5±4.6% vs. E<sup>-</sup>=34.8±2.0%, P<0.001; pA<sub>2</sub> AP39 E<sup>+</sup>=12.20±0.35 vs. E<sup>-</sup>=9.40±0.80, P<0.001; NaHS E<sub>max</sub> E<sup>+</sup>=84.0±7.7% vs. E<sup>-</sup>=47.1±5.7%, P<0,01), the addition of L-NAME (AP39 E<sub>max</sub> 27.0±4.5%, P<0,001; AP39 pA<sub>2</sub> 9,90±0,85, P<0,001; NaHS E<sub>max</sub> 44.0±7.0%, P<0,001; NaHS pA<sub>2</sub> 11.20±0.40, P<0,001) or ODQ (AP39 E<sub>max</sub> 22.9±3.4%, P<0,001; NaHS E<sub>max</sub> 33.0±4.4%, P<0,001; NaHS pA<sub>2</sub> 9.6±0.35, P<0,05) but not Indo. The expression of three major H<sub>2</sub>S-producing enzymes were detected by Western blot analysis and the addition of AOAA resulted in altered AP39 pA<sub>2</sub> (10,9±0,3, P<0,05). K<sup>+</sup> channels are also involved in the response to both H<sub>2</sub>S-donors; with TEA, AP39 E<sub>max</sub>=38.6±4.6%, P<0,001 and NaHS E<sub>max</sub>=45.8±4.7%, P<0,01; with Gli, NaHS E<sub>max</sub>=42.5±7.6%, P<0,01 and NaHS pA<sub>2</sub>=9.50±0.90, P<0,05). **Conclusions:** The vasorelaxant effects of the mitochondria-targeted H<sub>2</sub>S donor AP39 depend on endogenous H<sub>2</sub>S and endothelial NO but not K<sub>ATP</sub> channels. Taken together, our results and the previously published reports on the antioxidant effects of AP39 and its beneficial bioenergetic effects, support the potential use of AP39 for treatment of cardiovascular disorders. **Financial Support:** FAPESP, CNPq and CAPES. Ethics committee for animal experimentation license (CEUA/ICBN° 7759060218). **References:** CHATZIANASTASIOU, A. et al., J. Exp. Phar. Exp. Ther., 358, 431, 2016 CHENG, Y. et al., Am. J. Phys. 287, 2316, 2004 DONOVAN, K. et al., Vasc. Phar., 93, 20, 2017 GHEIBI, S. et al., Bioch.Phar., 149, 42, 2018 MANI, S. et al., Circ., 127, 2523, 2013 MOAT, S. et al., Eur. J. Phar., 530, 250, 2006 MULVANY, M. et al., Circ. Res., 41, 19, 1977 SZCZESNY, B. et al., N.O., 41, 120, 2014 TOMASOVA, L. et al., N.O., 46, 131, 2015 ZHAO, W. et al., Am. J. Phys. 283, 474, 2002

**06.037 Anti-inflammatory and vasodilatory effects of inosine in a hypercholesterolemic model: Crucial role of eNOS activation and NF-κB inhibition.**

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<sup>1</sup>UFF, <sup>2</sup>UFRJ

**Introduction:** Atherosclerosis is characterized as a chronic process closely related to inflammatory and proliferative responses of the endothelium after injury (Mutchler, Nitric Oxide, 15: 8-15, 2015). Inosine, an analog of adenosine, results of adenosine deamination by adenosine deaminase (Nishikura, Annu Rev Biochem., 79: 321-349, 2010). Adenosine and its analogs can change a variety of inflammatory diseases mediated by the immune system and has shown important effects at different models. The present study aims to evaluate the pharmacological properties of inosine, administered sub chronically in a hypercholesterolemic model in rats. **Methods:** The animal protocols were approved by the Ethics Committee for Experimental Research of the Federal Fluminense University (CEUA/UFF 858/2016). Male Wistar rats (200-250g) were randomly divided into four groups (n= 8, for each group): control group (C) and control group + inosine (C+INO) fed standard chow diet, hypercholesterolemic diet group (HC) and hypercholesterolemic diet group + inosine (HC+INO). At 31<sup>o</sup> diet day, was performed the sub chronic treatment with inosine (10 mg/kg/orally) once daily, totalizing 15 days. The animals were euthanized under ketamine and xylazine anesthesia. Blood samples were collected for ELISA and biochemical analysis. Thoracic aortas were excised for vascular reactivity and western blot assays. Data were analyzed using one-way ANOVA followed by a post-hoc Bonferroni Multiple Comparison Test, p<0.05. **Results:** We observed that the intake of hypercholesterolemic diet promoted an increase in total cholesterol (HC: 352.60 ± 73.81 mg/dl x C: 86.56±8.70mg/dl), Triglycerides (HC: 192,50 ± 45.98 mg/dl x C: 5 3.06 ± 6.75 mg/dl), VLDL (HC: 51.20 ± 11.14 mg/dl x C: 12.42 ± 1.94 mg/dl), LDL (HC: 458.70 ± 107.7 mg/dl x C: 86.56 ±8.7 mg/dl) serum levels and aorta lipid peroxidation (C: 5.11 ± 0.24 nmol/mg protein<sup>-1</sup> x HC: 6.89 ± 0.24 nmol/mg protein<sup>-1</sup>). However, inosine treatment significantly reduced the lipid profile (p<0.05), as well as aorta lipid peroxidation (2.37 ± 0.30 nmol/mg protein<sup>-1</sup>). Furthermore, the HC diet increased IL-6 (C: 41,32 ± 1,25 pg/ml x HC: 110,70 ± 5,44 pg/ml) and TNF-α (C: 30.93 ± 2.93 pg/ml x HC: 42.68 ± 2.34 pg/ml) serum levels, decreased the maximum relaxation induced by acetylcholine (83.02 ± 4.07 %) when compared to control group (89.17 ± 1.36%) and increased NF-κB expression, leading to an increase of iNOS expression and to a consequent decrease of proteins involved in the eNOS pathway (p<0.05). The treatment with inosine reduced IL-6 (HC+INO: 47,96±4,17 pg/ml) and TNF-α (HC+INO: 27.55±4.90 pg/ml) serum levels, iNOS, VCAM-1 and NF-κB protein expression in aortas of hypercholesterolemic rats (p<0.05). Inosine increased the maximum relaxation promoted by acetylcholine (98.23 ± 2.21%), through eNOS phosphorylation, PKA and PKG protein expression (p<0.05). **Conclusion:** This study demonstrated the ability of the hypercholesterolemic diet to promote vascular damages through increasing pro-inflammatory cytokines and proteins. On the other hand, we also showed that the treatment with inosine was able to improve vascular function, probably by increasing PKA and PKG expression and the activation of eNOS, culminating in a decrease of the inflammatory process through NF-κB inhibition. This study provides results that indicate inosine as a potential drug for the treatment of cardiovascular disorders such as atherosclerosis. **Funding:** CNPq, CAPES, PROPPI-UFF, FAPERJ.

**06.038 Inhibition of acetylcholinesterase promotes alterations on cardiovascular autonomic control and aortic vascular reactivity in L-Name-induced hypertensive rats.** Cavalcante GL<sup>1</sup>, Ferreira FN<sup>1</sup>, Sousa MF<sup>1</sup>, Lima JVO<sup>2</sup>, Furtado MM<sup>1</sup>, Alves Filho FC<sup>1</sup>, Silva MTB<sup>1</sup>, Arcanjo DDR<sup>1</sup>, Sabino JPJ<sup>1</sup> <sup>1</sup>UFPI, <sup>2</sup>UNIFSA

**Introduction:** Sympathetic overactivity and its outcome in hypertension have been thoroughly investigated to determine the focus of pharmacologic approaches targeting the sympathetic nervous system in the treatment of this pathophysiological condition. On the other hand, therapeutic approaches aiming to protect the reduced cardiac parasympathetic function, such as anticholinesterase administration, have not received much attention. Objective: Evaluate the effect of anticholinesterase drugs administration with central and peripheral (donepezil) or only peripheral (pyridostigmine) action on mean arterial pressure (MAP), heart rate (HR), baroreflex, chemoreflex, cardiac sympathetic and parasympathetic tonus, intrinsic heart rate (I-HR), and vascular reactivity in L-NAME hypertensive rats. Methodology: Male Wistar rats were divided into 4 groups: I) Control (H<sub>2</sub>O), II) L-NAME (70 mg/kg), III) L-NAME + donepezil (1,4 mg/kg) and IV) L-NAME + pyridostigmine (22 mg/kg/day). The oral administrations were performed for 2 weeks. Then, the systolic arterial pressure (SAP) was measured by tail plethysmography on 0, 2, 7 and 14 days. After the end of treatments, the femoral artery and vein were catheterized with polyethylene tubing under the anesthesia for recording MAP, HR and for intravenous drug administration. The baroreflex sensitivity was evaluated by phenylephrine and nitroprusside administration, while to the parasympathetic tonus was administered methylatropine. The *in vitro* vascular reactivity in aortic rings was assessed by determination of concentration-response curves for phenylephrine, acetylcholine and sodium nitroprusside. Results: Rats with L-NAME-induced hypertension did not presented changes in HR and I-RH. On the other hand, L-NAME hypertensive animals presented reduction of baroreflex sensitivity, parasympathetic tonus and vascular reactivity. Pyridostigmine and donepezil promoted reduction of MAP, as well as promoted an increase of baroreflex sensitivity and parasympathetic tonus. Moreover, concentration-response curves for acetylcholine or sodium nitroprusside presented a significant rightward shift when compared with L-NAME hypertensive control. **Conclusion:** The inhibition of acetylcholinesterase with either donepezil or pyridostigmine proved to be an important pharmacological approach, which could be used to increase parasympathetic function and various cardiocirculatory parameters, furthermore attenuated vascular dysfunction in rats with L-NAME-induced hypertension. Indicating that the inhibition of acetylcholinesterase produces beneficial effects for antihypertensive therapy. License Number of ethics committee: 495/18. **Financial support:** CNPq (409109/2018-5) and CAPES.

**06.039 Antiatherogenic effects of cyclic nucleotide modulators through NF- $\kappa$ B and p38 MAPK Inhibition in aortas of hypercholesterolemic rats.** Motta NAV<sup>1</sup>, Lima GF<sup>1</sup>, Lopes RO<sup>1</sup>, Mendes ABA<sup>2</sup>, Aufran LJ<sup>1</sup>, Brazão SC<sup>1</sup>, Marques EBM<sup>1</sup>, Kümmerle AEK<sup>3</sup>, Barreiro EJB<sup>2</sup>, Scaramello CBVS<sup>1</sup>, Brito FCF<sup>1</sup>UFF, <sup>2</sup>UFRJ, <sup>3</sup>UFRRJ

**Introduction:** Atherosclerosis is a chronic inflammatory disease and is closely associated with inflammation, thrombogenesis and oxidative stress. Due to its complexity, single-target drugs usually fail to treat this multifactorial disease. Since phosphodiesterases (PDEs) are associated with many physiological functions, several PDEs inhibitors have been studied in several cardiovascular diseases. Previous reports have demonstrated that cilostazol and thienylacylhydrazone LASSBio-788 exert antiatherogenic effects (Motta, NAV. J. Pharmacol. Sci. 123: 47, 2013; Motta, NAV. Fundam Clin Pharmacol 30: 327, 2016). Thus, this study aims to investigate the pharmacological properties of cyclic nucleotides modulators, such as cilostazol and LASSBio-788 derivative in aortas of hypercholesterolemic rats to characterize their molecular mechanisms and multi-target effects. **Methods:** The use of animals was according to Ethics Committee (CEUA 858/16). Male Wistar rats (150-200g) were randomly divided into 5 groups: C (control group) has received normal rat chow for 45 days. HCD (hypercholesterolemic diet) group, HCD+SIMV (simvastatin group), HCD+788 (compound LASSBio-788 group) and HCD+CIL (cilostazol group) have received hypercholesterolemic diet (HCD) for 45 days. At 31<sup>o</sup> diet day, was performed the chronic treatment with simvastatin (10mg/kg/day), LASSBio-788 (100  $\mu$ M/kg/day) and cilostazol (30 mg/kg/day) once daily, totalizing 15 days of treatment. The animals were euthanized under anesthesia (ketamine and xylazine). Blood samples were collected, the thoracic aortas were excised for biochemical, functional and molecular analysis. Data were analyzed using one-way ANOVA followed by post-hoc Bonferroni Multiple Comparison Test,  $p < 0.05$ . **Results:** The HCD increased serum lipids levels and reduces HDL levels in the HCD group ( $p < 0.05$ ). The HCD group showed an increase of blood pressure (systolic  $170.40 \pm 4.70$  x  $130.80 \pm 4.80$  mm/Hg), a decrease of maximal relaxation induced by acetylcholine ( $83.40 \pm 1.4$  x  $94.70 \pm 2.2\%$ ), besides increased the lipid peroxidation in aortas ( $63.41 \pm 5.70$  x  $33.20 \pm 0.50$  nmol/mg protein) of HCD group when compared to C group. The HCD group showed an increase of TNF- $\alpha$ , TXA<sub>2</sub> ( $p < 0.05$ ) and a decrease of cyclic nucleotide levels in aortas (cAMP:  $84.0 \pm 1.23$  x  $102.70 \pm 0.64$ ; cGMP:  $21.95 \pm 1.32$  x  $63.47 \pm 1.14$  pmol/mg protein). HCD promoted PLC- $\gamma$ , PKC- $\alpha$ , NF- $\kappa$ B, p38 phosphorylation and increased iNOS and VCAM-1 protein expression. Moreover, the HCD inhibited eNOS, I $\kappa$ B- $\alpha$ , PKA and PKG protein expression ( $p < 0.05$ ). The sub chronic treatment with LASSBio-788 and cilostazol reduced serum lipids and increased HDL levels, besides decreased lipid peroxidation in aortas ( $p < 0.05$ ). Furthermore, both reduced the blood pressure induced by HCD (systolic HCD+788:  $118.60 \pm 1.30$  and HCD+CIL:  $120.50 \pm 0.89$  mm/Hg) and increased the maximal relaxation induced by acetylcholine when compared to HCD group ( $p < 0.05$ ). LASSBio-788 and cilostazol also inhibited TNF- $\alpha$ , TXA<sub>2</sub> and increased cyclic nucleotide levels in aortas ( $p < 0.05$ ). The cyclic nucleotides modulators inhibited PLC- $\gamma$ -PKC- $\alpha$ -p38-NF- $\kappa$ B and activated eNOS-NO-GC-PKG pathway in aortas ( $p < 0.05$ ). **Conclusion:** The sub chronic treatment with LASSBio-788 and cilostazol presented antiatherogenic effects in vivo. This study provides a new insight of vascular mechanisms of LASSBio-788 and cilostazol to explain their clinical protective effects in atherosclerosis, in addition to elucidate the role of new multi-targeted drugs for the treatment of cardiovascular diseases. **Financial support:** CAPES, FAPERJ, PROPPi/UFF

#### **06.040 Influence of perivascular adipose tissue in vascular dysfunction of sepsis.**

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**Introduction:** Perivascular adipose tissue (PVAT) has more than a structural function, since it participates in the regulation of vascular tonus by the release of vasoactive mediators such as nitric oxide (NO), leptin and adiponectin in normal physiology (Brown NK, *Arterioscler Thromb Vasc Biol* 34: 1621, 2014). In some pathologies however, including hypertension and obesity, there are changes in the mediators released by PVAT that affects vessel smooth muscle, eliciting important vascular dysfunction (Lee H, *Atherosclerosis* 230: 177, 2013). Sepsis is a complex pathological condition in which cardiovascular dysfunction, characterized by hypotension e hyporesponsiveness to vasoconstrictors, plays a fundamental role (Angus DC, *N Engl J Med* 369: 840, 2013). If, in one hand, dysfunctional endothelium is a relevant player in sepsis vascular dysfunction of sepsis, on the other hand, PVAT putative role in this scenario is unknown. Therefore, the present work was designed to evaluate how PVAT contributes to the altered contraction of aorta and the superior mesenteric artery during sepsis. **Methods:** Wistar female rats weighting 200-250 g were anesthetized and septic shock was induced by cecal ligation and puncture (CLP) model. Twenty-four hours after sepsis induction, aorta and superior mesenteric arteries were gently collected with (PVAT+) or without PVAT (PVAT-), cut in rings and then mounted in tissue organ baths to record vascular reactivity to vasoactive agent using the software PowerLab® and LabChart®; the same was made incubating vessels with NOS inhibitor, L-NAME (200 µM). For the molecular analysis, PVAT were collected 12 and 24 h after sepsis induction, frozen in Tissue-Tek medium and then cut in slices for the evaluation of NO, ROS and NOS expression through fluorescent assays. Image capture was performed in a fluorescence microscope and the quantification was done by ImageJ® software. Analysis was made using one- or two-way ANOVA and Bonferroni as post hoc. **Results:** PVAT presented anticontractile effect in aorta, but not in superior mesenteric arteries of naïve female rats. In the late phase of sepsis, PVAT increased the vascular hyporesponsiveness to noradrenaline in both studied arteries. The difference in reactivity to noradrenaline was abolished by the use of L-NAME. There was an increase in NO production only in aorta PVAT and increase of ROS production for both aorta and superior mesenteric arteries PVAT. Also, neuronal isoform of NOS was increased during sepsis progression in aorta and mesenteric PVAT. **Conclusions:** The presence of PVAT increases the hyporesponsiveness in aorta and mesenteric artery during septic shock. Our results indicate that this effect may be mediated by NO, since 1) NO production inhibition abolished these differences to noradrenaline contraction between PVAT+ and PVAT- arteries; 2) there is an increase in nNOS expression of PVAT during sepsis progression, although NO production seems to be different depending on the location. Therefore, PVAT should be considered as a new and relevant player in sepsis vascular dysfunction. **Financial support:** CNPq and CAPES. Research approved by the Institutional Animal Ethical Committee: CEUA/UFSC nº2264190617.

**06.041 IL-1RI contributes to endothelial dysfunction, vascular remodeling and oxidative stress in Angiotensin II-induced hypertension.** Fedoce AG, Pereira CA, Aguiar CAS, Parente JM, Gonzaga NDA, Tostes RCA, Carneiro FS FMRP-USP

**Introduction:** Hypertension is associated with chronic low-grade inflammation, and systemic and vascular levels of cytokines such as interleukin 1 beta (IL-1 $\beta$ ) are elevated, while the levels of anti-inflammatory cytokines including interleukin10 (IL-10) are decreased in this disease [1,2]. IL-1 $\beta$  leads to endothelial dysfunction upon short-term incubation in rat mesenteric arteries, and it is also involved in vascular remodeling by increasing vascular smooth muscle migration, proliferation, and reactive oxygen generation (ROS)[3,4,5,6]. Thereby, the role of IL-1 $\beta$  receptor, the interleukin 1 type receptor (IL-1RI) still needs further elucidation to its role in those processes. Therefore, we hypothesized that IL-1RI promotes endothelial dysfunction and remodeling in Angiotensin II- (ANGII)-induced hypertension. **Methods:** Subcutaneous infusion of ANGII (1 $\mu$ g/kg/min, for 14 days), via osmotic mini-pump, was used to induce arterial hypertension in male mice aged 12 weeks, while controls had sham surgery. C57BL/6 control mice (WT) and IL-1RI knockout mice (IL-1RI KO) were divided into four groups: 1) WT; 2) WT + ANGII; 3) IL-1RI KO; 4) IL-1RI KO + ANGII. Mean arterial pressure (MAP) was measured by intra-arterial catheter method. Vascular function and structure were accessed by isometric tension and histological analysis, respectively, using second-order mesenteric resistance arteries MRA. ROS was measured in MRA by lucigenin. IL-1 $\beta$  and IL-10 plasma levels were detected using ELISA. Results: IL-1RI KO and WT mice had similar MAP (WT: 116.9  $\pm$  2.7 vs IL-1RI KO: 118.5  $\pm$  3.4; mmHg; n=7-9), while MAP increase was not prevented in IL-1RI KO mice two weeks after ANGII infusion (IL-1RI KO + ANGII: 155.4  $\pm$  4.7 vs WT + ANGII: 159.9  $\pm$  1.8; mmHg; n=7). However, the reduction in acetylcholine-induced vascular relaxation in WT mice infused with ANGII was prevented in IL-1RI KO mice (WT + ANGII: 75  $\pm$  7; vs IL-1RI KO + ANGII: 99  $\pm$  6; % of relaxation; n=12). The media-lumen ratio (M/L) was increased in WT infused with ANGII compared to its control and the IL-1RI + ANGII mice prevented this vascular remodeling alteration (WT: 13.1  $\pm$  1.1; vs WT + ANGII: 15.9  $\pm$  1.1; vs IL-1RI + ANGII: 12.9  $\pm$  0.8; n=4-5). The ANGII-induced hypertension doubled the IL-1 $\beta$  plasma levels in WT mice but not in IL-1RI KO mice (WT + ANGII: 228.9  $\pm$  20; vs IL-1RI KO + ANGII: 111.8  $\pm$  17; pg/ml, n=6-8). Surprisingly, the IL-1RI KO + ANGII increased the IL-10 plasma levels compared to WT + ANGII mice (IL-1RI KO + ANGII: 20.6  $\pm$  3.4; vs WT + ANGII: 1.20  $\pm$  0.6; pg/ml, n=6-7). Furthermore, the WT + ANGII mice increased the anion superoxide generation compared to its control (WT: 49,7  $\pm$  10,7 vs WT + ANGII: 267,0  $\pm$  42,1; URL), while IL-1RI + ANGII did not show a significant increase (IL-1RI KO + SHAM: 124,0  $\pm$  15,2 vs IL-1RI KO + ANGII: 172,2  $\pm$  18,6; URL, n=4-5). **Conclusion:** These results indicate that IL-1RI absence prevents endothelial dysfunction, remodeling and ROS generation in MRA from ANGII-induced hypertension in mice, but not MAP elevation. IL-1RI pharmacological inhibition may represent a new approach to reduce vascular damage and inflammation in arterial hypertension. **References:** 1. BOMFIM, G. F.; et al. British journal of pharmacology, v. 176, n. 12, p. 2028-2048, 2019. 2. CAILLON, A.; SCHIFFRIN, E. L. Current hypertension reports, v. 18, n. 3, p. 1-9, 2016. 3. BIVOL, L. M. et al. American journal of physiology-regulatory, integrative and comparative physiology, v. 294, n. 2, p.447, 2008. 4. VALLEJO, S. et al. Cardiovascular diabetology, v. 13, n. 1, p. 158, 2014. 5. JIMÉNEZ-ALTAYÓ, F. et al. Journal of Pharmacology and Experimental Therapeutics, v. 316, n. 1, p. 42-52, 2006. 6. AGUADO, A. et al. Journal of hypertension, v. 34, n. 2, p. 253, 2016. **Financial Support:** This work was supported by grants from CNPQ, CAPES and FAPESP (2016/11988-5). All experimental procedures were approved by the Ethics Committee on Animal Research of the Ribeirao Preto Medical School, University of Sao Paulo (protocol 179/2017).

**06.042 The flavonoid luteolin alters the production of prostanoids and nitric oxide by the venous endothelium.** <sup>1</sup>Assunção HCR, Cruz YMC<sup>2</sup>, Bertolino J<sup>1</sup>, Fernandes L<sup>2</sup>

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**Introduction:** Luteolin is a flavonoid present in a variety of vegetables, fruits and herbs, and several studies have indicated its antioxidant and anti-inflammatory properties. Recently, positive cardiovascular effects have been also attributed to luteolin, however, little is known about its specific actions at the venous endothelium. Changes in venous tone can induce physiological consequences similar to those promoted by acute changes in blood volume and, therefore, can significantly alter cardiac output and consequently all blood circulation. The aim of the present study was to investigate the effects of luteolin on the production of prostanoids (PGI<sub>2</sub>, PGF<sub>2α</sub> and TXA<sub>2</sub>) and nitric oxide (NO) in cultured endothelial cells obtained from rat inferior vena cava. **Methods:** Endothelial cultures of rat vena cava were subcultured in DMEM (10% FBS), 5%CO<sub>2</sub>, 37°C. Cells were incubated with different concentrations of luteolin [10, 20 and 50 μM], and control groups were performed by untreated cells. Cell viability was tested after 24 hours incubation by MTT assay (n=3). Prostanoids measurements in supernatants were performed by immunoenzymatic assay 24 hours after luteolin incubation (n=5-6). NO production was detected by spectrofluorometry in alive cells that were previously seeded in dark plates, pre-treated with a specific fluorescent probe for NO (DAF-2DA 10 μM, for 30 minutes) and stimulated for 10 minutes with luteolin (n=7). In another set of experiments, cells were previously seeded in coverslips, pre-treated with DAF-2DA (10 μM, 30 minutes), stimulated with luteolin for 10 minutes, fixed (PFA 4%) and observed in a confocal microscope. Fluorescence intensity was quantified by densitometry (n=6). Medium values of absorbance (for prostanoids) or fluorescence (for NO) from control groups were set as 1, and data from experimental groups were normalized as fold change, expressed in arbitrary units. **Results:** After 24 hours of incubation, luteolin had no effect on the cell viability of cultured endothelial cells. Luteolin [20 μM] decreased PGF<sub>2α</sub> levels (0.65 ± 0.04\*), but significantly increased TXA<sub>2</sub> in all tested concentrations [(10 μM = 1.22 ± 0.06\*), (20 μM = 1.37 ± 0.09\*), (50 μM = 1.49 ± 0.10\*)], while no significant changes were observed in PGI<sub>2</sub> levels. Spectrofluorometry assays detected a significant increase in NO production by cells pre-incubated with 50 μM of luteolin (3.73 ± 0.35\*). This result was confirmed by confocal microscopy images, where luteolin consistently increased NO production in comparison to basal levels [(20 μM = 1.99 ± 0.18\*), (50 μM = 2.32 ± 0.08\*)]. (\*P<.05). **Conclusion:** The flavonoid luteolin exhibits vasoactive properties in a concentration-dependent manner. Luteolin increases NO production, induces the release of TXA<sub>2</sub> and reduces PGF<sub>2α</sub> levels. These effects may account for changes in the venous tonus with important consequences for the whole venous capacitance and cardiac output. CEP UNIFESP 69782003 Supported by CNPq and FAPESP (2017/22028-5)

**06.043 Are echocardiographic changes addressed to neonatal leptin treatment related to different biochemical profiles in female Wistar rats?** Souza KP, Pedro SS, Rocha NN, Scaramello CBV UFF

**Introduction:** Literature describes that neonatal leptin treatment leads to an increased cardiovascular risk and an age-dependent cardiac dysfunction in male *Wistar* rats (Marques *et al.*, *Int J Cardiol.* 181C: 141, 2015) along to hypothalamic resistance and intermediary metabolic parameters changes in adulthood (Tosteet *et al.*, *Bri J Nutrit.* 95: 830-837, 2006). As sex differences may affect cardiovascular disease presentation as well as its, diagnosis and prognosis (Arain *et al.*, *Circ J.* 73(10): 1774-1782, 2009), the aim of this work was to evaluate cardiometabolic parameters in adult female *Wistar* rats submitted to neonatal leptin treatment. **Methods:** At postnatal day 1 offspring were randomly divided into 2 groups -Leptin and Control, 5-6 individuals per group, that received respectively leptin (8µg/100g sc) and saline (NaCl 0,9% sc) daily for the first 10 days of lactation. At postnatal day 21 both groups were weaned being offered water and commercial chow *ad libitum*. All animals were evaluated till postnatal day 150. Besides anthropometric, nutritional and echocardiographic analysis, blood samples were collected to determine serum glucose concentration and lipids profile. Data are presented as standard error and its mean, analyzed by Student t test being considered statistically different if  $P < 0.05$  (\*) compared to respective control. **Results:** No differences between groups were noticed about nutritional parameters. Despite similar body weights, leptin neonatal treatment leads to higher chest-to-abdominal circumference ratio ( $1.18 \pm 0.03 \times 1.27 \pm 0.01$ \*) and body mass index ( $0.51 \pm 0.01 \times 0.57 \pm 0.01$ \* g/cm<sup>2</sup>). Echocardiographic data points to a greater left ventricle internal diameter in systole ( $0.24 \pm 0.02 \times 0.34 \pm 0.03$ \*cm). Biochemical analysis showed increased concentration of serum glucose ( $88.52 \pm 9.38 \times 124.10 \pm 6.26$ \*mg/dL) and total cholesterol ( $47.16 \pm 1.18 \times 56.30 \pm 2.85$ \*mg/dL). **Conclusion:** As observed in males these data suggest that leptin neonatal treatment increases body fat and consequently cardiovascular risk in females. According to literature changes in biochemical profile and left ventricle dilatation are expected in females with higher body mass index (Serrano *et al.*, *Arq B Cardiol.* 4: 464-72, 2010; Bazzano *et al.*, *ClinCardiol* 34(3)153-9). **Financial Support:** FAPERJ, CNPq, CAPES, PROPPI/UFF. **Ethics Committee Approval Number:** CEUA/UFF812-16.

**06.044 Effect of carvacrol on monocrotaline induced pulmonary hypertension in rats.** Alves RMFR, Medeiros IA, Oliveira JCPL, Maciel PMP, Silva GNA, Santos PF, Azevedo FLAA, Gonçalves TAF UFPB

**Introduction:** Pulmonary hypertension (PH) is a chronic, progressive and rare disease that is characterized by increased pulmonary vascular resistance, right ventricular hypertrophy and elevated right ventricular systolic pressure (Alencar, BJP, v.169, p. 953, 2013). Carvacrol (CRV) is a phenolic monoterpene found in essential oils produced by aromatic plants that has displayed vasorelaxant, anti-inflammatory and antioxidant properties (Suntres, Crit. Ver. Food. SciNutr., v.55, 304, 2014). The aim of this study was to evaluate the effects of carvacrolin preventing monocrotaline (MCT)-induced PH in rats.

**Methods:** Male Wistar rats were injected subcutaneously with saline 0,9% (control group - CTL) or monocrotaline(60mg/Kg) to develop PH. They were divided into the following groups: CTL; MCT; MCT+CRV 50mg/Kg; MCT+CRV 100 mg/Kg; and MCT+SILD (sildenafil 50mg/Kg). 24 hours later, rats were treated daily with oral administration for 28 days. The following parameters were evaluated: right ventricular systolic pressure (RVSP), right ventricular weight to left ventricular plus septum weight ratio (Fulton index), vascular reactivity and production of superoxide anions.All the experimental protocols were approved by CEUA-UFPB (number: 1253040418 - ID 000266).Results: The MCT group presented an increased RVSP ( $37\pm 3$  mmHg; n= 4) compared to the CTL group ( $20\pm 2$  mmHg; n= 4). RVSP was significantly attenuated in the MCT+CRV 50mg/Kg ( $24\pm 1$  mmHg; n= 4) and MCT+SILD ( $21\pm 4$  mmHg; n= 4) groups. Regarding to index of right ventricular hypertrophy (RVH) the MCT group ( $0,38 \pm 0,02$  g; n= 4) showed an increase compared to the CTL group ( $0,23 \pm 0,04$  g; n= 4). RVH was significantly reduced in the MCT+CRV 50mg/Kg ( $0,26 \pm 0,02$ g; n= 4) and MCT+CRV 100mg/Kg ( $0,26 \pm 0,05$ g; n= 4) groups. In isolated pulmonary arteries, contractions to phenylephrine (Phe) as well as vasodilation to acetylcholine (ACh) or sodium nitroprusside (SNP) were significantly reduced ( $E_{max}= 66 \pm 5\%$ ; n= 6,  $E_{max} = 44 \pm 8\%$ ; n= 6, or  $E_{max}= 78 \pm 3\%$ ; n= 8, respectively) in the MCT group compared to control group. On the other hand, treatment with CRV (MCT+CRV 50 mg/kg or MCT+CRV 100 mg/kg) significantly improved contractions to Phe ( $E_{max}= 98 \pm 9\%$ ; n= 6, or  $E_{max}= 88 \pm 8\%$ ; n= 6, respectively) or vasodilations to ACh ( $E_{max}= 75 \pm 8\%$ ; n= 7, or  $E_{max}= 130 \pm 13\%$ ; n= 5, respectively). Nevertheless, no significant alterations were observed to SNP, excepting that vasodilation was improved in the MCT+CRV 50mg/kg group ( $E_{max}= 90 \pm 2\%$ ; n= 8).The analysis of oxidative stress in rat pulmonary arteries revealed a high fluorescence intensity in the MCT group ( $216 \pm 22\%$ ; n= 5). Treatments with MCT+CRV 50mg/Kg ( $119 \pm 8\%$ ; n= 5); MCT+CRV 100mg/Kg ( $68 \pm 6\%$ ; n= 5) or MCT+SILD ( $96 \pm 7\%$ ; n= 5) significantly attenuated the oxidative stress in pulmonary arteries of PH rats. **Conclusion:** The results obtained so far demonstrate that carvacrolhas been shown to be a promising substance for the treatment of PH since it attenuates pulmonary arterial pressure, right ventricular hypertrophy, improves endothelial dysfunction, and reduces tissue oxidative stress. Financial support: CAPES/FAPESQ.

**06.045 Perivascular tissue regulates the relaxation of umbilical cord veins in different nutritional states.** Machado MR, Servian CDPS, Oliveira SCM, Filgueira FP, Costa RM, Lobato NS UFG

**Introduction:** Although perivascular tissue has already been structurally characterized, there are no studies evaluating its influence on the functional response of the umbilical cord veins, as well as its role in the impairment of these vessels in obesity. We have previously demonstrated the presence of adipocytes in the perivascular tissue of the umbilical cord veins, as well as the influence of overweight/obesity in pregnant women on the structural characteristics of this tissue. The objective of the present study was to investigate the role of perivascular tissue in the reactivity of umbilical cord veins as well as the influence of overweight and excessive weight gain during the gestational period on the functional properties of this tissue. **Methods:** Functional parameters of the umbilical veins from pregnant women with normal weight (eutrophic group), overweight/obesity at the beginning of gestation and excess weight gain (EWG) during pregnancy were evaluated. The study was evaluated and approved by the Research Ethics Committee of the UFG (CAAE 62155316.0.0000.5083) and is in accordance with Resolution 466/2012. Segments of umbilical cord veins (4mm) with total removal of the perivascular region or preserved perivascular region were used in the present study. A clean vessel ring was incubated with solution obtained from previously incubation of vessels with perivascular tissue. Concentration-effect curves were performed with the insulin and NPS in vessels pre-contracted with KCl (40 mmol/L). **Result:** In vessels with preserved perivascular tissue obtained from eutrophic pregnant women, the relaxation response to insulin was similar to that observed in control vessels, however, vessels incubated with perivascular solution presented a greater response when compared to either control veins and veins with preserved perivascular tissue. In vessels from overweight/obese pregnant women, the presence of perivascular tissue induced a more pronounced relaxation when compared to either vessels without perivascular tissue or vessels that were incubated with perivascular solution. In EWG pregnant women, the vessels with preserved perivascular tissue showed reduced relaxation than either control vessels and those incubated with perivascular solution. The relaxation evoked by NPS was similar in umbilical veins from eutrophic, overweight/obese and EWG pregnant women. Additionally, no significant difference was observed in the response to this agonist in vessels with perivascular tissue and vessels incubated with perivascular solution when compared to control vessels. Pregnant women with overweight/obesity at the beginning of gestation and EWG displayed a greater relaxing response to insulin when compared to eutrophic pregnant women. Overweight/obese pregnant women at the beginning of gestation presented a significant increase in NPS-induced relaxation when compared to pregnant eutrophic and EWG. **Conclusion:** The perivascular tissue of the umbilical cord vein modulates vascular function promoting a relaxing effect. Nutritional status influences the relaxing response of the umbilical cord veins and also impacts the modulatory effect of the perivascular tissue.

**06.046 Combined inhibition of AT1 receptor and advanced glycation end products in the diabetic nephropathy.** Flores EEI, Pereira ENGS, Silveiras RR, Rodrigues KL, Araújo BPD, Martins CSM, Daliry A Fiocruz

Diabetes mellitus (DM) is a major public health problem worldwide. Current global estimates indicate that this condition affects 415 million people and is set to escalate to 642 million by the year 2040. Nephropathy is the renal microvascular complication of diabetes and the major cause of kidney failure. It is characterized by the presence of the decreased albuminuria and glomerular filtration rate with thickening of the glomerular basement membrane, glomerular sclerosis and proximal tubular cells atrophy. Since angiotensin II activation of AT1 causes afferent artery vasoconstriction impairing renal hemodynamics, and the accumulation of advanced glycation end products (AGEs) causes intracellular glycototoxicity of mesangial, podocytes and tubular cells, we tested the ability of the isolated or combined treatment of olmesartan and pyridoxamine in conferring improvement in diabetic nephropathy when compared to isolated treatments. For this, DM was induced in 20 C57BL/6 mice by administration of intraperitoneal streptozotocin 50 mg/kg/day for five consecutive days. Control animals (n= 5) received intraperitoneal sodium citrate injection. Diabetic status was confirmed with fasting blood glucose levels higher than 300 mg/dL and albuminuria greater than 300 mg/dL. Subsequently the animals were divided into four groups: control (CTL, n= 5), diabetic (DM, n= 5) diabetic treated with olmesartan 20 mg/kg/day (DM+OLM, n= 5), diabetic treated with pyridoxamine 400 mg/kg (DM+PYR, n= 5) and diabetic animals submitted to combined treatment of olmesartan and pyridoxamine (DM+PYR+OLM, n= 5). After 16 weeks of treatment, biological samples (serum, urine and tissue) were collected for metabolic and morphological analysis. Diabetic animals showed an increase in fasting blood glucose levels, which was not altered by any treatment. There was a significant increase in the serum creatinine and albuminuria levels in the DM group when compared to control animals, and all treatment protocols were able to decrease these parameters. Increased levels of urinary content of glycated proteins fructosamine and AGEs were observed in diabetic animals when compared to controls. Pyridoxamine and olmesartan treatments or the adjunct therapy were able to decrease the AGEs and fructosamine content in the urine. We conclude that treatment with pyridoxamine and/or olmesartan decreases the urinary glycated proteins levels and may thus minimize the intracellular glycototoxicity in glomerular cells. In addition, the decrease in serum creatinine and albuminuria may indicate an improvement in renal hemodynamics by inhibiting angiotensin II action on AT1 receptors. No additional effect of the combination therapy was observed in the present study. All experimental procedures were conducted in accordance with internationally accepted principles for the Care and Use of Laboratory Animals (License L-012/2018 A1). This work was funded by CNPQ, FAPERJ and PAPES / FIOCRUZ.

**06.047 Chronic ouabain administration modulates cardiac and renal membrane lipid content in rats.** Quintas LEM<sup>1</sup>, Garcia I<sup>2</sup>, Feijó P<sup>1</sup>, Araújo W<sup>2</sup>, França-Neto A<sup>3</sup>, Rossoni L<sup>3</sup>, Barbosa L<sup>2</sup>, Santos H<sup>2</sup> <sup>1</sup>UFRJ, <sup>2</sup>UFSJ, <sup>3</sup>USP

**Introduction:** Na<sup>+</sup>/K<sup>+</sup>-ATPase (NKA) is an active membrane transporter that carries Na<sup>+</sup> and K<sup>+</sup> against their electrochemical gradients and is important for renal reabsorption of Na<sup>+</sup> and water and cardiac performance, as well as regulation of blood pressure. Ouabain, an endogenous cardiotonic steroid, induces systemic hypertension in rats after chronic administration (Rossoni et al., Life Sci, 79: 1537, 2006). We have shown that administration of low doses of ouabain increased renal and cardiac NKA expression and renal NKA activity (Feijó et al., 15<sup>th</sup> International Conference on Na,K-ATPase and Related Transport ATPases, Otsu, Japan, 2017). Membrane lipids have different physico-chemical properties and changes in membrane fluidity may affect NKA function. Here we evaluated the effect of chronic ouabain in kidney and heart membrane lipid composition. **Methods:** Male Wistar rats (6 weeks) were administered with ouabain (OUA, 8 µg/day s.c.) or vehicle (VH) for 5 weeks. Kidneys and heart ventricles were dissected, weighed and stored at -80°C. They were homogenized and ultracentrifugation pellets were resuspended. Lipid fractions were extracted with chloroform: methanol mixture (2: 1 v/v) in order to measure phospholipids, determined from the amount of Pi released by acid hydrolysis, and cholesterol, determined by acetic acid/ferric chloride method. Values are expressed as mean ± SEM and statistical analysis was performed by Student t test (p<0.05 was considered statistically significant). **Results:** Chronic ouabain administration induced a significant increase in systolic blood pressure (135.5 ± 0.6 vs. 118.3 ± 0.7 mm Hg, respectively; n=6, p<0.05), but no change in cardiac and renal mass and in total protein content. On the other hand, in both organs cholesterol content was significantly elevated compared to VH (30%, kidney; 59%, heart; n=6, p<0.05) but total phospholipids were reduced (21%, kidney; 27%, heart; n=6, p<0.05), giving a phospholipid/cholesterol ratio 35% (kidney) and 55% (heart) lower when compared to VH. **Conclusions:** Our results demonstrate that ouabain regulates cardiac and renal cell cholesterol and phospholipid content. Phospholipid/cholesterol ratio may be inversely proportional to NKA function and might be a way to at least partially override the direct NKA inhibition promoted by ouabain. **Financial support:** FAPESP, CAPES, FAPERJ and CNPQ. Approved by the Ethics Committee on Animal Experiments of ICB/USP (protocol: 034/2012).

**06.048 Anticontractile effect of perivascular adipose tissue is enhanced by stimulated hydrogen sulfide formation and is ATP-sensitive potassium channel-dependent in hypertensive pregnant rats.** Tozzato GPZ, Polonio LCC, Paula ES, Dias Júnior CA Unesp-Botucatu

**Introduction:** It has been suggested that the perivascular adipose tissue (PVAT) modulates the vascular tone mainly through the releasing of diffusible adipocyte-derived relaxing factors (ADRFs) (Gao, J.Y; Br J Pharmacol, v 151-3, p.323-331, 2009). Also, ATP-sensitive potassium channels ( $K_{ATP}$  channels) are involved in the modulation of the vascular tone, possibly through its activation by hydrogen sulfide ( $H_2S$ ) (Beltowski, J; Molecules, v. 22-1, 2016). In addition, PVAT is impaired in hypertension (Lee, Y.C; Circulation, v.124-10, p.1160-71, 2011), however, it is not yet well elucidated during hypertension in pregnancy. Therefore, we aimed to investigate the involvement of PVAT-derived  $H_2S$  to modulate the vascular tone in aorta from normotensive and hypertensive pregnant rats and its relationship with  $K_{ATP}$  channels. **Methods:** Female Wistar rats (250g) were mated and then allocated in individual cages. Animals were divided into normal pregnant (Norm-Preg) and hypertensive pregnant (HTN-Preg; DOCA-Salt model - i.p. administration of desoxycorticosterone acetate (12.5 mg/Kg and 6.25 mg/Kg) and drinking water replaced by 0.9% saline solution). Thoracic aorta segments from Norm-Preg and HTN-Preg rats were divided into four rings as follow: +PVAT + endothelium (E), +PVAT -E, -PVAT +E, -PVAT -E. Rings were set up in organ bath, under basal tension of 1.5g. Preparations were challenged with cumulative concentrations of Potassium Chloride ( $KCl$   $10^{-3}$ mol/L –  $12 \times 10^{-3}$ mol/L compensated by the sodium reduction in solution) and Phe ( $10^{-9}$ – $10^{-4}$  M) which was constructed in absence or in presence of  $1 \mu M$  Glibenclamide, 2mM Pyridoxal 5-Phosphate, 10mM L-cysteine or 1mM DL-Propargylglycine (PAG). Vascular tone modifications were recorded by isometric force transducers and expressed as concentration-response curves. Log of  $EC_{50}$  and maximal response  $R_{max}$  (n=10) were compared by two-way ANOVA/Tukey (significance when  $P < 0,05$ ). Maternal blood pressure and  $H_2S$  concentrations, in plasma and placenta, were also assessed. CEUA IBB/UNESP 1083-2018. **Results:** DOCA-Salt model increased Systolic blood pressure during pregnancy in the HTN-Preg group. Both Norm-Preg and HTN-Preg groups showed no significant differences in KCl-induced contractions. We observed that PVAT (+PVAT-E) decreased phenylephrine-induced contraction and that stimulated  $H_2S$  formation with Pyridoxal 5-Phosphate and L-Cysteine enhanced the anticontractile effect of PVAT (+PVAT-E) from Norm-Preg and HTN-Preg rats.  $H_2S$  synthesis inhibition with PAG in aortas with and without PVAT/endothelium showed similar responses. However, blockade of ATP-sensitive potassium ( $K_{ATP}$ ) channels with glibenclamide eliminated the anticontractile effect of PVAT (+PVAT-E). Also, increased  $H_2S$  levels were found in PVAT, placental and plasma but not in aorta without PVAT. **Conclusions:** We suggest that PVAT presents anticontractile effect in hypertension in pregnancy, and this effect is enhanced by stimulated  $H_2S$  synthesis and is  $K_{ATP}$  channels-dependent. **Acknowledgements:** FAPESP, CAPES and CNPq.

**06.049 The acute hypotensive and vasorelaxation effects of S-nitrosoglutatione has no involvement of cyclooxygenase pathway.** Paula TC, Ferreira GC, Batista RIM, Santos JET FMRP-USP, USP

**Introduction:** The S-nitrosogluthathione (GSNO) is a NO donor and also indicated as nitrosant chemical specie. The nitrosation process is able to change the activity of some enzymes. The cyclooxygenase (COX) pathway is important to production of prostanoids involved in the vasomotor tonus and blood pressure control. Some reports demonstrate the possibility of nitrosation in elements of COX pathway. Our goal in this work was evaluate if COX pathway is involved in acute hypotensive and vasorelaxant effects of GSNO. **Methods:** In some experiments Male Sprague Dawley rats were treated by 7 days with L-NAME (1g/l) in the drinking water. On the day before the experiment the femoral artery was cannulated by insertion of polystyrene cannula in the lumen of artery. On the day of experiment the non-selective cox inhibitor (Ibuprofen, 50 mg/kg) was orally administered 2 hours before of GSNO. Next GSNO (15mg/Kg) was orally administered and mean and systolic blood pressures were recorded during 20 min. In another set of experiments normotensive Male Sprague Dawley were euthanized the aorta was collected cut in 4 mm rings and mounted in organ bath to test the vascular reactivity. The aortic rings were incubated or not with guanilil cyclase bloker ODQ (1 umol/l) or ODQ plus IBU (10 umol/l) by 30 min. Next concentration effect curve to GSNO (0.1nmol - 10µmol/l) were performed under stable contraction to phenylephrine (0.1 µmol/l). Ethics committee: 005/02-2019. **Results:** The GSNO induced a maximum reduction in systolic (GSNO  $-35.7 \pm 1,57$  mmHg, n=4 vs veicule  $4.03 \pm 2.06$  mmHg n=3) and mean (GSNO  $-28.42 \pm 2.05$  n=4 vs veicule  $3.5 \pm 2.25$  mmHg n=3) blood pressure at 3 min. The Ibuprofen did not change the effect of GSNO in systolic ( $-46.40 \pm 13,53$  n=3 mmHg) and mean ( $-42.56 \pm 4.70$  mmHg n=3) at 3 min or in other time evaluated. The GSNO induced a concentration-effect curve with maximum effect ( $101.50 \pm 1.29\%$  n=3) that was abolished by ODQ ( $1.55 \pm 6.17\%$  n=3). The co-incubation of ODQ plus IBU ( $5.67 \pm 6.22\%$  n=5) did not change the effect of ODQ alone. **Conclusion:** The acute hypotensive and vasorelaxant effect of GSNO has no involvement of cyclooxygenase. **Financial support:** FAPESP, CNPQ and CAPES.

**06.050 Hyperglycemia induces apoptosis of HEK 293 cells by reducing Nrf2 activity and oxidative stress.** Costa RM<sup>1</sup>, Silva JLM<sup>1</sup>, Alves JV<sup>2</sup>, Tostes RCA<sup>2</sup> <sup>1</sup>UFG, <sup>2</sup>FMRP-USP

**Introduction:** Individuals with metabolic diseases such as type 2 diabetes mellitus, obesity and metabolic syndrome are prone to the development of nephropathy. A common feature in these diseases is hyperglycemia, which can generate cascades of events that result in the production of proinflammatory cytokines, advanced glycation end products and oxidative stress. Oxidative stress is associated with increased apoptosis of renal cells. Several cell types have developed adaptive programs to counteract the 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 hyperglycemia reduces the Nrf2 activity in renal cells, favoring reactive oxygen species accumulation and apoptosis. **Methods:** HEK 293 (Human Embryonic Kidney) cells were cultured in normoglycemic (5.6 mM glucose, NG) and hyperglycemic (25 mM glucose, HG) medium for 24 and 48 hours. Under these conditions, HEK 293 cells were treated with vehicle or L-sulforaphane (SFN, Nrf2 activator) for 3 hours. Vehicle or SFN were added in culture medium at the 21 and 45 hours for the final stimulus conditions of 24 and 48 hours, respectively. The Nrf2 activity, reactive oxygen species generation and cell death markers were evaluated. The results were compared by Two-way ANOVA with Bonferroni post-test. Data are presented as mean  $\pm$  standard error of the mean. Experimental  $n=6-7$ . **Results:** Cells cultured in HG medium for 24 hours did not show changes in Nrf2 activity [NG\_Vehicle: 100 (%) vs. HG\_Vehicle:  $99.5 \pm 0.04$  (%)], however, cells cultured in HG medium for 48 hours showed a reduction in Nrf2 activity [NG\_Vehicle: 100 (%) vs. HG\_Vehicle:  $77.6 \pm 0.06$  (%)]. Treatment with SFN reversed the reduction in Nrf2 activity [HG\_Vehicle:  $77.6 \pm 0.06$  (%) vs. HG\_SF:  $133.9 \pm 0.04$  (%)]. Cells cultured in HG medium for 24 and 48 hours showed an increase in superoxide anion generation [24 hours – NG\_Vehicle:  $625.5 \pm 3.2$  (AU) vs. HG\_Vehicle:  $1022.4 \pm 4.8$  (AU); 48 hours – NG\_Vehicle:  $631.9 \pm 4.1$  (AU) vs. HG\_Vehicle:  $1330.7 \pm 5.8$  (AU)]. Treatment with SFN reversed the increase in superoxide anion generation [24 hours – HG\_Vehicle:  $1022.4 \pm 4.8$  (AU) vs. HG\_SF:  $624.1 \pm 3.9$  (AU); 48 hours – HG\_Vehicle:  $1330.7 \pm 5.8$  (AU) vs. HG\_SF:  $656.1 \pm 2.9$  (AU)]. Cells cultured in HG medium for 24 hours did not show changes in hydrogen peroxide generation [NG\_Vehicle:  $7.33 \pm 0.02$  (AU) vs. HG\_Vehicle:  $8.91 \pm 0.08$  (AU)] and lipid peroxidation [NG\_Vehicle:  $0.47 \pm 0.02$  (AU) vs. HG\_Vehicle:  $0.54 \pm 0.04$  (AU)], however, cells cultured in HG medium for 48 hours showed an increase in hydrogen peroxide generation [NG\_Vehicle:  $8.21 \pm 0.03$  (AU) vs. HG\_Vehicle:  $19.93 \pm 0.07$  (AU)] and lipid peroxidation [NG\_Vehicle:  $0.44 \pm 0.01$  (AU) vs. HG\_Vehicle:  $0.85 \pm 0.04$  (AU)]. Treatment with SFN reversed the increase in hydrogen peroxide generation [HG\_Vehicle:  $19.93 \pm 0.07$  (AU) vs. HG\_SF:  $9.48 \pm 0.06$  (AU)] and lipid peroxidation [HG\_Vehicle:  $0.85 \pm 0.04$  (AU) vs. HG\_SF:  $0.38 \pm 0.06$  (AU)]. Cells cultured in HG medium for 24 hours did not show changes in cell viability [NG\_Vehicle:  $99.4 \pm 0.8$  (%) vs. HG\_Vehicle:  $98.2 \pm 0.9$  (%)], however, cells cultured in HG medium for 48 hours showed a reduction in cell viability [NG\_Vehicle:  $99.9 \pm 0.7$  (%) vs. HG\_Vehicle:  $78.4 \pm 0.9$  (AU)]. Treatment with SFN reversed the reduction in cell viability [HG\_Vehicle:  $78.4 \pm 0.9$  (%) vs. HG\_SF:  $96.2 \pm 0.8$  (AU)]. Cells cultured in HG medium for 48 hours showed an increase in annexin V [NG\_Vehicle: 100 (%) vs. HG\_Vehicle:  $136.1 \pm 1.2$  (%)] and caspase-3 expression [NG\_Vehicle:  $4.1 \pm 0.08$  (AU) vs. HG\_Vehicle:  $6.7 \pm 0.07$  (AU)]. Treatment with SFN reversed the increase in annexin V [HG\_Vehicle:  $136.1 \pm 1.2$  (AU) vs. HG\_SF:  $99.3 \pm 2.2$  (AU)] and caspase-3 expression [HG\_Vehicle:  $6.7 \pm 0.07$  (AU) vs. HG\_SF:  $3.9 \pm 0.09$  (AU)]. **Conclusions:** The data indicate that hyperglycemia reduces the Nrf2 activity, favoring the reactive oxygen species accumulation, lipid peroxidation and consequent cellular apoptosis. **Financial support:** FAPESP, CAPES, CNPq.

#### **06.051 Hypotensive and vasorelaxant effect of (-)-myrtenol in hypertensive rats.**

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**Introduction:** Hypertension is a serious public health problem and the lack of control of this pathology contributes to the increase in the occurrence of fatal cardiac events. Spontaneously hypertensive rats (SHR) are the model of hypertension that most closely approximates the characteristics presented in human essential hypertension. Despite the efforts, the current treatment presents serious limitations, the main one of them, in the occurrence of some adverse effects that favor the abandonment of the use by the patients. Thus, the identification of natural substances that possess therapeutic properties for hypertension, becomes important. The aim of this study is to present the hypotensive and vasorelaxant effect of (-)-myrtenol in hypertensive rats evidencing or suggesting the mechanisms of action involved. **Methods:** male SHR rats aged 12-14 weeks weighing between 250-300g with free access to water and feed and light / dark cycle of 12-12 hours were used in two experimental approaches. The activity on systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) was measured using two catheters implanted in the artery and femoral vein after anesthesia (ketamine 80mg / kg and xylazine 10mg / kg) for implantation surgery, (-) myrtenol was administered in doses of 3; 5; 10, 15 and 20 mg / kg (i.v.) and the effect was recorded. The vasorelaxant effect was evaluated in the upper mesenteric artery in a bath system for an isolated organ with 10 mL vats containing Tyrode solution and carbogen at 37°C, the rings were initially stimulated with KCl 70 mM after a stabilization period, the endothelium functionality was measured and cumulative relaxation curves were stimulated with (-)-myrtenol ( $10^{-7}$ - $3 \times 10^{-4}$  M). Data were plotted in graphs and statistical tests were used when appropriate (t-student test and ANOVA of one way, followed by Bonferroni post-test), values of  $p < 0.05$  were significant. **Results:** The (-)-myrtenol, at all doses administered, triggers dose-dependent hypotensive effect on SBP and DBP. In HR the substance elicited a biphasic response characterized by tachycardia at the lowest doses 3 and 5 mg/kg and bradycardia at the highest doses 15 and 20 mg/kg. Ganglio blockade with hexamethonium (30 mg/kg for 30 minutes) reversed tachycardia and reduced (-)-myrtenol-induced bradycardia. In addition, (-)-myrtenol triggers a vasorelaxant effect in hinger mesenteric artery rings, contracted with phenylephrine, KCl 80 mM and Serotonin, similar maximum effect ( $E_{max}$ ). In the contraction with phenylephrine the rings with present endothelium presented lower  $pD_2$  value, indicating additional effect. After blocking the calcium influx with Bayk8644 the vasorelaxation was changed. **Conclusion:** (-)-mirtenol triggers the hypotensive effect, in part, by reducing peripheral vascular resistance, by blocking calcium channels sensitive to L-type voltage. In HR the substance promotes a biphasic effect by means of central mechanisms of blood pressure control. **Financial support:** Capes. **Ethical committee number:** 63/2016.

**06.052 Potential effects of Matrix Metalloproteinase (MMP)-2 on the Sarcoplasmic Reticulum Calcium ATPase (SERCA) in hypertension-induced vascular dysfunction.** Silva PHL<sup>1</sup>, Mello MMB<sup>1</sup>, Parente JM<sup>1</sup>, Schulz R<sup>2</sup>, Castro MM<sup>1</sup> <sup>1</sup>USP, <sup>2</sup>University of Alberta

**Introduction:** Increased matrix metalloproteinase (MMP)-2 activity results in vascular smooth muscle cells (VSMCs) migration, proliferation and hypertrophy, which contributes to hypertension-induced chronic vascular remodeling and dysfunction mainly by extracellular matrix degradation. However, recent studies showed that MMP-2 is also capable to cleave intracellular proteins that are important in the contractile function of VSMCs. A previous study of our research group showed that increased activity of 72 kDa MMP-2 in aortas is related to a decrease in calponin-1 levels, which results in VSMCs proliferation and arterial remodeling of hypertensive rats. Sarcoplasmic reticulum calcium ATPase (SERCA) is an important protein that controls cytosolic calcium concentrations of VSMCs. It has been shown that protein levels and activity of SERCA can be reduced in arterial hypertension, thus leading to vascular dysfunction. The hypothesis of this study is that increased activity of MMP-2 contributes to reduce SERCA level and activity, thus resulting in vascular hypercontractility. **Methods:** Male Sprague-Dawley rats were previously euthanized and its aortas were removed. Then, the aortas were incubated for 6, 12 and 24 hours with angiotensin II (AngII, 0.1  $\mu$ M) in the presence or absence of ONO-4817 (10  $\mu$ M), an MMP inhibitor, in DMEM culture medium. After that, vascular reactivity of aortas was analyzed by a concentration-effect curve to phenylephrine in an organ bath and SERCA levels and MMP-2 activity were analyzed in the aorta extracts by Western blot and gel zymography, respectively. MMP-2 activity was also evaluated by *in situ* zymography and co-immunoprecipitation assays of MMP-2 with SERCA were also performed to analyze whether both proteins are co-localized in the aorta. Statistical analysis was done by two-way ANOVA and *t*-test. The Ethics Committee in Animal Research of University of Alberta approved all protocols (#269). **Results:** MMP-2 was immunoprecipitated with SERCA in the aortas treated with AngII for 6 hours. MMP-2 activity was increased in the presence of AngII during 6, 12 and 24 hours and treatment with ONO-4817 significantly decreased it on *in situ* zymography ( $p < 0.05$ ). Even though 6 hours incubation with Ang II contributed to the increase of 72 kDa MMP-2 activity in the aorta, there is still no vasoconstriction or SERCA proteolysis, although alterations in its activity are possible to occur. Furthermore, it was observed that MMP-2 is co-localized to SERCA in the aorta ( $p < 0.05$ ). A previous study has shown that recombinant SERCA can be cleaved by MMP-2 *in vitro*, thus SERCA may be a potential proteolytic target for MMP-2 *in vivo*. **Conclusion:** AngII incubation increases MMP-2 activity in aortas and MMP-2 is also co-localized to SERCA, suggesting that SERCA may be a potential proteolytic target for MMP-2 in the aortas and this may contribute to vascular alterations. However, more studies must be performed to better elucidate that. **Financial support:** CAPES, CNPq and FAPESP.

**06.053 Protective effect of (-)-alpha-bisabolol in a model of acute kidney injury by ischemia-reperfusion *in vitro*.** Costa IV, Silva BP, Sampaio TL, Magalhães EP, Menezes RRPPB, Martins AMC UFC

**Introduction:** Acute Kidney Injury (AKI) is characterized by a decrease on glomerular filtration rate, as well as morphological and functional changes related to tubular damage. An important model of AKI is by induction of ischemia and reperfusion (I/R), which causes ROS production, mitochondrial damage, changes in the electron transport chain, decrease on ATP production and cell death. (-)- $\alpha$ -bisabolol (BIS) is an alcohol sesquiterpene present on many plants, as chamomile (*Matricaria chamomilla*), and whose nephroprotective effect has been previously described. So, the aim of this work was to evaluate the effect of BIS on *in vitro* I/R model using human kidney cells.

**Methods:** Human kidney tubular (HK-2) cells were submitted to I/R *in vitro* by anaerobic chamber method with DMEM without glucose, pyruvate and fetal bovine serum for 24 hours, followed by reoxygenation for 3 hours in standard conditions. After this period, the treatment with BIS (250-31.25 $\mu$ M) was performed, and the cell viability was measured by MTT reduction assay. After this, the cells treated with BIS (62.5 $\mu$ M) and untreated I/R cells were submitted to flow cytometry to evaluate the percentage of necrotic/apoptotic events (using 7-AAD and annexin V labeling), ROS production (using DCFH-DA) and mitochondrial transmembrane potential (using the cationic fluorochrome Rhodamine 123). Finally, the cells were observed by scanning electron microscopy (SEM) to analyze ultrastructural changes. All experiments were performed in triplicate (n=3). The data were expressed as mean  $\pm$  standard error mean and the comparison between groups was performed by one-way ANOVA followed by Bonferroni post-test (p <0.05), using GraphPad Prism 5.0 software. Results: BIS was able to increase cell viability in damaged I/R cells, mainly at 62.5 $\mu$ M, when compared to the untreated I/R group (77.5%  $\pm$ 1.9 vs 50.7%  $\pm$ 2.1). In flow cytometry assays, it was observed increase on apoptotic events in I/R groups, and BIS was able to reduce them (35.8%  $\pm$ 1.2 vs 26.3%  $\pm$ 0.6). In addition, BIS reduced ROS production when compared to the I/R group (relative fluorescence values of 1.07 $\pm$ 0.02 vs 1.31 $\pm$ 0.01) and reduced mitochondrial damage (relative fluorescence values of 0.51 $\pm$ 0.01 vs 0.83 $\pm$ 0.02). When analyzed by SEM, BIS reduced ultrastructural alterations induced by I/R, such as cellular retraction and apoptotic bodies formation. **Conclusion:** The results demonstrate that BIS protects HK-2 renal tubular cells against I/R damage by reducing apoptotic damage, ROS production and mitochondrial damage, evidenced by increased cell viability and reduction of induced morphological changes. We thank to CNPq, and CAPES for their financial support.

**06.054 Evaluation of the activity of SPRM12 on  $\alpha$ -adrenergic receptors and sex-related differences.** Silva SB, Alves SML, Silva WFP, Anjos JV, Araújo AV UFPE

**Introduction:** Despite the great number of anti-hypertensive pharmacological agents, a large number of patients do not achieve an adequate control of blood pressure. Therefore, it is essential the search for new therapeutic tools for the control of hypertension. Furthermore, since there are differences between the sexes in the pathophysiology of hypertension and in the response to drugs, the inclusion of both sexes in biological assays is mandatory. For the current study, it was synthesized the 2-morpholino-6-oxo-4-fluoro-1,6-dihydro-pyrimidine-5-carbonitrile (SPRM12), by a multicomponent reaction. This compound has a structure that is similar to some antihypertensive drugs (such as terazosin and prazosin). Thus, we aimed to characterize the action of SPRM12 on alpha-adrenergic receptors in aorta of male and female rats and to verify the differences of its effects between the sexes. **Methods:** Aortic artery rings with and without endothelium of male and female *Wistar* rats (2-3 months) were mounted in an organ bath. Responses were recorded by an acquisition system (AVS). Cumulative concentration-effect curves for phenylephrine were constructed in the absence or in the presence of SPRM12 (0.1 mmol/L). Statistical analyzes were performed by using the software Prism (GraphPad Software, version 6.0). Student's *t*-test for independent samples or one-way ANOVA, followed by Newmann-Keuls, were used to access the differences among the groups. The significance level was considered  $p < 0.05$ . The experimental protocols were submitted and approved by the Committee on Ethics in Animal Use - CEUA of UFPE (Processes nº 021446/2013-12 e 0032/2018). **Results:** At the concentration of 0.1 mmol/L, SPRM12 did not alter the phenylephrine-induced contraction in endothelium-denuded aortic artery rings of male nor female rats. However, in the presence of the endothelium, SPRM12 reduced the maximal effect of phenylephrine-induced contraction in rings of both sexes (males: Maximum Effect (ME) control =  $1.97 \pm 0.18$ g,  $n=7$ ; ME SPRM12 =  $1.20 \pm 0.20$ g,  $n=7$ ,  $p < 0.05$ ; Females: ME control =  $2.33 \pm 0.17$ g,  $n=6$ ; ME SPRM12 =  $1.09 \pm 0.18$ g,  $n=8$ ,  $p < 0.001$ ) and  $pEC_{50}$  only in male rat rings ( $pEC_{50}$  control =  $7.44 \pm 0.21$ ,  $n=7$ ;  $pEC_{50}$  SPRM12 =  $6.68 \pm 0.16$ ,  $n=7$ ,  $p < 0.05$ ). In addition, there was no difference in the  $EC_{50}$ -induced contraction of phenylephrine before and after the curve in the presence of the SPRM12. **Conclusion:** The results suggest that SPRM12 is not  $\alpha_1$  receptor antagonist, but its effect seems to involve the release of relaxing endothelial mediators. SPRM12 is not toxic at this concentration for the studied tissues. In addition, the sensitivity of the male rat rings to this molecule is greater than that of females. More studies are needed to confirm the mechanism of action of SPRM12. **Financing:** FACEPE e CNPq. **Acknowledgment:** We thank the Foundation for Science and Technology Support of Pernambuco (FACEPE) and the National Council for Scientific and Technological Development (CNPq) for financial support. BARRETO, M. S. et al. **Esc Anna Nery**, v. 20, n. 1, p. 114-120, 2016. CLAYTON, J.A. et al. **Clin cardiol**, v. 41, n. 2, p. 179-184, 2018. DESCHEPPER, C. F. et al. **Hypertension**, v. 49, n. 3, p. 401-407, 2007. GUDMUNSDOTTIR, H. et al. **Ther Adv Chronic Dis**, v. 3, n. 3, p. 137-146, 2012. NATIONAL INSTITUTES OF HEALTH. Disponível em: <https://orwh.od.nih.gov/sex-gender/nih-policy-sex-biological-variable>. Acesso em: 07 de mai. de 2019. NELSON, S. A. E. et al. **The J of CHypert**, v. 13, n. 2, p. 73-80, 2011. NOGUEIRA, D. et al. **Rev Panam Salud Publica**, v. 27, p. 103-109, 2010. WENGER, N.K. et al. **N Engl J Med**. v.329, n.4, p.247-256, 1993.

**06.055 Different pharmacological effect of SPRM09 on  $\alpha$ -adrenergic receptors of male and female rats.** Alves SML, Silva SB, Silva WFP, Anjos JV, Araújo AV UPE e UFPE

**Introduction:** The Systemic Arterial Hypertension is the main risk factor for the development of cardiovascular diseases. However, despite advances in antihypertensive therapy, a considerable number of patients do not have their blood pressure levels controlled. Furthermore, there are differences in the therapeutic response between men and women. The synthesis of compounds containing the pyrimidinone ring may provide new candidates for antihypertensive drugs. For the current study, a series of 2-morfolino-6-oxo-4-aryl-1,6-diidro-pirimidina-5-carbonitrilas, by multicomponent reaction, was performed. These compounds are similar to some  $\alpha_1$ -adrenergic receptors antagonists (that are anti-hypertensive drugs), such as prazosin. SPRM09 is one of these compounds with the substitution of the aryl radical by toluene. The aim of this study was to characterize the effect of SPRM09 on  $\alpha_1$ -adrenergic receptors in aortas of male and female rats. **Methods:** Aortic artery rings with or without endothelium of male and female Wistar rats (2-3 months) were mounted in an organ bath. The responses were recorded by an acquisition system (AVS). Cumulative concentration-effect curves for phenylephrine were constructed in the absence or in the presence of SPRM09 (0.1 mmol/L) and/or L-NAME (0.1 mmol/L). Statistical analyzes were performed by using the software Prism (GraphPad Software, version 6.0). Student's t-test for unpaired samples or one-way ANOVA, followed by Newman-Keuls, were used to access the difference among the groups. The significance level was considered  $p < 0.05$ . The experimental protocols were submitted and approved by the Committee on Ethics in Animal Use - CEUA of UFPE (Protocols nº 021446 / 2013-12 and 0032/201). Financing: FACEPE and CNPq. **Results:** SPRM09 had no effect in male endothelium-denuded aorta (Control: ME =  $2.27 \pm 0.16$ g,  $pD_2 = 7.67 \pm 0.65$ ,  $n = 7$ ; +SPRM09: ME =  $2.18 \pm 0.23$ g,  $pD_2 = 7.93 \pm 0.35$ ,  $n = 7$ ,  $p < 0.05$ ). SPRM09 reduced the Maximum Effect (ME) of the phenylephrine in male aortas with endothelium (Control: ME =  $2.27 \pm 0.16$ g,  $pD_2 = 7.67 \pm 0.65$ ,  $n = 7$ ; +SPRM09 = ME =  $1.13 \pm 0.18$ g,  $pD_2 = 7.32 \pm 0.32$ ,  $n = 7$ ,  $p < 0.05$ ), and this effect was abolished by the presence of L-NAME (Control: ME =  $1.71 \pm 0.22$ g,  $n = 7$ ;). In female rats, SPRM09 did not reduce the ME of phenylephrine in rings with endothelium (Control: ME =  $2.33 \pm 0.17$ g,  $pD_2 = 6.95 \pm 0.12$ ,  $n = 6$ ; +SPRM09: ME =  $1.90 \pm 0.24$ g,  $pD_2 = 7.36 \pm 0.18$ ,  $n = 8$ ,  $p < 0.05$ ) or in endothelium-denuded rings (Control: ME =  $1.91 \pm 0.11$ g,  $pD_2 = 7.67 \pm 0.10$ ,  $n = 6$ ; +SPRM09: ME =  $1.83 \pm 0.09$ g,  $pD_2 = 7.67 \pm 0.11$ ,  $n = 6$ ). **Conclusion:** SPRM09 acts through the nitric oxide synthase-induced NO production, possibly as a  $\alpha_2$ -adrenergic receptor agonist. Furthermore, its effect is observed only in male aortic rings. **Acknowledgements:** This study was supported by research grants from the National Council for Scientific and Technological Development (CNPq) and the Foundation for Science Support of Pernambuco (FACEPE). **References:** GOVERNO DO BRASIL. Disponível em: [www.brasil.gov.br/saude/2015/04/hipertensao-atinge-mais-de-30-milhoes-de-pessoas-no-pais](http://www.brasil.gov.br/saude/2015/04/hipertensao-atinge-mais-de-30-milhoes-de-pessoas-no-pais) NELSON, SAE et al. The J Clin Hypert, v. 13, n. 2, p. 73-80, 2011. BARRETO, MS et al. Esc Anna Nery, v. 20, n. 1, p. 114-120, 2016. CLAYTON, JA. et al. Clin Cardiol, v. 41, n. 2, p. 179-184, 2018. BROWN, DJ et al. Interscience Publishers, p. 31-115, 1962. BERNHART, CA, et al. Bioorg Med Chem Lett, v. 4, p. 157-162, 1994; SOCIEDADE BARSILEIRA DE CARDIOLOGIA. Arq Bras Cardiol, v. 107, p. 45.

#### **06.056 Pharmacological effects of $\beta$ -methylphenylethylamine on isolated rat aorta.**

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**Introduction:** Although marketed as products of natural origin, several products popularly known as thermogenic contain synthetic compounds derived from  $\beta$ -phenylethylamine ( $\beta$ -PEA). The amphetamine isomer  $\beta$ -methylphenylethylamine ( $\beta$ -MPEA) is one of these compounds. Such products have been widely introduced as dietary supplements with the purpose of reducing body fat. The aim of the present study was to investigate the pharmacological effects of  $\beta$ -PEA and  $\beta$ -MPEA on aorta obtained from Wistar rats (250-300 g). **Methods:** The experiments were performed in isolated preparations mounted in bath chambers and recordings of the contractile responses made by a data acquisition system. **Results:** At resting tonus,  $\beta$ -MPEA and  $\beta$ -PEA (1 - 1000  $\mu$ M) produced concentration-dependent contractile responses (maximal effect [in % of a reference 60 mM KCl-induced contraction] of  $111.9 \pm 11.2\%$  and  $95.8 \pm 7.5\%$  in endothelium-intact preparations, respectively. In endothelium-denuded aorta, the contractile effects corresponded to  $120.5 \pm 5.9\%$  and  $100.9 \pm 2.9\%$ , respectively. The removal of the endothelial layer did not change the maximal contractile effect of  $\beta$ -MPEA or  $\beta$ -PEA ( $p > 0.05$ ). However, the EC<sub>50</sub> values for the contractile effects of  $\beta$ -MPEA and  $\beta$ -PEA were significantly decreased ( $p < 0.05$ ) in endothelium-denuded aortic rings (136.9 [100.7 – 173.4] and 67.9 [46.1 – 97.5]  $\mu$ M, respectively) in comparison with the respective values in endothelium-intact preparations (229.2 [162.0 – 310.3] and 168.2 [120.8 – 220.5]  $\mu$ M, respectively). In Ca<sup>2+</sup>-free medium, the contractile effect of  $\beta$ -MPEA (10 – 300  $\mu$ M) did not occur. In the presence of 1 nM of the  $\alpha$ 1-antagonist prazosin, the EC<sub>50</sub> value of 222.6 [177.4-271.9] was significantly higher than 136.9 [100.7-173.4]  $\mu$ M recorded in its absence ( $p < 0.05$ ). The presence of the  $\alpha$ 2-antagonist yohimbine (30 nM) was unable to change the EC<sub>50</sub> value of  $\beta$ -MPEA. When endothelium-intact aortic rings were pre-contracted with 1  $\mu$ M phenylephrine the increasing addition of  $\beta$ -MPEA (1 – 3000  $\mu$ M) promoted a relaxing response with EC<sub>50</sub> value of 674.2 [583.7-772.1]  $\mu$ M. In endothelium-denuded aorta, the relaxing effect of  $\beta$ -MPEA revealed an EC<sub>50</sub> of 1157 [1003-1325]  $\mu$ M ( $p < 0.05$ ). In conclusion,  $\beta$ -MPEA and  $\beta$ -PEA promote contractile responses when rat aortic rings were at basal tonus. The contraction induced by  $\beta$ -MPEA depended on the extracellular Ca<sup>2+</sup> with probable recruitment of  $\alpha$ 1-adrenoceptors. In contrast, when aortic rings were pre-contracted with phenylephrine,  $\beta$ -MPEA exerted relaxing effects, although at concentrations higher than those able to induce contractions, which probably resulted from interaction of  $\beta$ -MPEA with other targets than  $\alpha$ -adrenergic receptors. Both contractile and relaxing effects of  $\beta$ -MPEA were influenced by the integrity of the endothelial layer, suggesting that part of the effects caused by  $\beta$ -MPEA is endothelial of origin, being predominantly inhibitory. **Acknowledgments:** Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support. **References:** SCHINDLER, Charles W. et al. The Supplement Adulterant  $\beta$ -Methylphenethylamine Increases Blood Pressure by Acting at Peripheral Norepinephrine Transporters. JPET, [s.l.], v. 369, n. 3, p.328-336. 2019. ZHAO, Jianping et al. Detection and quantification of phenethylamines in sports dietary supplements by NMR approach. J PharmBiomedSci[s.l.], v. 151, p.347-355, 2018.

**06.057 Indol-3-Carbinol lowers blood pressure and improves vascular function in hypertensive rats.** Arruda AVD<sup>1</sup>, Cabral B<sup>2</sup>, Gonçalves TAF<sup>1</sup>, Rezende MSA<sup>1</sup>, Oliveira JCPL<sup>1</sup>, Azevedo FDLAA<sup>1</sup>, Veras RC<sup>1</sup>, Medeiros IA<sup>1</sup>, Araújo IGA<sup>1</sup> <sup>1</sup>UFPB, <sup>2</sup>UFRN

**Introduction:** Increasing vegetable consumption has been widely recommended as a key component of a healthy diet to reduce the risk of major chronic diseases such as cancer (Liu X, *The Breast*, 22, 309, 2013). Indole-3-carbinol (I3C) is a alkaloid extracted from cruciferous vegetables and is well known for its anti-cancer, antioxidant and anti-inflammatory effects (Jiang Y, *Environ Toxicol Pharmacol*, 7, 70, 2019). This study investigated the protective effect of I3C on hypertension in rats. **Methods:** Twelve-week-old spontaneously hypertensive rats (SHR) were treated with I3C 50 mg/Kg/day (SHR-I3C) and vehicle (SHR-CTL), intragastrically, for four weeks. *Wistar-Kyoto* rats were used as aged-matched, normotensive controls (WKY-CTL). Direct blood pressure was measured in non-anaesthetized rats. For *in vitro* experiments, rat mesenteric artery rings were suspended by cotton threads for isometric tension recordings in a Tyrode's solution at 37 °C, gassed with 95% O<sub>2</sub> and 5% CO<sub>2</sub> at pH 7.4, resting tension 0.75 g. The protocols were approved by CEUA-UFPB nº 066/2017. **Results:** SHR-I3C treatment was able to decrease the mean arterial pressure (MAP) (160.6 ± 3.2 mmHg, n=6, p<0.05) when compared to that in the SHR-CTL (191.8 ± 2.4 mmHg, n=5). The heart rate was not changed with treatment. In isolated rat mesenteric artery, the contractile response induced by phenylephrine (Phe) (10<sup>-9</sup>-10<sup>-5</sup>M) was increased in SHR-CTL (MR = 96.6±5.8%, n=6, p<0.05) when compared to that in the WKY-CTL (MR = 79.1±5.6 %, n=5). SHR-I3C treatment attenuated the hypercontractility induced by Phe (MR = 77.3±4.6 %, n=6, p<0.05). Acetylcholine (ACh) (10<sup>-10</sup>-10<sup>-5</sup>M) induced lower relaxation in the mesenteric artery of the SHR-CTL (MR = 76.5±4.0%, n=6, p<0.05) when compared to that in the WKY-CTL (MR = 100.5±5.5%, n=6). This effect was reversed in SHR-I3C treatment (MR = 117.6±7.1%, n=6, p<0.05). In addition, the protective effect to fSHR-I3C treatment in the endothelial function involves increased NO-mediated component of relaxation, this can be verified by the decrease of the vasorelaxant response to ACh in presence of L-NAME (100 µM) in SHR-I3C (MR = 77.2±9.2%, n=6). Similarly to the observed response to ACh, SNP induced lower relaxation of the SHR-CTL (MR = 76.5 ± 4.0%, n=6, p < 0.05) when compared to that the WKY-CTL (MR = 100.5 ± 5.5%, n=6). In the SHR-I3C treatment, SNP-induced relaxation response was restored (MR = 121.0 ± 8.7%, n=6). **Conclusion:** Therefore, this study demonstrates that treatment with I3C lowers blood pressure, reduces endothelial dysfunction and smooth muscle cell hypercontractility in hypertensive rats. **Financial support:** This work was supported by the Pró-Reitoria de Pesquisa (PROPESQ-UFPB), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).