06. Cardiovascular and Renal

06.001 **The effect of simvastatin on cardiovascular changes and the bone loss induced by periodontitis**. Machado WM[1], Olchanheski Jr LR[1], Mendes RT[2], Prestes AP[1], Costa TP[1], Fernandes D[1]
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Introduction: Individuals with periodontitis have been reported to have a significantly increased risk of developing cardiovascular disease. It has been proposed that bacteria can access systemic circulation leading to systemic inflammation and endothelial dysfunction. Some studies have demonstrated that statins have antiinflammatory effect and can clearly improving endothelial function.The objective of this study was to evaluate the effect of simvastatin on systemic inflammation, endothelial function and alveolar bone loss induced by periodontitis. **Methods:** On day zero Wistar rats were divided into four groups of seven animals: G1: with bandages in the first molars and second molars + simvastatin (10 mg / kg / day, vo), G2: false-operated, and the bands were placed immediately removed + simvastatin; G3: ligature + vehicle; G4: sham-operated + vehicle. Treatment with simvastatin or vehicle was given from day 8 to day 14. On day 14, rats were prepared for analysis of blood pressure, heart rate and blood pressure response to acetylcholine and sodium nitroprusside. Blood was obtained by cardiac puncture for analysis of lipid profile, C-reactive protein and interleukin-6. The mandible and maxilla were removed for analysis of alveolar bone loss. **Results:** Alveolar bone loss was observed in the maxillary and mandible molars in the ligated rats. Additionally, we show a reduced response to endothelium-dependent vasodilatation (acetylcholine), a hallmark of endothelial dysfunction, in animals with periodontitis. The response to sodium nitroprusside was not altered at any time evaluated. The simvastatin treatment reduced alveolar bone loss and endothelial dysfunction in treated groups. In addition, the ligature-induced periodontitis increased plasma cholesterol levels, LDL, VLDL and triglyceride levels. **Discussion:** Our results show that simvastatin reduced alveolar bone loss, systemic inflammation and endothelial dysfunction induced by periodontitis. From this simvastatin may be regarded as a promising drug for the treatment of periodontal disease and prevent cardiovascular complications. Keywords: periodontitis, endothelial dysfunction, simvastatin. **Financial suport:** Decit/SCTIE through the support of CNPq and Fundação Araucária; CAPES and CNPq**.** This study was approved by the Ethics Committee of Animal Use (CEUA) present in the Dean of Research and Graduate Studies, State University of Ponta Grossa / Paraná / Brazil under protocol number 02590/2012.

06.002 **RAS blockade minimizes proteinuria in 2K-1C renal hypertensive rats**. Corrêa JWN[1,2], Girardi ACC[2], Salles T[2], Yogi A[3], Callera GE[3], Briones AM[3], Din Cat AN[3], He Y[3], Touyz RM[3], Bendhack LM[4], Krieger JE[2] – [1]UFAM – Physiological Sciences [2]InCor-HC-FMUSP [3]University of Ottawa – Kidney Research, [4]FCFRP-USP – Pharmacology

Introduction: Leakage of albumin from the plasma into urine is the first sign of renal injury. Many pathological conditions such as hypertension can lead to proteinuria. This work aimed to evaluate the effects of combined therapy with enalapril and losartan on the proteinuria in 2K-1C hypertensive rats. **Methods:** 2K-1C and sham-operated (2K) were induced by surgery in male Wistar rats. After six weeks, rats were treated with losartan (30mg/Kg/d), enalapril (20mg/Kg/d), losartan+enalapril (20+30mg/Kg/d) or saline by gavage for 14 days. The rats were housed in metabolic cages for urine collection and determination of renal function parameters. Plasma and kidney samples were collected in the day of the sacrifice. All the experimental procedures are in accordance to Ethics Committee (CEUA-USP-RP 07.1.607.53.1). **Results:** Systolic blood pressure (SBP, mmHg) was higher in 2K-1C (247±5) than in 2K (129±2, P<0.0001). Losartan (197±6, P<0.05) and enalapril (205±7, n = 8, P<0.05) progressively reduced 2K-1C SBP, with an additional hypotensive effect in the group treated with both drugs (173±16mmHg, P<0.001). The kidney hypertrophy index (kidney/tibia lenght) was higher in the right kidney of 2K-1C compared to 2K (0.61±0.04 vs 0.41±0.01, P<0.001) and it was not changed by the treatments. The glomerular filtration rate (GFR, L/Kg/day), was reduced in 2K-1C control rats as compared with 2K control rats (8.94±1.86 n = 6 vs. 15.21±3.15, n = 6, P<0.05). The values of GFR were not changed by the treatments. Plasma levels of sodium, potassium, creatinine, Ang II and TBARS were similar in 2K and 2K-1C rats and were not changed by the treatments. Total proteinuria (mg/mL/Kg/24h), together with microalbuminuria (protein/creatinin ratio), were highly increased in 2K-1C control rats when compared to 2K control rats (15.47±3.81 n = 6 vs. 0.96±0.09, n = 6, P<0.01 and 151,.50±27.57 n = 7 vs. 10.97±2.76, n = 6, P<0.001, respectively). Treatment of 2K-1C rats with losartan, enalapril or combined therapy similarly reduced the total protein and albumin excretion observed when compared to the hypertensive control group. Lucigenin-derived chemiluminescence was not different in the right and left kidneys of 2K and 2K-1C rats. Treatments had no effect upon NADPH oxidase activity. **Discussion:** 2K-1C hypertensive rats presented proteinuria which is minimized by the treatment of losartan or enalapril together with blood pressure. Combined therapy does not seems to induce additional effects on proteinuria, but cause a significant additional reduction of blood pressure. The mechanism of proteinuria reduction does not involve inhibition of ROS. Further studies are necessary to demonstrate how these drugs act on tissues in order to produce reversion of proteinuria. Supported by FAPESP, CAPES, CNPq, CIHR and Heart and Stroke Foundation of Canada.

06.003 Mechanisms underlying the vasorelaxant action of the labdane ENT-3-acetoxy-labda-8(17),13-dien-15-oic acid in the rat aorta. Simplício JA[1], Ambrósio SR[2], Batalhão ME[3], Carnio EC[3], Tirapelli CR[4] [1]FMRP-USP – Pharmacology, [2]Unifran – Sciences and Technology, [3]EERP-USP – General and Specialized Nursing, [4] EERP-USP – Psychiatric Nursing and Human Sciences

Introduction: Diterpenoids are the main constituents of plant extracts that are used in folk medicine for the treatment of hypertension. Labdane-type diterpenes are described to exert antispasmodic and relaxant action in vascular tissues. The mechanisms underlying the cardiovascular action of labdanes are related to their chemical structure. Thus, the identification of new compounds provides the best strategy and solution to increase the productivity in drug discovery and development. **Objectives:** The present investigation aims to evaluate the mechanisms underlying the cardiovascular effects displayed by the labdane *ent*-3-acetoxy-labda-8(17),13-dien-15-oic acid. **Materials and methods:** Male Wistar rats weighting between 200 and 250 g were used in accordance with the Ethical Animal Committee from the Campus of Ribeirão Preto—University of São Paulo (Protocol: 09.1.1007.53.0).The thoracic aorta was isolated for vascular reactivity experiments. cAMP and cGMP were measured by enzyme immunoassay (EIA) whereas nitrate measurement was performed by chemioluminescence. Statistical analysis was performed using one-way analysis of variance (ANOVA). P values of less than 0.05 were considered significant. **Results:** The E_{max} values for phenylephrine in endothelium-intact (E^+) rings were reduced in the presence of labda-15-oic acid at 100 µmol/L. In endothelium-denuded (E^-) rings, labda-15-oic acid reduced phenylephrine-induced contraction at 50 and 100 µmol/L. The E_{max} values for serotonin in E^+ and E^- rings were reduced in the presence of the labdane at 10, 50 and 100 µmol/L. The Labda-15-oic acid inhibited the contraction induced $CaCl_2$ in E^- rings in the concentration of 10, 50 and 100 µmol/L, after stimulation with phenylephrine (0.73±0.07g, n = 6; 0.79±0.12g, n = 5; 0.41±0.06g, n = 6), compared to control (1.28±0.10g, n = 6), or with KCl (0.91±0.13g, n = 5; 0.78±0.14g, n = 7; 0.67±0.05g, n = 6), compared to control (1.42±0.06g, n = 8). The labdane did not alter the contraction of Ca^{2+} induced by phenylephrine or caffeine from intracellular stores. The labdane induced relaxation in E^+ and E^- rings pre-contracted with phenylephrine (101.4±7.0%, n = 11 and 94.6±9.4%, n = 9) or KCl (111.8±7.03%; n = 9 and 113.9±2.0%; n = 7). In E^+ rings pre-contracted with phenylephrine, the E_{max} values for relaxation were reduced in the presence of L-NAME, ODQ, haemoglobin and RP-8-Br-Pet. There was a rightward displacement of the concentration–response curve for labda-15-oico in the presence of L-NAME, ODQ, haemoglobin, 7-nitroindazole, Rp-8-Br-Pet, thapsigargin and tetraethylammonium when compared to control. On the other hand, indomethacin, wortmannin, LY294002, atropine, propranolol, H89 and SQ22536 did not have a significant effect on labdane induced relaxation. The labdane increased the levels of cGMP and nitrate but not cAMP in E^+ rings. **Discussion:** The labda-15-oic acid acts on vascular smooth muscle where it blocks Ca^{2+} influx through interference with both voltage and receptor-operated channels. The relaxant action of the labdane is also partly mediated by the activation of endothelial NO-cGMP pathway and opening of K^+ channels. **Financial support:** CAPES and FAPESP.

06.004 **Vascular effects of chronic ethanol consumption, alone or in association with stress, in adult rats: Role of cyclooxygenase and nitric oxide pathway**. Cordellini S[1], Baptista RFF[1], Chies AB[2]
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Background: Although the literature reports cardiovascular changes induced by ethanol consumption (EtOH) and stress (ST) alone, there are few studies focusing on the cardiovascular effects of these conditions in association. Thus, the aim was to evaluate the vascular risk of ethanol consumption and stress, isolated and in association, in male adult rats. **Methods:** Male Wistar rats (100-day-old) were divided into control, EtOH (received 20% ethanol in drinking water for 6 weeks), ST (submitted to immobilization stress 1h day/5 days a week during 6 weeks), EtOH/ST (submitted to both EtOH and ST). These animals were killed and rings (3-4mm) of thoracic aorta were obtained to be set up in organ bath containing Krebs-Henseleit solution, 37°C, pH 7.4, gassed with 95% O_2 and 5% CO_2. Concentration-effect curves to noradrenaline (NA), in the absence or presence of yohimbine, L-NAME or indomethacin, and phenylephrine (PHE) were obtained in aortas with (+E) and without (-E) endothelium. Log of EC_{50} (pD_2) and maximum response (MR) (n = 8-12) were compared by two way ANOVA/Bonferroni ($P<0.05$). This study was approved by the Ethics Committee on Animal Experimentation, IB-UNESP-Botucatu, Protocol nº 295-CEEA. **Results:** Stress exposure determined a similar increase in NA maximum response of intact aortas from rats submitted or not to ethanol consumption (control 2.22 ± 0.12^a; EtOH 2.35 ± 0.10^a; ST 2.72 ± 0.11^b; EtOH/ST 2.78 ± 0.15^b). This effect was abolished by the removal of endothelium and the presence of both indomethacin and yohimbine, but not by the presence of L-NAME. None of the protocols altered the pD_2 values of aortas +E or –E and the MR of aortas –E to NA. Moreover, none of the protocols altered the responsiveness to PHE of aortas +E or -E. **Conclusion:** The hyperreactivitity to NA induced by St was shown to be dependent on endothelium integrity and is probably a consequence of endothelium-derived contracting prostanoids release through alpha-2 adrenoceptor stimulation. The absence of synergistic vascular effects between ST and EtOH in adult rats suggests that there is a reasonable safety in the association of these cardiovascular risk factors considering the adult life. Finally, these studies advance understanding of the vascular effects of ST and EtOH and could aid in the development of proper therapies for cardiovascular diseases caused by these conditions. **Support:** CAPES

06.005 **Consequence of the abstinence syndrome to ethanol on vascular reactivity and behavior of animals tested in the EPM**. Gonzaga NA[1], Padovan CM[2], Tirapelli CR[3] [1]FMRP – Farmacologia, [2]FFCLRP-USP – Psicologia, [3]EERP-USP – Enfermagem Psiquiátrica e Ciências Humanas

Introduction: Alcohol Withdrawal Syndrome (AWS) is a short-lasting, but potentially severe complication of alcohol dependence characterizing psychiatric symptoms and changes in autonomous and nervous systems. Even a single moderate dose of ethanol ingestion in non-alcoholic subjects causes significant changes in cardiovascular system characterizing by changes in heart rate, systolic blood pressure, cardiac output and total peripheral resistance and promotes behavioral changes such as increased anxiety. This study aimed to investigate the behavioral and cardiovascular effects associated with AWS and the mechanisms involved in this response. **Methods:** Male *Wistar* rats were divided into three groups: Control: animals received water ad libitum for 23 days; Ethanol: chronic treatment with ethanol was started with a solution of 3% ethanol (vol./vol.) being gradually increased every three days to 6% (day 4) and 9% (on day 7), maintaining this concentration up to 21 days; Abstinence of ethanol: the animals were treated the same way as the animals in the ethanol group until the 20^{th} day, and then the ethanol solution 9% was removed and returned the next day (day 21) for only two hours. After that, the animals received only water until the test day (23 days), thereby ensuring the framework of abstinence for 48 hours. Vascular reactivity experiments were performed on isolated thoracic aorta with intact endothelium (E^+) or denuded endothelium (E^-). Cumulative concentration-response curves to acetylcholine (ACh), phenylephrine (Phe), sodium nitroprusside (SNP), serotonin (5-HT) or KCl were obtained in isolated aortas. The behavioral test was performed in the elevated plus maze (EPM). All protocols were approved by the Ethical Committee (Protocol: 11.1.1212.53.5). **Results:** In E^- rings the AWS significantly alter the vascular contraction to Phe (1.3±0.1g, n = 7) and KCl (1.1±0.2g; n = 4) when compared to the respective control groups (Phe = 1.85g±0.1; n = 9; KCl = 1.9±0.1g; n = 8). In E^+ rings, there was no significant difference in Phe or KCl-induced contraction. The chronic use and/or withdrawal of ethanol did not alter 5-HT-induced contraction (E^+: $F_{2:21}$ = 2,9; E^-: $F_{2:25}$ = 2,6; p>0,05) compared with control. No significant change in the percentage of relaxation induced by Ach ($F_{2:19}$ = 1,4; p>0,05) or SNP ($F_{2:16}$ = 0,5; p>0,05) were observed. AWS promoted change in the exploratory behavior of animals tested in the EPM. Animals of group AWS present a significant decrease in percentage of entries (%EOA = 19,8±5,4; n = 7; $F_{2;20}$ = 25,9; p<0,05) and time spent in open (%TOA = 5,2±2,0; n = 7; $F_{2;20}$ = 32,9; p<0,05) compared with animals of control group (%EOA = 35,7±3,6; %TOA = 21,5±5,9; n = 7). No differences were found on the number of enclosed arms entries ($F_{2;20}$ = 2,8; p>0,05). **Discussion:** Our results show that AWS promotes changes in vascular reactivity to agents that induce vascular contraction and this effect is independent of the endothelium. Our results also suggest that the ethanol withdrawal promotes anxiogenic effects in animals tested in the EPM. **Financial Support:** CNPq

06.006 **P1 and P2 receptors modulate the inotropism and chronotropism in isolated right atrium (RA) from normotensive (NWR) and hypertensive rats (SHR)**. Rodrigues JQD[1], Silva Junior ED[1], Alves GA[2], Câmara H[1], Caricati-Neto A[1], Jurkiewicz NH[1], Jurkiewicz A[1] [1]Unifesp – Farmacologia, [2]Unifesp – Biofísica

Introduction : The P1 and P2 receptors are expressed in cardiac tissue and activated by purines (ATP) and pyrimidines (UTP), released by sympathetic neurons, endothelial cells and cardiomyocytes (Burnstock , G 2008). However, the role of these receptors in physiological or pathological conditions in the heart is still unclear. Therefore, this study proposed to investigate the role of P1 and P2 receptors on inotropic and chronotropic effects exerted by ATP or UTP in isolated right atria (RA) from NWR and SHR. **Methods:** RA from NWR and SHR (4-6 months) were isolated and mounted in isolated organ bath. The RA presented spontaneously beatings and the frequency between 180 and 380 bpm was considered as inclusion criterion. We study the effect of ATP and UTP (1 mM to 1 mM) on strength and frequency of RA in the absence and presence of the P2X desensitization agonist α, β-methylene ATP (30 μM / pre-incubated for 30 min). The results were analyzed by unpaired t test and one-way ANOVA. In accordance to the UNIFESP ethic committee (n° 0778/11). **Results:** ATP (1 mM to 30 μM) produced an initial negative chronotropic effect (NCE) that lasted 60-90 s, followed by a negative inotropic effect (NIE). After this time, the chronotropism gradually increased, showing a positive chronotropic effect (PCE) that lasted 400 s followed by positive inotropic effect (PIE). ATP 100 μM exerted in 40% of the experiments a biphasic effect. ATP 100 μM and 300 μM induced only a NCE in 60% and 100 % of the experiments, respectively. ATP 1 mM abolished the atrial contraction. The α-β-methylene blocked the NCE produced by ATP (300 μM) only in SHR resulting in a decrease of 44.5 ± 2.1% of the chronotropic effect. ATP (300 μM) produced a NIE with amplitude of 36.2 ± 3.6% in NWR and 30.9 ± 2.8% in SHR ($p<0.05$), in relation to the baseline value. After this time, atrial inotropism gradually increased, reaching the plateau at 180-200s, showing a PIE of 37.5±1.9% in NWR and 44.6 ± 2.1% in SHR ($p<0.05$), in relation to the final value of the NIE. After α, β-methylene ATP the effect produced by ATP (300 μM) was 46.2 ± 1.0% for NWR and 61.0 ± 2.6% for SHR. UTP (1 mM to 1 mM) initially produced a NCE lasted about 60 to 90s followed by a NIE. After this time it was observed a positive chronotropic effect (PCE) that lasted 400s. The α-β-methylene increased the PCE produced by UTP (300 μM) in NWR and SHR, showing an enhancement of more than 200 % in relation to control. UTP produced an initial NIE with an amplitude of 17.1 ± 1.9% in NWR and 12.4 ± 0.6% in SHR ($p<0.05$) compared with baseline. After this time, atrial inotropism increased gradually, reaching the plateau at 150-180 min, with a value of 25.4 ± 1.1% in NWR and 34.5 ± 2.1% in SHR ($p<0.05$). After α, β-methylene ATP the effect produced by UTP (300 μM) was 53.4 ± 1.0% for NWR and 73.3 ± 1.6% for SHR. **Discussion**: The results suggest that P2Y receptors modulated the inotropic and chronotropic effect in RA of NWR and SHR, suggesting modification in hypertensive animals. **Sources of Research Support:** CAPES and FAPESP.

06.007 **Acute ethanol intake increases the production of superoxide anion in mesenteric bed**. Hipolito UV[1], Callera GE[2], Touyz RM[2], Tirapelli CR[3] [1]FMRP-USP – Farmacologia, [2]Universidade de Ottawa, [3]EERP-USP

Introduction: Literature data show that the metabolism of ethanol leads to formation of reactive oxygen species (ROS), which may be the initial step in the cardiovascular dysfunction induced by ethanol. **Methods:** The experimental protocols were approved by the Ethical Committee from USP (10.1.235.53.0). Male Wistar rats initially weight 200-250 g were randomly divided into 4 groups: Control, Control + Vitamin C (250 mg/kg for 5 days), Ethanol (1g/Kg; 10 mL/Kg of 13% ethanol diluted in water) and Ethanol + Vitamin C. Activity of NAD(P)H was measured in mesenteric arteries by lucigenin assay. Western blotting was used to asses: phospho-p38MAPK (Thr180/Tyr182), total p38MAPK, phospho-ERK1/2 (Thr202/Tyr204), total ERK1/2, phospho-SAPK/JNK (Thr183/Tyr185), total SAPK/JNK, phospho-Akt (Ser173), total Akt, phospho-eNOS (Ser1177), total eNOS and NOX 1. Cytosol/Membrane fraction to check p47phox, RHO A and RHO Kinase translocation was performed in mesenteric arteries. **Results:** The lucigenin-derived luminescence was significantly higher in ethanol group (1363.9 ± 83.8, n = 5) when compared to control group (399.8 ± 53.3, n = 7) The pre-treatment with vitamin C (5 days) prevented the increase in ROS generation induced by ethanol (479.0 ± 90.3, n = 5) ($p<0,05$/ANOVA). Western blotting showed that protein phosphorylation of p38, JNK, ERK1/2, Akt and eNOS did not differ among the groups. Similarly, NOX 1 expression in the 4 experimental groups was similar. Translocation (cytosol/membrane) assay showed that ethanol significantly increased p47phox, RHO A and RHO kinase translocation. The pre-treatment with vitamin C (5 days) prevented this response ($p<0.05$/ANOVA). **Discussion:** Acute ethanol intake increases superoxide anion generation in the mesenteric bed. This response may contribute to functional alterations that serve as a causative factor for cardiovascular diseases. **Supported by:** FAPESP.

06.008 **Effect of a potentiator of bradykinin from *Caudisona durissus cascavella* in normotensive and renovascular hypertensive rats**. Martins PL[1], Gomes Jr NE[1], Galeno DML[1], Santos CF[1], Carvalhos KM[2], Cardi BA[2], Fonteles MC[1], Nascimento NRF[1] [1]ISCB – Fisiofarmacologia Cardiovascular e Renal, [2]ISCB – Toxinologia e Farmacologia Molecular

Introduction: New bioactive peptides, such as natriuretic and bradykinin potentiating peptides (BPPs), have been isolated from venom of some snakes and amphibians. The BPPs are natural inhibitors of angiotensin-converting enzyme (ECA). This aim of the present work was to pharmacologically characterize the BPP isolated from Caudiosona *durissus cascavella* (BPP-Cdc). **Methods:** Rats were anesthetized with ketamine and xylazine (90 and 10 mg / kg, respectively, i.p.) for cannulation of the carotid artery and femoral vein. Rats were made hypertensive by renal artery clamping (2K-1C Goldblatt model) and evaluated 3 and 4 weeks after the procedure. The rats received i.v. injection of either saline, BPP-Cdc (50µg) or BPP-9a (50µg) in bolus and then bradykinin (BK; 250ng) and angiotensin I (Ang I, 10ng) at 15 minute intervals. This project was approved by the Ethics Committee for Animal Use of the State University of Ceará with protocol number 084393384-0. **Results and Discussion:** The hypotensive effect of BPP-Cdc, -19.1 ± 3.7 mmHg, was higher than of the BPP-9a, -5.2 ± 0.9 in normotensive rats. The amplitude of this hypotensive response of BPP-Cdc increased in 3- weeks hypertensive rats reaching a peak at 107 minutes (-33.0 ± 7.3 mmHg). Similar result was found in hypertensive 4 weeks. BPP-Cdc showed sustained BK potentiating effect and inhibited the pressoric effect of ANG I in hypertensive animals. In addition, the duration of the hypotensive response to BK was greater than in normotensive rats (115.3 ± 19.8s vs. 36.2 ± 4.8 s, $p<0.001$) when compared to 3-week hypertensive rats and 178 ± 22.3s for 4-week rats. The amplitude of the pressoric response to Ang I was reduced after the administration of BPP-Cdc with a maximal inhibition values in 3-week hypertensive rats from a control of 40.2 ± 4.7 mmHg vs 10.4 ± 2.3 mmHg and the same ocurred in 4-week hypertensive rats (control 33.0 ± 4.4mmHg vs 10.0 ± 4.3 mmHg). BPP-Cdc has both BK potentiating and AGI inhibitory properties and should be further evaluated for its antihypertensive effects by chronic administration through osmotic pumps in animal models of hypertension. This work was supported by institutions Coordination of Improvement of Higher Education (CAPES) and the National Council for Scientific and Technological Development (CNPq).

06.009 **Anti-platelet activity of the haem-independent soluble guanylyl cyclase activator BAY 60-2770 in human washed platelets**. Mendes-Silvério CB, Morganti RP, Anhê GF, Mónica FZT, De Nucci G, Antunes E Unicamp – Pharmacology

Introdution: Nitric oxide-independent soluble guanylyl cyclase (sGC) activators are reported to reactivate the haem-oxidized enzyme in vascular diseases[1]. This study was undertaken to investigate the anti-platelet mechanisms of the haem-independent sGC activator BAY 60-2770 in human washed platelets. The hypothesis that sGC oxidation potentiates the anti-platelet activities of BAY 60-2770 has been tested. **Methods:** The experimental protocols were approved by the Human Ethics Committee of the State University of Campinas (UNICAMP; n° 487/2011). Human washed platelet aggregation and adhesion assays were performed. Intracellular calcium levels were monitored in platelets loaded with a fluorogenic calcium-binding dye (FluoForte). Flow cytometry was performed using monoclonal αIIbβ3 antibody (PAC-1). **Results:** BAY 60-2770 (0.001–10 μM) produced significant inhibition of collagen (2 μg/mL)- and thrombin (0.1 U/mL)-induced platelet aggregation that was markedly potentiated by prior incubation with the sGC inhibitor 1H-[1,2,4]oxadiazolo[3,4-a]quinoxalin-1-one (ODQ, 10 μM). In fibrinogen-coated plates, BAY 60-2770 significantly inhibited platelet adhesion, an effect potentiated by ODQ. BAY 60-2770 increased the cGMP levels and reduced the intracellular Ca^{2+} levels, both of which were potentiated by ODQ. The cAMP levels were unchanged by BAY 60-2770. Thrombin- and collagen-induced platelet $\alpha_{IIb}\beta_3$ activation was markedly inhibited by BAY 60-2770 that was further inhibited in the presence of ODQ. In contrast, the inhibitory effects of the NO donor sodium nitroprusside (3 μM) in platelet aggregation, adhesion, cGMP generation, intracellular Ca^{2+} levels and integrin $\alpha_{IIb}\beta_3$ activation were fully prevented by ODQ. **Discussion:** Oxidation of sGC haem moiety with ODQ potentiates the inhibitory effects of BAY 60-2770 in aggregation, adhesion, cGMP production, intracellular Ca^{2+} levels and $\alpha_{IIb}\beta_3$ activation. This could be of therapeutic interest in cardiovascular diseases associated with thrombotic complications. **References:** Evgenov, O.V., *Nat Rev Drug Discov*, 5, 755, 2006. **Financial support:** CNPq and FAPESP.

06.010 **The role of aldosterone in the development of albuminuria and podocyte injury in 2K,1C hypertensive rats**. Singulani JL[1], Coimbra TM[2], Francescato HDC[2], Costa RS[3], Silva GEB[3], Coelho LTER[4], Coelho EB[1] [1]FMRP-USP – Farmacologia, [2]FMRP-USP – Fisiologia, [3]FMRP-USP – Patologia, [4]FMRP-USP – Clínica Médica

Introduction: Stenosis of the renal artery is the most common cause of renovascular hypertension. In this setting, pharmacologic treatments and renal artery angioplasty have been unsatisfactory to prevent kidney disease. Recent evidence points that aldosterone may contribute to evolution of kidney injury. Considering that the model of 2 kidney,1 clip (2K,1C) is characterized by high activity of the renin-angiotensin-aldosterone system and that present pathologic characteristics that resemble those of human renal artery stenosis, the objective of this study is to assess the role of aldosterone in renal injury in this model. **Methods:** The experimental protocols were in accordance with the Ethical Committee from USP (148/2007). Male Wistar rats were divided into 4 groups: sham (n = 10); 2K,1C (n = 8); 2K,1C that received 20 mg/Kg/day of spironolactone (n = 10) and 2K,1C that received 7 mg/Kg/day of amiloride (n = 12). Albuminuria was determined by electro-immunoassay in agarose gel. Systolic blood pressure (SBP) was measured by tail-cuff plethysmography. Immunohistochemistry was performed by the avidin-biotin-peroxidase technique, using primary antibody monoclonal anti-desmin (1:100) and secundary antibody anti-mouse (1:200). Data were analyzed by ANOVA nonparametric (Kruskal-Wallis test) followed by Dunn's post-test and expressed as median and percentile (25, 75%). SBP was analyzed by ANOVA followed by Bonferroni post-test and expressed as mean ± SEM. **Results:** Albuminuria in the 2K,1C rats was significantly higher than in the sham rats at 6 week after surgery (42.80; 31.54-77.30 vs 0.00; 0.00-0.10, *P*<0,001). Treatments with spironolactone (13.86; 4.30-28.58, *P*<0,05 vs sham, *P*<0,05 vs 2K,1C) and amiloride (18.13; 9.02-34.91, *P*<0,01 vs sham, *P*<0,05 vs 2K,1C) significantly reduced albuminuria. SBP was greater in 2K,1C rats than in sham rats (223±6.3 vs 132±3.7, *P*<0,001 vs sham), and treatment with spironolactone (225±7.3, *P*<0,001 vs sham) or amiloride (227±3.7, *P*<0,001 vs sham) had no significant effect on SBP. In sham rats, the expression of desmin was found restricted to mesangial cells and smooth muscle cells. In contrast, expression was observed in the podocytes of kidneys in the 2K,1C rats, which was significantly elevated in the nonclipped kidney (0.82; 0.36-1.05, *P*<0,05 vs sham) and discrete in the clipped kidney (0.01; 0.00-0.07). Treatment with spironolactone (0.44; 0.06-1.51), but not amiloride (0.85; 0.35-1.45, *P*<0,05 vs sham), attenuate the expression of desmin in podocytes in nonclipped kidney. **Discussion:** These findings indicate that aldosterone participates in the development of the albuminuria and podocyte injury of 2K,1C rats. This effect is independent of SBP and can be partly dependent of effect on epithelial sodium channel (ENaC). **Financial support:** FAPESP, CNPq.

06.011 Intracapsular LPA treatment recovers renal glomerular function of wistar rats subjected to kidney ischemia-reperfusion. Gonsalez SR[1], Leal AC[1], Verdoorn KS[2], Beiral HJV[2], Einicker Lamas M[2], Lara LS[1] [1]UFRJ – Farmacologia, [2]UFRJ – Biofísica

Introduction: The injury caused by ischemia-reperfusion (I/R) is an important cause of acute renal failure (ARF). Lysophosphatidic acid - 1-acyl-2-Lyso-sn-glycero-3-phosphate (LPA) is an endogenous phospholipid generated during the process of renal ischemia, whose effects are mediated through specific receptors coupled to G protein. Since no pharmacological approach used in clinical medicine was effective in preventing renal injury mediated by I/R, the LPA emerged as a potential target of study. This work aimed to determine the effect of LPA intracapsular treatment on renal function during the I/R. **Methods:** All procedures used were approved by the Committee for Experimental and Animal Ethics at the Federal University of Rio de Janeiro (IBCCF087). Adult male Wistar rats were divided into three groups (n = 11): (a) sham-operated, (b) I/R: ischemia was induced by applying a non-traumatic vascular clamp to the two renal arteries for 30 min . Then the clip was removed to reperfusion occurs and the scar closed, and (c) I/R+LPA: 1mg /Kg LPA was injected in the renal capsulae during the ischemic process. After the surgery, the rats were housed on metabolic cages for 24 h to collect urine for the measurement of the renal function. The rats were sacrificed by decapitation where the blood and the kidneys were collected. Paraffin embedded kidney sections (3µm) were stained with periodic acid-Schiff for glomeruli analysis. **Results:** The blood nitrogen urea increased 141% in I/R and the LPA treatment prevented this increase without changing the high levels of proteinuria. The glomerular filtration rate decreased from 11.09 µl /min to 4.20 µl/min ($P<0.05$) in I/R, returning to control values with LPA, this profile was parallel the Na^+ filtered load. The 50 % reduction in glomeruli number during I/R was prevented by LPA. By the other hand, decrease in the urinary Na^+ excretion and fractional excretion observed during I/R was not prevented by LPA treatment. These data is correlated to the balance between the increase in the cortical (Na^++K^+) ATPase activity (52 %) and the decrease in the cortical Na^+-ATPase (46 %) in I/R and which was not modified by the LPA treatment ($P<0.05$). **Discussion:** The LPA treatment recovers the glomerular function of Wistar rats subjected to the I/R process, without modifying the renal tubular function and Na^+ transport. The maintenance of the intratubular fluid levels during LPA treatment due to the return of the glomerular function suggests a possible mechanism to prevent acute tubular necrosis commonly observed in the I/R episodes. **Financial support**: CNPq; Faperj.

06.012 **Cardiometabolic risk evaluation in rats submitted to neonatal leptin treatment**. Marques EB, Oliveira GF, Silva RM, Graça RO, Scaramello CBV LAFE-UFF

Introduction: Studies have shown a strong correlation between stressful events (nutritional/hormonal) in early life and development of adult diseases such as obesity and cardiovascular (CV) failure (Trevenzoli *et al*. J Physiol, v.580, p.629, 2007). Rats submitted to leptin treatment during lactation presented higher body weight as observed in the offspring of mothers under energetic restriction in this period (Toste *et al*. Br J of Nutrition, 95: 830, 2006). The aim of this work is to study anthropometrical parameters and markers of obesity in rats submitted to neonatal leptin treatment correlating them to cardiometabolic risk. **Methods:** Pups were divided into two groups. During lactation (days 1-10) leptin (8µg/100g sc; L group) or saline injections (C group) were performed. After weaning, body weight and food intake were monitored until 5 months-old. Body length as well as overweight predictor indexes, such as Lee and Body Mass Indexes (BMI), were determined at 1 and 5 months-old. Based on food consumption and body weight gain, feed efficiency ratio was calculated between 21-30, 30-90 and 90-150 days-old. Serum glucose and lipid profile were determined using Labtest kit. Castelli's atherogenic ratios I and II were also assessed. Data were presented as mean and standard error of the mean (at least 3 observations), analysed by Student *t* test and considered statistically different if $P<0.05$(*). The use of animals was according to Ethics Committee (CEPA/UFF00123-09). **Results:** Body weight and food intake were significant higher in L group compared to C group since 76th days-old. There were no differences in body length but BMI (C = 0.730±0.006 x L = 0.758±0.005*) and Lee index (C = 0.311±0.001 x L = 0.316±0.001*) were higher in L group at 5 months-old. Body weight gain was higher in L group all over the investigated period (21-30: C = 57.8±1.7 x L = 65.1±1.5*; 30-90: C = 224.9±5.0 x L = 240.7±4.1*; 90-150: C = 51.9±4.3 x L = 65.6±4.3*) while feed efficiency ratio was higher just between 21-30 days-old (C = 0.43±0.01 x L = 0.50±0.02*). Food consumption was superior in L group between 30-90 (C = 1544.3±34.2 x L = 1657.4±26.1*) and 90-150 days-old (C = 1497.6±24.9 x L = 1682.8±35.5*). Although leptin treatment do not affect serum glucose levels, total cholesterol was higher in L group at 1 month-old (C = 62.5±0.8 x L = 76.5±2.3*). At 5 months-old HDL-c was smaller in L group (C = 26.7±1.1 x L = 11.1±0.2*) while VLDL-c (C = 7.3±2.5 x L = 22.6±2.2*) and triglycerides (C = 38.3±11.3 x L = 113.2±11.0*) were higher. Neonatal leptin treatment increases Castelli's ratio I (C = 2.49±0.05 x L = 4.26±0.12*) and II (C = 1.40±0.06 x L = 2.88±0.10*) at 5 months-old. **Discussion:** Body weight and food intake programming are ascribed to hyperleptinaemia in early life as well as leptin/insulin resistance which is related to metabolic syndrome, a CV risk factor (Toste *et al*. Br J of Nutrition, v.95, p.830, 2006; Zimmet *et al*. Ann N Y Acad Sci, v.892, p.25, 1999). Rat obesity may be easily estimated through BMI and alterations in this index are associated with dyslipidemic profile, as observed in this work, and oxidative stress (Novelli *et al*. Laboratory Animals, v.41,p.111, 2007). In conclusion, the present results show that neonatal leptin treatment increases cardiometabolic risk, reinforcing our previous data (Marques *et al*., FeSBE2011, panel 24.033). **Financial Support:** FAPERJ, CNPq, CAPES, PROPPI/UFF.

06.013 **Resveratrol improves the endothelium-dependent vasorelaxation in 2K-1C hypertension**. Scalabrini AC[1], Oliveira JC[2], Antonietto CRK[1], Talita SM[1], Restini CBA[3] [1]Unaerp – Ciências Farmacêuticas, [2]Unaerp – Nutrição, [3]Unaerp – Medicina

Introduction *High blood pressure* is a cardiac chronic medical condition and important cause of death in the world due to cardiovascular complications [Rev. Bras. Hipert. 17:6, 2010]. This condition triggers excessively production of reactive oxygen species (ROS) [Landmesser. Coron Artery Dis, 12:461,2001]. Relevant damages caused by oxidative stress underlying hypertension and correlated diseases are the impairment of endothelium-dependent vasorelaxation mechanisms [Higashi, The New Engl. J of Med, 346:1954, 2002], which is generally related to the reduced generation or bioavailability [Harrison, Clin Cardiol, 20:11, 1997] of a potent vasodilator released from endothelium: nitric oxide (NO) [Furchgott. Nature, 288:373, 1980]. The reduced NO bioavailability has been attributed to the oxidative inactivation of NO by the excessive generation of O_2^- in the vascular wall [Kojdaa. Cardiovasc Res, 43:562,1999]. Resveratrol (RESV) presents antioxidant properties and potential protection against damage caused by oxidative stress [Zhang. *Arterioscler Thromb Vasc Biol*, 29:1164,2009]. Thus, RESV can reduce the incidence of cardiovascular disease as well as improves relaxation of vascular smooth muscle by interfering in the synthesis and releasing of NO from endothelium [Sinclair. Nat Rev Drug Discov, 5: 493, 2006]. Since the renovascular hypertension (2K-1C) is associated with changes in NO production through interaction with ROS, especially O_2^- [Lerman. Hypertension, 37: 541, 2001] our hypothesis is that RESV is able to mitigate the harmful actions of ROS improving relaxation vascular on isolated aorta from 2K-1C rats. **Aim:** Investigate the effects of treatment with RESV on relaxation stimulated by acetylcholine (ACh) in aortic rings isolated from rats 2K-1C. Male Wistar rats (180g) through ethical approval (007/2010), were submitted to surgery to induce hypertension 2Kidney-1Clip. Control animals (sham) were exposed to the fictitious surgery. Systolic blood pressure (SBP) was measured by an indirect tail-cuff method, one day before the surgery, and weekly after. Hypertensive rats: SBP higher than160 mm Hg. Treatment with 20 mg/Kg RESV by gavage was initiated one day after surgery, three times a week, for six weeks. Vascular reactivity was investigated in aorta with intact endothelium by developing concentration-effect curves for ACh. **Results**: RESV attenuated the increased SBP (179.13±4.9 mmHg, n = 23) compared to 2K1-C untreated (196.66±6.06 mmHg, n = 15). It was obtained lower maximum relaxation in aorta from 2K-1C untreated (85.97±0.69%, n = 6) compared to the untreated sham (105.52±1.31%, $p < 0.0001$, n = 9). Maximum relaxation stimulated by ACh in treated 2K-1C: 116.63±1.72%, $p < 0.0001$, n = 12. We also studied the role of NO production by the inhibition of the NO Synthase. Incubation with L-NAME (10^{-6}M) an inhibitor of NOS, reduced the relaxation to ACh in all groups. However, in 2K-1C treated (76.94 ± 2.62%, n = 7), compared to 2R-1C untreated (89.58 ± 4.49%, $p<0.05$, n = 7), the minor relaxation caused by L-NAME was attenuated by the RESV. **Conclusion**: Treatment with RESV can reduce blood pression in hypertensive animals and improves endothelium-dependent relaxation and the production of NO. Financial Support: CNPq, UNAERP.

06.014 **Nitric oxide diminishes matrix metalloproteinase-9 expression in endothelial cells**. Meschiari CA[1], Izidoro-Toledo TC[1], Gerlach RF[2], Tanus-Santos JE[1] [1]FMRP-USP – Pharmacology, [2]FORP-USP – Morphology, Stomatology and Physiology

Introduction: Impaired nitric oxide (NO) bioavailability and imbalanced matrix metalloproteinase (MMP) activity are key pathogenetic mechanisms involved in cardiovascular diseases. However, there is little evidence supporting a direct link between these mechanisms, although NO is known to interfere with nuclear factor kappa B (NFkB) activity, an important modulator of MMP-9 expression. Moreover, it is not known whether the possible effects of NO on MMPs are dependent on cyclic GMP formation. **Objective:** We examined the effect of NO donors on MMP-9 production by endothelial cells, and if this effect is dependent on cGMP formation or NFkB activation. **Methods:** Human umbilical vein endothelial cells were cultured in appropriate medium and treated for 24 hours with 10 nM phorbol myristate acetate (PMA; a MMP-9 inducer) and other drugs: NO donors (S-nitroso-N-acetylpenicillamine; SNAP, or DetaNONOate), 1H-[1,2,4]Oxadiazolo[4,3-a]quinoxalin-1-one (ODQ; a soluble guanylyl cyclase inhibitor), BAY 11-7082 (a NFkB inhibitor), or their vehicles. Conditioned media were analyzed by enzyme-linked immunoabsorbent assay. **Results:** PMA increased MMP-9 concentration from 0.03±0.03 ng/mL to 1.17±0.40 ng/mL (P<0.05). Treatment with SNAP (320 μM) attenuated the increases in MMP-9 concentration induced by PMA (0.26±0.08 ng/mL *vs.* PMA 1.17±0.40 ng/mL, P<0.05). DetaNONOate (400 μM) exerted similar inhibitory effects on MMP-9 concentration (1.33±0.13 ng/mL *vs.* PMA 2.00±0.51 ng/mL, P<0.05). Conversely, treatment with ODQ (32 μM) had no effects on 320 μM SNAP-induced inhibition of PMA-stimulated MMP-9 activity (0.23±0.07 ng/mL *vs.* PMA 1.17±0.40 ng/mL P<0.05). Nevertheless, BAY 11-7082 (5 μM) decreased PMA-stimulated MMP-9 production (0.27±0.10 ng/mL *vs.* PMA 0.78±0.16 ng/mL, P<0.05). **Discussion**: Our results suggest that NO attenuates MMP-9 expression in endothelial cells. While this effect is independent of soluble guanylate cyclase activation, it apparently involves inhibition of NFkB activity. Financial agencies and Acknowledgements: FAPESP, FAEPA, CAPES and CNPq.

06.015 **Effect of the extract of *Euterpe oleracea* Mart. (AÇAI) on cardiovascular changes and oxidative stress in spontaneously hypertensive rats**. Cordeiro VSC[1], Carvalho LCRM[1], Costa CA[1], Bem GF[1], Souza MAV[1], Sousa PJC[2], Soares de Moura R[1], Resende AC[1] – [1]UERJ – Farmacologia e Psicobiologia, [2]UFPA – Farmácia

Aim: Hypertension is a major cause of premature death worldwide. Studies on the *Euterpe oleracea* Mart., a typical plant of Brazil, rich in polyphenols, have shown great therapeutic potential against hypertension, since its benefits can be associated with antioxidant, vasodilator and antihypertensive actions. Therefore, this study investigated the effect of chronic treatment with the hydroalcoholic extract of seeds of the açaí (ASE) on cardiovascular changes and oxidative stress in spontaneously hypertensive rat (SHR). **Methods:** The experiments were approved by the Ethics Committee of Animal Experiments of the UERJ (protocol: CEA/022/2010). Young male Wistar (W) and SHR (21 days old) were treated with ASE (200 $mg.kg^{-1}.day^{-}$) in drinking water, or vehicle, from 21 days to 4 months of age and systolic blood pressure (SBP, mm Hg) was measured by plethysmography. Renin levels (pg/mL) were measured in plasma samples by radioimmunoassay. The vasodilator effect of acetylcholine (ACh, 1-100 pmol) was studied in mesenteric arterial bed (MAB) pre-contracted with norepinephrine (30 mM). The antioxidant enzymatic activity of superoxide dismutase (SOD), catalase (CAT), and glutationa peroxidase (GPx) (U/mg protein) and protein carbonylation (nmol/mg protein) were assessed in samples of heart and MAB by spectrophotometry. The protein expressions of eNOS and SOD were evaluated by western blotting in MAB and vascular changes by the thickness of the tunica media in aorta (μm^2). **Results:** SBP was increased in SHR compared to controls (SHR:217±4 vs W:140±3 and W+ASE:137±7) and treatment with ASE reduced the hypertension (SHR+ASE:175±6;*P*<0.05). The increased levels of renin in SHR were reduced by treatment with ASE (W:2.6±0.3; W+ASE:2.5±0.4; SHR:4.6±0.8; SHR+ASE:2.4±0.2;*P*<0.05). The reduced vasodilator effect (% of relaxation) of ACh (100 pmol) in SHR was recovered by ASE (W:83±4; W+ASE:74±1; SHR:59±4; SHR+ASE:85±2;*P*<0.05). ASE decreased the levels of carbonyl protein in the heart of SHR (W:0.2±0.02; W+ASE:0.1±0.1; SHR:0.7±0.09; SHR+ASE:0.04±0.01;*P*<0.05), however there was no difference in samples of MAB (W:0.0016±0.0005; W+ASE:0.0016±0.0006; SHR:0.001±0.0007; SHR+ASE:0.0004±0.0003;*P*<0.05). Antioxidant activity of SOD was increased in heart and MAB of SHR which was reduced by treatment with ASE (Heart:W:24.8±4.7; W+ASE:13.9±2.7; SHR:148.8±35.6; SHR+ASE:32.9±3.2; MAB:W:9.2±1.6; W+ASE:9.4±0.8; SHR:28.6±5.6; SHR+ASE:9.6±1,6;*P*<0.05). The activities of GPx and CAT were not different between groups in MAB, as well as the activity of GPx in heart. However, the treatment of SHR with ASE increased the activity of CAT in the heart (W:0.7±0.2; W+ASE:0.8±0.3; SHR:0.07±0.1; SHR+ASE:4.1±1.1;*P*<0.05). The expressions of eNOS and SOD were increased in SHR and unchanged by treatment with ASE. The medial thickness of the aorta was increased in SHR, which was reduced by ASE (W:223.1±6.5; W+ASE:219.8±3.8; SHR:271.2±3.9; SHR+ASE:232.6±7.7;*P*<0.05). **Discussion:** The results demonstrate that chronic treatment with ASE reduces the hypertension in SHR. The improvement of endothelial function, vascular remodeling and the antioxidant activity induced by ASE may contribute to this beneficial effect of the extract in SHR. **Financial Support:** CAPES and FAPERJ

06.016 **Effect of aminoguanidine into the paraventricular nucleus of the hypothalamus on cardiovascular and autonomic modulation in conscious rats during LPS endotoxemia**. Matsumoto AK[1], Silva AMD[1], Abreu SB[1], Pinge-Filho P[2], Martins-Pinge MC[1] [1]UEL – Fisiologia, [2]UEL – Patologia

Objectives: Sepsis induces excessive production of inflammatory mediators such as nitric oxide (NO), and cause cardiovascular changes. A section of the central nervous system active in case of endotoxemia is the paraventricular nucleus of the hypothalamus (PVN). Data from our laboratory showed the involvement of PVN in cardiovascular and autonomic modulation during endotoxemia by lipopolysaccharide (LPS). The presence of NO synthase in the PVN suggests that NO may play a role in endocrine and autonomic regulation of cardiovascular responses. The objective of this study was to evaluate the involvement of nitric oxide from iNOS pathway in the PVN on mean arterial pressure (MAP), heart rate (HR) and spectral analysis of HR variability (HRV) after administration of LPS in conscious rats. **Methods and Results:** Male Wistar rats were anesthetized for implantation of bilateral guide cannulae to the PVN, stereotactic coordinates used as a reference suture bregma (AP = -1.6 mm, L = 0.5 mm, DV = 6.6 mm). After 5 days the rats were anesthetized by Tribromoethanol 2.5% for chronic cannulation of the femoral artery and vein, with the objective of blood pressure monitoring and drug administration, respectively. 24 hours after catheterization, animals are subjected to the record of baseline blood pressure, followed by the experimental protocol. We performed the bilateral microinjection of 100 nl of saline or aminoguanidine (250 pmol) into the PVN of conscious rats, followed by LPS administration (iv). Cardiovascular parameters were analyzed by 2 hours. Only animals that had marked the sites in the area of the PVN were considered in the statistical analysis. Saline microinjection into the PVN did not modify the baseline parameters and after LPS was observed hypotension and tachycardia (MAP = -24 ± 5 mmHg, HR = 85 ± 15 bpm, $p<0,05$). Aminoguanidine microinjection before LPS caused no alterations in MAP and the same increase in HR (MAP = -0.4 ± 1 mmHg, HR = 110 ± 11 bpm) when compared with control group. The HRV was abolished after LPS (Basal = 58.25 ± 15.95; Sepsis = 4.67 ± 1.61) and was not different between groups for systolic arterial pressure. After aminoguanidine the HRV and the variability of systolic arterial pressure during endotoxemia did not alter. **Conclusions:** Our results suggest that NO participate in the cardiovascular responses by modulating neurotransmission in the PVN, during the initial phase of endotoxemia by LPS. **Financial Support**: PIBIC/UEL. The license number of the Human Ethics Committees: 02/09

06.017 **Mechanisms of action vasorelaxant induced adrenomedullin in rat carotid**. Passaglia P[1], Tirapelli SD[2], Tirapelli CR[3] [1]USP – Clínica Médica, [2]USP – Cirurgia e Anatomia, [3]USP – Enfermagem Psiquiátrica e Ciências Humanas

Introduction: Adrenomedullin (AM) is a peptide produced by endothelial cells and vascular smooth muscle which exerts a direct action on the cardiovascular functions being able to induce vascular relaxation and hypotension. Considering that several experimental models of vascular injury use the carotid artery, becomes pertinent the receptor functional characterization for AM and its expression in rat carotid artery. **Objectives:** Investigate the mechanisms involved in vascular relaxation induced by AM in rat carotid and show the components expression of the AM system (pre-pro-AM, CRLR, ARPMs 1, 2 and 3) in this artery. **Methods:** Carotid artery of adult rats, aging between 40 and 50 days (200-250 g), was isolated for functional studies of vascular reactivity. Concentration-response curves for AM were obtained in the presence or absence of endothelium, AM antagonists, cyclooxygenase, K^+ channels and NO-cGMP pathway inhibitors. The AM system components expression (pre-pro-AM, CRLR, ARPMs 1, 2 and 3) was evaluated by real time PCR. Data were analyzed by ANOVA or Student t test, followed by Bonferroni post-test and $p < 0.05$ were considered significant. The protocols were approved by Ethical Animal Committee from the Campus of Ribeirão Preto, USP (Protocol: 10.1.200.53.2). **Results:** The carotid expresses all AM system components. The AM induced relaxation in phenylephrine pre-contracted carotid arteries with or denuded endothelium (Emax: 58.0 ± 4.3% n = 11 and 24.7 ± 2.9% n = 9, respectively). It was observed that CGRP also induced relaxation in arteries with and denuded endothelium (Emax: 49.6 ± 3.0% n = 6 and 32.3 ± 0.8% n = 6, respectively). In rings with endothelium, was observed a significant inhibition in the relaxation response amplitude induced by AM in the presence of 7-nitroindazole (Emax: 28.0 ± 0.7% n = 3), ODQ (Emax 6.2 ± 3.9% n = 4), Rp-8-Br-Pet (Emax: 22.5 ± 3.1% n = 7), H-89 (Emax: 16.9 ± 1.1%, n = 6) and indomethacin (Emax: 36.5 ± 1.5%, n = 9). There was a significant shift to the right in the AM curve with endothelium (pD2: 10.7 ± 0.4, n = 11) in the presence of indomethacin (pD2: 10.2 ± 0.4 n = 9), SC560 (pD2 : 12.9 ± 0.4, n = 8) and AM22-52 (pD2: 11.2 ± 0.6, n = 6), an antagonist of AM. AM induced relaxation was not affected by tetraethylammonium (Emax: 55.6 ± 8.0%, n = 8) and wortmannin (Emax: 66.9 ± 7.3%, n = 5). **Discussion:** The carotid artery expressed all components of AM system. The AM induced relaxation is mainly but not entirely, mediated by vascular endothelium and involves NO-cGMP pathway and cyclooxygenase. **Financial support:** FAPESP.

06.018 **The role of oxidative stress and inflammation during nitrate tolerance induced by sodium nitroprusside**. Diniz MC[1], Olivon VC[2], Tavares LD[3], Santos RAS[2], Souza DG[3], Bonaventura D[1] [1]UFMG – Pharmacology, [2]UFMG – Physiology and Biophysics, [3]UFMG – Microbiology

Introduction: Chronic therapy with nitroglycerin (GTN) leads to a nitrate tolerance that is characterized as a reduction of its hemodynamic effects. This study aimed to verify if an inorganic nitrate, sodium nitroprusside (SNP), induces tolerance, such as GTN. **Methods:** This project was approved by CETEA/UFMG (037/2010). Vascular reactivity was performed in thoracic aortas from Balb/c mice and maximal relaxation (Emax) and potency (pD2) of SNP were analyzed. *In vitro* tolerance was induced, in intact and denuded mice aorta, by SNP (EC50: 10nM) incubation for 15, 30, 45 and 60 minutes. After that, aortic rings were washed for 15 minutes and then precontracted with phenylephrine for SNP-relaxation studies. We also measured the superoxide concentration induced by SNP-toleranceusing a selective dye, DHE, by confocal microscopy. **Results**: Preincubation of denuded aorta to SNP for 60 minutes reduced the potency to SNP, characterizing SNP-tolerance (pD2-control: 8.40±0.06, n = 05; tol: 7.79±0.09, n = 04). Preincubation to SNP also induced cross-tolerance to GTN (Emax-control: 127.74±4.55, n = 5; tol:114.21±1.03). The preincubation with NPS+Tiron, O^{2-} scavenger, restored the SNP-potency to control arteries values (pD2-control+tiron: 8.72±0.13, n = 07; tol+tiron: 8.46±0,02. n = 07), showing involvement of O^{2-} on this phenomenon. The same results were observed in preexposition to SNP+atorvastatin or apocynin, NADPH oxidase inhibitors (pD2-control+atorv: 8.62±0,07, n = 06; tol+atorv: 8.44±0,14, n = 05; control+apo: 8.76±0,08, n = 05; Tol+apo: 8.71±0,07, n = 05), showing the involvement of this enzyme on SNP-tolerance. The O^{2-} production induced by SNP-tolerance was abolished in the presence of tiron or atorvastatin, showing that NADPH oxidase is one of the sources of O^{2-} in SNP-tolerance. The preincubation with NPS+L-NAME, NO synthase inhibitor, or NPS to L-arginine, NO syntase substrate, restored the SNP potency to control arteries values (pD2-control+L-NAME: 8.89±0.14, n = 06; tol+L-NAME: 8.69±0.13, n = 05, control+L-arg: 9.01±0.07, n = 04; tol+L-arg: 8.85±0.12, n = 05). Both results suggest the uncoupling of NO synthase in SNP-tolerance. The preincubation with SNP+ibuprofen, non-selective COX inhibitor, also restored SNP-potency (pD2-controle+IBU: 8.81±0.14, n = 5; Tol+Ibu: 8.62±0.05, n = 06). The same result was observed after preincubation with SNP+AH6809, $PGF_{2\alpha}$ receptor antagonist, or SQ29584, TXA_2 receptor antagonist (pD2-control+AH: 9.02±0.09, n = 05; tol+AH: 9.01±0.09, n = 05; pD2-control+ SQ: 8.80±0.07, n = 04; tol+ SQ: 8.70 ±0.05, n = 06), confirming the involvement of vasoconstrictor prostanoids on SNP-tolerance. **Discussion:** Taken together, our results demonstrated that, *in vitro*, SNP induces tolerance and this phenomenon is time dependent and endothelium independent. Furthermore, this SNP-tolerance involves NADPH oxidase activation and NO synthase uncoupling, leading to enhance in O^{2-} production. This enhancement in oxidative stress induces COX activation with enhancement in vasoconstrictor prostanoids, such as $PGF_{2\alpha}$ and TXA_2. **Financial Agencies**: CAPES, FAPEMIG and FAPESP.

06.019 Tolerance and cross-tolerance induced by nitroglycerin and by the new nitrite donor CIS-[RU(BPY)2(PY)(NO2)](PF6) in cava vein. Paulo M, Silva RS, Bendhack LM FCFR-USP – Physics and Chemistry

Introduction: Organic nitrates, such as nitroglycerin (GTN, glycerol trinitrate), are commonly used in clinical cardiovascular medicine in the acute treatment of stable-effort angina, unstable angina, acute myocardial infarction, chronic congestive heart failure, pulmonary edema, and severe arterial hypertension. The major clinical benefit of organic nitrates, including GTN, has been attributed to its potent venodilator effect, resulting in the reduction of venous return and cardiac preload and myocardial oxygen demand. Unfortunately, the chronic use of these drugs is limited by serious side effects such as nitrate tolerance and cross-tolerance. It leads to the reduction in vasodilatator and hemodymanic effects. The etiology of tolerance is still poorly understood, but it is believed that this process is multifactorial. The present study aimed to verify if the new nitrite donor *cis*-[$Ru(bpy)_2(py)(NO_2)](PF_6)$ (RuBPY) induces *in vitro* tolerance and cross-tolerance with acetylcholine in the rat cava vein and to compare this effect with the effect of GTN. **Methods:** We have analyzed the maximum relaxing effect (Emax) and the potency (pD_2) induced by GNT, RuBPY and acetylcholine in concentration-effect curves constructed in cava vein rings pre-contracted with endothelin-1 (ET-1). *In vitro* tolerance was induced by incubation with GTN (EC_{100}: 0.1mmol/L or EC_{50}: 4 µmol/L) or RuBPY (EC_{100}: 0.1µmol/L or EC_{50}: 2 µmol/L), or vehicle for 10, 30 and 60 min. *In vitro* cross-tolerance for acetylcholine was studied by constructing concentration-effect curves for acetylcholine in ET-1 pre-contracted cava vein rings after incubation for 60 min with GTN (4 µmol/L) or RuBPY (4 µmol/L). All these procedures were performed in accordance with the guidelines of the Animal Ethics Committee, University of São Paulo, Brazil (11.1.828.53.2). **Results and Discussion:** Our results demonstrate that after incubation for 10 min with GNT, Emax and pD_2 were not changed as compared to control for GTN EC_{50} and EC_{100} (Emax: 75.3 ± 2.2%, n = 6; pD_2 = 6.40 ± 0.38, n = 6) or RuBPY EC_{50} and EC_{100} (Emax: 92.85 ± 4.2%, n = 7; pD_2 = 7.21 ± 0.4, n = 7). After incubation for 30 min with RuBPY (EC_{50} and EC_{100}) were not changed in Emax and pD_2 as compared to control. For GTN, the EC_{50} induced no change in Emax and pD_2 compared to control. The Emax of GTN was lower than the control (75.3 ± 2.2% n = 6 to 53.2 ± 1.8% n = 6) after a 30 min incubation with the EC_{100}. After 60 min incubation with GTN (EC_{50} and EC_{100}) Emax was reduced (Control 75.3 ± 2.2% n = 6 to EC_{50} 45.4 ± 2.2%, n = 6 and to EC_{100} 39.2 ± 1.4%, n = 6). After 60 min incubation with RuBPY (EC_{50} and EC_{100}) Emax was reduced (Control: 92.8 ± 4.2%, n = 7 to EC_{50} 48.0 ± 2.3%, n = 7 and to EC_{100} 30.1± 1.2%, n = 7). Therefore, we found that tolerance for GTN is induced after 30 min (for EC_{100}) and 60 min (for EC_{100} and EC_{50}) of prior exposure to GTN. The tolerance for RuBPY is induced after 60 min of prior exposure to RuBPY for EC_{100} and EC_{50}. GTN significantly reduced the acetylcholine-induced relaxation, indicating a marked degree of cross-tolerance for acetylcholine for GTN. Supported by FAPESP and CNPq.

06.020 **A new vasodilator does not induce tolerance in rat aorta**. Banin TM[1], Da Silva RS[1], Bendhack LM[1] [1]FCFRP-USP – Physics and Chemistry

Introduction: The new compound $[Ru(BPY)_2(PY)NO_2](PF_6)]$, (RuBPY), releases NO inside the vascular smooth muscle cell in a tissue dependent manner. Long-term treatment of the patients with NO donors such as nitroglycerin, leads to the development of tolerance characterized by the rapid loss of vasodilator effects. It is believed that the tolerance is a multifactorial process and it involves increased production of vascular reactive oxygen species (ROS), decreased activity of soluble guanylyl-cyclase (sGC) and increased expression and activity of phosphodiesterases. Therefore, we have hypothesized that RuBPY would induce tolerance in intact endothelium rat aorta. The aim of the present study was to investigate whether exposure of rat aortic rings with or without endothelium, with RuBPY (EC_{100}:10 µmol/L) induces tolerance to this NO donor. **Methods**: Male Wistar rats (200-250g) were killed under anesthesia and the thoracic aorta was quickly cut into rings that were placed between two stainless-steel stirrups and connected to an isometric force transducer to measure the tension. The aortic rings were placed in an organ chamber with Krebs solution maintained at pH 7.4 and gassed with carbogen, at 37°C. Endothelium-intact and endothelium-denuded tissues were pre-contracted with phenylephrine (EC_{50}: 100 µmol/L). After reaching a stable and maintained contraction, RuBPY (3 nmol/L–5 µmol/L) was added cumulatively to the organ bath. Experiments were conducted after 5 min incubation (tolerance) with RuBPY (10 µmol/L) followed by 20 min or 60 min of wash-out, or in the absence (control) of RuBPY. The parameters of maximum effect (Emax) and Potency (pD_2) were analyzed. Results are expressed as mean ± SEM. Statistical significance was determined by using the Student's *t* test. In all cases, probability levels of less than 0.05 ($P<0.05$) were taken to indicate statistical significance. All pharmacological studies were performed in accordance with the Ethical Animal Committee of the University of São Paulo (2012.1.134.53.12). **Results**: The compound RuBPY induced concentration-dependent relaxation in aortas with endothelium (pD_2:7.81 ± 0.18; Emax: 101.6 ± 1.4%, n = 7, $P<0.05$) and without endothelium (pD_2:7.54 ± 0.13; Emax: 103.4 ± 0.4%, n = 6, $P<0.05$). It was observed that the incubation with RuBPY EC_{100}, for 5 min followed by 60 min of washing did not affect the maximum relaxation or potency induced by the compound in intact endothelium aorta (pD_2:7.99 ± 0.18; Emax: 98.5 ± 1.6%, n = 5, $P<0.05$) and in denuded arteries incubated with RuBPY (pD_2:7.89 ± 0.18, Emax: 100.0 ± 0.4%, n = 6, $P<0.05$). Similarly, 5 min incubation with RuBPY EC_{100} followed by 20 min did not alter the maximum relaxation or potency in intact endothelium aorta (pD_2:7.92 ± 0.27; Emax: 101.1 ± 1.1%, n = 3, $P<0.05$) or in denuded arteries incubated with RuBPY (pD_2:7.34 ± 0.25, Emax: 99.4 ± 4.5%, n = 4, $P<0.05$) compared with control aortas with intact endothelium (pD_2:8.21 ± 0.41; Emax:101.1 ± 1.1%, n = 3, $P<0.05$) and without endothelium (pD_2:7.39 ± 0.05; Emax:100.8 ± 1.8%, n = 3, $P<0.05$). **Conclusion**: Our data demonstrate that incubation with the NO donor RuBPY (EC_{100}) for 5 min does not induce tolerance. **Financial Support:** CNPq and FAPESP.

06.021 **Do mitochondria modulate the positive inotropic effect produced by ATP and UTP in isolated left atrium from normotensive and hypertensive rats?** Câmara H[1], Rodrigues JQD[1], Silva Junior ED[1], Alves GA[2], Jurkiewicz NH[1], Jurkiewicz A[1] [1]Unifesp – Farmacologia, [2]Unifesp – Biofísica

Introduction and Aims: The P1 and P2 purinergic receptors are expressed in cardiac tissue and are activated by Adenosine-5'-triphosphate (ATP) and Uridine-5'-triphosphate (UTP) released by sympathetic neurons, endothelial cells and cardiomyocytes (Burnstock , G.; 2008). ATP and UTP induced a negative inotropic effect (NIF) followed by a positive inotropic effect (PIE) in left atria (LA). The PIE exerted by these nucleosides is related to increase of cytosolic calcium concentration. Several studies reported that calcium handling by calcium channels, sarcoplasmatic reticulum and mitochondria is altered in hypertension. Therefore, we decided to evaluate the role of mitochondrial calcium in the PIE exerted by ATP and UTP in LA from normotensive (NWR) and hypertensive rats (SHR). **Materials and Methods:** LA from NWR and SHR male rats (4-6 months) were isolated and mounted in organ bath system and submitted to transmural electrical stimulation (2 Hz, 5 ms, 8-12 V). To study the influence of calcium from mitochondria on PIE induced by ATP (300 mM) and UTP (300 mM), we used CCCP 100 nM (mitochondria-depolarizing agent). The results were expressed by means±SEM and analyzed by one-way ANOVA. All experiment procedures were approved by UNIFESP ethic committee (n° 0193/12). **Results:** ATP (300 µM) produced an initial negative inotropic effect (NIE). However, the inotropism gradually increased, showing a positive inotropic effect (PIE). ATP produced a NIE with amplitude of 37.5 ± 2.0% in NWR and 29.5 ± 2.7% in SHR ($p<0.05$), in relation to the baseline value. Thereafter, atrial inotropism gradually increased, reaching the plateau at 180-200s, showing a PIE of 28.5 ± 2.0% in NWR and 37.5 ± 1.3% in SHR ($p<0.05$), in relation to the final value of the NIE. In the presence of CCCP, the PIE effect induced by ATP was reduced at about 43% (28.5 ± 2.0% to 16.2 ± 1.2%, $p <0.05$) in NWR and 60% (37.5 ± 1.3% to 22.8 ± 1.7%, $p <0.05$) in SHR. UTP produced an initial NIE with an amplitude of 15.4 ± 1.8% in NWR and 9.8 ± 1.0% in SHR ($p<0.05$) compared to baseline. Thus, atrial inotropism increased gradually, reaching the plateau at 150-180 min, with a value of 26.4± 0.7% in NWR and 36.5 ± 1.1% in SHR ($p<0.05$). In the presence of CCCP, PIE induced by UTP was reduced by 31% (26.4± 0.7% to 8.2 ± 0.7%, $p <0.05$) fot NWR and 53% (36.5 ± 1.1% to 19.3 ± 0.9%, $p <0.05$) for SHR. **Conclusion**: The results showed that PIE by ATP and UTP were inhibited after pre-treatment with CCCP, suggesting that mitochondria are involved in the PIE induced by ATP and UTP in LA of NWR and SHR. Furthermore, the PIE produced by ATP and UTP were increased in SHR and more susceptible to blocking with CCCP. **Sources of Research Support:** CAPES and CNPq.

06.022 **Opioid receptors and exercise-induced cardioprotection *in vivo*.** Borges J, Tibiriçá E, Lessa MA Fiocruz – Investigação Cardiovascular

Introduction: *In vitro* studies of the myocardial tolerance against ischemia-reperfusion (I-R) injury induced by exercise has suggested the involvement of the Opioid system. However, the link between exercise-induced cardioprotection and the opioid system remains an area where further clarification of the mechanisms involved is warranted. The present study aims to verify an *in vivo* model of exercise-induced cardioprotection against I/R injury in rats. **Methods:** The surgical procedures and protocols were approved by the Institutional Animal Care and Use Committee at the Oswaldo Cruz Foundation (CEUA - license number LW-4/11). Male Wister rats (250-300g) were first divided into 2 groups: trained and sedentary. The trained group underwent 4 consecutive days of treadmill training (60 min at 70% of maximal velocity obtained in a graded exercise test). In the sedentary group the rats were placed on a non-moving treadmill 60 min during 4 consecutive days. The trained rat group was then divided into 2 groups: an Exercise I-R group (Exe I-R; n = 7) and a Naloxone (a non-selective opioid receptor antagonist) + Exercise I-R group (Nal + Exe I-R; n = 7). Non-trained animal were also divided into 2 groups: Sedentary I-R (Sed I-R; n = 10) and Sedentary Sham I-R (S-Sed I-R; n = 9). To induce the I-R injury, anesthetized animals were submitted to a left thoracotomy and a 30 min interventricular coronary occlusion followed by 60 min of reperfusion. The hemodynamic parameters were recorded and the infarct size was determined by double staining using triphenyltetrazolium/Evans blue and expressed as a percentage of the area at risk (AAR). **Results:** The Sed I-R group had a 43.5% larger infarct area when compared to the Exe I-R group (38.6 ± 5.0 and 21.8 ± 4.5% of the AAR respectively, $p<0.05$). Naloxone pretreatment completely blocked the exercise-induced cardioprotection (37.6 ± 3.1% of the AAR). Hypotension elicited by the I-R injury was not seen in the Exe I-R group. The S-Sed I-R group showed no infarct. **Conclusions:** Our results indicate that opioid system is involved in cardioprotective effects of the aerobic exercise in the anesthetized rat. New protocols are ongoing to determine the role of the opioid receptors subtypes in this exercise-induced cardioprotection. **Financial support**: FAPERJ, CNPq, FIOCRUZ.

06.023 **Increased sympathetic tone may contribute to the cardiovascular dysfunction of sepsis**. Favero AM[1], Sordi R[1], Nardi GM[2], Assreuy J[1] [1]UFSC – Farmacologia, [2]UNOESC

Introduction: The cardiovascular dysfunction of sepsis/septic shock is characterized by hypotension, tachycardia/bradycardia and hyporesponsiveness to vasoconstrictors. The hypotension and low tissue perfusion triggers an increase in sympathetic tone in an attempt to restore blood pressure to normal levels. The persistently higher sympathetic stimulation may thus create a vicious circle contributing to the vascular hyporesponsiveness to adrenergic stimulation. Therefore, the aim of this work was to evaluate the vascular response to adrenergic agonists during severe sepsis and the effects of an early inhibition of the sympathetic tone in sepsis-induced cardiovascular dysfunction. **Methods:** Sepsis was induced by cecal ligation and puncture (CLP) surgery in female Wistar rats. Six hours after CLP procedure, isoprenaline (a non selective beta-adrenergic agonist), terbutaline (a selective beta-2 agonist) and norepinephrine (a preferential alpha-1 agonist) were administered in anaesthetized rats to evaluate the vascular response and heart rate to agonists. In other groups, animals were treated with hexamethonium (HEX, 18,3µmol/kg, s.c.) one hour after sepsis onset. Again, vascular response to norepinephrine, isoprenaline and terbutaline were evaluated 6 h after CLP surgery. Naïve (CT) animals were used as controls. All procedures were approved by our Institutional Ethics Committee (PP00631/CEUA-UFSC) and are in accordance with NIH Animal Care Guidelines. **Results:** Six hours after CLP surgery, sepsis resulted in hypotension (CT 93.1 ± 2.9; CLP 57.6 ± 5.9 mmHg) and increased heart rate (CT 208.1 ± 8.9; CLP 257.2 ± 13.6 bpm). There was a significant reduction in the response to isoprenaline (1nmol/kg, CT -30.4 ± 2.6; CLP -12.2 ± 4.9 mmHg; $p < 0.05$), terbutaline (10nmol/kg, CT -30.5 ± 0.9; CLP -2.32 ± 1.1 mmHg; $p < 0.05$) and norepinephrine (10nmol/kg, CT 50.3; CLP 30.6 ± 7.8 mmHg; $p < 0.05$). Hexamethonium treatment significantly improved heart rate of septic animals (CT 218 ± 10.8; CLP 294.4 ± 4.1; CLP + HEX 254.8 ± 15.8 bpm; $p < 0.05$). It also improved the responses to norepinephrine (CT 50.3; CLP 30.6 ± 7.8; CLP + HEX 52.5 ± 4.0 mmHg; $p < 0.05$), but did not affect significantly the responses to isoprenaline (CT -33.4 ± 4.9; CLP -5.73 ± 2.11; CLP + HEX -12.5 ± 4.3 mmHg) or terbutaline (CT -30.5 ± 0.9; CLP -2.3 ± 1.1; CLP + HEX -5.1 ± 0.9 mmHg). **Discussion:** Our results demonstrate that is sepsis there is a loss of alpha- and beta-adrenergic responses. Our data indicates that the higher sympathetic tone present in sepsis may contribute to the hyporesponsiveness to alpha-adrenergic agonists. However, since ganglionic blockage improved only norepinephrine but not isoprenaline or terbutaline responses in septic animals, it appears that the mechanisms causing alpha and beta-adrenergic hyporesponsiveness in sepsis are different. **Financial Support**: CAPES, CNPq, FAPESC, FINEP.

06.024 Kinin B1 receptor modulates L-arginine uptake and nitric oxide generation in endothelial cells. Torres TC, Tudela RC, Loiola RA, Freitas JAM, Assunção NA, Pesquero JB, Fernandes L Unifesp

Introduction and Objective: B_1 receptor knockout mice ($B_1^{-/-}$) present impaired vascular response to endothelial-dependent agents, and reduced nitric oxide (NO) levels [Loiola *et al*, Peptides (32):1700, 2011]. These alterations are not elucidated at the endothelial cell level. The present study investigated the NO production in cultured endothelial cells from $B_1^{-/-}$, analyzing in parallel the L-arginine (L-arg) uptake and the cationic amino acid transporter CAT-1 expression. **Methods:** Endothelial cells obtained from lung explants of $B_1^{-/-}$ and Wild Type (WT) mice were sub-cultured over 100 passages and immortalized. NO production was determined in cells (n = 3-4) pre-incubated (30 min) with a NO fluorescent dye (DAF-2 DA, 10 µmol/L) and stimulated with Acethylcoline [ACh (1 mmol/L)] in the presence or absence of L-arg (1 mmol/L). Fluorescence was detected during 300 seconds after stimulation and analyzed by optic densitometry. For L-arg uptake assays, culture media were analyzed 15 hours after cell incubation (n = 4), in the presence or absence of the CAT-1 blocker N-ethylmaleimide [NEM (1mmol/L)] using reverse-phase HPLC (340 nm) under a gradient of solutions: A, 90% Acetate buffer (10 mmol/L, pH 5), 9.5% Methanol and 0.5% tetrahydrofuran; B, 100% Methanol. L-arg was derivatizated (OPA); retention time was used to identify peaks of interest and L-arg decay was deducted from its initial concentration in the stock media and normalized by total protein. CAT-1 expression was determined by immunocytochemistry; cells were seeded on coverslips (n = 3) and incubated overnight (4°C) with primary polyclonal rabbit antibody anti CAT-1 [1:50], washed in PBS and incubated with the secondary bovine antibody anti-rabbit IgG – Texas Red conjugated [1:100] for 2 hours (37°C). Coverslips were observed in a fluorescence microscope and images (20 fields / assay) were analyzed by optic densitometry. Ethics Committee of the UNIFESP: protocol number 1913/11. **Results**: In both WT and $B_1^{-/-}$ cells, ACh produced a gradual and consistent increase in fluorescence. Cellular NO release was lower in $B_1^{-/-}$ during the 300 seconds of observation. In all experiments, maximal responses were observed 270 seconds after ACh; for this reason, this point of the time curve was chosen to be compared [arbitrary units (a.u.)]: 66.9 ± 3.2 for WT *vs* 35.8 ± 3.1* for $B_1^{-/-}$. L-arg supplementation had no effect in both WT (51.1 ± 11.9) and $B_1^{-/-}$ (26.2 ± 8.5*). HPLC method was successfully developed; detection and quantification limits were estimated at 10 and 30 µmol, respectively. L-arg uptake (µg/mg protein) was markedly decreased in $B_1^{-/-}$ (83.8 ± 2.3*) *vs* WT (114.7 ± 5.5). CAT-1 blockade induced by NEM decreased L-arg uptake in WT (51.1 ± 6.3*), but not in $B_1^{-/-}$ (104.5 ± 5.7). Positive staining for CAT-1 was detected in both groups; protein expression (a.u.) was significantly higher in $B_1^{-/-}$ (23.9 ± 1.3*) *vs* WT (12.0 ± 1.4) *P<.05. **Conclusions**: Endothelial B_1 receptors interfere with the L-arg transport, probably by regulating CAT-1 functioning. Higher levels of CAT-1 detected in $B_1^{-/-}$ cells are not able to reverse the impaired L-arg uptake and NO release. These results can explain the endothelial dysfunction observed in $B_1^{-/-}$ vessels and reveal an important role of B_1 receptors on the vascular physiology related to NO. **Support**: CAPES, FAPESP 07/59039-2, 11/18129-4

06.025 **A new nitric oxide (NO) donor induces similar vasodilatation in aorta from normotensive and renal hypertensive rats**. Araújo LMPC, Silva RS, Bendhack LM FCFR-USP – Physics and Chemistry

Introduction: Nitric oxide (NO) is the main endogenous vasodilator that regulates vascular tone. There is a great interest in the development of compounds that may serve as vehicle to NO release in biological systems, mainly when the endogenous NO production is impaired, such as in hypertension. The nitrosyl ruthenium is a new class of NO donors. RuBPY is a new nitrosyl ruthenium complex synthesized in our laboratory that releases NO in the presence of the vascular tissue only. Thus, the aim of present work was to investigate the effects of NO donors RuBPY in thoracic aorta from normotensive (2K) and renal hypertensive (2K-1C). **Methods:** The RuBPY complex was synthesized as previously reported and characterized by UV-Visible spectroscopy as well as cyclic voltammetry experiments. Male Wistar rats (180–200 g) were used in the present study. Renovascular hypertension (2K-1C) was produced by placing a silver clip on the left renal artery while rats were under anaesthesia (tribromoethanol 2.5%, 250mg/kg). Control rats (2K) were subjected only to laparotomy. Rats were considered to be hypertensive when systolic arterial pressure was higher than 160 mmHg at 6 weeks after surgery. Following arterial pressure recordings, rats were killed by decapitation. The thoracic aorta was isolated, cut into rings and mounted between two steel hooks to measure the isometric tension. The rings were placed in organ chambers containing Krebs' solution maintained at pH 7.4 and gassed with 95% O_2 and 5% CO_2 at 37 ºC. The maximal relaxation (E_{max}), potency (pEC_{50}) and time course of RuBPY were analyzed. To avoid interference of endogenous NO, the experiments were performed on endothelium-denuded. Aortic rings were pre-contracted with EC_{50} of phenylephrine (100 nmol/L). When the contractions reached a plateau, the RuBPY, prepared in pH 7.4 phosphate buffer (1 mmol/L), were added cumulatively (0.1 nmol – 10 mmol/L) to the organ bath. The time-course for the relation induced by RuBPY was evaluated when RuBPY (10 mmol/L) was added to the organ chamber after a stable contraction in response to EC_{50} of phenylephrine. Statistical significance was determined by using the Student's *t* test. In all cases, probability levels of less than 0.05 ($P<0.05$) were taken to indicate statistical significance. All pharmacological studies were performed in accordance with the Ethical Animal Committee of the University of São Paulo (11.1.1050.53.5). **Results and Discussion:** Vascular reactivity experiments showed that the RuBPY induced a relation in denuded aortic rings, and the E_{max} and potency triggered by RuBPY was similar from 2K (E_{max} = 112.7 ± 34.6%, pEC_{50} = 7.37 **+**0.11, n = 8) and 2K-1C rats (E_{max} = 114.6 ± 6.3%, pEC_{50} = 7.19 **+**0.12, n = 5). The time to reach maximum relaxation was similar to RuBPY in 2K and 2K-1C (4 min, n = 5). Taken together, our results demonstrated that RuBPY is a potential vasodilator with effect above 100% and this effect is similar in isolated aortic rings from 2K and 2K-1C rats. Supported by FAPESP and CNPq.

06.026 **Long-lasting effect of nitric oxide on platelet aggregation**. Gonçalves MC, Assreuy J UFSC – Farmacologia

Introduction: Non-physiological platelet aggregation is present in many physiopathological syndromes as atherosclerosis, sepsis, and diabetes. Physiological inhibitors of platelet aggregation maintain the homeastasis to avoid indiscriminate intravascular aggregation. One of these inhibitors is nitric oxide (NO) that acts through soluble guanylate cyclase enzyme (sGC) and seems to be one of the most important mechanisms. Despite of well described acute inhibition of platelet aggregation effect of NO, its long-term action is unknown. Thus, in this study we aimed to study the profile of NO donors on platelet aggregation. **Methods**: The experiments were done by using a microplate reader. Inducers of platelet aggregation were ADP (5 μM) and collagen (4 μg/mL) in platelet rich plasma (PRP; 2 x 10^8 platelets per well) from male rats (300-350g). We used GTN (glyceryltrinitrate) and GSNO (S-nitrosoglutathione) as NO donors and ODQ (1H-[1,2,4]oxadiazolo[4,3,-a]quinoxalin-1-one) as a sGC inhibitor. The basic protocol was the incubation of NO donor with PRP and then stimulation of platelet aggregation. The other protocol was incubation with GTN (1 mM), centrifugation of PRP, removal of plasma, ressuspension of platelets in autologous fresh plasma and aggregation assay. All the procedures have been approved by our institutional Animal Ethics Committee (PP00595/CEUA/UFSC). **Results:** GTN inhibited platelet aggregation [300 and 1000 μM] after 15 and 60 minutes incubationat 37°C. GSNO on the other hand, inhibited pletelet aggregation in all concentrations tested [30, 100, 300 and 1000 μM]. ODQ [100 μM] prevented the platelet inhibitory effect caused by GTN. Platelets incubated with NO donor and ressuspended in fresh plasma still presented ~50% of aggregation. Even when tested 2 hours after NO donor removal, at least 50% of the inhibitory effect were present yet. **Discussion**: The long-lasting anti-aggregating effect of NO may be explained by S-nitrosylation of proteins present in platelets. The present findings suggest that this prolonged anti-aggregating effects of NO may be an extra benefit of organic nitrates used in patients with cardiovascular disorders. **Financial support:** CNPq, CAPES, FAPESC and FINEP. **Keywords**: Nitric oxide, platelet aggregation, gliceryl trinitrate.

06.027 **Effects of the adipokine chemerin on the vascular reactivity: analysis in the rat aorta.** Neves KB[1], Lobato NS[2], Lopes RAM[3], Zanotto CZ[3], Filgueira FP[2], Tostes RC[3], Oliveira AM[1] [1]FCFRP-USP, [2]UFG, [3]FMRP-USP

Adipose tissue is a critical regulator of vascular function, which until recently had been virtually ignored. Almost all blood vessels are surrounded by perivascular adipose tissue, which is actively involved in the maintenance of vascular homeostasis by producing "vasocrine" signals such as adipokines. The effects of the novel adipokine chemerin on the vascular function as well as the mechanisms by which chemerin may interfere with the vascular function are not fully understood. To address the effects of chemerin in vascular reactivity, isometric contraction was recorded in endothelium-intact and endothelium-denuded rat thoracic aortic rings incubated with chemerin (0.5ng/mL or 5ng/mL; 1 hour) or vehicle (PBS 0,1% BSA) and responses to endothelin-1 (ET-1), phenylephrine (PhE), acetylcholine (ACh) and sodium nitroprusside (SNP) were determined. The adipokine chemerin increased the contractile responses to ET-1 (pD_2: vehicle = 9,4±0,1; chemerin 0,5 ng/mL = 10,3±0,1; chemerin 5ng/mL = 10,2±0,01) and PhE (pD_2: vehicle = 6,5±0,1; chemerin 0,5ng/mL = 7,1±0,05; chemerin 5ng/mL = 7,2±0,1). The ERK1/2 inhibitor PD98059 (1 μM) inhibited the effects of chemerin (pD_2 PD98059 = 9,2±0,03; chemerin 0,5ng/mL+PD98059 = 8,90±0,1; chemerin 5ng/mL+PD98059 = 9,3±0,04). Chemerin also increased the phosphorylation of MEK1/2 [arbitrary units (a.u.): vehicle = 0,8±0,1; chemerin = 1,4±0,1; ET-1 = 1,4±0,05; chemerin+ET-1 = 2,1±0,1) and ERK1/2 (a.u.: vehicle = 0,8±0,03; chemerin = 1,2±0,02; ET-1 = 1,3±0,05; chemerin+ET-1 = 2,1±0,1), and protein expression of ETA [a.u.: vehicle = 1±0,01; chemerin = 1,1±0,01] and ETB [a.u.: vehicle = 1±0,01; chemerin = 1,2±0,04] receptors. Chemerin effects on PhE-induced vasoconstriction was reverted by ETA [pD_2; vehicle = 6,9±0,1; chemerin = 8,0±0,1; BQ123 = 6,8±0,05; BQ123+chemerin = 6,9±0,1] and ETB [pD_2: vehicle = 7,9±0,1; chemerin = 8,4±0,1; BQ788 = 8,1±0,05; BQ788+chemerin = 7,9±0,1] antagonists. Chemerin decreased vasodilatation to ACh [Emax: vehicle = 93,06±11,05; chemerin 0,5ng/mL = 66,1±3,1; chemerin 5ng/mL = 63,8±12,6] and SNP [pD_2:vehicle = 8,6±0,1; chemerin 0,5ng/mL = 7,4±0,1; chemerin 5ng/mL = 6,9±0,1]. Chemerin also induced eNOS uncoupling, since the eNOS dimer-monomer ratio was reduced in vessels incubated with this adipokine (a.u.: vehicle = 1±0,01; chemerin = 0,5±0,1). The eNOS cofactor tetrahydrobiopterin (BH_4) and the superoxide anion (O_2^-) scavenger Tiron reverted chemerin-induced decreased ACh vasodilation (Emax: vehicle = 103,3±2,5; chemerin = 67,8±5,2; BH_4 = 92,3±1,3; BH_4+chemerin = 92,6±3,3; Tiron = 95,6±0,9; Tiron+chemerin = 92,8±2,9), suggesting that oxidative stress contributes to the effects of chemerin. Our studies may contribute to a better understanding of the role of factors released by the visceral adipose tissue on vascular function and, consequently, on the vascular dysfunction in obesity and obesity-associated diseases. **Financial Support:** FAPESP. **Protocol of the Animal Use Ethic:** 10.1.1295.53.7.

06.028 **Functional characterization of the relaxation induced by the soluble guanylate cyclase activator**, **BAY 60-2770 in isolated pulmonary artery from rabbit**. Faria W, Caipisco JA, Antunes E, de Nucci G, Mónica FZ Unicamp – Farmacologia

Introduction: The nitric oxide (NO)-independent class of drugs, soluble guanylate cyclase (sGC) stimulators (BAY 41-2272, BAY 41-8543 and BAY 63-2521) and activators have been used to distinguish between reduced heme-containing and heme-free sGC. BAY 60-2770, BAY 58-2667, HMR 1766 increases the catalytic activity of the enzyme in a NO- and heme-independent manner, which is further increased by the addition of the sGC inhibitor, ODQ (Stasch et al., 2002, Knorr, 2008). The aim of the present study was to characterize functionally the relaxation induced by BAY 60-2770 in isolated pulmonary artery from rabbit. **Methods**: The experimental protocols were approved by the Animal Ethical Committee of UNICAMP (2720-1). The rabbit was exsanguinated by the abdominal aorta and the pulmonary artery was isolated and placed in Krebs solution, continuously aerated with carbogen (95% O_2:5% CO_2) at 37^{o}C. Concentration response curve to the soluble guanylate cyclase (sGC) activator (BAY 60-2770, 0.001-30 nM) were obtained in tissues pre-contracted with phenylephrine (1 μM) in the absence (control) and in the presence the sGC inhibitor (ODQ, 10 μM), the nitric oxide synthase inhibitor (L-NAME, 100 μM), the phosphodiesterase type 5 inhibitor (tafalafil, 100 nM). In some set of experiments the endothelium was removed and the relaxation of BAY 60-2770 was also carried out. The integrity of the endothelium was assessed by addition of acetylcholine (1 μM). The potency (pEC_{50}) and maximal response (E_{max}) values were evaluated. **Results:** In endothelium-intact (E^+) pulmonary artery rings, addition of the heme-independent sGC activator BAY 60-2770 concentration-dependently relaxed isolated pulmonary artery with a pEC_{50} and E_{max} values of 9.83 ± 0.08 and 105 ± 0.6%, respectively. Incubation with ODQ or tadalafil produced leftward shifts in the BAY 60-2770-induced relaxations (8.9- and 13.4-fold, respectively, $P<0.05$) without changing the E_{max} values (104 ± 2 and 105 ± 1.5%, respectively). Surprisingly, the addition of L-NAME (100 μM) also potentiated the relaxation induced by BAY 60-2770 (3.7 fold, $P<0.05$), whereas in endothelium-denuded rings the pEC_{50} (9.81 ± 0.07) and E_{max} (113 ± 3%, $P>0.05$) values of BAY 60-2770 did not differ in comparison with E^+ rings. **Discussion:** Our results show that the relaxation induced by BAY 60-2770 was significantly potentiated by sGC or PDE5 inhibition, showing that the oxidation of sGC or the increase levels of sGMP favor this response. Moreover, the NOS inhibition by L-NAME increased the potency of BAY 60-2770, suggesting to authors that the vasodilator response to BAY 60-2770 are not dependent of the presence of endogenous NO. One may speculate that the absence of NO would favor the binding and thus activation of sGC by BAY 60-2770. **References:** *Stasch* et al. BJP, 136, 773, 2002; Knorr et al., Liver Therapeutics, 58, 71, 2008. **Financial Support: CNPq**

06.029 Involvement of RHO-A/RHO-KINASE pathway in the renal vascular hyperreactivity to vasopressin in endotoxemic shock. Guarido KL, da Silva-Santos JE UFSC – Farmacologia

Introduction: The maintenance of renal vascular tone seems to be involved in sepsis-mediated acute renal failure. This study aimed to evaluate the responses to vasopressin (AVP) and the functionality of Rho-kinase (ROCK) in the renal vascular bed of rats treated with lipopolysaccharide (LPS). **Methods:** All procedures were approved by the Institutional Ethics Committee from UFSC (protocol 463). Male Wistar rats (230-280g) received either saline (1 ml/kg, i.p.) or LPS (10 mg/kg, i.p.), and 6 or 24 h after these treatments it were anesthetized with ketamine/xylazine (100/20 mg/kg) and had their left kidney cannulated, removed and attached to a perfusion system under constant flow (4 ml/min) of physiologic salt solution (PSS), kept at 37 °C and constantly bubbled with O_2 (95%). Changes in the renal perfusion pressure (RPP) were recorded by a digital polygraph. The kidneys were perfused with PPS containing either phenylephrine (PE, 1 μM) or AVP (3 nM), or dose-response curves to AVP (3, 10 and 30 pmol) were performed before and after perfusion with PSS containing Y-27632 (30 μM), a ROCK inhibitor. The effect of AVP was also evaluated in isolated aortic rings. In another set of experiments, the mean arterial pressure (MAP) and renal blood flow (RBF) from anesthetized rats were measured. The effects of AVP (3, 10 and 30 pmol/kg, i.v.) on MAP and RBF were analyzed before and after administration of Y-27632 (0,1 mg/kg, i.v.). The expression of Rho-A, ROCK and both total and phosphorylated myosin phosphatase (MYPT-1 subunit, a main target for ROCK) were measured in the medulla of kidneys from rats. **Results:** The continuous perfusion or acute administration of AVP promoted enhanced effects on RPP of kidneys of endotoxemic rats. For instance, RPP increased by 27.5 ± 4.7, 95 ± 12.8 and 150.6 ± 11.4 mm Hg after administration of 10 pmol of AVP, in control, LPS 6h and LPS 24h groups, respectively. Nevertheless, aortic rings from LPS groups displayed significant hyporeactivity to AVP. The effects of AVP on RPP of kidneys continuously perfused with Y-27632 remained unchanged in preparations obtained from control and LPS 24h groups, but it was reduced by 20% in kidneys from LPS 6h group. Systemic administration of AVP (10 and 30 pmol/kg) increased the MAP by 29.5 ± 3.9 and 51.2 ± 5.5 mm Hg in control and by 51.5 ± 6.5 and 69.7 ± 4.2 mm Hg in LPS 24h group, respectively. AVP also produced enhanced ($p < 0.05$) reduction in the RBF in LPS 6h and LPS 24h groups, as measured by laser Doppler. Administration of Y-27632 decreased the reduction of RBF elicited by all tested doses of AVP in control rats, but did not change the enhanced effect of the highest dose of AVP in both LPS 6 and LPS 24h. Although homogenates of kidney medulla have presented unchanged expression levels of RhoA, ROCK I and II and total MYPT-1, increased levels of phospho-MYPT-1 were found in the kidney medulla of LPS 6h group. **Discussion:** This study shows that the vasoconstriction induced by AVP in the renal vascular bed, but not in aortic rings, is increased in both stages of endotoxic shock in rats. Taken together, our results suggest that the enhanced effects of AVP seen in both *in vitro* and *in vivo* experiments involve an increased activity of components of the Rho-A/Rho-kinase pathway. **Sources of research support:** FAPESC; CNPq

06.030 **Cyclic nucleotide modulators reduce vasoconstrictor, oxidative and inflammatory profile in Wistar rats fed hypercholesterolemic diet**. Motta NAV[1], Fumian MM[1], Castro J[1], Miranda ALP[2], Kümmerle AE[3], Barreiro EJ[2], Brito FCF[1] [1]UFF – Farmacologia Experimental, [2]UFRJ – Avaliação e Síntese de Substâncias Bioativas, [3]UFRRJ – Química

Introduction: An important role has been demonstrated to phosphodiesterases (PDEs) activities in various cardiovascular disease models. Since PDEs are associated with many physiological functions, several PDEs inhibitors have been studied in cardiac or vascular-related diseases. Thus, this study aims to investigate the pharmacological properties of cyclic nucleotides modulators, such as cilostazol and thienylacylhydrazone LASSBio-788 derivative in hypercholesterolaemic rats. Studies have demonstrated that cilostazol and LASSBio-788 reduces inflammatory markers, platelet aggregation and reactive oxygen species production (Lee JH. J.Pharmacol. Exp.Ther. 313: 502, 2005; Brito, FCF. Eur.J. Pharmacol. 638:5, 2010). We have hypothesized that cilostazol and LASSBio-788 may exert beneficial effects on atherosclerosis. This work describes their anti-inflammatory, antiplatelet and vasodilatory properties in hypercholesterolaemic rats. **Methods:** The use of animals was according to Ethics Committee (CEPA/UFF00116/09). Male Wistar rats (150-200g) were randomly divided into 4 groups: C (control group) has received normal rat chow for 45 days. HCD (hypercholesterolaemic diet group), HCD+788 (compound LASSBio-788 group) and HCD+CIL (cilostazol group) have received hypercholesterolemic diet (HCD) for 45 days. To C and HCD groups it was administered vehicle (tween 80: ethanol: H2O, 0.05 ml/Kg, *p.o.* or *i.p.*). To HCD+788 group it was administered LASSBio-788 (100 µmol/Kg *p.o.)* and to HCD+CIL group it was administered cilostazol (30mg/Kg *i.p*.). The animals received its treatment once daily for 15 days. The animals were euthanized under anesthesia. Blood samples were collected and thoracic aortas were excised. Platelet aggregation, aorta isolated studies, cytokine and malondialdehyde levels were evaluated, as well as nitric oxide synthase expression. Data were analyzed using one way analysis of variance (ANOVA) with the Bonferroni´s test ($p < 0.05$). **Results**: Cilostazol and LASSBio-788 reduced the malondialdehyde production (HCD+CIL: 3.3±0.5; HCD+788:4.8±0.3; HCD: 9.4±0.5 nmol/ml). They also reduced the potency of platelet agonists, such as adenosine diphosphate (CE_{50}: HCD+CIL: 0.3±0.02; HCD+788:1,0±0,1; HCD:0.06±0.02 µM) and collagen (CE_{50}: HCD+CIL:0.6±0.02; HCD+788:4.2±0.8; HCD:0.1±0.02 µg/ml). Cilostazol and LASSBio-788 promoted a decrease in contractile response to phenylephrine (CE_{50}: HCD+CIL:$1.2x10^{-6}$; HCD+788:$3.1x10^{-7}$; HCD:$7.4x10^{-8}$ M) as well as they have promoted an improvement in endothelium-dependent vasorelaxant response (CE_{50}: HCD+CIL:8.6x10-8; HCD+788:$3.2x10^{-8}$; HCD: $1.3x10^{-6}$ M). In western blot assay, both cyclic nucleotide modulators increased the expression of nitric oxide synthase when compared with HCD group. They also decreased inflammatory markers serum levels. **Discussion:** Our results suggest that cilostazol and LASSBio-788, presented an antiatherogenic effect *in vivo*, exerting antiplatelet, anti-inflammatory, vasodilatory, antioxidant and lipid lowering properties. These results contribute to elucidate the role of new multi-targeted drugs candidates for the treatment of atherosclerosis. **Sources of research support:** CAPES, FAPERJ, PROPPi/UFF

06.031 Controlled delivery of vascular endothelial growth factor from polymeric microparticles induces tissue revascularization and positive heart remodeling in a rat myocardial infarction model. Formiga FR[1,2], Pelacho B[3], Gavira JJ[3], Abizanda G[3], Prósper F[3], Blanco-Prieto MJ[1] [1]University of Navarra – Pharmacy and Pharmaceutical Technology [2]UPE – Biotecnologia, [3]University of Navarra – Hematology, Cardiology and Cell Therapy

Introduction: Myocardial infarction (MI) is a major health concern worldwide. Vascular Endothelial Growth Factor (VEGF) has been identified as factor involved in cardiac repair after MI. However, its therapeutic value has important limitations *in vivo,* related to its short-lived effect and high instability after systemic delivery (reviewed in [1]). **Methods:** We evaluated the therapeutic potential of VEGF encapsulated into Poly (lactic-*co*-glycolic acid) (PLGA) microparticles (MP) to repair the myocardium after a MI. First, we developed PLGA-MP by Total Recirculation One-Machine System (TROMS) [2]. MP was characterized in terms of VEGF loading and *in vitro* release of VEGF by ELISA and Western blot assays. Also, the bioactivity of the VEGF released from the MP was evaluated by determining the proliferative capacity of an endothelial cell line (HIAEC). Next, we compared the effect of delivery of VEGF-MP with free VEGF or control non-loaded microparticles (NL-MP) in a rat model of ischemia-reperfusion. A histological and morphometric study was conducted. **Results and Discussion:** MP with a diameter of 5 μm was found to be compatible for intramyocardial administration. PLGA-MP released bioactive VEGF in a sustained manner for up to 28 days *in vitro*. An increase in vascularization was observed in animals treated with VEGF-MP, but not in the NL-MP or free-VEGF groups at 30 days follow-up (NL-MP: 579.5 ± 33.8; VEGF-MP: 704.9 ± 31.7, $P<0.05$; Free-VEGF: 571.6 ± 37.3, P = NS, capillaries/mm^2). Correlating with this data, a positive remodeling of the heart was detected in the VEGF-MP group with a significantly greater left ventricle wall thickness (NL-MP: 1.07 ± 0.02 mm; VEGF-MP: 1.30 ± 0.05 mm, $P<0.01$; Free-VEGF: 1.07 ± 0.10 mm, P = NS). Of note, a tendency toward lower tissue fibrosis was identified in the VEGF-MP (NL-MP: 32 ± 2.6%; VEGF-MP: 25.5 ± 2.3, P = 0.08; Free-VEGF: 27.3 ± 8.4%, P = NS). Importantly, no hemangioma formation or leaking vessels were detected in the VEGF-MP group in the analysis of hematoxylin-eosin stained sections and confocal 3D analysis of caveolin-1$^+$ stained vessels (where non-leaking vessels were detected). In summary, we have demonstrated that VEGF could induce neovascularization when delivered *in vivo* in a sustained manner, which translates into positive remodeling of the heart. **References:** [1] F.R. Formiga *et al.* Heart Fail. Rev. 17:449 (2012) [2] F.R. Formiga *et al*. J. Control. Release 147:30 (2010) **Financial Support and Acknowledgments:** European Union Framework Project VII (INELPY), Ministerio de Ciencia e Innovación de España, Agencia Española de Cooperación Internacional para el Desarrollo (AECID), Instituto de Salud Carlos III, Comunidad de Trabajo de los Pirineos (CTP). All animal procedures were approved by the University of Navarra Institutional Committee on Care and Use of Laboratory Animals as well as the European Community Council Directive Ref. 86/609/EEC.

06.032 High salt intake increases the activity of the RhoA/RHO-kinase pathway in rat aorta and small mesenteric arteries. Crestani S[1], Marques MCA[1], Webb RC[2], Da Silva-Santos JE[3] [1]UFPR – Farmacologia, [2]GHSU – Physiology, [3]UFSC – Farmacologia

Introduction: The Rho-kinase (ROCK), activated by Rho-A, inhibits myosin phosphatase contributing to vascular contraction (Hilgers and Webb 2005). High-salt intake is putatively associated with hypertension and cardiovascular diseases (Orlov and Mongin 2007). The Rho-A/ROCK pathway has been associated with hypertension (Wirth 2010), but its role in vascular changes induced by sustained high-salt exposition was scarcely investigated. This study aimed to evaluate the functionality of ROCK in rat aortic and mesenteric rings isolated from rats exposed to high amounts of salt. **Methods:** Male Wistar rats (21 days old) were subjected to food containing NaCl at 2, 4, or 8% (the control received regular food). After 6 weeks the rats were killed and had their aorta and mesenteric artery mounted *in vitro* to evaluate its functionality. Concentration response curves to acetylcholine (ACh) or Y-27632 (both 1 nM-10 μM) were obtained in vessels contracted by phenylephrine (PE). The vessels were also exposed to PE (1 nM-100 μM), either in the presence or absence of Y-27632 (incubated for 15 min). Protein expression of RhoA, RhoE, ROCK I and II, MYPT1, pMYPT1, MLC and pMLC were investigated in aorta from 4% NaCl group. These procedures were approved by the Institutional Ethics Committee of UFPR (number 345) and by the Institutional Animal Care and Use Committee of Georgia Health Sciences University (numbers 2009-0226; 2009-0227, and 2011-0353). **Results**: The high-salt intake for 6 weeks did not result in any change in the maximal contraction (MC) induced by KCl and PE in rat aortic or mesenteric rings. In addition, the relaxation induced by ACh (a muscarinic agonist) and Y-27632 (a selective ROCK inhibitor) did not differ between the groups. Incubation of Y-27632 (1 μM) reduced the MC elicited by PE from 94.7 ± 3.3 to 39.4 ± 12.6% (compared MC induced by KCl) in control aortic rings, but had no effect in vessels obtained from high-salt groups (e.g. in NaCl 4% group, the MC to PE was 77.2 ± 9.2 and 77.2 ± 16.1%, before and after Y-27632 incubation, respectively). Similarly, MC induced by PE in presence of Y-27632 (3 μM) was inhibited by 45.8% in small mesenteric rings from control, but not in 4% NaCl group. The total protein levels of RhoA, RhoE, ROCKI and II expressed in the aorta of animals exposed to the high-salt diet were not altered. However, the translocation of RhoA to the membrane increased by 53% in aorta from 4% NaCl group when compared to control, an event that was augmented by ~200% in PE-stimulated aorta. In addition, the expression levels of pMYPT1 and MLC were increased by 217% and 118%, respectively, in samples of aorta obtained from 4% NaCl group, without changes in MYPT1 and pMLC. **Discussion**: These data reveal that long-term exposition to high-salt amounts increased Rho-A/ROCK activity in both conductance and resistance vessels of normotensive rats, disclosing a potential involvement of this pathway in the deleterious effects elicited by excessive ingestion of sodium on the cardiovascular system. **Financial support:** CAPES and CNPq (482214/2007-4). **References:** Hilgers, R. H. and R. C. Webb (2005). "Molecular aspects of arterial smooth muscle contraction: focus on Rho." *Exp Biol Med* (Maywood)230(11): 829-835. Orlov, S. N. and A. A. Mongin (2007). "Salt-sensing mechanisms in blood pressure regulation and hypertension." *Am J Physiol Heart Circ* Physiol293(4): H2039-2053. Wirth, A. (2010). "Rho kinase and hypertension." *Biochim Biophys* Acta1802(12): 1276-1284.

06.033 **Chronic captopril treatment significantly attenuates erectile dysfunction in doca-salt hypertensive rats**. Neves NCV[1], Mendes HO[2], Damasceno EC[1], Felipe-Batista K[1], Guimarães HN[3], Rodovalho GV[2], Grabe-Guimarães A[1], Santos RAS[3], Leite R[1] [1]UFOP – Ciências Farmacêuticas, [2]UFOP, [3]UFMG

Introduction: Hypertension affects about 25% of the population and the structural and functional vascular damages are implicated with the development of male erectile dysfunction (ED). The purpose of this study was to evaluate the beneficial effects of increasing levels of circulating Angiotensin-(1-7) [Ang-(1-7)] usually observed in chronic oral treatment with angiotensin converting enzyme (ACE) inhibitors, such as captopril, on the ED commonly reported in DOCA-salt hypertensive rats. **Methods and Results:** Male Wistar rats (200-220 g) were uninephrectomized and implanted with a subcutaneous silicone pellet containing DOCA (200 mg/kg) under ketamine/xylasine (100/14 mg/100g) anesthesia (Protocol nº 2010/53). Animals were maintained on drinking water containing 1.0% NaCl and 0.2% KCl for 4 weeks. Uninephrectomized rats were used as controls and had free access to drinking water. Systolic blood pressure was measured by standard tail-cuff procedures. The rats received through drinking water daily treatment with captopril (25 mg/Kg) for 4 weeks. To evaluate erectile function, the animals were anesthetized by ketamine/xylazine, had the major pelvic ganglion isolated and electrically stimulated. Intracavernosal pressure (ICP) and mean arterial pressure (MAP) were measured and presented as an index of erection (ICP/MAP). Frequency-response curves (1-12 Hz, 4V, 5ms pulse and 30 seconds for each frequency) were obtained from control (n = 9) and DOCA-salt (n = 7) rats. Erectile function was severely reduced in hypertensive when compared to normotensive rats. Captopril treatment did not affect the ganglionic induced erectile response in control rats, but significantly improved ($p<0.05$, ANOVA and Bonferroni post test), almost to the normal level, the impaired responses observed in DOCA-salt hypertensive rats. **Discussion:** Our data indicate that chronic oral treatment with ACE inhibitors such as captopril, that has been known to increase the circulating levels of Ang-(1-7) improve erectile function in DOCA-salt hypertensive rats. We speculate that the high levels of Ang-(1-7) could be, at least in part, responsible for the improvement of the erectile response in hypertensive rats. These results suggest that strategies that increase Ang-(1-7) can be clinically useful to improve ED in hypertensive man. **Financial support:** FAPEMIG, CNPq, CAPES and UFOP.

06.034 **Enhanced aorta reactivity after sepsis**: **Involvement of RHO kinase pathway**, **calcium sensitization and oxidative stress**. de Souza P[1], da Silva LM[1], Marques MCA[1], da Silva-Santos JE[2]
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Introduction: Sepsis is an emergent public health problem caused by an overwhelming immune response to infection. Due the complexity of the systemic changes that occur in sepsis, only about 30 to 50% of people diagnosed survive, recover and are discharged. Epidemiological studies reveal a high mortality rate among patients that are discharged after an episode of sepsis, when compared to age-matched people. To investigate if changes in the functionality of cardiovascular system contribute to this high mortality rate, we assessed the responses of aortic rings from 3 groups: control and sepsis induced by cecal ligation and puncture, 30 (S30), and 60 (S60) days after recovery. **Methods:** Male *Wistar* rats were anesthetized with ketamine/xylazine (100/20 mg/kg, ip). After antisepsis of the abdominal area, a midline incision was performed. Then, the cecum was exposed and ligated (comprising 75% of the cecum) followed by a puncture with a needle (14 G, one hole; enough to generate a mortality rate around 30%). The wound was closed by applying interrupted sutures to the abdominal musculature and skin. Finally, the animals received a postoperative fluid resuscitation by injecting saline solution (3 ml per 100 g of body weight) subcutaneously. Naive animals did not undergo any manipulation. Thirty or 60 days after, aortas obtained from the surviving sepsis and naive rats were subjected to different vasoactive stimuli to evaluate the responsiveness to classical targets of vascular tonus maintenance. In addition, the antioxidant enzymes activities (superoxide dismutase – SOD, glutathione reductase - GSH), and the levels of lipid hydroperoxides (LOOH) were evaluated in homogenates of aortic tissue obtained from S60 group. All procedures were approved by the Institutional Ethics Committee of UFPR (authorization number 527). **Results and Discussion:** The effects of potassium, phenylephrine, angiotensin I, calcium chloride, caffeine, acetylcholine, and sodium nitroprusside were not changed in vessels from sepsis-surviving rats. However, in S60 group angiotensin II (AII)- and vasopressin (AVP)-induced vasoconstriction were increased by 70 and 78%, respectively, when compared to control. It was accompanied by reduced relaxation in response to Y-27632, a Rho-kinase inhibitor, with EC50 of 0.43 (0.33-0.55) and 1.03 (0.76-1.40) μM in control and S60 groups, respectively. AII and AVP induced augmented contractile responses in aortic rings from S60 rats were also found in calcium-free solution, an event fully reversed by thapsigargin (an inhibitor of SERCA). In spite of an augmented activity of glutathione reductase (GSH levels increased by 98 ± 3.9% when compared to control), the activity SOD was decreased by 45 ± 3%, while LOOH generation was increased by 41 ± 12% in homogenates of aorta obtained from S60 group, when compared to samples obtained from control animals. Our study discloses that aortic rings from sepsis-surviving rats display augmented reactivity to AII and AVP by mechanisms involving changes in calcium mobilization and sensitization, associated with an impaired activity of antioxidant enzymes. **Research support:** Priscila de Souza receives a fellowship from CAPES/Brazil.

06.035 Characterizing a malnutrition model based on a high fat diet to study cardiovascular effects of molecules with therapeutic potential and nutraceuticals. Miranda R[1], Marques EB[1], Oliveira GF[1], Rocha NN[2], Scaramello CBV[1] [1]UFF – Laboratório de Farmacologia Experimental, [2]UFF – Fisiologia e Farmacologia

Introduction: Feeding is part of our routine, however, not always is performed properly. Currently the term "malnutrition" is also being used to describe a wide range of nutrients deficiency or excess resulting in adverse effects on body composition and function (Saunders and Smith. Clin Med. 10(6): 624, 2010). High fat intake is one of many factors that can cause hypercholesterolemia, which may drive to cardiac dysfunction due to direct action on myocytes' membrane fluidity, enzyme activities and cation transporters activity or indirectly as a consequence of ischemic heart disease (Saini *et al*. Can J Cardiol. 20: 333, 2004). **Methods:** The use of animals was according to Ethics Committee (CEPA/UFF00099/2011). After weaning male Wistar rats were randomly divided and submitted to 30 or 60 days of experiment: G1- fed with commercial chow (Nuvilab: 56% carbohydrate,19% protein, 3.5% lipids, 4.5% fibers, 5% vitamins/minerals = 4.1kcal/g); G2-fed with high-fat diet (HFD: 39.4% carbohydrate,17% protein, 17% lipids, 3% fibers, 4% vitamins/minerals = 4.3kcal/g). Body weight and length, abdominal/thoracic circumference ratio as well as body mass index (BMI) were determined. Feed efficiency ratio was also calculated (Novelli *et al*., Lab Anim. 41(1):111, 2007). The animals were anesthetized and hearts were removed, weighed and the rates of hypertrophy (RH) were measured. Serum glucose and lipid profile were determined using Labtest kit. Echocardiographic studies were performed according to Lang *et al*. (J Am Soc Echocardiogr. 18(12):1440, 2005). Data are presented as mean and standard error of the mean (at least 4 observations), analyzed by Student *t* test and considered statistically different if $P<0.05$(*). **Results:** As G2 weight gain has been higher than G1 while calories consumption was smaller, HFD fed animals presented a higher feed efficiency ratio after 30 (G1 = 0.092±0.003;G2 = 0.112±0.003*g/kcal) and 60 days (G1 = 0.057±0.002;G2 = 0.078±0.002*g/kcal) of experiment. Castelli's index has been different between groups since 30 days of HFD (G1 = 2.10±0.12;G2 = 2.53±0.11*), however, BMI (G1 = 0.631±0.021;G2 = 0.728±0.019*g/cm^2) and abdominal/thoracic circumference ratio (G1 = 1.138±0.022;G2 = 1.191±0.001*) were distinct just after 60 days. No differences were noted about serum glucose (data not shown). G2 heart RH was smaller than G1 just after 60 days of experiment (G1 = 2.35±0.03; G2 = 2.13±0.03*). Echocardiographic studies showed no difference between groups after 30 days of HFD. **Discussion:** Our data suggest that G2 presented an inferior energy expenditure compared to G1 and a profile consistent to obesity after 60 days of HFD. Rat obesity may be easily estimated through BMI and alterations in this index are associated with dyslipidemic profile, as observed in this work and oxidative stress (Novelli *et al*., Lab Anim. 41(1):111, 2007). The data suggest that HFD was able to imprint a cardiometabolic risk (Pinheiro *et al*. *Arq Bras Cardiol*. 93(3): 400, 2009). In addition, the smallest heart RH presented by 60 days HFD fed rats points to a cardiac mass disproportionate to BMI. Literature shows that in cases of protein-calorie malnutrition the mean cardiac mass falls, causing a significant cardiac atrophy that is reflected in decreased cardiac output and slightly reduced contractility (Fioretto *et al.*, *Am J Physiol Heart Circ Physiol* 282: H1327, 2002). Echocardiographic studies are being made with rats after 60 days of HFD to evaluate cardiac function. **Financial Support :**CAPES, CNPq, FAPERJ, PROPPi/UFF

06.036 Does LASSBio1425 modulate cardiac and renal P-type ATPases in a diet-induced hypercholesterolaemia model? Marques EB[1], Oliveira GF[1], Carvalho NPR[1], Fumian MM[1], Motta NAV[1], Maia RC[2], Barreiro EJ[2], Brito FCF[1], Scaramello CBV[1] [1]UFF –Farmacologia Experimental, [2]UFRJ – Avaliação e Síntese de Substâncias Bioativas

Introduction: Tumor necrosis factor-α (TNFα), a pro-inflammatory cytokine, influences the balance of blood lipid composition and has been identified as a player in several atherosclerosis complications (Branen *et al*. Arterioscler Thromb Vasc Biol. 24:2137, 2004). The anti-inflammatory and immunomodulatory properties of thalidomide (Th), a phtalimide derivative, are due to its ability to inhibit TNFα. However, its use is limited to the treatment of diseases where there are no alternative therapies conditioned to the monitoring of neurological effects during treatment and to the use of contraceptive methods in women. Research efforts have been devoted to the search for new analogues with improved pharmacotherapeutic and safety profile (Barbosa *et al*., Braz J Pharm Scie. 47: 427, 2011). The aim of this work is to investigate the *in vivo* cardiorenal effects of LASSBio1425, a new phtalimide derivative, analogous to Th, in a diet-induced hypercholesterolaemia model. **Methods:** The use of animals was according to Ethics Committee (CEPA/UFF00116/09). Male Wistar rats (200g) were randomly divided and submitted to 45 days of experiment: G1-normal diet; G2-high-fat diet (HFD); G3-HFD and 100 µmol/kg of LASSBio1425 p.o. during the last 15 days of experiment. HFD was prepared according to Ramesh *et al*. (Fund & Clin Pharmacol. 22: 275, 2008). The animals were anesthetized and the hearts(H)/kidneys(K) were removed, weighed and the rates of hypertrophy (RH) were measured. Then tissues homogenates were obtained (Bambrick *et al*., *J Pharmacol Meth*. 20: 313, 1988). Serum lipid profile and TNFα concentration were determined using commercial kits. Homogenates' protein concentration and P-type ATPases activity were determined according to Lowry *et al*. (J Biol Chem. 193:265,1951) and Fiske & Subbarow (J Biol Chem. 66: 375, 1925), respectively. Data are presented as mean and standard error of the mean (3-12 observations) and considered statistically different if $P<0.05$ (*vs G1 and †vs G2). **Results:** LASSBio1425 was able to reduce TNFα level increased by HFD (G1 = 30.9±2.9;G2 = 42.0±2.7*;G3 = 31.8±2,1†pg/mL) and improved lipid profile (data not shown). LASSBio1425 reversed HFD effect on renal (G1 = 7.7±0.3;G2 = 6.4±0.08*;G3 = 7.1±0.1†mg/g) but not cardiac (G1 = 2.7±0.1;G2 = 2.2±0.05*;G3 = 2.3±0.08*mg/g) RH. HFD decreased both cardiac (G1 = 2399±231;G2 = 1427±261* nmolPi/mg/h) and renal Na^+/K^+ATPase activity (G1 = 6267±97;G2 = 4285±374*nmolPi/mg/h) but LASSBio1425 just restored this activity in the K (G3 = 6631±114†nmolPi/mg/h). Na^+ATPase activity was increased by HFD without LASSBio1425 visible influence (H: G1 = 2048±159;G2 = 4211±253*;G3 = 3735±154*nmolPi/mg/h; K: G1 = 3426±434;G2 = 4907±273*;G3 = 5455±166*nmolPi/mg/h). **Discussion:** As LASSBio1425 reduced serum TNFα level and improved lipid profile, it may mitigate atherosclerosis harm. However, so far, LASSBio1425 was able to mitigate HFD effects just in the K. Renal Na^+/K^+ATPase drives active Na^+ reabsorption throughout the nephron and is involved in the regulation of extracellular volume and blood pressure (Feraille & Doucet, Physiol Rev. 81:345, 2001). Cardiac Na^+/K^+ATPase impairment is associated to Ca^{2+} overload and tachyarrhytmias (Vié *et al*., J Pharmacol Exp Ther. 330:696, 2009). Changes in Na^+ATPase activity are expected to compensate intracellular Na^+ level while Na^+/K^+ATPase activity is decreased (Reyes *et al*., Physiol Res. 58:693,2009). **Financial Support:** CAPES, FAPERJ, PROPPi/UFF

06.037 The NADPH oxidase inhibitor apocynin ameliorates the erectile dysfunction in middle-aged rats. Silva FH[1], Bau FR[1], Brugnerotto AF[2], Mónica FZT[1], Priviero FBM[1], Toque HA[1], Antunes E[1]
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Introduction: Erectile dysfunction (ED) is highly associated with aging, which has been related to an unbalance between reactive-oxygen species (ROS) production and antioxidant capacity of tissues (Teles et al., 2008; Frey et al., 2009). However, few studies have investigated the ED in middle-age and the importance of oxidative stress in corpus cavernosum (Hosogai et al., 2003; Gür et al., 2005). Therefore, we have undertaken functional and molecular studies to evaluate the importance of superoxide anion in ED of middle-aged rats. **Material and Methods:** The experimental protocols were approved by the Animal Ethical Committee of UNICAMP (n° 2110-1). Male Wistar rats were divided into two groups, namely young and middle-aged rats (2.5 and 10 months, respectively). The erectile function was assessed by measuring the intracavernous pressure (ICP) following cavernous nerve electrical stimulation. Rat corpus cavernosum (RCC) relaxations induced by acetylcholine (ACh), sodium nitroprusside (SNP) and electrical field stimulation (EFS) in phenylephrine (10 μM)-pre-contracted tissues, as well as determination of cGMP in tissues were stimulated with SNP. The mRNA expression for $gp91^{phox}$ and SOD-1 in RCC were also evaluated. **Results:** A significant decrease in ICP was observed in middle-aged compared with young rats (6 Hz: 15.6±3 and 25.8±3 mmHg, respectively; $P<0.05$). The maximal relaxant response (E_{max}) elicited by ACh, SNP and EFS (32Hz) were significantly lower in middle-aged RCC (E_{max}: 37±2%, 88±2% and 36±3%, respectively, $P<0.05$) compared with young rats (E_{max}: 70±1%, 103±2% and 53±1%, respectively). Pre-incubation of RCC with the NADPH oxidase inhibitor apocynin (100 μM) or superoxide dismutase (SOD, 75U/mL) fully restored the relaxant responses elicited by ACh, SNP and EFS in middle-aged RCC with no changes in the RCC relaxations in young rats (n = 5-8, $P<0.05$). The basal cGMP content in the erectile tissue was 64% lower ($P < 0.05$) in middle-aged group compared with the young group, as well as in tissues stimulated with SNP (10 μM) was 50% lower in middle-aged RCC. Pre-incubation of RCC with apocynin (100 μM) or SOD (75 U/mL) fully restored SNP-induced increases in cGMP levels in middle-aged RCC with no changes in the young RCC (n = 4). In separate groups, young and middle-aged rats were treated orally with apocynin, given in tap water for 4 weeks. This treatment also restored the relaxant responses elicited by ACh, SNP and EFS in middle-aged RCC, without changing the relaxations in young rats (n = 5-8). The mRNA expression for $gp91^{phox}$ in cavernosal tissues was increased by approximately 64% in middle-aged group compared with young group, whereas no changes in the mRNA expression for SOD-1 were. **Discussion:** Our findings that apocynin treatment ameliorates the ED in middle-aged rats and that mRNA expression for $gp91^{phox}$ is increased in RCC indicate that increased generation of superoxide anion greatly contributes to this disorder. **References:** Frey RS *et al.,* Antioxid. Redox. Signal. 11:791, 2009. Gür S *et al.,* Int. J. Urol. 12:821-8, 2005. Hosogai N *et al.,* Eur. J. Pharmacol. 18:65, 2003. Teles AG *et al.,* J. Sex. Med. 5:1317, 2008. **Financial Support**: FAPESP

06.038 **Pharmacological induced sympathetic overactivity in ApoE deficient mice**: **Relationship between sympathetic hyperactivity**, **metabolic syndrome and atherosclerosis**. Nascimento AR[1], Doras C[2], Greney H[2], Niederhoffer N[2], Tibiriçá E[1], Bousquet P[2] [1]Fiocruz – Investigação Cardiovascular, [2]Université de Strasbourg – Neurobiologie et Pharmacologie Cardiovasculaire

Introduction: Glucose intolerance and insulin resistance, proinflamatory and prothrombotic state, visceral obesity, dyslipidemia and hypertension are individual risk factors for atherosclerosis. When associated, the incidence of cardiovascular events is substantially increased. The metabolic syndrome is a pathologic entity that clusters all these disorders. It is now established that increase in sympathetic activity and catecholamines levels is positively correlated to atherosclerosis severity in diabetic and obese patients. As there is a link between metabolic syndrome and sympathetic activity, we wondered if sympathetic dysfunction could generate or aggravate atherosclerosis. **Methods:** We used male C57BL/6 ApoE$^{-/-}$ and NET knockout mice in conformity with the guidelines of the Animals Care and Use Comitee of the University of Strasbourg, France (Autorization Nº. 67-249). The animals were housed within the Medicine Faculty facilities and maintained under normal diet (Safe;Augy, France) or a high-fat diet (D08100201: with 40% fat; Research Diet New Brunswick, USA). The weight, food consumption and arterial blood pressure were measured all over the protocol. The C57BL/6 ApoE$^{-/-}$ mice were treated with desipramine (10mg/kg/j), a norepinephrine transporter inhibitor, for 16 weeks. Plasma catecholamines and atherosclerotic plaques (Paigen's histological quantification) were assessed post-mortem. **Results:** Compared to controls, ApoE$^{-/-}$ mice treated with desipramine presented an increase in plasma glycaemia, norepinephrine level and exaggerated atherosclerosis formation (1587.0±131.2 *vs* 900.3±85.4 x10^3 µm^2 n = 10, p<0.001). Knockout NET mice, an experimental model of constitutive sympathetic hyperactivity, presented a spontaneous development of atherosclerosis which was substantially enhanced by the high-fat diet when compared to the wild-type (NET$^{+/+}$ 68.0±18.5 *vs* NET$^{-/-}$ 150.9±19.3 x10^3 µm^2 p<0.01). WT animals from the same genetic background had no atherosclerotic lesions. Systolic arterial pressure was normal in both models. However, desiprimine caused moderate tachycardia in ApoE$^{-/-}$. **Discussion:** The present study confirmed that sympathetic hyperactivity causes or aggravates atherosclerosis despite of very weak cardiovascular and metabolic changes.

06.039 **Histological characterization of nitric oxide synthesis after 6 months of the end of treatment**. De Paula DCC[1], Bianchini-Silva LS[1], Silva MDA[1], Carneiro C[1], Guimarães HN[2], Saúde-Guimarães DA[1], Grabe-Guimarães A[1] [1]UFOP – Farmácia, [2]UFMG – Engenharia Elétrica

Introduction: Inhibition of nitric oxide (NO) in rat model causes significant changes at the cardiovascular system, due to a generalized vasoconstriction and alterations of cardiac function and morphology. These alterations are well described soon after the NO inhibition or a few days after. The present study aimed to measure the structural alterations induced by the NO synthesis inhibition by *L*-NAME 6 months after the end of treatment **Methods:** All the procedures were approved by the UFOP Ethical Committee under number 2010/64. Male Wistar rats (150-180 g) were treated with *L*-NAME 60 mg/kg for 7 days (I.P.) or vehicle. The animals were maintained under observation for six months and at the end of this period they were anesthetized and had the arterial pressure (AP), left ventricular systolic pressure (LVSP) and ECG signals obtained. After that they were euthanized and the hearts were removed for histological analysis. The cell nuclei and the area of collagen were measured in 20 random images obtained from slides stained with hematoxylin-eosin (HE) and Masson trichrome, respectively, assessed by heart fragment. It was calculated the ratio of heart weight and body weight. **Results:** The AP and LVSP was similar between the groups (SAP control 122 mmHg ± 2,8 X L-NAME 107 mmHg ± 9,8; DAP control 84 mmHg ± 2,4 X *L*-NAME 75 mmHg ± 6,3; LVSP control 127 mmHg ± 5,1 X *L*-NAME 108 mmHg X 8,3) and the ECG parameters the interval PR and QT were reduced the *L*-NAME group (PR control 64,7 ms ± 0,56 X *L*-NAME 60,6 ms ± 0,83; QT control 81,4 ms ± 1,67 X *L*-NAME 75,2 ms ± 2,05). The images from HE stained heart sections showed significant reductions cell nuclei number for *L*-NAME treated group compared to the control (control 162 ± 3,5 X *L*-NAME 113 ± 2,3). Sections stained with Masson trichrome showed significant increase in collagen content in *L*-NAME group (control 4404 mm^2 ± 287,3 X *L*-NAME 5197 mm^2 ± 230,2). Although the heart weight was not different between the groups, the weight ratio was significantly lower in *L*-NAME group, due to its higher weight at the end of the 6 months. **Discussion:** The decline in total number of myocytes, represented in this study by the total number of cell nuclei, suggests that the rate of loss of cardiomyocytes appears to be higher than the rate of proliferation models *L*-NAME (Gomes-Pessanha & Mandarim-De-Lacerda, 2000). This difference can be related to the analysis time 6 months after the end of L-NAME treatment. The presence of collagen is characteristic of *L*-NAME experimental models, caused by fibrosis and cardiac muscle disorder (Moreno et al., 1996). Thus, the changes observed are indicative of typical of heart failure, but the absence of hemodynamic, heart weight and ECG alterations indicates an initial or completely compensated heart failure state. **Financial Support:** UFOP, Capes, FAPEMIG. Gomes Pessanha M, Mandarim-De-Lacerda CA. Influence of the chronic nitric oxide synthesis inhibition on cardiomyocytes number. Virchows Archives; 437 (6), 2000. Moreno H, Metze K, Bento A. Chronic nitric oxide inhibition as a model oh hypertensive heart muscle disease. Basic Research Cardiology; 91: 249-55, 1996.

06.040 **Antiplatelet and antithrombotic activity of new nitric oxide donors**: **E-CAOx and NTHF**. Santos PC[1], Maciel PMP[1], Assis VA[2], Queiroz TM[2], Pita JCR[2], Alustau MC[2], Furtado FF[3], Medeiros IA[1], Veras RC[1], Athayde Filho PF,[4] [1]DCF-CCS-UFPB, [2]CCS-UFPB, [3]ETSC-CFP-UFCG, [4]CCEN-UFPB

Introduction: In cardiovascular system, a reduced production of Nitric Oxide (NO) or NOS activity can be the responsible to endothelial dysfunction, a condition present in some Cardiovascular Diseases (CVD) (LEFER, *Card.Res*., 36, 743, 1996; JOHN, Cur.Hypert. Rep., 5, 199, 2003).Platelets aggregation is a central process in the development of ischemic complications in various vessels (GURBEL. Exp. Rev. *Cardiovasc. Ther*. 2, 535, 2004). To supply this condition, the use of NO donor can be an important alternative. The purpose of this study was to evaluate the effect antiplatelet and antithrombotic of two NO donors: an oxime derivative, E-cinnamaldehyde oxime (E-CAOx), and nitrate tetrahydrofurfuryl (NTHF), an organic nitrate obtained from cane sugar reject. **Methods**: The action of E-CAOx and NTHF was evaluated using *in vivo* and *in vitro* protocols. For *in vivo* testing, was evaluated coagulation time (CT) and bleeding time (BT) in mice. The groups were divided: control and treated with E-CAOx (50 and 100 mg/Kg) or NTHF (25, 50 and 100 mg/Kg) groups. For *in vitro* test it was induced platelet aggregation with collagen (100 µg/mL) and ADP (200 µM), in the presence and absence of E-CAOx using a Net Lab agregometer. **Results and Discussion**: The administration of E-CAOx (50 mg/kg, i.p.) produced no significant change on TC, while E-CAOx (100 mg/kg, i.p.) caused a significant increase on TC when compared to control or vehicle (Tween 80, 0.5%). E-CAOx (50 mg/kg) significant increased the BT, an effect not observed with dose of 100 mg/kg, when compared to control or vehicle. On the other hand, NTHF (25 mg/kg, 50 mg/kg or 100 mg/kg) did not promoted any significant change in CT mice when compared to control and vehicle (cremophor®). NTHF (100 mg/kg) caused a significant increase in BT, which was not observed at a dose of 25 mg/kg and 50 mg/kg. The extension of the BT caused by NTHF and E-CAOx may be due to the inhibition of aggregation induced by serum factors, such as arachidonic acid, thrombin or ADP, but may be related to NO production, which could be a positive effect in prolonging BT, for being a known antiplatelet agent, whose action takes place preferentially by the activation of sGC (soluble guanylyl cyclase) (MILLER, *BJP*, 151, 305, 2007). Since the CT is much more related to events secondary hemostasis, in which the main components are the coagulation factors. The E-CAOx significantly inhibited platelet aggregation *in vitro* induced by ADP and collagen agonists, suggesting an investigation of the mechanism by which this effect occurs. In conclusion, it was possible to demonstrate inhibition of the activity of primary and secondary hemostasis of two potential NO donors, and show an antiplatelet activity *in vitro* to E-CAOx, suggesting that E-CAOx and NTHF may be new agents for the antiplatelet and antithrombotic therapy. **Financial support**: CNPq,CAPES and Laboratório de Análises Clínicas

06.041 The role of renin-angiotensin system and oxidative stress in development of experimental preeclampsia induced by L-NAME. Amaral TAS[1], Carvalho LCRM[1], Ognibene DT[2], Rocha APM[3], Soares de Moura R[1], Resende AC[1] [1]UERJ – Farmacologia e Psicobiologia, [2]UEZO – Ciências Biológicas e da Saúde, [3]Unirio

Introduction: Preeclampsia (PE), a systemic syndrome of pregnancy characterized by proteinuria and hypertension, is associated with significant morbidity and mortality to both mothers and fetuses, however its causes have not been completely clarified. Despite an expressive increase in renin-angiotensin system (RAS) activity in the normal pregnancy, blood pressure does not increase. Nevertheless, the role of RAS in PE is not well known. In PE, the reduction in intrauterine perfusion pressure promotes an increased release of reactive oxygen species, which may contribute to hypertension in pregnancy. In the present study, we investigated the role of the vascular RAS and the plasmatic and placental oxidative stress to maternal cardiovascular regulation on normal pregnancy and in an animal model of preeclampsia which was induced by L-NAME. **Methods:** These experiments were approved by the Ethics Committee of UERJ (protocol: CEA/023/2010). Pregnant Wistar rats were treated with L-NAME (60 mg/kg/day, orally, LNP) or vehicle (CP) from day 13 to day 20 of pregnancy and non-pregnant rats were treated with L-NAME (LNNP) or vehicle (CNP) during 7 days. Systolic blood pressure (SBP) was measured by plethysmography. The vasodilator effect of acetylcholine (ACh), nitroglycerine (NG), angiotensin II (Ang II) and angiotensin 1-7 (Ang 1-7) as the vasoconstrictor effect of Ang II and Phenylephrine (Phe) were studied in perfused mesenteric arterial bed (MAB). We determined the maternal body weight, pups body weight, placental weight, number of fetus alive and death fetus for each mother, serum estradiol, plasma renin, Ang II, Ang 1-7 and bradykinin (BK). Oxidative damage and antioxidant enzyme activity: superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were measured in plasma and placenta by spectrophotometry. **Results:** SBP was increased in animals which received L-NAME. LNP rats had fat loss during pregnancy, increased number of death fetus, decreased number of fetus alive, lower total placental mass and lower pups weight. The vasodilator effect of ACh and Ang II was reduced in LNP and LNNP, otherwise, the vasodilator effect of NG and the vasoconstrictor effect of Phe and Ang II were increased. The plasma levels of renin and BK were increased in LNP rats, while Ang 1-7 was reduced. The serum estradiol was increased in CP and LNP rats. The levels of malondialdehyde and protein carbonyls were increased and activities of antioxidant enzymes SOD and GPx were lower in LNP and LNNP animals, but CAT did not show significant difference among the four groups. **Discussion:** The reduced vasodilator response of Ang II associated to increased vasoconstrictor response of this peptide, suggest that RAS contribute to development of preeclampsia-like characteristics in this model of PE. Similarly, the increase of lipid peroxidation and protein oxidation, as the reduction of antioxidant activity suggest the involvement of a deficient mechanism of antioxidant defense and an increased oxidative damage contributing to development of hypertension, endothelial dysfunction, high fetus mortality and intrauterine growth restriction observed in this model of PE. **Financial Support:** CNPq and FAPERJ.

06.042 **Oral administration of AVE-0991, a nonpeptide angiotensin(1-7) receptor agonist, facilitates erectile response in conscious and in anesthetized rats**. Felipe-Batista K[1], Costa-Gonçalves AC[2], Lopes IM[1], Neves NCV[1], Damasceno EC[1], Guimarães HN[3], Rodovalho GV[1], Grabe-Guimarães A[1], Santos RAS[2], Leite R[1] [1]UFOP – Farmácia, [2]ICB-UFMG, [3]UFMG – Engenharia

Introduction: Previous data from our group have shown that Ang-(1-7), infused into the cavernosal tissue or subcutaneously injected, significantly improves male rat erectile function. The aim of this study was to evaluate the potentiating effect of the oral administration of AVE-0991, a nonpeptide compound that mimics Ang-(1-7), on the erectile response studied in models of conscious and anesthetized rats. **Methods and Results:** Ganglionic-induced erectile response in anesthetized rats was evaluated in male Wistar rats (270-300 g) under ketamine/xylazine (100/14mg/100g, IM) anesthesia. Intracavernosal pressure (ICP) and mean arterial pressure (MAP) were measured and presented as an index of erection (ICP/MAP). Frequency-response curves (1-12 Hz, 4V, 5ms pulse and 30 seconds for each frequency) and voltage-response curves (0.5 - 4.0 V, 12Hz, 5ms pulse and 30 seconds for each voltage) were obtained in anesthetized vehicle (control) or AVE-0991 (1mg/Kg by gavage 60 minutes before) treated rats. For the conscious model of studying erectile response, male Wistar rats (270-300 g) were treated with vehicle or AVE-0991 (1mg/Kg by gavage 60 minutes before) and received a subcutaneous injection of apomorphine (25,5 nmol/100g). The rats were then placed in a glass box and recorded for 30 minutes. The number of erections induced by apomorphine was counted. Erectile function was not affected by vehicle, but significantly potentiated ($p<0,05$, ANOVA and Bonferroni post test) by AVE-0991 in both conscious and anesthetized rats. **Conclusion:** Our data indicate that oral administration AVE-0991, a non-peptidic Ang-(1-7) mimetic has a great potential for treatment of for the development of new approaches to treat male erectile dysfunction. **References:** Costa-Gonçalves, ACC. *et al*. Am. J. Physiol Heart Circ Physiol 293: H2588-2596(2007). Marques FV, *et al*. Hypertension 57:477-483(2011). Wiemer, G et al. *Hypertension*, 40, 847-52(2002). **Financial support:** FAPEMIG, CNPq, CAPES and UFOP. The experimental protocols performed were approved by the Ethics Committee on Animal Use of UFOP Protocol (CEUA UFOP-in. 53/2010)

06.043 **Cardiovascular responses to *Bothrops atrox* venom in anesthetized rats**. Rodrigues MAP, Dial L, Neves RC, Brunieri LVP, Rennó AL, Stroka A, Hyslop S Unicamp – Farmacologia

Introduction: *Bothrops atrox* is an important species responsible for venomous snakebites in the Brazilian Amazon (Pardal PP *et al.,Trans. R. Soc. Trop. Med. Hyg.***98,** 28, 2004). While some studies have investigated the biochemical and biological activities of *B. atrox* venom, including its local effects, little is known of the cardiovascular responses to this venom. In this study, we investigated the changes in blood pressure, heart rate, electrocardiogram (ECG) and respiratory rate caused by *B. atrox* venom in rats. We also examined the histological alterations caused by the venom in selected organs. **Methods:** Male Wistar rats (300-400 g) were anesthetized with isoflurane (2% in 98% air; flow rate of 1 L/min) and maintained with this anesthetic throughout the experiment. A carotid artery was catheterized for continuous blood pressure measurements (PowerLab data acquisition system, ADInstruments) and venom (0.4, 0.5, 0.6 or 0.7 mg/kg; one dose/rat; n = 4 each) was administered via a femoral vein in a fixed volume of 100 µL that was washed in with a further 100 µL of 0.9% NaCl. The changes in cardiovascular parameters were monitored for 120 min, after which the rats that survived to this time point were killed with an overdose of anesthetic. Heart, lung, liver and kidney tissue samples were collected and processed for histological analysis. The results (mean ± SEM) were analyzed statistically with ANOVA followed by the Tukey-Kramer test, with $p<0.05$ indicating significance. This work was approved by the institutional Committee for Ethics in Animal Research (CEEA/UNICAMP, protocol no. 2181-1). **Results:** The intravenous injection of venom produced significant ($p<0.05$) immediate hypotension that was maximal within 60 s (decrease in blood pressure from 100±5 to 55±3, 57±5 and 68±5 mmHg for 0.4, 0.5 and 0.6 mg/kg, respectively; n = 4), followed by gradual recovery to basal values; there were no significant differences in the hemodynamic responses to these three doses of venom. There were also no significant changes in heart rate, ECG parameters, body temperature or respiratory rate with these three doses. In contrast, at the highest dose (0.7 mg/kg) the rats died of irreversible shock and respiratory failure within 20 min. Macroscopic examination at death revealed hematuria, as well as focal hemorrhage in the lungs that was confirmed histologically. There were no histological alterations in the heart or liver. In the kidneys, doses of 0.4-0.6 mg/kg resulted in the glomerular deposition of proteinaceous material in renal cortex tubules and Bowman's capsular space; there were no renal alterations with 0.7 mg of venom/kg, probably because of the early death of rats injected with this dose. **Conclusion:** These results show that *B. atrox* venom causes hemodynamic alterations predominantly through a vascular mechanism, with no marked effect on cardiac electrical activity. Histological analysis confirmed that the venom caused important systemic damage in the lungs and kidneys. **Financial support:** CAPES, CNPq, FAPESP

06.044 Cardiac alterations caused by *Lachesis Muta* (Bushmaster) snake venom in rat isolated perfused heart. Dias L[1], Rodrigues MAP[1], Brunieri LVP[1], Rennó AL[1], Sousa NC[1], Stroka A[1], Melgarejo AR[2], Hyslop S[1] [1]Unicamp – Farmacologia, [2]IVB – Zoologia Médica

Introduction: Systemic envenoming by bushmasters (*Lachesis* spp.) results in coagulopathy, bradycardia, hypotension and "neurotoxicity" involving activation of the autonomic system (Jorge MT *et al., Toxicon***35,** 545, 1997; Pardal PP *et al.,Trans. R. Soc. Trop. Med. Hyg.***98,** 28, 2004). However, the cardiovascular responses to this venom and the mechanisms involved have not been extensively studied. In this work, we examined the cardiac alterations caused by Peruvian *L. muta* venom in rat isolated perfused heart. **Methods**: Hearts were rapidly removed from heparinized (500 IU, i.p.) male Wistar rats (200-300 g) anesthetized with 2% isoflurane and perfused retrogradely (constant pressure: ~80 mmHg; basal perfusion: 15 ml/min) with modified Krebs-Henseleit solution (KHS) at 37°C; the solution was aerated continuously with 95%O_2-5%CO_2, pH 7.4). A latex balloon was inserted into the left ventricle for left ventricular pressure measurements and the electrocardiogram (ECG) was measured with wire electrodes. Direct (heart rate, perfusion pressure, left ventricular systolic and end diastolic pressures and ECG) and indirect (dP/dt) cardiac parameters were recorded continuously (PowerLab system; ADInstruments). Coronary flow was measured manually. Creatine kinase-MB (CK-MB) and lactate dehydrogenase (LDH) release was measured using commercial kits. After stabilization for 15 min, venom (3, 4.5 or 6 mg) was injected in 200 ml over 1 min and the changes in cardiac parameters were monitored for 45 min. The results (mean±SEM) were analyzed using ANOVA followed by the Tukey-Kramer test, with $p<0.05$ indicating significance. These experiments were approved by the institutional Committee for Ethics in Animal Research (CEEA/UNICAMP, protocol no. 2182-1). **Results:** Three milligrams of venom (n = 5) caused no significant alterations compared to control hearts perfused with KHS alone whereas 6.0 mg caused cardiac arrest within 5 min (n = 2). An intermediate amount of venom (4.5 mg, n = 5) significantly ($p<0.05$) reduced the ventricular pressure from 71±6 mmHg to 41±7, 38±4 and 31±6 mmHg at 15, 30 and 45 min, respectively. The Max dP/dt decreased significantly from 2277±527 to 1420±252, 1224±218, 1229±277 and 784±126 at 5, 15, 30 and 45 min, respectively, while the Min dP/dt increased significantly from -1695±206 to -878±127, -834±112 and -639±104 at 15, 30 and 45 min, respectively. There were no significant changes in the ECG, heart rate and coronary flow. CK-MB release was significantly elevated throughout the experiment, with a peak at 5 min (from 0.4±0.01 to 2.9±0.2 U/ml; n = 5); LDH was significantly elevated at 15 min and 30 min from 26±4 (basal) to 109±21 and 90±16 U/ml, respectively (n = 5). Histological analysis of cardiac tissue showed necrosis with 4.5 mg of venom compared to the control group. **Conclusion:***Lachesis muta* venom reduces the contractility of rat isolated perfused hearts without affecting electrical activity and coronary flow. The release of marker enzymes suggested tissue damage that was confirmed by histological analysis. **Financial support:** CAPES, CNPq, FAPESP

06.045 **Time course involvement of metaloproteinases and oxidative stress in the progression of renovascular hypertension-induced cardiac hypertrophy**. Rizzi E[1], Ceron CS[1], Guimarães DA[1], Prado CM[2], Rossi MA[2], Gerlach RF[3], Tanus-Santos JE[1] [1]FMRP-USP – Farmacologia, [2]FMRP-USP – Patologia, [3]FORP-USP – Morfologia, Estomatologia e Fisiologia

Introduction: Imbalanced matrix metalloproteinase (MMP) activity is associated with left ventricular hypertrophy (LVH). Increased reactive oxygen species (ROS) formation enhances MMP activity and decrease tissue inhibitor of MMP (TIMP) activity. We examined the temporal relationship between ROS, MMP-2, TIMP-4 levels and gelatinolytic activity in the heart during the progression of LVH in two-kidney, one-clip (2K1C) hypertension. **Methods:** Sham or 2K1C hypertensive rats were studied after 15, 30, and 75 days of hypertension. Systolic blood pressure was monitored weekly. Structural changes and fibrosis in left ventricles were carried out in hematoxylin/eosin and picrosirius red stained-sections, respectively. Cardiac MMP-2 levels were determined by immunofluorescence and gelatin zymography. TIMP-4 levels were evaluated by western blotting. Gelatinolytic activity was determined by *in situ* zymography. Cardiac superoxide production was evaluated by dihydroethidium probe. Procedures were approved by the local Ethical Committee (protocol number: 123/2011). **Results:** Systolic blood pressure increased with time in 2K1C rats: 167 ± 3, 201 ± 1, and 203 ± 2 mmHg after 15, 30, and 75 days of hypertension (all $P<0.05$). Hypertension induced LVH in a time-dependent manner ($P<0.05$). Collagen deposition increased by 60% in hypertensive rats after 2 weeks and was maintained after 75 days of hypertension ($P<0.05$ *vs.* respective sham). Cardiac MMP-2 expression (immunofluorescence) increased from 13 ± 1 (sham) to 19 ± 2, 17 ± 1, and 20 ± 2 arbitrary units (AU) after 15, 30, and 75 days of hypertension, respectively (all $P<0.05$ *vs.* sham). The same results were observed by gelatin zymography. Cardiac TIMP-4 levels increased by 325 ± 68% ($P<0.05$) only after 75 days of hypertension. Cardiac gelatinolytic activity increased by 129 ± 4%, 150 ± 6%, and 152 ± 6% after 15, 30, and 75 of hypertension, respectively (all $P<0.05$ *vs.* sham). Cardiac ROS levels increased by 137 ± 5%, 154 ± 15%, and 130 ± 10% after 15, 30, and 75 days of hypertension, respectively (all $P<0.05$ *vs.* sham). **Discussion:** These results indicate that LVH in is an early process associated with imbalanced MMP-2 and TIMP-4 levels in renovascular hypertension, thus resulting in increased gelatinolytic activity and fibrosis, which may be due to oxidative stress. **Financial Agencies:** FAPESP, CNPq AND FAEPA.

06.046 **Nebivolol attenuates the hypertrophic remodeling in the 2-kidney, 1-clip model of renovascular hypertension**. Ceron CS[1], Rizzi E[2], Guimarães DA[2], Martins-Oliveira A[2], Gerlach RF[3] [1]USP – Farmacologia, [2]USP – Farmacologia, [3]USP – Morfologia e Estomatologia

Introduction: Nebivolol (Nebi) is a third generation β-1 receptor antagonist with vasodilator and antioxidant properties. Vascular remodeling is associated with increased reactive oxygen species (ROS) and Matrix metalloproteinases (MMPs) activity. MMPs are enzymes involved in cardiovascular remodeling, and the upregulation of these enzymes has been associated with increased ROS levels. It is possible that Nebi reverses the increase in MMP levels and vascular remodeling associated with 2K-1C hypertension through its antioxidant activity. **Methods:** Hypertension was induced in male Wistar rats by clipping the left renal artery. Six weeks after surgery, hypertensive and sham rats were treated with Nebi (10 mg/kg/day), metoprolol (Meto; 20 mg/kg/day) or vehicle for four weeks. Systolic blood pressure (SBP) was monitored weekly by tail-cuff plethysmography. Structural changes of the aortic wall were studied in hematoxylin/eosin sections. MMPs levels and activity were determined by zymography and in situ zymography. NADPH oxidase activity and ROS production were evaluated by luminescence and dihydroethidium. The license number of the Animal Ethics Committe: 123/2011. **Results:** Similar reductions in SBP were found with both Meto or Nebi treatments (156 ± 8 mmHg and 151 ± 9 mmHg, respectively, versus 206 ± 7 mmHg in hypertensive controls; both $P<0.05$). However, only Nebi (all $P<0.05$) reversed aortic hypertrophy (aortic cross sectional area x 10^4 = 89±5, 68±3, and 80±8 μm^2, respectively, in the 2K1C, 2K1C+Nebi, and 2K1C+Meto groups), the increases in aortic MMP-2 levels (0.18±0.04, 0.07±0.02, and 0.12±0.06 arbitrary units; AU, respectively in the 2K1C, 2K1C+Nebi, and 2K1C+Meto groups), in aortic MMP activity (21071±700, 16217±818, and 18848±1396, respectively in the 2K1C, 2K1C+Nebi, and 2K1C+Meto groups), in aortic NADPH oxidase activity (253887±13712, 143765±15642, and 232465±14352 AU, respectively in the 2K1C, 2K1C+Nebi, and 2K1C+Meto groups) and in aortic ROS levels (8057±800, 4400±480, and 6230±1190 AU, respectively, in the 2K1C, 2K1C+Nebi, and 2K1C+Meto groups). No significant differences were found in the Sham groups. **Discussion:** Our results suggest that lower vascular NADPH oxidase activity associated with Nebi treatment may explain the attenuation of oxidative stress levels, the reduction of MMPs activity, and the improvement of hypertrophic remodeling observed in 2K1C animals. **Supported by:** FAPESP, CNPq, CAPES.

06.047 **Vascular hyporesponsiveness to vasoconstrictors**: **The involvement of no reservoirs**. Benedet PO, Ramos GC, Assreuy J UFSC – Farmacologia

Introduction: Nitric oxide (NO) plays an important role in the regulation of vascular tone. However, NO overproduction is involved in pathological processes such as hyporesponsiveness to vasoconstrictors, an important feature observed in septic shock. NO reacts to thiol groups of cysteine residues in a process called S-nitrosylation producing S-nitrosothiols. In the presence of free –SH groups, NO can be exchanged from a S-nitrosothiol, a reaction called transnytrosilation. The aim of the present study is to investigate the role of S-nitrosothiols as a storage form of NO, which may account for its long-lasting effects in the vasculature. **Methods**: Female Wistar rats were anesthetized with isoflurane and sodium nitroprusside (SNP) was infused over 30 min (250 nmol/kg/min-1i.v.). Six and 12h after SNP infusion, animals were prepared for invasive blood pressure measurements. We also compared the vascular responses to L-cysteine (as a free thiol group; 0,01-100 mg/kg-1i.v.) of naïve (CTR) and NO-loaded (SNP) animals. Moreover, methylene blue (MB 10 mg/kg-1i.v.) was used as an inhibitor of soluble guanylyl-cyclase (sGC). All procedures were approved by our institutional Animal Ethics Committee (PP00631/CEUA/UFSC). **Results**: Six hours after SNP infusion we observed a pronounced hyporesponsiveness to phenylephrine (10 nmol/kg; 27.8 ±2.8 mmHg SNP group compared to 44.7 ± 2.1 mmHg in CTR group; $p < 0.05$, $n = 7$). Basal MAP did not differ between groups (CTR 89.1±5.4 and SNP 87±3.5 mmHg). L-cysteine dose-dependently caused a hypotensive effect in SNP loaded but not in CTR animals (0,1 mg/kg cysteine caused 0,6 ± 0,6 and 16,1 ± 2.9mmHg; $n = 6$, reduction in MAP of CTR and SNP animals, respectively). The L-cysteine-induced vascular relaxation was reduced 80%by MB, thus suggesting involvement of sGC. **Discussion**: Our results demonstrate the NO donor SNP infusion induces long-lasting hyporesponsiveness to phenylephrine in rats. The NO-induced hyporesponsiveness to phenylephrine appears to depend on the availability of sulphydryl groups present in proteins. L-cysteine induces hypotension in NO-loaded animals indicating the formation of S-nitrosothiols storage and release. NO released from this stores causes hypotension soluble guanylate cyclase. The understanding of the role of nitrosylation of cysteine residues can help the development of therapeutic strategies to avoid hyporesponsiveness to vasoconstrictors during septic shock. **Financial support**: CNPq, CAPES, FAPESC and FINEP.

06.048 Contractile response induced by phenylephrine is modulated by eNOS phosphorylation and by hydrogen peroxide production in renal hypertensive rat aorta. Silva BR[1], Pernomian L[1], Grando MD[2], Bendhack LM[2] [1]FMRP-USP, [2]FCFRP-USP

Introduction: The endothelium plays important role on the vascular tone control. In cardiovascular diseases such as hypertension, the production of reactive oxygen species (ROS) induced by contractile agonists is increased in blood vessels, mainly in endothelial cells. **Aim:** This study aimed to evaluate the role of the endothelium and EROs to the contractile response induced by phenylephrine (PE) in isolated aorta from renal hypertensive rat (2K-1C) as compared to normotensive sham-operated rat (2K). **Methods:** Concentration-effect curves for PE were constructed in intact endothelium rat aorta (E+) and in denuded rat aorta (E-), in the absence or in the presence of the superoxide scavenger (Tiron 0.1 and 1 mmol/L) or Catalase (30, 90, 150 and 300U). The potency (pD_2) and efficacy (Emax) of PE in inducing contraction were evaluated. The expression of Ser^{1177} phosphorylated endothelial nitric oxide sintase (eNOS) was evaluated by Western Blotting. In isolated aortic endothelial cells, it was measured the fluorescence intensity (FI) of the ROS sensitive dye (DHE) by flow citometry. The endothelial cells were stimulated with 0.1 µmol/L PE for 10 min and the FI was measured in the absence (control) and presence of Tiron 0.1 and 1 mmol/L, or the enzyme that catalyses the degradation of hydrogen peroxide (H_2O_2), Catalase 300 and 3000U, or the combination of both Tiron and Catalase. In organ chamber, aortas (E+) and (E-) were stimulated or not (basal) with 0.1 µmol/L PE and H_2O_2 production was measured using Amplex red H_2O_2 assay kit. This study was approved by the Ethical Committee of the University of São Paulo (156/2009). **Results:** The contractile response induced by PE was not different between aortas E- from 2K (Emax: 2.5±0.2g, pD_2: 7.93±0.10; n = 8) and 2K-1C (Emax: 2.6±0.2g; pD_2: 7.89±0.08; n = 9). However, the contractile response induced by PE was less potent in aortas E+ than in aortas E-, in both 2K and 2K-1C. In aortas E+, the Emax was decreased only in 2K-1C (1.2±0.2g, n = 7; $p<0.001$). Pre-incubation with Catalase, partially reversed the decreased contractile response induced by PE in 2K-1C E+ (1.9 ± 0.1g, n = 4; $p<0.001$). PE induces higher Ser^{1177} phosphorylation of the eNOS in 2K-1C aorta than in 2K aorta. The basal FI to DHE was not different between the endothelial cells from 2K (43,388.5 ± 869.8; n = 4) and 2K-1C (37,373.0 ± 536.2; n = 4). PE increased the FI only in endothelial cells from 2K-1C (68,911.5 ± 4,146.3, n = 4, $p<0.001$). The increase in EROs was reduced to the basal levels by the antioxidants. In both 2K and 2K-1C, the basal H_2O_2 production was higher in aortas E+ as compared with aortas E-. However, PE stimulated higher H_2O_2 production in 2K-1C (E+ or E-) as compared with 2K (E+ or E-), respectively. **Conclusion:** Our results indicate that the reduced contractile response induced by PE in intact endothelium rat aorta from 2K-1C is due to high eNOS-Ser^{1177} phosphorylation and H_2O_2 production in the endothelial cells. Supported by FAPESP, CAPES and CNPq.

06.049 **The vascular relaxation induced by the nitric oxide donor and cyclooxygenase inhibitor compound (NCX2121) is potentiated by the endothelium**. Paula TD[1], Silva BR[2], Bendhack LM[1]
[1]FCFRP-USP – Physics and Chemistry, [2]FMRP-USP – Pharmacology

Introduction: The vascular endothelium plays important role on the control of vascular tone due to the release of contractile factors (EDCFs) as well relaxant factors from endothelial cells (EDRFs). In some cardiovascular diseases such as hypertension the mechanisms of balance between EDCFs and EDRFs are impaired with augmented levels of reactive oxygen species (ROS) and EDCFs. ROS can alter the signaling, production and/or bioavailability of the major EDRF, nitric oxide (NO). In addition, ROS increases the concentrations of prostanoids like prostaglandins (PGs) and thromboxane (TXs), major EDCFs, produced by cyclooxygenase (COX). The renal hypertensive rats 2 kidney-one clip (2K-1C) present augmented levels of angiotensin II (Ang II) that activates AT1 receptors and production of ROS like O_2^- and H_2O_2, activation of the enzyme NADH/NADPH oxidase, the main ROS production pathway in the endothelial cells. The compound NCX2121 can act as NO donor and COX inhibitor. The present study aimed to characterize the relaxation induced by the compound NCX2121 in 2K-1C rat aorta and to investigate the role the endothelial cells in this response. **Methods:** Renovascular hypertension was induced by implantation of silver clip in the left renal artery of the anesthetized rats (weighing 180–200 g). Another rats group was submitted to similar procedures but without the renal-artery clip placement (normotensive group or 2K). After 6 weeks, the systolic arterial pressure (SAP) was measured and the rats with SAP ≥ 160 mmHg were considered hypertensive. The rats were killed under anesthesia with isoflurane and aortic rings (4mm length) were removed and mounted in organ chambers for isometric tension measurements. The integrity of the endothelium was tested with acetylcholine (1 mmol/L). Cumulative concentration-effect curves were constructed for NCX2121 (1 nmol/L-10 mmol/L) in aortic rings with endothelium (E+) or without endothelium (E-). The pharmacological values of potency (pD_2) and efficacy (maximum effect, ME) were analyzed. All the procedures were performed in accordance to the Ethics Committee of the University of São Paulo (CEUA No. 12.1.120.53.0) **Results:** NCX2121 induced relaxation in a concentration- dependent way, and the presence of the endothelium increased the pD_2 in 2K (E+): (6.94 ± 0.70 n = 3) *vs* (E-): (4.68 ± 0.30 n = 3) and in 2K-1C (E+) (7.38 ± 0.37 n = 4) *vs* (E-) (3.87 ± 0.12 n = 4). The relaxation was not different in 2K-1C and 2K aortic rings (E+). On the other hand, in the aortic rings (E-) the ME was lower in 2K-1C (8.5 ± 3.1% n = 4) than in 2K (29.2 ± 6.2%, n = 3). **Conclusion**: Taking together, these results show that the compound NCX2121 produces aortic relaxation in both normotensive and renal hypertensive rat aorta in a similar way in the presence of vascular endothelium. However, in denuded aortic rings, the relaxation is impaired in 2K-1C rat aorta. **Financial support**: CNPq and Fapesp.

06.050 **Role of B1 kinin receptor and nitric oxide in arterial coronary reactivity of angiotensin II hypertensive rats**. Ceravolo GS[1,2], Soares AG[1], Silva MA[2], Tostes RC[1], Fortes ZB[1], Carvalho MHC[1]
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Coronary blood flow is highly regulated to ensure an adequate matching of coronary perfusion to meet the metabolic demands imposed by beating heart. The endothelium, mainly by nitric oxide (NO) release, is able to adjust the coronary flow and it function can be modified in some cardiovascular diseases, such as hypertension. Angiotensin II (A II) is a pleiotropic factor involved in the regulation of multiple systems. Of prime importance is the pro-inflammatory effect of A II in the vasculature, which leads to vascular dysfunction. The inducible kinin B1 receptor (B1R) has been described to be also important in cardiovascular homeostasis and inflammation. Because B1R is expressed only in response to injury, as A II infusion, we hypothesize that it could play a role in the development of coronary dysfunction induced by A II. Methods: Male Wistar rats were infused with A II (400ng/kg/min, 14 days, A II rats), saline (C rats) or A II (400ng/kg/min) plus B1R antagonist des-Arg9-Leu8-bradykinin (350ng/Kg/min, DAL rats), via osmotic minipumps subcutaneously implanted. Afterwards, the septal coronary artery (CA) was mounted on a wire myograph and the acetylcholine (ACh, 100pM-100μM) and sodium nitroprusside (SNP, 100pM-10μM) relaxation were evaluated. In another series of experiments, the thromboxane mimetic (TxA2, 100pM-100μM)-induced contraction in CA was evaluated in the presence or absence of L-NAME (100μM), L-NIL (1μM), catalase (100U/mL), DAL (1μM) or HOE140 (1μM). The cardiac eNOS, iNOS and nNOS mRNA levels were determined by real time RT-PCR. The results are expressed as mean±SEM; (n) is the number of animal/group. Statistical analyses: ANOVA ($p<0.05$; *vs. C; #vs. A II). The procedures were approved by ethics committee of the Institute of Biomedical Sciences-USP (n.98). Results: ACh-induced relaxation was decreased in CA of A II and DAL as compared to C (Emax: C: 87±4 vs. A II: 74±5* vs. DAL: 73±4*% relaxation; n = 10/group). SNP-induced relaxation was similar among groups. The TxA2-induced vasoconstriction was reduced in CA of A II when compared with DAL and C (Emax: C: 160±4 (10) vs. A II: 84±10* (8) vs. DAL: 151±7# (5)%). LN incubation increased TxA2 contraction in CA of all groups [C: 201±15 (9) vs A II: 206±10# (12)%]. The incubation either with LNIL [C: 167±5.2% (10); A II: 176±7# %(5)]; catalase [C: 159±5% (6); A II: 161±10# % (5)] or B1R antagonist [C: 150±2.6 (6)%; A II: 164±23# (5)%] increased the vasoconstriction in CA of A II but did not interfere with C. The B2R antagonism increased contractile response in C and A II groups [C: 174±7.6*% (6); AII: 186±19#% (8)]. The eNOS mRNA expression was reduced in A II and DAL heart when compared with C [C: 2.6 ± 0.6 (8) vs A II 1.06±0.2* (5) vs 1.09±0.1* (10)]. The iNOS [C: 1.1±0.2 (10) vs A II: 2.9±0.6* (9) vs DAL 1.6±0.2*# (6)] and nNOS [C: 2.8±0.2 (5) vs A II: 15±2.3* (5) vs DAL 9.8±2.5*# (6)] mRNA expression in A II and DAL's heart was increased vs C's heart. Discussion: We observed that the response to a vasoconstrictor was decreased in CA of A II rats, this decreased response can be related with increased: B1R expression; NO produced by iNOS or nNOS and hydrogen peroxide production. Support: FAPESP/CNPq.

06.051 **Rats with heart failure induced by myocardial infarction display erectile dysfunction *in vivo***. Rodrigues FL, Doi MG, Tostes RC, Carneiro FS FMRP-USP – Pharmacology

Introduction: The indices of erectile dysfunction (ED) in heart failure (HF) are alarming and different mechanisms have been proposed as causes of ED in HF. However, no study to date has evaluated the effects of HF on the cavernous tissue. In the present study, we evaluated the erectile function, as well as the cavernosal contractility and relaxation in rats with HF. **Methods**: All procedure were reviewed and approved by the Ethics Committee in Animal Research of the School of Medicine of Ribeirão Preto (protocol n^{o} 119/2011). Myocardial infarct was induced by coronary artery ligation. Changes in the ratio of intracavernosal pressure/mean arterial pressure (ICP/MAP) after electrical stimulation of cavernosal nerve were determined *in vivo*. Cavernosal contractility was induced by electrical field stimulation (EFS) and phenylephrine (PE). In addition, nonadrenergic-noncholinergic (NANC) and sodium nitroprusside (SNP)-induced relaxation was determined. **Results**: Rats with HF display a significant decrease in maximal ICP/MAP responses (HF: 0.50 ± 0.04 vs. control: 0.74 ± 0.02 at 16 Hz, $p<0.05$). Contractile responses to EFS or PE were not different between cavernosal strips from HF or control rats. However, in the presence of L-NAME (10^{-4} mol/L) plus atropine (10^{-6} mol/L), contractile responses to EFS were increased in HF rats (HF: 760.30 ± 123.60 vs. control: 537.40 ± 80.10 mN/g of dry tissue, at 32 Hz, $p<0.05$). HF tended to increase the SNP-induced relaxation (pD_2 in HF: 8.47 ± 2.87 vs. control: 6.73 ± 0.28), although the differences were not statistically significant. NANC-induced relaxations were increased in HF rats at low frequencies (HF: 21.77 ± 6.34 and 40.17 ± 6.94 vs. control: 6.38 ± 1.31 and 17.48 ± 6.68 percentage of relaxation, at 2 and 4 Hz respectively, $p<0.05$). **Conclusion**: HF rats display ED *in vivo* and the increase in sympathetic activity can contribute to ED in this model. However, they exhibit changes in cavernosal reactivity that suggests the existence of compensatory mechanisms in cavernosal tissue. **Financial Support:** CAPES, FAPESP and CNPq.

06.052 **Functional cardiac analysis in endotoxemic rats**: **Gender-linked differences**. Gonçalves RPM, Guarido KL, Assreuy J, da Silva-Santos JE UFSC – Farmacologia

Introduction: Several studies have been realized about heart function during sepsis however possible gender-specific differences of cardiovascular dysfunctions have been scarcely investigated. **Methods:** We evaluated the *in situ* cardiac function in male and female Wistar rats by using the pressure-volume catheter (SPR-901, Millar Instruments Inc.). The rats were injected with *E. coli* lipopolysaccharide (LPS, 10 mg/kg, i.p; n = 7) 6 or 24 h before the experiment. The anesthetic protocol used was xylazine (2mg/Kg, i.p.) followed by isoflurane (1-3%, inhalation) and further lidocaine (surgical sites). The catheter was inserted into the left ventricle by the right carotid artery. The data were obtained for baseline and intravenous injection of phenylephrine (PE; 30 nmol/Kg) analysis. This study was approved by the local Animal Care and Use Committee (protocol PP00566). Statistical comparison among the groups was performed using two-way ANOVA followed by Bonferroni's post hoc test. **Results:** Male (M) and female (F) rats from LPS 6 h groups presented similar increased heart rate when compared to control groups (CTR) (333 ± 42 and 357 ± 22 *vs* 254 ± 40 and 279 ± 32 bpm, respectively, for M and F; $p<0.001$). However, the augmented heart rate persisted only in F-LPS 24 h group. Intrinsic myocardial depression observed in F-LPS 6 h was associated with reduced ejection fraction (EF = 41% *vs* 79% in F-CTR) and reduced dP/dt_{max} index (40%), $p<0.001$. In addition, F-LPS 6 h showed that values are increased in maximum and minimum volume, end-systolic volume, $V@dP/dt_{max}$ and $V@dP/dt_{min}$ (34%, 167%, 133%, 19% and 128%, respectively; $p<0.05$). All of these parameters presented similarly increasing in M-LPS 6 h but without statistical significance. The cardiac output remained increased by 67% ($p<0.01$) although the basal contractile parameters as well as the basal left ventricle volume data observed in M and F-LPS 24 h groups were similar to their respective CTR groups. The overload pressure developed after PE injection induced new intraventricular volume congestion in all LPS groups (i.e. M and F-LPS 6 and 24 h) but only F-LPS groups were statistically significant (V_{max}, V_{min}, V_{es}, $V@dP/dt_{max}$ and $V@dP/dt_{min}$; $p<0.05$). PE injection also induced reduction of ventricle performance parameters as maximum pressure (111 ± 8 *vs* 138 ± 7 and 112 ± 3 *vs* 130 ± 6 mm Hg), developed pressure (114 ± 8 *vs* 139 ± 6 and 112 ± 3 *vs* 132 ± 6 mm Hg), intraventricular end-systolic pressure (105 ± 8 vs 132 ± 7 and 109 ± 4 *vs* 127 ± 7 mm Hg) and $P@dP/dt_{max}$ (63 ± 7 *vs* 80 ± 4 and 62 ± 2 *vs* 77 ± 2 mm Hg) ($p<0.05$) in both LPS 6 h genders (M and F, respectively). While there was no difference in arterial elastance ($p>0.05$) over PE injection in all groups, M and F-LPS 6 h showed reduced afterload increasing. **Discussion:** According to our results, female rats appear to be more susceptible than male rats in developing cardiac dysfunction in early stages of endotoxic shock. Nevertheless, in late stages both genders improved their cardiodynamic profile; the PE injection significantly impaired the cardiac function in female, but not in male rats. The better understanding about the mechanisms underlying gender differences in the cardiac performance during the endotoxic shock could be helpful to improve the clinical management of septic patients. **Research support:** CNPq and FAPESC

06.053 **Mechanism of action of the total poison *Apis mellifera* in ring of isolated aorta**. Rodrigues FA[1], Sousa PCP[1], Brito TS[1], Sousa DF[1], Magalhães PJC[1], Toyama MH[2], Costa PHS[1], Monteiro HSA[1], Havt A[1] [1]UFC – Physiology and Pharmacology, [2]UNESP – Biochemistry

Introduction: The bee venom is rich in peptides. PLA2 is a major immunogenic component of bee venom and can contribute to the overall toxicity in poisoning by a synergistic interaction with melittin (SCHUMACHER *et al,.Arch. Intern. Med.,* 155, p. 2038, 1995; GRISSOTO *et al.,Toxicon*, vl 48, p. 44, 2006). However, hydrolysis products of the PLA2 can serve as precursors of the mediators, such as leukotrienes and prostaglandins (DOTIMAS, *Bee World*, 68, p. 51, 1987). Some experiments have reported hypotension after inoculation of the venom of *Apis mellifera* (VAM). Pathophysiological functions of melittin are: hemolytic activity, depolarization of the heart muscles, causing necrosis, facilitating the entry of other poison components in the circulatory system of the victim. However, depending on the venom concentration in the tissue, this may cause vasoconstriction or vasodilatation (OWNBY *et al.,***J** *Toxicon* 37, p. 411 1997). **Methods:** This study examined the possible changes in contractions of rat aorta ring using different concentrations of the whole bee venom aiming to investigate its mechanism of action. Aortic rings of male Wistar rats (250 - 300 g) were kept in Krebs-Henseleit (pH 7.4, 37° C, continuously aerated with carbogen mixture, baseline tension = 1 g). Isometric contractions were recorded by force transducers, connected to the digital system of data acquisition (PowerLab, ADI, Australia). To verify a possible mediating mechanism of the poison's vascular action, tests were performed in endothelium intact aortic rings with the administration of an alpha-adrenergic antagonist (Phentolamine 5μM, n = 05), Calcium Channel blocker (verapamil 5μM, n = 05) and an inhibitor of phospholipase-C (10μM U-73122, n = 05). After the equilibration period of the tissue, the VAM was added cumulatively (0.1 - 50 mg/mL) in a concentration-dependent manner. Data was analyzed by ANOVA and Bonferoni test as post test ($p < 0.001$). (This study was approved by Ethics Committee (protocol no. 01/12). **Results and discussion:** VAM (50 mg/mL) caused a contraction corresponding to 92,6471 ± 5, 5512 % (n = 5) of the contraction induced by 60 mM of K+. Another effect was also observed with VMA + phentolamine (26,8028± 5, 629332 %) (n = 5); VMA+Verapamil (21,7321± 3,347021%) (n = 5) and VMA + U-73122 (38,4277± 13,7485 %) (n = 5). This smooth muscle's kind of contraction is already well known by means of the opening of voltage-gated Ca2+ channels (SOMLYO & SOMLYO, **Nature,** 372, p. 231, 1994) and by activating receptor-operated G-protein-dependent cascades that culminate in inositol-triphosphate (IP3) and diacylglycerol (DAG) production (HASHIMOTO *et al.,* J. **Physiol**. 370, p. 605, 1986). It was observed a significant reduction in the contractile effect of the whole venom in the aortic ring. This suggests the involvement of alpha-adrenergic receptors in the tissue contraction via phospholipase-C. **Financial support**: CNPq, RENORBIO/UECE, and UFC.

06.054 **Reduced cardiovascular alterations of arthemeter loaded in pcl nanocapsules**. Vidal-Diniz AT[1], Andrade RS[1], Guimarães HN[2], Grabe-Guimarães A[1], Mosqueira VCF[1] [1]Cipharma-UFOP, [2]UFMG – Engenharia Elétrica

Introduction: Despite the technological and scientific development, malaria remains a major health problem in Brazil and worldwide. Modern strategies for the control of the disease include the development of new therapeutic agents and the optimization of the activity of drugs already used (Santos-Magalhães e Mosqueira, 2010). Arthemeter (ATM) is effective and can improve malaria control. However, it has short half-life and is used only by intramuscular route. Aditionally, it presents frequently QT interval prolongation on electrocardiogram (ECG) (Brewer *et al.*, 1994; White, 2007). In this context, the ATM loading in nanocapsules could be useful to reduce the dose number, improve efficacy and reduce toxicity. **Objective:** The main goal of the present work was to assess and evaluate ECG and arterial blood pressure (AP) changes induced by intravenous (IV) administration of a single high dose ATM base loaded-nanocapsules or arthemeter solution in anaesthetized Wistar rats. **Methodology:** The NC containing ATM was prepared by the nanoprecipitation method and characterized. Free ATM preparation was obtained by dissolution in a Tween/PEG/glucose solution. The NC containing ATM was administrated IV to male Wistar rats (80.0 mg/kg). APECG (limb lead II) signals were obtained for two hours, and the cardiovascular parameters were compared to animals that received intravenous injection of ATM in free form or empty nanocapsules. All procedures related to the use of animals in these studies were approved by the local ethics committee under number 03/2011. **Results:** NC obtained showed 250nm mean size and <0.25 polidispersity index indicating homogenous and monodispersed populations. The ATM association with NC induced no size change but zeta potential was reduced in 5.8mV, indicatingthat part of drug could be located in the surface of the the NC. Animals that received free ATM it was observed a significant decrease in systolic and diastolic pressure (42% and 48%, respectively). PR, QRS and QT intervals of ECG were increased 9.9, 6.1 and 32%, respectively. This effect was reduced when ATM was administered entrapped in nanocapsules – 13 and 15% ofsystolic and diastolic pressure decrease and 5.8, 5.2 and 18.6% of PR, QRS and QT intervals increase respectively. **Discussion:** The ATM base loaded-nanocapsules showed reduced cardiotoxic profile when compared to the free form. Our group has already demonstrated that drugs encapsulation can reduce the cardiotoxicity (Leite *et al.*, 2007, Vidal *et al.*, 2010 and Maciel *et al.*, 2010). These findings show that the encapsulation of ATM reduces the QT interval prolongation of ECG in rats and suggest that a modification of drug distribution, possible by using nanocapsules. ATM encapsulation was the main factor responsible for the significant reduction in cardiac toxicity observed. **Financial Support**: Coordenação Geral do Programa Nacional de Controle da Malária – CGPNCM, Ministério da Saúde/MS, NANOBIOMG/FAPEMIG network, CAPES.

06.055 **Effects of the hydroalcoholic extract of *Euterpe oleracea* Mart (açaí) on glucose metabolism and oxidative damage in C57BL/6 mice fed a high fat diet**. Oliveira PRB[1], Costa CA[1], Rocha APM[2], Bem GF[1], Amaral TAS[1], Cordeiro VSC[1], Carvalho LCRM[1], Conceição EPS[3], Soares de Moura R[1], Resende AC[1] [1]UERJ – Farmacologia e Psicobiologia, [2]UNIRIO, [3]UERJ – Fisiologia

Introduction: Metabolic syndrome (MS) is identified as an association of risk factors, which is strongly associated with a high cardiovascular morbidity and mortality. Insulin resistance appears to play a central role in the pathogenesis of MS and lipid disorder is underlying the etiology of this syndrome. Many aspects of MS can be induced in experimental models such as C57BL/6 mice by manipulation of the diet given. Among the various substances extracted from plants, polyphenols have shown great therapeutic potential, since it reduces the incidence of cardiovascular disease and its benefits may be associated with antioxidant action, vasodilator and antihypertensive. The *Euterpe oleracea* Mart (açaí) is a typical plant of the tropics, rich in polyphenols. Thus we evaluated the effect of the açai seed extract (ASE, 300 mg / kg / day) on changes in lipid and glucose metabolism and oxidative stress in C57BL/6 mice fed a high-fat diet. **Methods:** These experiments were approved by the Ethics Committee of UERJ (protocol: CEA/025/2010). Male mice C57BL/6 with 30 days old were divided into four groups and received the following diets for 12 weeks: control group (C): standard diet; control group ASE + (C + ASE): ASE + standard diet; group lipids (H): high-fat diet; group lipids ASE + (H + ASE): ASE + fat diet. We evaluated body weight, plasma and liver lipids, blood glucose, plasma insulin, hepatic lipid peroxidation, expression of SOD-2, IRβ, IRS1, IRS1 phosphorylated (pIRS1), PI3-K, AKT, phosphorylated (pAkt) and tubulin protein in liver homogenate. **Results:** Body weight was increased in group H compared with the control group and decreased in group H + ASE compared to group H. It was observed that the C57BL/6 mice fed a high-fat diet also showed hyperglycemia, hyperinsulinemia, glucose intolerance, insulin resistance (index calculated by HOMA) and hyperlipidemia (plasma and liver) and the ASE significantly reduced all of these parameters. In addition, was observed a decreased expression of proteins IRβ, IRS1, IRS1 phosphorylated (pIRS1), PI3-K, AKT, phosphorylated (pAkt) and SOD2 in H group and the treatment with ASE promotes a significantly increase in expression of these proteins. The levels of malondialdehyde (MDA) were higher in liver samples from group H, and the ASE decreased these levels in group H + ASE. **Discussion:** The data together show that the ASE protected C57BL/6 mice fed a high-fat diet against weight gain, dyslipidemia and insulin resistance. The antioxidant activity and increased insulin sensitivity may be contributing to these benefits effects of ASE, suggesting a possible use of ASE as a tool in the treatment of these components that characterize MS. **Financial Support:** CNPq and FAPERJ.

06.056 **Role of inducible nitric oxide synthase in the pathophysiology of experimental preeclampsia**. Amaral LM[1], Palei AC[2], Pinheiro LC[1], Sertorio JT[3], Guimarães DA[1], Portella RL[1], Tanus JE[1] [1]FMRP-USP – Pharmacology, [2]University of Mississippi – Physiology and Biophysics, [3]FCM-Unicamp – Pharmacology

Introduction: The pathophysiology of preeclampsia (PE) is not entirely known. However, increased oxidative stress possibly leading to impaired nitric oxide activity has been implicated in the critical condition. Increased oxidative stress with increased levels of highly reactive species including superoxide may generate peroxynitrite. We examined the role of inducible nitric oxide synthase (iNOS) and oxidative stress in the reduction uterine perfusion pressure (RUPP) preeclampsia experimental model. **Methods:** All experimental procedures executed in this study were in accordance with Ethics committee in animal (n.159/2010**)** at Faculty of Medicine of Ribeirao Preto, Brazil. RUPP was induced in wistar rats. Pregnant rats in the RUPP group had their aortic artery clipped at day 14 of gestation. After a midline incision, a silver clip (0.203 mm) was placed around the aorta above the iliac bifurcation; silver clips (0.100 mm) were also placed on branches of both the right and left ovarian arteries that supply the uterus. Sham-operated (pregnant control rats) and RUPP rats were treated with oral vehicle or 1 mg/kg/day 1400W (iNOS inhibitor) for 5 days. Mean arterial pressure (MAP) and plasma levels of thiobarbituric acid-reactive species (TBARS) and total radical-trapping antioxidant potential (TRAP) were measured determined. Aortic iNOS expression (Western blotting) and reactive oxygen species (ROS; assessed by fluorescence microscopy with dihydroethidium-DHE) were measured. **Results:** We found increased mean arterial pressure in RUPP compared with pregnant control rats (MAP = 128±1 *vs.* 100±1.8 mmHg, respectively; $P<0.05$) and 1400W exerted antihypertensive effects (MAP = 114±2 *vs.*128±1 mmHg in RUPP treated and untreated rats, respectively; $P<0.05$). Higher reactive oxygen species (ROS) concentrations were found in RUPP compared with pregnant control rats (7.1±0.5 *vs.* 5.1±0.5 arbitrary units (A.U.), respectively; $P<0.05$) and 1400W decreased ROS production to 5.8±0.02 A.U. in RUPP treated rats, $P<0.05$. In addition, 1400W attenuated iNOS expression in RUPP rats (0.29±0.02 *vs.* 0.55±0.8 A.U. in RUPP treated and untreated rats, respectively; $P<0.01$) and had no effects on plasma TBARS and TRAP levels. **Discussion:** Our results suggest that 1400w exerts antihypertensive effects in the RUPP model and suppresses ROS formation. **Financial support**: Fapesp

06.057 **NTHF**: **An organic nitrate with cardiovascular action without tolerance induction**. Furtado FF[1], Veras RC[2], Silva TAF[2], Queiroz TM[3], Alustau MC[3], Machado NT[3], Oliveira-Filho AA[3], Santos AF[3], Athayde-Filho PF[3], Medeiros IA[2] [1]CFP-ETSC-UFCG, [2]DCF-CCS-UFPB, [3]CCS-UFPB

Introduction: Tetrahydrofurfuryl nitrate (NTHF) is an organic nitrate obtained through of synthetic route from sugar cane. Usually, an organic nitrate promotes vasorelation due NO donation and soluble Guanylyl Cyclase (sGC)/Protein GMPc dependent (PKG) pathway activation. This activity also was observed with NTHF in endothelium-denuded superior mesenteric artery rings. A critical limitation in clinical application of the organic nitrates is the development of tolerance. Thus, is very important to determinate whether NTHF develops tolerance in chronic administration. The purpose of this study was to evaluate the cardiovascular effects and hemodynamic tolerance to NTHF using a combined *in vivo* and *in vitro* approach. **Methods**: All protocols were approved by CEPA/UFPB (protocol nº: 0310/09). For *in vivo* experiments, Wistar rats (250-300 g) were anesthetized with sodium thiopental (45mg/kg, i.v.). Abdominal aorta and inferior vena cava were cannulated for pressure recordings and administration of drugs, respectively. For *in vitro* experiments, isolated rat superior mesenteric rings (1-2 mm) were suspended by cotton threads for isometric tension recordings in a Tyrode's solution at 37 ºC, gassed with a 95% O_2 and 5% CO_2, under a resting tension of 0.75g. **Results and discussion:** Intravenous injection of NTHF (10, 20, 30, 40 e 50 mg/kg, randomly) produced a dose-dependent hypotension (-6.8±1.7; -13.8±3.9; -24.9±8; -44.7±5.5 and -56.8±4.6) and caused a significant bradycardia (-3.5±2; -8,9±0.6; -23.8±1.8; -80±3.7 and -88.8±2%). The effect of NTHF was unchanged by the blockade of ganglionic autonomic neurotransmission with hexamethonium (30 mg/Kg, i.v.), but was attenuated in the presence of inhibitor of sGC, methylene blue (MB, 3 mg/Kg i.v.). Rats treated with NTHF (unique dose of 200 mg/Kg, v.o.) for three days obtained similar responses to rats no-treated, when submitted to acute application (in bolus, i.v.) of different doses of NTHF. In isolated superior mesenteric rings of treated-rats, NTHF had same effect that rings from no-treated rats, suggesting that NTHF, in this concentration, was not promoted vascular tolerance. Furthermore, after prolonged presence (per one hour, followed by washing) of isolated concentrations of NTHF (10^{-6}, $3x10^{-6}$, 10^{-5}, $3x10^{-5}$ and 10^{-4} M), the vasorelaxant effect was not changed, opposite to result obtained with nitroglycerin (10^{-6} and 10^{-5} M). Our results demonstrate that NTHF induces a potent hypotensive and bradycardic effect MB-sensible. NTHF seems no induce tolerance as *in vitro* as *in vivo*. Such investigations in global are very important, suggesting that NTHF can be a organic nitrate that not cause tolerance but have cardiovascular effects by the pharmacodynamic action of NO. **Financial support:** CNPq and CAPES

06.058 **Vascular effects of spironolactone in an experimental model of type 2 diabetes mellitus**.
Silva MAB[1], Cau SBA[1], Lopes RAM[1], Bruder-Nascimento T[1], Manzato CP[1], Touys RM[2], Tostes RC[1]
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The renin-angiotensin-aldosterone system has been linked to hyperglycemia-associated microvascular and tissue injury. Aldosterone decreases nitric oxide production leading to endothelial dysfunction and other vascular abnormalities, similar to those induced by hyperglycaemia and diabetes-associated metabolic abnormalities. In addition, aldosterone levels are correlated with insulin resistance in patients and experimental models with diabetes. Thus, the aim of this study was to determine whether treatment with the mineralocorticoid receptor antagonist, spironolactone, is able to reduce or abolish the vascular dysfunction exhibited by mice with type 2 diabetes mellitus. Male db/db mice (C57BLKS/J^{Lepr}), a model of type 2 diabetes, and their nondiabetic controls (12-14 weeks old) were treated with spironolactone 50mg/kg/day or vehicle (ethanol 1%) by gavage for 6 weeks. At the end of the treatment, the animals were anesthetized for blood collection and euthanized by cervical dislocation. Mesenteric arteries were used in experiments of vascular reactivity (acetylcholine [Ach], sodium nitroprusside [SNP], phenylephrine [Phe], and insulin). Body weight, systolic blood pressure by tail cuff plethysmography, nonfasting glycemia, total cholesterol, triglycerides, sodium and potassium were also evaluated. The study was approved by the Animal Experiments Ethics Committee (CETEA/FMRP/USP) under the protocol nº 052/2012. At the end of the treatment period, db/db mice exhibited increased body weight [(g) 50.89±3.94 vs. 27.75±0.69, $p<0.05$], higher levels of plasma glucose [(mg/dL) 382.00±31.00 vs. 164.00±6.24, $p<0.05$) and total cholesterol [(mg/dL) 146.00±27.28 vs. 43.00±12.72, $p<0.05$) vs. control mice, clearly characterizing a metabolic disturbance secondary to obesity. None of these parameters were modified by the treatment with spironolactone. There were no significant differences in the values of systolic blood pressure, serum triglycerides and sodium between the experimental groups. Treatment of db/db mice with spironolactone increased serum potassium [(mEq/L) 6.60±0.18] vs. vehicle-treated db/db (5.60±0.50) and control mice (4.90±0.21), which is related to the potassium-sparing effect of this drug. There were no significant differences in Phe-induced contraction in vessels with or without endothelium between the experimental groups. However, endothelium-independent vascular relaxation mediated by SNP was greatly decreased in db/db mice treated with vehicle compared to controls (pD_2 = 6.25±0.06 vs. 7.21±0.04, $p<0.05$), and the treatment with spironolactone significantly reversed this dysfunction in db/db mice (pD_2 = 7.13±0.18, $p<0.05$ vs. vehicle-treated db/db). Insulin-induced vascular relaxation was severely impaired in db/db mice (E_{max} = 56.68±6.15 vs. 87.44±4.81, $p<0.05$). Spironolactone treatment reversed vascular resistance to insulin in db/db mice (85.05±8.02, $p<0.05$). Additionally, preliminary data suggest that the vascular relaxation induced by Ach, which is impaired in db/db animals compared to controls (pD_2 = 7.14±0.41 vs. 7.70±0.07) was restored in spironolactone-treated db/db mice (pD_2 = 7.50±0.20 vs. vehicle-treated db/db). Our results suggest that spironolactone improves vascular function in db/db mice without significantly altering the physiological and biochemical parameters. **Financial support**: FAPESP/CNPq.

06.059 **Impaired *in vitro* reactivity of corpus cavernosum of rats exposed to high-sodium diet**. Leitolis A, Linder AE, da Silva-Santos JE UFSC – Farmacologia

Introduction: Erectile dysfunction (ED) is a common disorder affecting millions of middle- aged men. Recently, it has been suggested that ED may precede and be used as an earlier indicative of susceptibility for other systemic and potentially deleterious cardiovascular diseases. Although the excessive ingestion of sodium has been putatively associated with cardiovascular diseases, mainly hypertension, the contribution of high salt intake on ED has never been investigated.

Methods: Male Wistar rats (2 months old) were exposed to high salt diet (4% NaCl) for 12 or 24 weeks (HS group). Control animals received regular chow (containing 0.27% NaCl). After this period the animals were anesthetized and killed, the penis was removed, and strips of corpus cavernosum (CC) were obtained. Each strip was placed in organ bath containing physiological saline solution (37 °C, aerated with 95% O_2/5% CO_2), and kept at a basal tension of 250 mg for isometric tension recording. After the stabilization period (60 min) the following experimental protocols were performed and the tension recorded: i) tissues were depolarized with KCl (80 mM) and washed, ii) strips were contracted by phenylephrine (PE, 1 mM) and washed, iii) concentration-response curves (CRC) to PE (10 nM to 100 mM) were obtained in the presence or absence of Y-27632 (1 mM, a Rho-kinase inhibitor), or, iv) a CRC to Y-27632 (30 nM to 1 mM) was obtained in PE-contracted preparations. All experimental procedures were approved in our Institutional Ethics Committee for Animal Experimentation (CEUA-UFSC; PP00706). **Results**: The CC of HS group (12 weeks of treatment) exhibited a decreased contraction in response to KCl (17.26% lesser than in control group). The incubation of Y-27632 similarly inhibited PE-induced contraction of CC in both control and HS group (12- and 24- week-treatment). However, the excessive ingestion of salt for 24 weeks resulted in a parallel rightward shift in the CRC to Y-27632 in PE-contracted CC, when compared to the effects obtained in CC from control group. In these experiments, the EC50-value was 74.2 (3.8-143.9 nM) and 1.2 (0.53-2.8 mM) in control and HS group, respectively. In addition, the magnitude and duration of contraction evoked by PE (1 mM) were increased in CC of HS group, when compared to those obtained in the control group. **Discussion**: Our results suggest that excessive and continuous intake of sodium can impair the functionality of the RhoA/Rho-kinase in CC of rats, an event that may have contributed to the increased responses to PE seen after 24 weeks of exposition to HS. Since the increased activity of the RhoA/Rho-kinase has been previously implicated in several models of experimental ED, the development of new studies may allow a better understanding regarding the relationship between excessive sodium intact and the development of ED. **Support**: Amanda Leitolis receives fellowship from CNPq for her graduate studies.

06.060 **Renal and cytotoxic effects promoted by venom total of snake *Bothrops pauloensis*.** Marinho AD, Jorge RJB, Morais ICO, Jorge ARC, Menezes RRPPB, Martins AMC, Monteiro HSA UFC – Fisiologia e Farmacologia

Snake envenoming is an important health problem widespread in tropical countries. According to the Department of Health of Brazil, the genus *Bothrops* are the main involved in snakebites in the country and acute renal failure (ARF) is a serious complication of snake poisoning. The study protocol was approved by ethics committees from the federal university of Ceará, in Fortaleza Brazil (n° 79/08). This study investigated the effects of the *Bothrops pauloensis* venom (vBp) in the renal perfusion system and in cultured renal tubular cells of the type MDCK (Mardin-Darby canine kidney). Isolated kidney from Wistar rats weinghing 250 to 300g. were perfused with Krebs-Henseleit solution containing 6%w/v of albumin serum albumin previously dialyzed. The effects of *Bothrops pauloensis* venom (10µg/mL) were studied on the Perfusion Pressure (PP), Renal Vascular Resistance (RVR), Urinary Flow (UF), Glomerular Filtration Rate (GFR), Percentage of Sodium (%TNa+), Potassium (%TK+) and Chloride (%TCl-) Tubular Transport. The treatment with vBp caused decrease in cell viability to the lowest concentration tested with an IC50 of 4,18 µg/mL. *B. pauloensis* venom (10 µg/mL) reduction the PP (PP30: 96.23**+** 4.44; PP90: 60.28 **+** 7.94*; PP120: 58.81 **+** 9.54*) and RVR (RVR30: 4.72**+** 0.60; RVR90: 3.06**+** 0.55*; RVR120: 3.16**+** 0.68*) at 90 and 120 min. The UF increased at 120 min (UF30: 0.14**+** 0,01; UF120: 0.34**+** 0,05*). The GFR decreased at 60 min (GFR30: 57 **+** 0,05; GFR90: 0.32 **+** 0,04*). It was also observed a decrease on percentual tubular transport of sodium (%TNa^{+}); of chloride (%TCl^{-}) and potassium (%TK+) at 60, 90 and 120 min (% TNa^{+}30: 80.48**+** 1.51; % TNa^{+}60: 64.48**+** 2.63*; % TNa^{+}90: 55.56**+** 4.70*; % TNa^{+}120: 62.32**+** 2.98*; (% TK^{+}30: 79.54**+** 1.47; % TK^{+}60: 63.46**+** 2.58* ; % TK^{+}90: 55.31**+** 4.70*; % TK^{+}120: 62.33 **+** 2.98*;% TCl^{-}30: 65.09**+** 3.71; % TCl^{-}60: 50.17**+** 3.83*; % TCl^{-}90: 50.51**+** 4.72*; % TCl^{-}120: 57.55**+** 2.98*). Histological analysis of kidneys perfused with vBp showed the presence of significant morphological changes such as accumulation of proteins in tubular and glomerular spaces. The venom of *B. pauloensis* (VBp) was able to decrease cell viability under the study conditions, by showing cytotoxicity effect up to 3.12 mg/mL (IC_{50} = 4.18 mg / mL), and promoting an inhibition on the cell growth in a dependent-concentration. The venom of *B. pauloensis* (VBp) suggests a nephrotoxicity. Other studies, with fractions of VBp will be conducted to ascertain which is the fraction involved in the role of the toxic effects in the kidney and in isolated and MDCK culture cells and also to investigate the mechanisms involved in cell death.Financial Support from CNPq.

06.061 Orchidectomy enhances the expression of endothelin-1 and ET_B receptors in rat portal vein. Rossignoli PS[1,2], De Labio RW[3], Payão SLM[3], Pereira OCM[1], Chies AB[2] [1]IB-USP – Pharmacology, [2]FAMEMA – Pharmacology, [3]FAMEMA – Genetics

Introduction: A previous functional study has shown that orchidectomy induces increase of the phenylephrine contractile effects in rat portal veins, which is completely prevented in presence of both ET_A and ET_B receptors antagonists (Rossignoli et al., Clin Exp Pharmacol Physiol, 37, 368, 2010). In this sense, the aim of the present study was to verify if orchidectomy increases the local expression of ET-1 as well as ET_A and ET_B receptors in rat portal vein. **Methods:** The study was approved by the Research Ethics Committee of the School of Medicine at Marília (protocol 268/09). Male Wistar rats (350-400g) were sham-operated (CONT) or orchidectomized (ORX). In the 23th day, the treatment (for 3 weeks, with 5-day intervals between the doses) was started with vehicle (CONT and ORX) or testosterone propionate (ORX+T) (10mg/kg, i.m.). The effectiveness of the orchidectomy and testosterone replacement was evaluated by plasma testosterone level and the wet weight of sexual accessory hormone-dependent organs (Matsuda et al., Am J Physiol, 267, H887, 1994). The expression of ET-1, ET_A and ET_B receptors in portal veins taken from CONT, ORX and ORX+T animals were determined by Real Time RT-PCR (Tirapelli et al., *Br J Pharmacol*, 146, 903, 2005). **Results:** Orchidectomy significantly reduced plasma testosterone level in ORX animals and, moreover, the hormone replacement treatment not only restored but even promoted supraphysiological levels of this hormone in ORX+T animals. In addition, orchidectomy reduced significantly the wet weight of sexual accessory hormone-dependent organs whereas the testosterone replacement treatment reverted completely this orchidectomy-induced atrophy. Orchidectomy induced a significant increment of the ET-1 and ET_B receptor expression in rat portal veins, which was completely reversed by the testosterone replacement treatment. **Discussion:** The results of plasma testosterone level and wet weight of sexual accessory hormone-dependent organs reinforce the effectiveness of our experimental model, thereby supporting the results obtained by real time RT-PCR that show orchidectomy-induced increment of the ET-1 expression in portal vein. This increment of the ET-1 expression was reversed by testosterone treatment, confirming the pivotal role of this hormone in this phenomenon (Takahashi et al., Naunyn Schmiedebergs Arch Pharmacol, 366, 166, 2002; Takahashi et al., BJU Int, 92, 803, 2003). In addition, orchidectomy increases the expression of ET_B receptors in portal veins but it does not imply in modification of responsiveness to exogenous ET-1. Perhaps it indicates that there is a counterbalance between ET_B receptors present in endothelium (Mazzuca et al., Biochem Pharmacol, epub ahead of print, 2012) and ET_B receptors overexpressed by orchidectomy in the smooth muscle cells. Thus, the findings of the present study support the previously proposed hypothesis that orchidectomy promotes an increment of the local expression of ET-1, thereby increasing the α_1-adrenoceptor-mediated contractile effects of phenylephrine on the portal vein. **Financial Support:** FAPESP (Proc. 09/08012-2).

06.062 The effect of exercise on microvascular rarefaction and hypertension in rats under long-term high-fat-diet. Machado MV[1], Vieira AB[2], Nascimento A[1], Conceição FG[1], Santos S[1], Bonomo I[1], Lessa MA[1], Tibiriçá E[1] – [1]IOC-Fiocruz – Cardiovascular Investigation, [2]IOC-Fiocruz – Laboratory of Inflammation

Introduction: Increased visceral fat is associated with several metabolic and cardiovascular disorders. Moreover, obesity and glucose intolerance are related to different microvascular alterations, such as the reduction in the density of microvessels in the skeletal muscle. The exercise has been used to reverse the risk factors associated with obesity. However, the optimal level of exercise needed to prevent and treat these abnormalities is not yet fully defined. This study aimed to investigate the influence of the training session duration, the number of weekly sessions and the intensity of exercise on microvascular rarefaction and hypertension in obese rats. **Methods:** Eighty Wistar rats were submitted to normal (CON, n = 8) or high fat diet (HFD n = 72) during 32 weeks. Animals that fed HFD were divided in sedentary (HFD SED) and 8 exercise training groups, divided in weekly frequency (3 or 5 times), duration (30 or 60 minutes) and intensity (60 or 80% the maximal incremental test), as: 1) HFD+TR 3.30.60; 2) HFD+TR 3.30.80; 3) HFD+TR 3.60.60; 4) HFD+TR 3.60.80; 5) HFD+TR 5.30.60; 6) HFD+TR 5.30.80; 7) HFD+TR 5.60.60; 8) HFD+TR 5.60.80. Aerobic training was performed in the last 12 weeks of study. At the end of training period, systolic blood pressure was evaluated using a tail-cuff system and functional capillary density was evaluated in the gracilis muscle using intravital videomicroscopy after intravenous injection of fluoresceine coupled to dextran. All procedures involving care and use of laboratory animals were approved by the Ethics Committee of the Fiocruz (license number LW-21/10). **Results and Discussion:** HFD induced a reduction in the number of spontaneously perfused capillaries in the skeletal muscle of the HFD SED group (228.0 ± 19.18 capillaries/mm^2) compared with CON group (264.6 ± 23.26 capillaries/mm^2). However all exercise training protocols reversed this rarefaction (240.8 ± 29.18 to 261.0 ± 77.52 capillaries/mm^2). Trained rats also showed a decrease in systolic blood pressure (151.73 ± 9.12 vs. 133.68 ± 9.35 to 124.77 ± 12.80 mmHg, $p<0.05$). These results suggest that all protocols of exercise training reverse skeletal muscle capillary rarefaction in association with decreases in blood pressure in obese rats. Financial Support: FAPERJ and CNPq.

06.063 **Atorvastatin and sildenafil attenuate the 2K1C-hypertension-induced MMP-2 upregulation through antioxidant effects**. Guimarães DA[1], Rizzi E[1], Ceron CS[1], Martins-Oliveira A[1], Gerlach RF[2], Tanus-Santos JE[1] [1]FMRP-USP – Farmacologia, [2]FORP-USP – Morfologia, Estomatologia e Fisiologia

Introduction: Hypertension-induced vascular hypertrophy is associated with oxidative stress and matrix metalloproteinase (MMP) up-regulation. Atorvastatin (ATORVA) and sildenafil (SILD) exert beneficial effects on cardiovascular diseases through their pleiotropic effects. We evaluated the effects of ATORVA and SILD on vascular changes induced by MMPs and on oxidative stress and MMP activity in 2 kidney-1 clip (2K1C) hypertension. **Methods:** Sham and 2K1C rats were treated with vehicle, ATORVA (50 mg/kg), SILD (45 mg/kg) or both for 8 weeks. Systolic blood pressure was assessed weekly throughout the experiment period by tail-cuff plethysmography. Morphometry of structural changes in the aortic wall were studied in hematoxylin/eosin sections. Aortic MMP levels/activity were determined by gelatin and *in situ* zymography and by imunofluorescence. We studied whether these drugs directly inhibit human recombinant MMP-2 (hrMMP-2) *in vitro* activity. Superoxide production and plasma malondialdehyde levels were evaluated. Procedures were approved by the local Ethical Committee (Protocol number: 153/2010). **Results:** ATORVA, SILD, or both drugs exerted antihypertensive effects (systolic blood pressure: 148±6, 156±2, and 138±4 mmHg, respectively, *vs.* 200±4 mmHg in 2K1C untreated rats; $P<0.05$). All treatments prevented the increases in the aortic cross-sectional area and media/lumen ratio in 2K1C rats ($P<0.05$). Aortas from 2K1C rats showed higher MMP-2 levels when compared with sham and all treatments attenuated 2K1C hypertension-induced increases in MMP-2 levels (both $P<0.05$). Increased gelatinolytic activity (20.6±0.9 *vs.* 13.9±1.2 A.U.; arbitrary units) co-localized with upregulated aortic MMP-2 expression (8.1±0.3 *vs.* 5.4±0.4 A.U.) in 2K1C *vs.* control rats. ATORVA, SILD, or both drugs lowered gelatinolytic activity to 14.6±0.5, 14.6±1.0 and 14.5±0.8 A.U. and lowered the aortic MMP-2 expression to 5.8±0.3, 5.9±0.5 and 5.5±0.5 A.U., respectively. However, these drugs had no *in vitro* effects on human recombinant MMP-2 activity ($P>0.05$). Hypertension induced oxidative stress assessed with dihydroethidium probe (7.9±1.2 *vs.* 16.5±1.5 A.U. in sham *vs* 2K1C), which was attenuated by ATORVA, SILD or both (9.9±0.3, 10.4±1.5 and 9.6±0.6 A.U., respectively). Plasma malondialdehyde levels paralleled dihydroethidium results. **Discussion:** Treatment with ATORVA or SILD, or both, exert antihypertensive effects and prevents 2K1C hypertension-induced vascular remodeling and oxidative stress. These effects were associated with lower MMP-2 levels possibly resulting of antioxidants effects. Our results suggest that ATORVA and SILD may prevent the vascular alterations of hypertension. Supported by: FAPESP and CNPq.

06.064 **Does "protein diet" modulate cardiac and renal P-type ATPases in female Wistar rats?**

Silva RM[1], Marques EB[1], Oliveira GF[1], Fernandes WO[1], Felberg MFS[1], Massucati-Negri M[1], Azeredo VB[2], Marostica E[1], Scaramello CBV[1] [1]LAFE-UFF Physiology and Pharmacology, [2]UFF – Nutrition and Dietetics

Introduction: Popular diets, particularly those low in carbohydrates, have challenged current recommendations advising a low-fat, high-carbohydrate diet for weight loss (Gardner *et al*., JAMA. 297(9):969, 2007). The Atkins diet, known as "Protein diet", restricts carbohydrates, but it does not restrict consumption of calories or proteins (Tonekaboni *et al*., Arch of Iranian Med. 13 (6):492, 2010). However, its potential benefits and risks have not been tested adequately. **Methods:** The use of animals was according to Ethics Committee (CEPA/UFF0027/08). Ninety days-old female Wistar rats were divided in four groups: C1- balanced ration with casein and E1-high protein/low carbohydrate/high fat ration ("Protein diet"), fed *ad libitum*; C2-balanced ration with casein and E2-"Protein diet", with energy-restricted intake (30%). After 60 days on diet, animals were anesthetized and the hearts/kidneys were removed, weighed and the rates of hypertrophy (RH) were measured. Then tissues homogenates were obtained (Bambrick *et al*., *J Pharmacol Meth*. 20: 313, 1988). Protein concentration and P-type ATPases activity were determined according to Lowry *et al*. (J Biol Chem. 193:265,1951) and Fiske & Subbarow (J Biol Chem. 66: 375, 1925), respectively. Data are presented as mean and standard error of the mean (at least 3 observations) and considered statistically different if $P<0.05$ (*vs C1; †vs C2; ‡vsE1). **Results:** Independently of caloric restriction, "Protein diet" raises renal RH (C1 = 5.6±0.3;E1 = 7.8±0.3*; C2 = 5.4±0.03;E2 = 7.3±0.03†mg/g). No statistical differences were observed in cardiac RH nor Na^+/K^+ATPase activity among the 4 groups (data not shown). However, "Protein diet" decreased renal Na^+/K^+ATPase activity (C1 = 9616±863;E1 = 6289±247*;C2 = 5732±441*;E2 = 4585±907*nmolPi/mg/h) and increased Na^+ATPase activity (C1 = 2338±162;E1 = 3101±156*;C2 = 2635±488; E2 = 3766±59*‡nmolPi/mg/h), effects apparently potentiated by caloric restriction. **Discussion:** So far, our data points that "Protein diet" affects directly just renal but not cardiac system and its effects may be potentiated by caloric restriction. As expected, "Protein diet" caused renal hypertrophy (Schrijvers *et al*., Kidney Int. 61(5):1600, 2002). According to Thomson *et al*. (*Am J Physiol Renal Physiol*. 286: F8, 2004), glomerular filtration rate (GFR) and kidney size are linked in a negative feedback arrangement. As a consequence of GFR increase, plasma renin activity and angiotensin II level may rise (Rosenberg *et al*. J Clin Invest. 85:1144, 1990; Brands & Labazi, Hypertension. 52:188, 2008). The work of Queiroz-Madeira *et al*. (Biochim et Biophys Acta, 1798: 360, 2010) indicates that Na^+ATPase is a key target during the development of hypertension and that its selective modulation by angiotensin II may be an important mechanism of extracellular fluid volume control. In addition, changes in Na^+ATPase activity are also expected to equalize intracellular Na^+ level while Na^+/K^+ATPase activity is decreased (Reyes *et al*., Physiol Res. 58:693,2009). **Financial Support:** CAPES, FAPERJ, PROPPi/UFF.

06.065 A new nitric oxide donor induces relaxation of mesenteric resistance artery from normotensive and hypertensive 2K-1C rats. Andrade FA[1], Restini CBA[2], da Silva RS[3], Bendhack LM[3]
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Introduction: Nitric oxide (NO) is a physiological modulator that plays important role in controlling vascular tone and blood pressure. Many studies have highlighted the development of compounds that may serve as vehicle to NO release in biological systems, mainly when the endogenous NO production is impaired such as in hypertension. The hypothesis of this study was that the NO donor *cis*-$[Ru(bpy)_2(py)(NO_2)](PF_6)$ (RuBPY) releases NO and it promotes relaxation of mesenteric resistance artery isolated from normotensive rats (2K) and hypertensive rats (2K-1C). This study aimed to investigate the release of NO and to evaluate whether the vasodilator effect of the compound RuBPY in mesenteric resistance artery isolated from 2K and 2K-1C rats is due to sGC activation. **Methods:** To induce renovascular hypertension, male Wistar rats (180–200 g) were anesthetized with tribromoethanol (2.5 mg/kg^{-1}, i.p.) and after a midline laparotomy a silver clip with an internal diameter of 0.20 mm was placed around the left renal artery (2K-1C) and 2K rats were only submitted to laparotomy. Six weeks after surgery, the rats with systolic blood pressure higher than 160 mmHg were considered hypertensive. 2K and 2K-1C were killed by decapitation and the second or third-order mesenteric arteries with internal diameter between 200 and 300 µm were removed for functional studies. Cumulative concentration-effect curves to compound RuBPY (0.001 nmol/L-10 µmol/L) were constructed in isolated denuded mesenteric resistance artery from 2K and 2K-1C rats pre-contracted with phenylephrine (PE, 10 µmol/L), in the absence or after incubation with sGC inhibitor, ODQ (1 µmol/L) for 30 minutes. The potency (pD_2) and the maximum effect (ME) were analyzed. To measure the cytosolic NO concentration ($[NO]_c$), mesenteric resistance arteries were loaded with the NO indicator DAF-2DA (50 µmol/L) for 40 min, at room temperature. $[NO]_c$ was evaluated by confocal microscopy. This study was approved by the Ethics Committee of the Faculty of Medicine Ribeirão Preto - University of Sao Paulo (CETEA- 044/2008). **Results:** RuBPY induced concentration-dependent relaxation in mesenteric beds from 2K and 2K-1C and this effect had similar potency and efficacy in both groups 2K (ME: 90.1±8.6% ; pD_2: 5.25±1.74, n = 5) and 2K-1C (ME: 88.5±7.0%; pD_2: 5.72±1.32, n = 6). However, the relaxation induced by RuBPY was attenuated in mesenteric beds from 2K (ME: 36.5±2.9%; pD_2: 4.97±0.57, n = 4) and 2K-1C (ME: 20.3±8.3%; pD_2: 8.86±0.9, n = 5) pre-incubated with ODQ. RuBPY increased the fluorescence intensity of DAF2-DA in mesenteric arteries from 2K and 2K-1C, which corresponds to an increase in the $[NO]_c$. However, this response was greater in mesenteric arteries from 2K-1C than 2K rats (2K: 43.9±14.4%, n = 3; 2K-1C: 134.2±15.82%, n = 3, $P<0.05$). **Discussion:** These findings suggest that the compound RuBPY releases NO inside the vascular smooth muscle cell. The released NO promotes relaxation in mesenteric resistance artery from 2K and 2K-1C rats that is due to sGC activation. Financial Support: FAPESP and CNPq

06.066 LASSBio-1425 – Antiatherogenic and anti-inflammatory activity of a new phtalimide derivate. Fumian MM[1], Motta NAV[1], Maia RC[2], Barreiro EJ[2], Brito FCF[1] [1]LAFE-UFF – Fisiologia e Farmacologia, [2]LASSBio-UFRJ – Fármacos

Introduction: Atherosclerosis is a major cause of cardiovascular diseases and is characterized by progressive deposition of lipid and fiber vessels in arteries. Atherosclerosis was considered a consequence of dyslipidemia, but recent investigations have revealed that chronic inflammatory processes associated with dyslipidemia and endothelial dysfunction are also important contributors to its development (ZHANG *et al., Trends Pharmacol.*, 28: 286, 2008). The compound LASSBio-1425 is a phtalimide derivative. Studies have demonstrated that LASSBio-1425 shows relevant anti-inflammatory activities (Dissertação de mestrado Milla Fumian, 2010, UFRJ). Other studies have demonstrated hypolipidemic activity of phtalimides derivatives (Chapman *et al*., J. Med. Chem., 26: 237, 1983; Wyrick *et al*., J. Med. Chem., 28: 286, 1984). Our hypothesis is that LASSBio-1425 plays a protective role in cardiovascular diseases. Thus, in this study we aimed to evaluate the pharmacological properties of LASSBio-1425, administered chronically in rats, which were fed with a hypercholesterolemic diet to investigate it's *in vivo* effects. **Methods:** The animal protocols were approved by the Ethics Committee for Experimental Research of the Federal Fluminense University (CEPA/UFF00116/09). Adult male Wistar rats (150-200g) were randomly divided into three groups (n = 10, for each group): C (control group) has received a normal rat chow for 45 days. AT (atherosclerosis group) and LASSBio-1425 (LASSBio-1425group) have received a hypercholesterolemic diet (HCD) for 45 days. A vehicle (tween-ethanol-water: 1:1:8) (0.05 ml/kg) was administered to C and AT groups whereas LASSBio-1425 (100 µmol/kg) was administered to the LASSBio-1425 group. The treatments were given orally, once a day, for 15 days. The animals were euthanized by cervical dislocation and decapitation under anesthesia. Blood samples were collected, the thoracic aorta, and liver were excised. Data were analyzed using one way analysis of variance (ANOVA) with the Bonferroni´s test ($p < 0.05$). **Results:** A high cholesterol diet for 45 days caused a dramatically significant increase in all lipid parameters. Treating hypercholesterolemic rats with LASSBio-1425 (100µmol/Kg) for 15 days significantly decreased the total levels of cholesterol (2.122±490.3 x 1.061±130.6 mg/dL), triglycerides (192.5±45.9 x 107.3±7.8 mg/dL), LDLc (873.7±64.05 x 665.0±67.06 mg/dL) and VLDLc (45.2±11.0 x 21.5±1.7 mg/dL) when compared to the atherosclerosis group. On the other hand, the HDL levels significantly increased (25.4±3.8 x 108.3±14.5 mg/dL) when compared to the AT group. The treatment with LASSBio-1425 showed a significant decrease in the expression of anti-inflammatory cytokine TNF-α (42.0±2.7 x 31.8±2.0). In the vascular reactivity, the LASSBio-1425 group showed a decrease in phenylephrine dependent contractions (CE_{50} = $6.9x10^{-7}$M), when compared with AT group (CE_{50} = $9.3x10^{-8}$M). In acetylcholine dependent relaxation, a statistical difference between the LASSBio-1425 group (CE_{50} $1.5x10^{-7}$M) and the AT group (CE_{50} 3.8 $x10^{-7}$M) was observed. In the platelet aggregation study, the treatment with LASSBio-1425 reduced the aggregation induced by collagen 5µM (59.2±5.0 x 45.5±3.0). **Discussion:** Antiatherogenic properties of LASSBio-1425 seem to be associated with the inhibition of TNF-α production , resulting in a decreased inflammatory response. These effects improve the endothelial dysfunction probably leading to an increased NO bioavailability**.** Therefore, the present results indicate LASSBio-1425 as a potential drug candidate for treating cardiovascular diseases. **Financial Agencies:** CAPES, PROPPI-UFF, FAPERJ.

06.067 **Renal effects of *Calotropis procera* protein fraction**. Costa PHS, Monteiro MCSA, Jorge RJB, Monteiro SMN, Jorge ARC, Alves NTQ, Clementino MAF, Fonseca MRB, Monteiro HSA, Alencar NMN UFC – Physiology and Pharmacology

Aim: *Calotropis procera* is widely distributed in arid and semi-arid regions, especially in the Northeast of Brazil. This plant is used in folk medicine to treat several diseases, as rheumatism, eczema and cutaneous infections. Some pharmacological effects have been described as antimicrobial, antifungal, anti-inflammatory, and hypotensive (AHMED, Phytotherapy Research, v.19, p.807, 2005). However, some studies have shown cardiotoxic effects of *C. procera* latex, which raised questions about its safety (LIMA, Toxicon, v.57, p. 183 2010). According to Aslani et al. (2004), *C. procera* (Cp) administration induces the Na+/K+-ATPase, causing irregular heartbeat. In this context, there are no studies regarding possible nephrotoxic effects of Cp latex. This work aimed to show the renal effects of Cp latex protein fraction *in vitro*. **Methods:** The protein fraction (FCp) was extracted from dialyzed *C. procera* latex. Isolated kidneys from adults Wistar rats, weighing 250 to 300g (n = 6), were perfused with a modified Krebs-Henseleit solution, containing 6% of BSA for 120 minutes. The effects of FCp (10µg/mL) were studied on glomerular filtration rate (GFR), urinary flow (UF), perfusion pressure (PP), renal vascular resistance (RVR) and percentage of sodium (%TNa+), potassium (%TK+) and chloride (%TCl-) tubular transport at 60, 90 and 120 minutes of experiment. All data were analyzed with unpaired t test and ANOVA (*$p<0.05$). In the treated group, the addition of the substance occurred 30 minutes after the beginning of each experiment. The first 30 minutes were used as internal control. Experimental protocol was approved by the Federal University of Ceará Animal Research Ethical Committee, license number 68/08. **Results and discussion:** The FCp decreased UF at time 120 (UF30: 0,129+0,011; UF120: 0,083+0,007* mL/g.min-1). The other parameters were not changed. Thus, we concluded that the dose of FCp used in this experiment showed no evidence of nephrotoxicity regarding to the parameters evaluated, although it showed a decrease in urinary flow after 90 minutes. It is necessary to investigate other doses to identify or exclude the possible nephrotoxic effects in this *in vitro* model. **Financial Support**: CNPq; CAPES and FUNCAP.

06.068 **"Protein diet" and vascular dysfunction**: **possible mechanisms**. Fernandes WO[1], Massucati-Negri M[1], Felberg MFS[1], Alfradique VAP[1], Boaventura GT[2], Azeredo VB[2], Marostica E[1] [1]UFF – Fisiologia e Farmacologia, [2]UFF – Nutrição e Dietética

Introduction: High protein/low carbohydrate/high fat diet (*protein diet*) is widely used by large segments of overweight or obese individuals which want a quick weight loss. Our previous studies in rats fed with *protein diet* showed a decrease in vascular reactivity in response to ACh *(Marostica et al., Bas. Clin. Pharmacol. Toxicol. p.23, 2010),* that can promote chronic vascular dysfunction. Thus, the purpose of this study is to investigate the mechanisms involved in vascular dysfunction observed with this diet, assessing potential changes in the biochemical profile, inflammatory parameters, oxidative stress and NOS expression. **Methods:** The use of animals was according to Ethics Committee (no. 027/08). 90-day-old female Wistar rats were divided in 4 groups and fed for 30 days as following:C1-balanced ration and E1-*protein diet*, fed *ad libitum*; C2-balanced ration and E2-*protein diet*, with energy-restricted intake (30%). The rats were selected in the same phase of estrous cycle and after anesthesia (thiopental,50 mg/kg) the blood was collected after 6:00 PM for plasma cortisol and biochemical measurement according to the manufacturer's instructions. The thoracic aortas from the different groups were used for the study of vascular reactivity *in vitro* and eNOS expression using western blot assay. Concentration-effect curves were obtained from aorta ring using phenylephrine (FNF) and acetylcholine (ACh). Endothelium-dependent relaxation was investigated by using L-NAME ($3x10^{-4}$M) and sodium nitroprusside. The data were express as % of the maximum effect and pD2 (-log EC50) was calculated. The values are mean±SEM and considered statistically different if $P<0.05$. **Results:** After 30 days on *protein diet*, the weight loss was observed only in C2 (-25.06±4.17g) and E2 (-11.43±2.02g). The vessel relaxation was decreased in E2 group (pD2:6.77±0.08) when compared to its control, C2 (pD2:7.48±0.12). ACh-induced relaxation was inhibit with L-NAME and recovered with sodium nitropusside, confirming to be endothelium dependent. The eNOS expression increased in the aorta of E1 and E2 groups. The biochemical profile showed an increase of plasma glucose in E1 and E2 (E1: 87.50±1.83, E2: 85.44±2.62 mg/dL) compared to controls (C1: 70.44±1.23, C2: 68.25±1.74 mg/dL), whereas cholesterol was lower in groups with restricted intake (C2: 36.87±2.79, E2: 46.75±2.63mg/dL) compared to *ad libitum* groups (C1:59.00±2.58, E1:68.62±2.71mg/dL). The E1 group also showed an increased level of MDA (13.96±1.86nmol/ml) by the TBARS assay when compared to the other groups (C1: 9.64±0.22, C2: 9.16±0.88, E2:9.26±0.37 nmol/ml). No significant difference was found in relation to inflammatory markers among different groups, but TNF-α and IL-6 showed a trend towards higher levels in E1 and in the plasma cortisol concentration of E2. **Discussion:** More than the *protein diet* is the caloric restriction that promotes greater weight loss. Furthermore, our results suggest that *protein diet* alters lipids and carbohydrate metabolism, increases oxidative stress that can compromise the cardiovascular system. Caloric restriction, in its turn, can increase the cortisol levels and reduce the inflammation and oxidative stress, playing a protective effect on the cardiovascular system. **Supported by:** CAPES, CNPq, FAPERJ, UFF.

06.069 **Influence of acute swimming exercise in relaxing response of the aorta in Wistar rat**. Brito AF[1], Souza ILL[2], Pereira JC[2], Carreiro JN[2], Silva AS[1], Silva BA[3] [1]DEF-CCS-UFPB, [2]CCS-UFPB, [3]DFP-CCS-UFPB

Introduction: The benefits conferred by exercise on the cardiovascular system are notorious and well documented, and the improvement in endothelial function is a major beneficial factors associated with aerobic exercise. However, no studies that correlate the use of different intensities of training and a possible change in the relaxing response to acetylcholine (Ach). **Objective:** Thus, this study aimed to evaluate the relaxant response to ACh in the aorta rats by means of concentration-response curves after swimming exercise at intensities of 3, 4 and 5% of its body weight. **Methods:** After project approval by the ethics committee for animal research with protocol number: 1101/11, started the search with a week of adaptation to swimming exercise, rats (*Rattus norvegicus*) weighing between 250 and 300g and aged 12 weeks, underwent a session of forced swimming for 1 hour, having stuck to its torso a metal ring corresponding to 3 (n = 5) 4 (n = 5) and 5% (n = 5) of its body weight. The exercise was performed in a tank made of polyethylene with water at 28 ± 1 °C (Chies, *Clin. Exp. Pharmacol.* Physiol., v. 30, p. 951, 2003). The control group (n = 5) of this study was subjected to the same stress that the exercised animals, by acclimation, which were wet at the same place that the animals were exercised. Immediately after the exercise, the animals were euthanized, the aorta removed and suspended in an organ bath (5 mL) in Krebs solution, under tension of 1 g at 37 °C and bubbled with carbogen. After a stabilization period of 1 hour, a contraction was induced with phenylephrine (FEN) (3×10^{-7} M) and during the tonic component was added ACh 10^{-6} M to verify the integrity of the endothelium. After washing, waited for 30 minutes, a further contraction induced by FEN and then obtained a cumulative curve to ACh (10^{-8} - 10^{-6} M). After being washed, it was expected 30 min, and a cumulative curve was obtained with FEN (10^{-8} - 10^{-4} M). **Results:** According to the data obtained, it was observed that all animals in groups 3 and 4% exhibited relaxation maximum effect (Emax) equal to 100%, however the group 5% showed Emax reduced to 93,7±0, 67. Interestingly, the animals were trained had relaxation to lower doses of ACh compared to the control group, which is shown by the pD_2 value = 7.9 ± 0.2; 7.8 ± 0.29; 7.9 ± 0.21; for 3, 4 and 5% respectively, compared with the control group (pD2 = 7.2 ± 0.04). We also observed that in all groups showed contraction Emax = 100%. Furthermore, animals showed no statistical difference for the vasoconstrictor response to FEN compared with the control group, which is demonstrated by the values of pD_2 = 7.6 ± 0.2, 7.2 ± 0.1, 7, 2 ± 0.2 to 3, 4 and 5% respectively, compared to control (pD^2 = 7.1 ± 0.1). **Discussion:** There was no statistical difference between the pD_2 values of the trained groups. Taken together, we can conclude that the exercise intensities tested did not influence the vasorelaxant response, however a single exercise session of swimming have shown to be enough to acutely improve endothelial function in rats. **Financial support:** CAPES, CNPq, PgPNSB/UFPB.

06.070 **Acute stress of restraint alters the vascular reactivity in rats and promotes anxiogenic effect**. Carda APP[1], Gonzaga NA[2], Padovan CM[3], Tirapelli CR[4] [1]EERP-USP, [2]FMRP-USP– Farmacologia, [3]FFCLRP-USP – Psicologia, [4]EERP-USP – Enfermagem Psiquiátrica e Ciências Humanas

Introduction: The physical or psychological stress is defined as the state of disharmony or threat to homeostasis that can be caused by psychological, environmental or physiological stressors. Studies show that a stressful event contributes to behavioral changes such as increased levels of anxiety, as well as the development of cardiovascular diseases such as hypertension. The cardiovascular changes associated with stress involve endothelial dysfunction. The aim of this study was to study the mechanisms underlying the vascular dysfunction induced by acute restraint stress (ARS). **Methods:** Male Wistar rats weighting between 250-300 g were divided into two experimental groups: non-stressed (control) and stressed (animals were stressed for a period of 2 hours one day before the experiments). The vascular reactivity was studied in isolate aortas. Aortic rings were mounted in organ chambers and concentration-response curves for the following vasoactive agents were obtained: sodium nitroprussiate (SNP), acetylcholine (ACh), phenylephrine (Phe), serotonin (5-HT) and KCl. The curves were obtained in endothelium-intact (E^+) or endothelium-denuded rings (E^-). For behavioral evaluation, the animals were subjected to elevated plus maze (EPM) 24 h after restraint and exploratory behavior was analyzed by frequency of entry into the closed (CA) and open arms (OA) and time spent in this latter. All protocols were approved by the Ethics Committee on Animal Use of Campus of RibeirãoPreto - USP (Protocol: 11.1.1267.53.4). **Results:** In stressed animals, the number of entries in OA ($t20 = 7.891$, $p < 0.001$) as well as the time spent in them ($t20 = 9.51$, $p < 0.001$) (% FA: 16.75 ± 0.67%; TA: 8.36 ± 0.57, n = 13) was decreased when compared to unstressed animals (FA%: 30.38 ± 1.86;% T: 21.50 ± 1.45, n = 9). There was a significant reduction ($P<0.05$, Student's t test) in the vascular relaxation induced by Ach in aortic rings from stressed animals (95.7 ± 3.8%, n = 8) when compared to control (110.5 ± 3.5% n = 6). Stress also increased Phe-induced contraction in E^+ aortic rings (1.5 ± 0.1 g, n = 8) when compared to control (1.1 ± 0.1 g, n = 7) ($P<0.05$, Student's t test). In E^- rings, there was no significant difference on Phe-induced contraction between the groups. There was a significant increase ($P<0.05$, Student's t test) in 5-HT-induced contraction in E^+ rings from stressed animals (1.3 ± 0.1 g, n = 8) when compared to control (0.9 ± 0.1 g, n = 6). This response was not observed in E^- rings. The restraint stress did not alter the reactivity to KCl or SNP. **Discussion:** Our results show that acute stress is associated with an anxiogenic behavior. ARS alters the vascular reactivity to agents that induce vascular contraction and relaxation being this effect endothelium-dependent. **Financial Support**: CNPq.

06.071 **Generation and characterization of spontaneously immortalized endothelial cell from mice**. Loiola RA[1], Torres T[1], Landgraf M[2], Landgraf R[1], Pesquero JL[3], Fernandes L[1] [1]Unifesp – Biologia Química, [2]USP – Imunologia, [3]Unifesp – Biofísica

Introduction: Primary cultured endothelial cells provide a valuable resource to investigate vascular physiology, however the isolation of endothelium is laborious and these cells undergo replicative senescence *in vitro* after a finite number of divisions. In order to overcome this limitation, the present study describes the generation and characterization of spontaneously immortalized endothelial cells obtained from primary cultured vascular endothelium of mice microcirculation. **Methods:** Lung explants (1x1 mm) were placed in a flask culture, covered with culture medium [DMEM low glucose containing fetal bovine serum (FBS, 20%)] and removed after 60 hours. The cells were maintained in culture during 1 year (100 passages), when monoclonal populations were isolated by serial dilution. At each passage, an aliquot of cell suspension was used to count trypan-blue-excluding cells in order to determine population doubling time (PDT). Expression of von Willebrand factor (vWF), angiotensin converting enzyme (ACE), endothelial nitric oxide synthase (eNOS) and binding of the Ulex europaeus lectin agglutinin I (UEA-1) were assessed by immunofluorescence. Expression of vascular endothelial-cadherin (VE-cadherin), endoglin, and vascular cell adhesion molecule-1 (VCAM-1) was assessed by flow cytometry. NO production was determined in cells stimulated with bradykinin (BK, 1 mmol/L) after incubation with a fluorescent dye for NO [DAF-2 DA (10 µmol/L)] during 30 minutes prior to the experiments. Cells were observed in a fluorescence microscope and analyzed by optic densitometry (arbitrary units, a.u). Ethics Committee of the UNIFESP: protocol number 1913/11. **Results:** Cultures were grown to confluence until 3rd passage, when cells reached senescence and lost their proliferative potential. The initial focus of spontaneously immortalized endothelial cells was obtained at this stage. The PDT was progressively reduced from isolation (64.1±7.9 hours, n = 5) to 100th passage (19.4±2.1 hours, n = 3). Monoclonal populations (3 clones) isolated after 100 passages maintained the growth rate over 20 passages (PDT of 17.2±0.1 hours, n = 3). The effect of serum concentration on the cellular proliferation was discrete, since clones cultured using medium supplemented with 10% (PDT of 20.0±3.6 hours, n = 3) and 5% (PDT of 21.6±2.9 hours, n = 3) of FBS presented similar growth rate. Both early-passage (3rd passage) and cloned cells exhibited typical cobblestone appearance at confluence and presented positive staining to vWF, eNOS, ACE and binding to UEA-1. Primary and cloned cells also presented constitutive expression of VE-cadherin, endoglin and induction of VCAM-1 upon activation with lipopolysaccharide. NO production after BK stimulation (a.u.) was slightly higher in early-passage (63.5±11.3, n = 3) when compared to cloned (42.3±1.2, n = 3) cells, but no statistic significance was detected. **Discussion:** Spontaneously immortalized endothelial cells described in the present study showed unlimited lifespan and retained specific endothelial characteristics, including functional ability to produce NO. This cell model can provide continuous supply of homogenous biological material that can be useful to investigate endothelial biology. **Financial support**: FAPESP (JP 2007/59039-2) and CAPES.

06.072 **Hemodynamic effects of recombinant human matrix metalloproteinase-2 in anesthetized lambs**. Ferraz KC[1], Rizzi E[2], Sousa-Santos O[2], Neto-Neves EM[2], Muniz JJ[1], Gerlach RF[3], Tanus-Santos JE[2] [1]FCM-Unicamp – Pharmacology, [2]FMRP-USP – Pharmacology, [3]FORP-USP – Morphology, Stomatology and Physiology

Introduction: Experimental and clinical evidence indicate that upregulation of matrix metalloproteinases (MMPs) play an important role in cardiovascular disorders (Chow *et al.*, Br J Pharmacol, 152, 189, 2007). Indeed, abnormal MMP-2 levels have been reported in atherosclerosis, hypertension, heart failure and ischemic heart diseases (Castro *et al.*, Pharmacol Res, 64(6), 551, 2011). Although some studies have shown that MMP-2 may affect the vascular tone and the cardiac contractility by modulating vasoactive peptides and cardiac contractile proteins levels, no previous study has examined the acute hemodynamic effects of MMP-2. Thus, we examined whether of recombinant human MMP-2 (rhMMP-2) would produce hemodynamic changes in anesthetized lambs at baseline conditions and during β-adrenergic cardiac stimulation with dobutamine, and if these changes would be attenuated by doxycycline, a non-selective MMPs inhibitor. **Methods**: Anesthetized lambs (ketamine 15 mg/kg and xylazine 0.1 mg/kg, intramuscular) pretreated with doxycycline (10 mg/kg, intravenously) or saline received infusions of rhMMP-2 (220 ng/kg/min over 60 min, intravenously) or saline at rest and during cardiac stress induced by dobutamine infusion (5 µg/kg/min over 180 min, intravenously). Hemodynamic evaluations were carried out every 30 min for four hours. Plasma and cardiac MMP-2 levels were assessed by gelatin zymography and gelatinolytic activity was assessed by spectrofluorimetry. Procedures were approved by the local Ethical Committee (protocol number: 020/2007). **Results**: We observed that rhMMP-2 infusion exerted no significant effects on cardiac index (CI) and left ventricular dP/dt_{max} (LV dP/dt_{max}). Dobutamine increased the CI by approximately 1.2 l/min/m^2 (p> 0.05) and LV dP/dt_{max} by approximately 1700 mmHg/s (p< 0.01). Interestingly, the co-infusion of rhMMP-2 was associated with lower dobutamine-induced increases in CI (from 0.8 l/min/m^2 to -0.7 l/min/m^2; p< 0.05) and LV dP/dt_{max} (from 4000 mmHg/s to 2700 mmHg/s; p< 0.05). The previous administration of doxycycline blunted the effects of rhMMP-2 on the dobutamine-induced increases in CI and LV dP/dt_{max} (p < 0.05). Furthermore, while the infusion of rhMMP-2 did not increase plasma and cardiac MMP-2 levels, it increased cardiac gelatinolytic activity, and doxycycline blunted this effect (p< 0.05). **Discussion**: Our findings show that rhMMP-2 exerts no major hemodynamic effects in lambs. However, rhMMP-2 impairs the responses elicited by activation of β-adrenoreceptors. These findings suggest that MMP inhibitors such as doxycycline can clearly attenuate cardiovascular dysfunction associated with increased MMP-2 activity. **Acknowledgments**: FAPESP, CAPES and CNPq.

06.073 **Pyrimidine *N*-acylhydrazone derivatives – LASSBio-1088 and LASSBio-1277 – exert vasodilatory activity by different mechanisms**. Rocha SO, Lopes AB, Silva LL, Barreiro EJ, Fraga CAM, Miranda ALP LASSBio-FF-UFRJ

Introduction: The regulation of vascular tone is crucial in the management of several cardiovascular diseases. The existing direct vasodilators don´t have specificity of action and presents side effects. Drugs presenting both vasodilator and antiplatelet properties are of interest for the treatment of cardiovascular diseases.*N*-acylhydrazone derivatives have being described as vasodilators also possessing antiplatelet activity (Silva, Bioorg Med Chem 13, 3431, 2005; Brito, *Eur J Pharmacol* 638, 5, 2010**).** This study focused on the evaluation of the vasodilator activity of a new series of pyrimidine *N*-acylidrazone derivatives. Two compounds in this series –LASSBio-1088 and LASSBio-1277- stand out and seems to exert their activities through different mechanisms. **Methods:** The vasodilator activity has been evaluated using rat thoracic aorta rings, with and without functional endothelium, contracted with phenylephrine (PHE) (10 μM). Briefly, Wistar rats of both sexes were used and the thoracic aorta was quickly removed, cut into rings, held under tension of 1g in oxygenated Krebs buffer at 37 °C. The endothelium viability was verified in aortas pre-contracted with PHE by observing a relaxation greater than 80% induced by acetylcholine (10 μM). Relaxation below 10% demonstrates the absence of endothelium. Cumulative concentration response curves (0.01-100 μM) were constructed in the screening step. Compounds were also tested against other contractile agonists: KCl (80Mm) and serotonin (10μM) for both, and endothelin (20nM) for LASSBio-1277. Functional studies were performed using ODQ (30μM), DDA (100μM), L-Name (100μM), atropine (50μM) and PDE5 inhibitor, zaprinast (0,01-100μM). Results were expressed as % of inhibition compared to DMSO (*$p<0.05$; n = 4-5 aorta rings). IC_{50} values were determined by non-linear regression using GraphPad Prism software. **Results and Discussion:** The derivatives were able to cause relaxation of aortic rings pre-contracted with phenylephine showing IC values, with and without functional endothelium respectively, of 1.3 μM (E_{max} = 91%) and 85.4 μM (E_{max} = 51%) for LASSBio-1088, and of 3.9 μM (E_{max} = 95%) and 4.1 (E_{max} = 100%) for LASSBio-1277. The latter showing an effect endothelium independent. LASSBio-1277 was able to inhibit by 50% the contraction induced by serotonin, KCl (IC_{50} = 5.1 μM) and endothelin-1(IC_{50} = 0.43 μM). Its effect was significantly changed by the addition of ODQ or DDA. LASSBio-1088 was effective on contractions induced by KCl (IC_{50} = 5.2 μM), but showed no activity against serotonin. Its effect was reduced by addition of ODQ and L-Name, but was not reversed by atropine. The concentration-response curve of zaprinast was shifted to the left by LASSBio-1088, which was not seem for LASSBio-1277. The pyrimidine N-acylhydrazone derivatives showed greater vasodilator effects and present themselves as promising prototypes of vasodilators. The results suggest different mechanisms of action for these compounds. LASSBio-1088 seems to exert its effect through the NO pathway while the effect of LASSBio-1277 was independent of endothelium-derived factors. **Financial support**: CNPq, FAPERJ, INCT-Inofar, PRONEX.

06.074 **Influence of the route of administration on the hemodynamic, electrocardiographic and blood gas responses to *Bothrops jararacussu* (Jaracuçu) venom in anesthetized rats**. Neves R[1], Rodrigues MAP, Dias L, Brunieri LVT, Hyslop S Unicamp – Farmacologia

INTRODUTION: *Bothrops* snake venoms can produce systemic effects, including coagulopathy, hypotension, internal bleeding and acute kidney injury. Although several studies have examined the cardiovascular actions of *Bothrops* venoms, there has been no systematic assessment of the influence of the route of venom administration on these responses. In this work, we investigated whether the route of administration influenced the hemodynamic, electrocardiographic (ECG) and blood gas alterations caused by *Bothrops jararacussu* snake venom in rats. **Methods:** Male Wistar rats (300-400 g) were anesthetized with 2% isoflurane in 98% air and the left carotid artery was catheterized for continuous blood pressure measurement (PowerLab system, ADInstruments) and arterial blood sampling. The ECG was determined with an electrocardiograph and respiratory rate was counted manually. For i.v. administration, venom (0.5 mg/kg; n = 5) was injected via the left femoral vein in 100 µl of 0.9% NaCl and washed in with a further 100 µl. For i.m. administration, the rats received venom (5 mg/kg, 100 µl) in the left gastrocnemius muscle while for i.p. administration venom (5 mg/kg, 100 µl) was injected in the lower left quadrant of the abdominal cavity. The changes in cardiovascular parameters were monitored immediately before venom (time zero, 0) and at 1, 5, 10, 20, 60 and 120 min post-venom. Blood gas parameters were analyzed with an ABL555 blood gas analyzer (Radiometer). The results (mean±SEM) were analyzed with ANOVA followed by the Tukey-Kramer test, with $p<0.05$ indicating significance. These experiments were approved by the institutional Committee for Ethics in Animal Use (CEUA/UNICAMP, protocol 2181-1). **Results**: Venom (0.5 mg/kg) injected i.v. caused immediate hypotension that was maximal after 5 min (from 92±8 to 46±4 mmHg for mean blood pressure, n = 5) and gradually returned to baseline over 60 min. When injected i.m. and i.p., a 10-fold higher dose of venon was required (5 mg/kg) but the time-scale and extent of the hemodynamic responses were not significantly different from those seen with i.v. injection, although recovery was slower with i.p. There were no significant changes in heart rate or respiratory rate with any of the routes. The ECG showed a significant increase in the P wave duration for venom given i.p. compared to i.v. (from 1.8±0.1 to 2.4±0.1 s) and in the QRS complex duration (from 2.0±0.6, i.v., to 5.5±0.2 s, i.m.); there were no changes with i.p. injection compared to other routes. Blood K^+ levels increased from 3.4±0.1, 3.8±0.1 and 4.0±0.2 (mM) for i.v. injection to 4.2±0.0, 4.4±0.1 and 4.7±0.1 (mM) for i.m. injection at 15, 30 and 120 min, respectively ($p<0.05$); there were no changes with i.p. injection compared to other routes. There were no significant differences in blood Na^+ levels, hematocrit, pH and blood gas parameters (pO_2, pCO_2, SBC, SBE, etc.) among the three routes of injection. **Conclusion**: These results show that the route of administration (i.m., i.p. or i.v.) does not significantly influence the pattern and extent of venom-induced changes in blood pressure, heart rate, ECG, respiratory rate and blood gas parameters in rats. **Financial support:** CAPES, CNPq, FAPESP.

06.075 **Involvement of muscarinic pathway in the cardiovascular effects of ayahuasca tea**. Moura MTD[1], Costa CDF[1], Herculano EA[1], Netto SM[2], Ribeiro EAN[1] [1]ESENFAR-UFAL, [2]Unifesp – Psicobiologia

Introduction: Ayahuasca tea is a psychoactive drink of South American origin, used in indigenous rituals and the Amazonian region and other Brazilian religions. It is prepared by decoction of the vine Banisteriopsis sp ("mariri") with the leaves of the shrub Psychotria sp ("chacrona"), the species *Banisteriopsis caapi* and *Psychotria viridis* are the most frequently used (Castello & Brito 1999; De Rios 2003; Grob 2002). In previous studies our group found hypotensive activity of ayahuasca tea in spontaneously hypertensive rats (SHR) (Herculano et al, 2011). The aim of this study was to investigate the acute cardiovascular effects of the by lyophilized ayahuasca tea (LYT) in rats, by *in vivo* approach. **Methods:** Male Wistar rats (250-350 g) were anesthetized with sodium pentobarbital (45mg/kg, i.p.) and polyethylene catheters were inserted into the lower abdominal aorta and into the inferior vena cava for blood pressure measurements and administration of drugs, respectively. **Results and Discussion:** In conscious SHR, LYT (5; 20; 30 e 60 mg.kg-1 i.v. n = 5) produced significant hypotension at all doses (-6,9 ± 1,3; -14,6± 2,2; -14,0 ± 2,8; -29,7 ± 3,0 mmHg, respectively) associated with a bradycardia at doses of 20, 30 e 60 (-15,0± 3,4; -14,9±1,6;-144,1±9,7 bpm, respectively). in order to investigate the role of endothelium-derived relaxing factors and derivatives prostanoids in the bradycardic and hypotensive responses, block was performed with NG-nitro-L-arginine methyl ester (L-NAME 20mg.kg-1 i.v. n = 5), a blocker of the enzyme NO synthase, and indomethacin (5mg.Kg, i.v. n = 5), a non-selective blocker of the enzyme cyclooxygenase, however, no significant changes in the hypotensive effect, increasing the bradycardic effect only at doses of 20 and 30mg.kg-1. Pretreatment with methylatropine (2mg/kg, i.v. n = 5) significantly reversed the LYT-induced hypotension at all doses (8,6±2,4; 9,2±3,2; 27,9±4,7; 24,9±3,9 mmHg, respectively) and bradycardic at a dose of 60mg.kg-1(7,2±1,1 bpm). In SHR rats anesthetized bradycardic effect was attenuated in a dose of 60mg. kg-1 (-40,7±22,3) , but the hypotensive response was enhanced at all doses (-36.2±3,1; -44,7±2,8; -50,8±1,6; -50,7±4,8). These results taken together, suggest that the hypotension and bradicardic LYT- induced is probably due to muscarinic receptor activation. The bradycardia appears partial dependent upon the participation of parasympathetic pathway. **Financial Agencies:** CAPES and CNPq. **License number of Animal Ethics Committees:** 0106/08. **References:** Castello,O. & Brito, G.S. (1999). “Ayahuasca (Hoasca): Histórico, Botânica, Fitoquímica, Farmacologia, Efeitos Clínicos e Neuropsicológicos”. Documento não publicado. Direitos Autorais Registrados na Fundação Biblioteca Nacional sob número 214.259 (livro: 373 folha: 419). De Rios, M. D. (2003). “LSD spirituallity and the creative process”. Park Street Press, California, 1st ed. Pág 167 a 169. Grob,C.S. (2002). “A Psicologia da Ayahuasca” em “Ayahuasca – alucinógenos, consciência e o espírito da natureza” de Ralph Metzner. Edição traduzida para o português. Editora Gryphus, Rio de Janeiro, 1a Edição. Pág 195 a 225. Herculano, E. A. ; Costa, C. D. F. ; Moura, M. T. D. ; Netto, S. M. ; Ribeiro, Ê. A. N. Avaliação dos Efeitos Cardiovasculares do Chá Liofilizado Ayahuasca. XX Congresso Italo-Latinoamericano de Etnomedicina, 2011, Fortaleza-Ceará, Brasil.

06.077 **A new model for adenine-induced chronic renal failure in mice, and the effect of gum acacia treatment thereon: Comparison with rats**. Ali BH[1], Al-Za´abi M[1], Waly M[2], Beegam S[1], Al-Lawati I[1], Al- Salam S[3], Nemmar A[4] [1]CMHS-Sultan Qaboos University – Pharmacology and Clinical Pharmacy, [2]CMHS-Sultan Qaboos University – Food Sciences, [3] CMHS-Sultan Qaboos University – Pathology, [4]CMHS-Sultan Qaboos University – Physiology

Introduction: Chronic renal failure (CRF) is on the increase and there is a need for studies into its pathophysiology and mechanisms, and also for the development of new effective therapeutic strategies, and for the development of appropriate and valid animal models which should simulate, as much as possible, the human renal disease in their natural course, as well as histological features, and can predict renal functional outcome, and responsiveness to clinically-used drugs. Adenine feeding in rats is an accepted animal model for CRF. This study aimed at comparing the effects of feeding rats and mice with adenine to induce a state of chronic renal failure (CRF), and to assess the effect of treatment of gum acacia (GM) thereon. **Methods**: After an acclimatization period of seven days, rats (Wistar) and mice (C57 BL-6) were each randomly divided into four equal groups. The first groups in the two species continued to receive the same diet without treatment until the end of the study (control group). The second groups were switched to a powder diet containing adenine (0.75% w/w in feed for four weeks) in rats, and 0.2 – 0.75% w/w in mice . The third groups in the two species were given normal food and GA in drinking water at a concentration of 15% w/v, for four weeks. The fourth groups in the two species were given adenine (0.2 % w/w in the feed) as in group two, plus GA in drinking water at a concentration 15% w/v, respectively, for four weeks. Urine, blood and kidneys were obtained from all animals after sacrifice, and used for measuring indices of renal functions. **Results**: Rats treated with adenine (0.75%, w/w) survived the treatment, but all treated mice died within 1 -2 days. The dosage in mice was reduced to c for 4 weeks, but again all treated mice died within 3-4 days. A further reduction in the dosage in mice to 0.2% w/w for 4 weeks, resulted in no mortality, and produced physiological, biochemical, and histopathological alterations broadly similar to that observed in rats fed adenine at a dose of 0.75% w/w for 4 weeks. GA (15% w/v in the drinking water for 4 weeks) given concomitantly with adenine ameliorated the severity of CRF to a similar extent. **Discussion:** Evidently, mice are more sensitive to adenine than rats, and a dose of adenine (0.2% w/w, 4 weeks) in mice is suggested as a model for CRF, and that at this lower dose (0.2% w/w), adenine can be used as a convenient and relatively inexpensive model of CRF. In both models, we have confirmed here that GA is an oral sorbent that can ameliorate CRF in the two species used here as models for this disease. **Acknowledgment**: This work was financed by The Research Council, and SQU, Oman. This project was reviewed and approved by the Institutional Review Board of the Animal Research Ethics Committee of the Sultan Qaboos University (SQU/COMHS # 423)

06.078 **Effect of chronic treatment with apocynin on arterial pressure**, **heart rate and *in vivo* responses to acetylcholine and to phenylephrine in spontaneously hypertensive rats (SHR)**. Antoniali C[1], Perassa LA[1], Lima MS[2], Potje SR[1], Graton ME[3], Munhoz FC[1], Callera JC[1] [1]FOA-UNESP – Basic Sciences, [2]UNIP-Araçatuba – Pharmacy, [3]UniSALESIANO – Pharmaceutical Sciences

Apocynin, a NOX/NADPH oxidase inhibitor, has been used in antioxidant therapies. Previous data from our laboratory demonstrated the chronic treatment of Spontaneously Hypertensive Rats (SHR) with the apocynin (30 mg/Kg, b.w.) prevented the development of hypertension. In this study, we evaluated the effect of chronic treatment with apocynin on mean arterial pressure (MAP), heart rate (HR) and *in vivo* responses stimulated by acetylcholine (ACh) and phenylephrine (Phe) in SHR. Male SHR was treated from the fourth to tenth week of life with apocynin (30 mg/Kg/day, b.w, diluted in the drinking water). At the end of the treatment, cannulae were implanted into the abdominal aorta, throughout the femoral artery, for blood pressure and heart rate recording and another cannula was inserted into the femoral vein for the administration of the drugs. The responses to ACh (doses: 2 and 10 µg/Kg, b.w) and to Phe (8 µg/Kg, b.w) were evaluated and expressed as DMAP, calculated based on the difference of the MAP values at basal condition and after the drugs administration. The results (mean ± SEM) were compared between Wistar rats and SHR and between SHR and SHR treated with apocynin (Test *t* of Student; $p<0,05$). In the tenth week, the MAP and HR values of the SHR treated with apocynin (129,5 ± 2,2 mmHg; 318,8 ± 5,8 bpm, n = 9) were reduced when compared to SHR (160,3 ± 2,7 mmHg; 362,7 ± 8,3 bpm n = 9). The hypotensive responses to 2 and 10 µg/Kg of ACh were less effective in SHR (D: -22,2± 2,1; -32,6±3,9 mmHg, respectively) than in Wistar rats (D: -31,1±1,5; -38,7±2,2 mmHg). However, the hypotensive effects of ACh were increased in SHR treated with apocynin in both used doses (D:-40,5 ± 3,4 mmHg and -52,7 ± 3,5 mmHg, respectively) when compared to Wistar or to SHR. The *in vivo* response to Phe was increased in SHR treated with apocynin (D:+50,9 ± 1,4 mmHg) when compared to SHR (+42,7 ± 2,0 mmHg) or to Wistar rats (D:+41,8 ± 1,1 mmHg). These results demonstrated that chronic treatment with apocynin prevents the development of hypertension, reduces the MAP and HR and also increases the hypotensive responses to ACh and pressor response to Phe in SHR. In the next studies, we will study the mechanisms involved in these effects. Approved by the local Ethics Committee (CEEA-FOA 01561-2011). **Financial support**: CAPES, FAPESP (Proc. 2011/04619-0; Proc. 2011/20998-0).