Hypertension 2015 Scientific Sessions Abstracts

001

Mutation in the PPARγ Ligand Binding Domain Impairs the Anti-inflammatory Action of PPARγ

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Peroxisome proliferator-activated receptor gamma (PPARγ) has been proposed to antagonize the activities of nuclear factor kappa B (NFκB) to regulate inflammation. Transgenic mice expressing dominant negative (DN) PPARγ specifically in vascular smooth muscle cells (SMC) exhibited exacerbated atherosclerosis but the mechanism remains unknown. We hypothesized that DN PPARγ promotes NFκB-induced inflammation in SMC. To test this, we cultured mesenteric SMCs from transgenic mice that would conditionally express wild-type (WT) or DN PPARγ (P467L) in response to adenovirus expressing Cre-recombinase (AdCRE). PPARγ expression remained silent in control SMC infected with AdGFP. TNF-α (0.05 ng/ml, 6 hr) induced NFκB target gene (MCP-1, iNOS and MMP9) expression to a greater extent in P467L-Cre compared to P467L-GFP. For example, MMP9 expression was induced 6.3±0.2-fold in P467L-Cre vs 3.2±0.5-fold in P467L-GFP ($p<0.01$). The NFκB subunit, p65, mRNA level was not altered in these cells. There was no induction of the PPARγ target aP2 in P467L-Cre, but it was induced 6.5±1.7-fold in WT-SMC infected with AdCRE (WT-Cre). The ability of TNF-α to induce NFκB target gene expression was blunted or abrogated in WT-Cre cells, and their expression was significantly reduced in TNF-α-treated WT-Cre compared to WT-GFP (MMP9: 0.7±1.2 vs 6.0±0.3, $p<0.01$). To examine mechanisms in vivo, we crossbred transgenic mice expressing WT PPARγ specifically in SMC (S-WT) with mice expressing luciferase under control of a NFκB-responsive promoter. TNF-α (500 ng/ml, 24 hr)-induced NFκB activity was decreased in aorta and carotid artery from S-WT mice compared to control mice (aorta: 4.7±1.1 v s 6.7±0.7, $p<0.05$, carotid artery: 4.0±0.6 vs 8.7±1.2, $p<0.01$). Finally, to assess the mechanism preventing anti-inflammatory activity by DN PPARγ, we assessed its interaction with p65 protein when co-expressed in HEK293T cells. WT PPARγ co-precipitated with p65, but the interaction between p65 and P467L PPARγ was severely impaired (n=6). Other mutants (R165T, V290M) could bind to p65, suggesting that loss of the ability is specific to P467L PPARγ. We conclude that SMC-PPARγ has anti-inflammatory effects mediated through inhibition of NFκB activity, which is abolished by P467L mutation.

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002

Dynamic T Cell-Antigen Presenting Cell Interactions and Direct T Cell Activation Within the Vascular Wall During Hypertension

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T cells are now known to be vital to the development of experimental hypertension. Hypertension is associated with significant accumulation of T cells into the perivascular fat surrounding the aorta and renal vasculature. While a hypertension-specific neoantigen has been implicated in T cell activation, it is not known whether vascular-infiltrating T cells recognize and are locally activated by an antigen within the vessel wall. We developed live-cell imaging of explanted aortas to identify whether cognate antigens are presented to T cells within the vessel wall of hypertensive mice, evidenced by slower T cell velocities and a greater number of T cells interacting with antigen presenting cells (APCs). Splenic T cells were isolated from normotensive vehicle-treated (nT cells) and hypertensive angiotensin II (Ang II)-infused (0.7mg/kg/day; 14 days; hT cells) C57BL6/J mice. Following anti-CD3/CD28 stimulation (48 hours), cells were fluorescently labelled and co-incubated simultaneously (16 hours) with explanted aorta from normotensive or hypertensive CD11c-YFP mice, where APCs are fluorescently labelled. In CD11c-YFP mouse aorta alone, we detected a ~2-fold increase in CCR5 ligand (CCL3, CCL4 and CCL5) secretion from hypertensive mouse aorta compared to vehicle-treated mouse aorta (*P<0.05; n = 4). Using 2-photon microscopy, we observed a greater number (~2-fold) of hT cells compared to nT cells within Ang II-infused mouse aorta (390 ± 113 Vs 198 ± 49). Importantly, time-lapse recordings of hypertensive mouse aorta revealed hT cells exhibited significantly slower velocity (hT cells 2.0 µm/min Vs nT cells 4.8 µm/min; **P<0.01, n=8-12), and a greater proportion of interactions with APCs (hT cells 4.4 ± 1.1 Vs nT cells 0.8 ± 0.4%). Moreover, activation of local vascular T cells by incubating hypertensive aorta with anti-CD3/CD28 antibodies (16 hours) augmented Ang II-induced endothelial dysfunction (67.5 ± 2.0 Vs Ang II alone 54.5 ± 3.7% maximal relaxation). We have the first evidence that vascular-infiltrating T cells are presented with cognate antigens by APCs within the vessel wall during hypertension; direct activation of these T cell infiltrates further impairs endothelial function, which may promote the development of hypertension.

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003

Selective Splenic Denervation Inhibits the Egression of T Cell From Spleen, Induced by Angiotensinii, and Protects From Hypertension

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It has been elucidated that immunity plays a role in both etiology and target organ damage of hypertension (HTN) induced by different stimuli (AngII, salt, etc.). In the very last year, we found that AngII activates a neuroimmune pathway, passing through the celiac ganglion (CG). Here, we aimed at dissecting this neuroimmune drive, in order to examine the specific role of splenic sympathetic nerve activity (SSNA) in the onset of HTN.

We tested the hypothesis that chronic AngII infusion activates SSNA, in order to recruit T cells. In splenic nerve bundles, we recorded SSNA with a bipolar electrode in mice infused with AngII for 3 days (before BP increase) or with vehicle. SSNA was higher in AngII mice, as compared to vehicle (355±18 vs 141±24 spikes/10min) ($p<0.001$). We next selectively denervated splenic nerve (SDN) by thermoablation, in order to evaluate whether the AngII-induced increase in SSNA could be detrimental for activation of immunity and onset of HTN. We confirmed the efficacy of SDN by injecting a retrograde neurotracer in the spleen, which was effective in labeling the CG neurons only in sham mice and not in SDN. Furthermore, the tyrosine hydroxylase innervation in the splenic artery of SDN mice was reduced as compared to sham, as well as noradrenaline content in the spleen (sham: 1000±274 vs SDN: 172±44 pg/mg tissue, $p<0.01$). After 28 days from AngII infusion, SDN mice were protected from HTN as compared to sham (SBP: 107±2 vs 129±3 mmHg) and vehicle treated mice (veh-sham 110±1 vs veh-SDN 106±1 mmHg) ($p<0.001$). Moreover, SDN hampered the T cell egression from splenic reservoir, evaluated as CD3+ cell content, induced by AngII. In the end, we assessed the amount of T cells infiltration, finding that AngII-SDN mice were significantly protected in aorta (CD8+/mm2: veh-sham 197±17; veh-SDN 143±14; AngII-sham 293±36*; AngII-SDN 156±8) and kidney (CD8+/mm2: veh-sham 40±4; veh-SDN 34±4; AngII-sham 231±27*; AngII-SDN 29±5), *$p<0.01$ vs all other groups. Similar results were found for CD4+ infiltrates.
Our results demonstrate that the neuroimmune drive activated by chronic AngII infusion is mediated by the activation of the SSNA, converging into T cells activation in the spleen and their egression toward target organs, where they contribute to the onset of HTN.

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004

Splenectomy Differentially Affects Angiotensin-2 and L-NAME Murine Models of Hypertension

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The immune system plays a major role in animal models of hypertension (HTN) and end-organ damage. However, few studies have assessed the role of lymphoid organs in the pathogenesis of HTN. We have shown previously that prior splenectomy (SPLX) significantly alters tissue inflammation; however the effect of SPLX on HTN remains unclear. Therefore, the objective of the current study is to determine whether prior SPLX influences the development of HTN in 2 different mouse models. Mice underwent SPLX or sham surgery 7 days prior to the induction of HTN using angiotensin-II (AngII, 400ng/kg*min s.c.) or nitric oxide synthase inhibition using L-NAME (30mg/kg*d in drinking water). Systolic blood pressure (SBP) was measured by tail-cuff manometer daily and mice were euthanized 14 days after induction of HTN. Heart weight/body weight (H/BW) ratios were calculated and kidney leukocyte infiltration was analyzed by flow cytometry. Mice with prior SPLX+AngII had significantly lower ($P=0.03$) SBP at both week 1 (148±7) and week 2 (135±7) as compared to Sham+AngII (174 and 173±7mmHg, n=5). Similarly, SPLX+AngII mice had significantly smaller ($P=0.007$) H/BW (4.3±0.3) as compared to

Sham+AngII treated mice (5.2±0.4mg/g BW). Interestingly, no difference was observed in renal CD45+ (9.8±3 vs 10.6±3x105 cells/g, P=0.64) or CD3+ T-cell infiltration (8.8±0.2 vs 9.6±0.1x104 cells/g , P=0.64) between the Sham+AngII and SPLX+AngII treated mice, respectively (n=4/5). Furthermore, SPLX did not appear to influence the development of L-NAME HTN. SPLX+L-NAME mice had similar (P=0.84) SBP (145±4mmHg) as the Sham+L-NAME group (146±4mmHg, n=6) after 2 weeks. Relative heart weights were also similar (P=0.45) between SPLX+L-NAME (4.9±0.2) and Sham+L-NAME treated mice (4.8±0.3mg/g BW). Our data suggests that the full pressor response to AngII is dependent on the spleen. However, the effect of the spleen appears to be independent of renal inflammation. Moreover, the protective effect of the spleen is specific to AngII-dependent HTN and does not appear to be generalizable to all mouse models of hypertension. Further studies are needed to understand the physiological link between lymphoid organs (such as the spleen), renal inflammation, and the development of chronic HTN.

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005

Axl Controls Survival of the CD4+ T Lymphocytes in Salt-dependent Hypertension

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Introduction: Axl, a receptor tyrosine kinase, is required for vascular and immune cell survival. We sought to investigate the effects of Axl on T lymphocyte survival during deoxycorticosterone acetate (DOCA)-salt hypertension in mice.
Methods and Results: We found significant reduction in systolic blood pressure (BP) after 5-6 weeks of DOCA-salt in RAG1-/- mice after adoptive transfer of CD4+ T cells from Axl knockout (Axl-/- →RAG1-/-) compared to transferred CD4+ T cells from wild type (Axl+/+ →RAG1-/-) mice. Media area of the mesenteric artery was significantly lower in Axl-/- →RAG1-/- ($4.2\pm0.7x10^3$ m^2) vs. Axl+/+ →RAG1-/- ($6.0\pm0.9x10^3$ m^2) or Axl+/+ ($6.8\pm0.6x10^3$ m^2) mice. There was significant decrease in interferon gamma production by the T cells from Axl-/- (396±23 ng/mL) compared to Axl+/+ (512±42 ng/mL) after T_h1-priming. The number of carboxyfluorescein succinimidyl ester-positive cells in 6th division was dramatically declined in Axl-/- (~0.3%) vs. Axl+/+ (~1.8%) in culture. Accordingly, we found lower number of lymphocytes in blood from Axl-/- ($4.5\pm0.7x10^9$) compared to Axl+/+ ($7.8\pm0.7x10^9$) mice. Blood leukocyte apoptosis was 2.5-fold higher in Axl-/- mice. We next investigated repopulation capacities of the hematopoietic cells from Axl-/- vs. Axl+/+ mice. There was significant decrease in Axl-/- CD3+ T cells (21±3 %) than Axl+/+ (49±3 %) in spleen after 8 weeks of competitive repopulation of bone marrow-derived cells. However, we found even greater reduction of Axl-/- T lymphocytes (15±1 %) vs. Axl+/+ T lymphocytes (52±6 %) in peripheral blood after 8 weeks of competitive repopulation. Finally, percentage of apoptotic cells was the greatest in the media (20±7 %) and adventitia (13±5 %) from Axl-/- →RAG1-/- mice compared to vascular apoptosis (6-14 % in media; and 6-9 % in adventitia) in other groups after 6 weeks of DOCA-salt.
Conclusions: Our data suggest that Axl-dependent survival of the T lymphocytes is

crucial for the late increase in BP in DOCA-salt hypertension.

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006

Distinct and Overlapping Roles of Cytokines IL-17A and IL-17F in Angiotensin II-induced Hypertension and End-organ Injury

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Recently, interleukin-17A (IL-17A) has been found to contribute to the renal and vascular dysfunction associated with hypertension, but the mechanisms involved are unknown. We and others found that IL-17F is upregulated in IL-17A deficient mice, suggesting a compensatory response. The goal of the present study was to determine the role of IL-17F in hypertension and the effect of IL-17A or IL-17F neutralization on blood pressure and renal/vascular inflammation. Twelve-week-old male C57BL/6J mice received angiotensin II (Ang II) (490 ng/kg/min, s.c. via 4 wk-osmotic minipump). After 2 weeks, the systolic blood pressure as measured by tail-cuff was 179.4 ± 3.7 mmHg, n=33. Mice were then randomly assigned to one of four treatment groups: (1) Mouse anti-IL-17A; (2) Rat anti-IL-17F; (3) Mouse IgG1 control; (4) Rat IgG1 control. Mice were injected intraperitoneally with 100 µg of antibodies twice weekly during the remaining 2 weeks of Ang II infusion. The administration of anti-IL-17A, but not anti-IL-17F, improved Ang II-induced hypertension (166.5 ± 7.4 mmHg, n=10 vs 181.8 ± 8.6 mmHg, n=7 respectively). Interestingly, both anti-IL-17A and anti-IL-17F blunted the increase in aortic and renal total leukocyte infiltration as quantified by flow cytometry in both organs (Fig. 1a). In addition, both treatments attenuated the increase in glomerular injury as monitored by albuminuria measurement (ELISA) during the Ang II infusion period (Fig. 1b). In conclusion, IL-17F neutralization does not lower blood pressure in response to Ang II, but both IL-17A and IL-17F are pro-inflammatory and orchestrate a major role in the end-organ damage associated with Ang II-induced hypertension.

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007

Interleukin-6 Inhibition Attenuates Hypertension and Associated Renal Damage in Dahl Salt-sensitive (SS) Rats

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Data from our lab indicates that infiltration of T lymphocytes into the kidney amplifies salt-sensitive hypertension and renal damage in SS rats. Interestingly, interleukin 6 (IL-6) mRNA is >50-fold higher in T cells isolated from the kidney in comparison to circulating T cells. Experiments were performed to assess the role of IL-6 in Dahl SS rats (n=13-14/group) fed low salt chow until 9 weeks of age and subsequently treated with goat anti-rat IL-6 neutralizing antibody (anti-rIL-6; 4 µg /day, IP; R&D Systems, Minneapolis, MN) or normal goat IgG control (4 µg /day, IP) during an 11 day period of high salt intake. The MAP and urine albumin excretion rates (Ualb) were not different between the

groups when fed low NaCl chow (MAP=121±1.6 mmHg versus 122±1.8 mmHg and Ualb=15±2mg/day versus 12±1.7 mg/day in the vehicle-treated versus anti-rIL-6-treated rats). Following 11 days of drug-treatment, the rats receiving anti-rIL-6 demonstrated a significant 47% reduction in IL-6 in the renal medulla compared to vehicle-treated SS. Moreover, the increase in MAP following 11 days of high NaCl intake was significantly attenuated in SS administered anti-rIL-6 (MAP=138±3 mmHg) compared with the control group (MAP=149±3 mmHg). The renal damage was also attenuated in SS administered anti-rIL-6; Ualb was significantly reduced in the treated (109±10 mg/day) compared to the control group (151±16 mg/day) and glomerular and tubular damage were also attenuated. To investigate potential mechanisms of action, a flow cytometric analysis of infiltrating immune cells in the kidney (n=4-5/group) was performed. The total number of leukocytes (CD45+) was significantly lower in the treatment vs the control group (4.8±0.5x106 vs 6.8±0.5x106 cells/kidney). Infiltrating monocytes and macrophages (CD11b/c+) were also significantly lower in the treatment vs the control group (3.7±0.3x106 vs 5.4±0.3x106 cells/kidney). The total number of infiltrating T and B lymphocytes was not different among the groups. The present studies indicate that IL-6 production may participate in the development of SS hypertension and end-organ damage by mediating the recruitment and infiltration of macrophages into the kidney.

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008

Early Life Stress Induces Renal Pro-inflammatory Immune Responses

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We previously reported that maternal separation (MatSep), an animal model of early life stress, sensitizes rats to pro-hypertensive stimuli in adulthood. We hypothesized that MatSep induces a renal pro-inflammatory immune response. Immune cell populations and expression of cytokines were assessed by magnetic bead isolation, FACS analysis, ELISA and RT-PCR in adult male MatSep and normally-reared littermate control rats. Circulating and renal mononuclear or T cell numbers were similar between control and MatSep rats (n=4-11/group, $p>0.05$). Both groups presented similar percentages of circulating macrophages and T_H, T_C, and T_{reg} cells (n=4, $p>0.05$). However, the percentage of circulating B cells was significantly decreased in MatSep rats (23.7±1.2% vs. 20.1±0.7%; n=4, $p<0.05$). Pro-inflammatory cytokine IL-1Beta was significantly elevated in kidneys from MatSep rats (4.4±0.5 vs. 7.9±1.0 pg/mg prot; n=7-8/group; $p<0.05$). However, IFN-gamma, IL-6, and IL-4 were not different between control and MatSep rats. To further assess the immune system in MatSep and control rats, we acutely challenged adult rats with lipopolysaccharide (LPS; 2 mg/kg; i.v., 14 h). LPS significantly elevated renal expression of pro-inflammatory chemokine receptors (CCR3, CCR4, CXCR4), cytokines (IFN-gamma,

CCL3, CCL4, IL-16), and activation markers (CD40, CD40lg) in MatSep rats (4 to 6 fold increase; n=5/group, p<0.05), suggesting that MatSep induces an exaggerated pro-inflammatory renal immune response to LPS. In conclusion, early life stress induces a renal pro-inflammatory status in adulthood that leads to sensitization to further immune challenges. Funded by P01 HL 69999 to JSP, NIH T32 DK007545 to CDM, F32 HL 116145 to DHH and K99/R00 HL 111354 to ASL.

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009

Vertical Sleeve Gastrectomy Reduces Mean Arterial Blood Pressure and Hypothalamic Endoplasmic Reticulum Stress Independent of Body Weight in Mice

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Bariatric surgery, such as vertical sleeve gastrectomy (VSG), results in remission of hypertension (HTN) and type 2 diabetes. The mechanism(s) by which this occurs remain elusive, but reduction in endoplasmic reticulum (ER) stress is a central concept. For example, VSG-induced reductions in ER stress in peripheral tissues contribute to improved insulin sensitivity. ER stress in the hypothalamus promotes development of HTN; however, brain ER stress has not been assessed in the context of bariatric surgery. Therefore, we hypothesized that VSG would ameliorate high fat diet (HFD)-induced HTN and this would be associated with reductions in hypothalamic ER stress. We have validated a mouse model of VSG that exhibits body weight-independent improvements in glucose homeostasis and peripheral ER stress. Here, male C57 mice (8 wks) were placed on a HFD (60%), which was maintained throughout the study. These mice underwent sham (n=4) or VSG (n=6) surgery and radiotelemeter implantation at 16 wks of age. Sham mice were food restricted to match their body weight to VSG mice (S-WM), to study the body weight-independent effects of VSG. At 2.5 months after surgery mice were fasted (6 hrs) and euthanized for tissue collection. Energy intake and body weight were reduced by ~25% after VSG compared with pre-operative values (39 ± 2 vs 30 ± 2g; P<0.01). Energy intake, body weight and adiposity did not differ between groups. Mean arterial pressure (MAP) was measured by telemetry at 2 and 6 wks after surgery. VSG mice exhibited lower MAP compared with S-WM (S-WM = 112.2 ± 1.4, VSG = 99.5 ± 2.3mmHg; P<0.01). Strikingly, the percent decrease in MAP from 2 to 6 wks after surgery was 4-fold greater in VSG compared with S-WM (%ΔMAP: S-WM = 9.3 ± 2.8, VSG = -5.2 ± 2.6; P<0.001). We assessed the PERK pathway of ER stress and inflammation in the hypothalamus by immunoblotting. Normalized PERKThr980 phosphorylation, downstream eIF2αSer51 phosphorylation and TNFα were reduced by 35%, 29% and 55% in VSG compared with S-WM, respectively (pPERK/PERK (AU): S-WM = 1.2 ± 0.2, VSG = 0.8 ± 0.1; peIF/eIF: S-WM = 1.2 ± 0.1, VSG = 0.8 ± 0.1; TNFα/tubulin: S-WM = 1.4 ± 0.3, VSG = 0.6 ± 0.1; P<0.05). Therefore, VSG produces body weight-independent reductions in MAP which may be due to VSG-induced reductions in hypothalamic ER stress.

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010

The Role of Angiotensin AT_{1A} Receptors in Leptin-sensitive Cells in Resting Metabolic Rate Control

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The brain renin-angiotensin system (RAS) and leptin contribute to the control of resting metabolic rate (RMR) and their receptors are co-expressed in areas of the brain critical for metabolic control; thus angiotensin and leptin may interact within the brain to regulate RMR and obesity. Inhibition of the brain RAS attenuates sympathetic nerve activity (SNA) responses to leptin, leading us to hypothesize that the brain RAS mediates the RMR effects of leptin. Mice lacking angiotensin AT_{1A} receptors in leptin receptor-expressing cells (ObRb-Cre x $AT_{1A}^{flox/flox}$; "KO") exhibited normal body weight (15 weeks of age: control n=28, 26.0 ± 0.7, vs KO n=35, 25.8 ± 0.6 g), food intake (control n=12, 3.1 ± 0.15, vs KO n=15, 3.4 ± 0.14 g) and RMR (control n=13, 0.15 ± 0.004, vs KO n=15, 0.16 ± 0.006 kcal/hr) on standard chow diet. Brown adipose SNA responses to acute leptin injection, however, were completely attenuated in KO mice. When maintained on a 45% high fat diet (HFD), KO mice gained significantly more fat mass (control n=35, 5.6 ± 0.4, vs KO n=31, 7.4 ± 0.5 g, $P<0.05$) and body mass (control, 27.4 ± 0.6, vs KO, 29.6 ± 0.6 g, $P<0.05$) due to a loss of diet-induced thermogenesis (control n=22, 0.18 ± 0.008, vs. KO n=12, 0.16 ± 0.004 kcal/hr, $P<0.05$). KO mice exhibited attenuated hypothalamic proopiomelanocortin (POMC) gene expression and partially attenuated RMR responses to alpha-melanocyte stimulating hormone (αMSH; control n=3, 0.25 ± 0.01, vs KO n=7, 0.2 ± 0.01 kcal/hr, $P<0.05$) indicating that the interaction between leptin and AT_{1A} modulates both αMSH production and action. To localize the site of the brain RAS-leptin interaction, we developed novel multi-transgenic mouse models which expresses GFP via the AT_{1A} promoter (NZ44, from GenSat) and/or conditional activation of a tdTomato reporter (ROSA-$stop^{flox}$-tdTomato) in cells expressing the leptin receptor (ObRb-Cre) or agouti-related peptide (AgRP-Cre). Immunohistochemical staining of adrenocorticotropin in brain tissue from NZ44 mice revealed no localization of AT_{1A} to POMC neurons; in contrast, AT_{1A} was strongly localized with AgRP promoter activity. Taken together, these data support a critical role for angiotensin AT_{1A} receptors on AgRP neurons in the arcuate nucleus in resting metabolic rate control.

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011

Chronic Renal-artery Infusion of Insulin+Glucose Increases Mean Arterial Pressure in Rats

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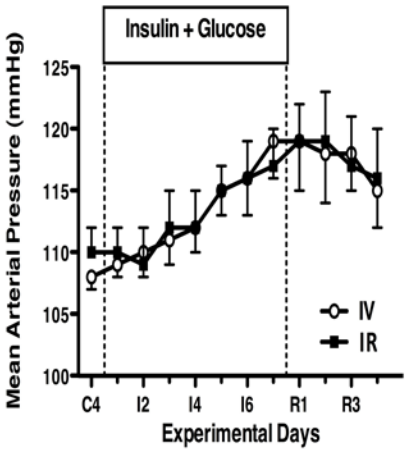
Obesity, metabolic syndrome, and Type 2 Diabetes are a continuum of hyperglycemia and hyperinsulinemia that is associated with hypertension. We have reported that inducing chronic hyperglycemia and hyperinsulinemia by continuous IV infusion increases blood pressure in rats. To test the hypothesis that a renal mechanism underlies the increase in blood pressure, we developed a method for chronic renal-artery (IR) infusion in rats. Male Sprague Dawley rats underwent right nephrectomy and a catheter was placed in the left renal artery. Artery and vein catheters and a DSI telemetry unit for 24 hr/day blood pressure also were implanted. Control IV and IR vehicle infusions were begun in all rats via dual-channel Instech swivels. Rats then were assigned randomly to receive either IV (n=11) insulin (1.5 mU/kg/min) and glucose (20 mg/kg/min) or IR (n=8) insulin and glucose at 20% of the IV doses. The alternate syringe in each group continued with the control vehicle solution. MAP averaged 108±1 and 110±2 mmHg in IV and IR rats, respectively, during control, increased progressively and significantly in both groups during 7 days of insulin+glucose infusion, and returned towards control during recovery. Cumulative sodium balance increased significantly in both groups, with no significant change in GFR in either group. Blood glucose did not change significantly and did not differ between groups. Plasma insulin (uU/ml) did not change significantly in the IR rats (8.3±2.6 to 4.8±1.2), but increased significantly in the IV group (13.3±5.5 to 27.9±4.9). These data suggest that chronic hyperinsulinemia and hyperglycemia increase arterial pressure through a direct action on the kidneys.



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012

Brain Endoplasmic Reticulum (ER) Stress Reduces Appetite and Increases Blood Pressure Independent of the Melanocortin-4 Receptor in Rats

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Although there is evidence that chronic endoplasmic reticulum (ER) stress affects hypothalamic pathways that regulate food intake, body weight and blood pressure (BP), the specific mechanisms are unclear. One key pathway for controlling energy balance and BP is the central nervous system (CNS) melanocortin system. However, the importance of this system in mediating the effects of ER stress on metabolic and cardiovascular function is unclear. In this study we examined the role of

the melanocortin-4 receptor (MC4R) in controlling blood pressure (BP) and metabolic functions during chronic brain ER stress. MC4R knockout ($MC4R^{-/-}$, n=5) and control wild-type Wistar Hannover rats (WT, n=5) were implanted with blood pressure (BP) telemetry transmitters and an intracerebroventricular (ICV) cannula was inserted into the third ventricle at 22 weeks of age. After 10 days of recovery, food intake, BP and HR were measured 24-hrs/day. After stable baseline measurements for 4 days, thapsigargin (TG, 5µg/5µl, ICV) was injected daily for 3 consecutive days to induce ER stress. At baseline, $MC4R^{-/-}$ rats ate 23% more food and were 41% heavier than WT rats. MAP was slightly higher (115±3 vs. 109±2 mmHg) and HR was lower (318±10 vs. 363±6 bpm) in MC4R-/- rats. Induction of brain ER stress decreased food intake (26%) while causing no changes in blood glucose levels (WT: 94±4 vs. 98±3 and MC4R-/-: 110±6 vs.116±4 mg/dl) or HR in both groups. Induction of brain ER stress also raised BP in both groups (7 and 5 mmHg, respectively for WT and $MC4R^{-/-}$ rats). These results suggest that chronic brain ER stress-induced reductions in appetite and increases in BP are independent of MC4R signaling. (NHLBI-PO1HL51971, NIGMS- P20GM104357 and AHA SDG5680016)

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013

Early Life Stress Increases Susceptibility to Develop Obesity and Metabolic Syndrome in a Sex-specific Manner

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Recent epidemiological studies demonstrate that women have a greater prevalence of metabolic syndrome with associated increases in fasting glucose not seen in men. Lifestyle factors including diet and physical activity contribute to the risk of developing metabolic disease; however, it has been reported that exposure to early life stress (ELS) has enduring emotional, immune, and metabolic disturbances resulting in increased risk for obesity and type II diabetes. To investigate the effects of ELS as an independent risk factor for metabolic disease, we expose C57Bl/6 mice to maternal separation (MSep), an established behavioral stress model during postnatal life. At weaning, mice were placed on a low-fat diet (LFD, n=6-10) or high-fat diet (HFD, 60% fat calories, n=10) for 16 weeks. Body weight (BW) gain was not different between MSep and control (C) mice when fed a LFD; however, only female MSep mice display higher fat mass compared to C (6.7 ± 0.5 vs. 5.2±0.5 g, $p<0.05$). Magnetic resonance spectroscopy revealed elevated levels of visceral fat in female MSep mice compared to C, suggesting that MSep increases central adiposity. HFD increased BW in male MSep mice vs C (54.2± 0.7 vs 51.3 ± 0.5, $p<0.05$); however, BW was dramatically exaggerated in female MSep mice vs. C (48.2± 1.3 vs 34.4 ±2.3 g, $p<0.05$). Accordingly, fat mass was increased in female MSep mice vs. C (18.2 ± 1.4 vs. 7.6 ± 0 g, $p<0.05$). Only female MSep exhibited significant impaired glucose tolerance (AUC: 18445±507 vs 22070± 696 AU, $p<0.05$), hepatomegaly, hypercholesterolemia (156±20 vs. 82 ± 2 mg/dl, $p<0.05$), hyperleptinemia and hyperinsulinemia ($p<0.05$) compared to C. Because impaired metabolic function has been linked to inflammation, we characterized splenocytes in female HFD-fed mice. We found a lower population of T-cells but no difference in B-cells in MSep mice vs. C.

Functional studies of T cell differentiation demonstrated a reduced capability of naïve T cells to differentiate into anti-inflammatory T regulatory cells in MSep mice vs. C (54± 4 vs. 66± 1 % FoxP3+/CD25+ cells, $p<0.05$, n=3). The ability to polarize into the pro-inflammatory Th17 phenotype remained intact. These data suggest that the mechanisms by which MSep primes the metabolic responses are sex-specific. NIH R00 HL111354

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014

Nox1-derived ROS Impairs Internal Pudendal Artery Function via Nrf2 and Rho Kinase in Diabetic Mice

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Oxidative stress plays an important role in vascular dysfunction in diabetes, an important risk factor for erectile dysfunction (ED). Functional and structural changes in internal pudendal arteries (IPAs), which provide blood supply to the corpus cavernosum, can lead to ED. We hypothesized that downregulation of Nrf2-regulated enzymes, consequent to increased NOX1-derived ROS, impairs IPAs function in diabetic mice. IPAs and cultured vascular smooth muscle cells (VSMC) from C57BL/6 (control) and NOX1 knockout (KO) mice were used; diabetes (DM) was induced by streptozotocin in C57BL/6 mice. Vascular function assessment, by wire myography, demonstrated that IPAs from diabetic mice displayed increased contractility to phenylephrine (Phe, Emax: control 138.5±9.5 vs. DM 191.8±15.5) and decreased endothelial-dependent relaxation to acetylcholine (ACh, Emax: control 98.3±2.65 vs. DM 76.1±4.5). These responses were corrected by incubation of IPAs with tiron (ROS scavenger), bardoxolone (Nrf2 activator), ML171 (NOX1 inhibitor) and Y27632 (Rho Kinase inhibitor). IPAs from diabetic mice exhibited decreased Y276732-induced vasodilatation (LogEC50: control 5.8±0.1 vs. DM 5.3±0.1). Cultured VSMC from IPAs, maintained in high glucose (HG) medium, displayed increased levels of superoxide anion (59.6%±10.43) and nitrotyrosine (40.8%±9.8), as well as decreased NO production (37.7%±10.42) and nuclear accumulation of Nrf2 (20.42%±3.6), effects not observed in VSMC from IPAs isolated from NOX1 KO mice. The expression of Nrf2-regulated genes was also decreased in VSMC from IPAs maintained in HG [catalase (25.6%±0.05), HO-1 (21%±0.1) and NQO-1 (22%±0.1)], an effect prevented by the incubation with ML171. HG decreased H_2O_2 levels in control VSMC (29%±5.0), but increased H_2O_2 in NOX1 KO VSMC (45.8%±7.1), effects not observed when cells were pre-incubated with GKT 137831 (NOX1/4 inhibitor). In conclusion, diabetes-associated IPAs dysfunction involves increased NOX-1 derived ROS, decreased expression of Nrf2-regulated enzymes and activation of the Rho kinase pathway. These data suggest that Nrf2 may be vasoprotective in diabetes-associated ED. Financial Support: FAPESP, Brazil.

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015

Increased 20-HETE Levels Contribute to Impaired Glucose Metabolism and Type 2 Diabetes in Cyp4a14 Knockout Mice Fed on High Fat Diet.

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20-HETE (20-Hydroxyeicosatetraenoic acid) is a cytochrome P450 ω-hydroxylase metabolite of arachidonic acid that promotes endothelial dysfunction, microvascular remodeling and hypertension. Previous studies have shown that urinary 20-HETE levels correlate with BMI and plasma insulin levels. However, there is no direct evidence for the role of 20-HETE in the regulation of glucose metabolism, obesity and type 2 diabetes mellitus. In this study we examined the effect of 20-SOLA (2,5,8,11,14,17-hexaoxanonadecan-19-yl-20-hydroxyeicosa-6(Z),15(Z)-dienoate), a water-soluble 20-HETE antagonist, on blood pressure, weight gain and blood glucose in Cyp4a14 knockout (Cyp4a14-/-) mice fed high-fat diet (HFD). The Cyp4a14-/- male mice exhibit high vascular 20-HETE levels and display 20-HETE-dependent hypertension. There was no difference in weight gain and fasting blood glucose between Cyp4a14-/- and wild type (WT) on regular chow. When subjected to HFD for 15 weeks, a significant increase in weight was observed in Cyp4a14-/- as compared to WT mice (56.5±3.45 vs. 30.2±0.7g, $p<0.05$). Administration of 20-SOLA (10mg/kg/day in drinking water) significantly attenuated the weight gain (28.7±1.47g, $p<0.05$) and normalized blood pressure in Cyp4a14-/- mice on HFD (116±0.3 vs. 172.7±4.6mmHg, $p<0.05$). HFD fed Cyp4a14-/- mice exhibited hyperglycemia as opposed to normal glucose levels in WT on a HFD (154±1.9 vs. 96.3±3.0 mg/dL, $p<0.05$). 20-SOLA prevented the HFD-induced hyperglycemia in Cyp4a14-/- mice (91±8mg/dL, $p<0.05$). Plasma insulin levels were markedly high in Cyp4a14-/- mice vs. WT on HFD (2.66±0.7 vs. 0.58±0.18ng/mL, $p<0.05$); corrected by the treatment with 20-SOLA (0.69±0.09 ng/mL, $p<0.05$). Importantly, glucose and insulin tolerance tests showed impaired glucose homeostasis and insulin resistance in Cyp4a14-/- mice on HFD; ameliorated by treatment with 20-SOLA. This novel finding that blockade of 20-HETE actions by 20-SOLA prevents HFD-induced obesity and restores glucose homeostasis in Cyp4a14-/- mice suggests that 20-HETE contributes to obesity, hyperglycemia and insulin resistance in HFD induced metabolic disorder. The molecular mechanisms underlying 20-HETE mediated metabolic dysfunction are being currently explored.

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016

Profound and Sustained Amplification of Circulating ACE2 Activity Does Not Protect Diabetic Mice from Kidney Disease

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ACE2 is a monocarboxypeptidase that by converting AngII to Ang1-7 should down-regulate the renin-angiotensin system and therefore provide a means to therapeutically target diabetic kidney disease, a condition where the kidney RAS is overactive. Previous work indicated that soluble human recombinant (r)ACE2 administration for 4 weeks attenuated kidney injury in diabetic Akita mice. Whether such effect of rACE2 can be confirmed and attributed to augmented ACE2 activity is uncertain because chronic use of human rACE2 in mice induces immunogenicity and the development of antibodies that neutralize serum ACE2 activity.
To examine the effect of chronic amplification of circulating ACE2 on kidney injury caused by STZ-induced diabetes and to circumvent the immunogenicity arising from xenogeneic ACE2, ACE2 of mouse origin was administered to mice using either daily i.p. injections (1 mg/kg) of mrACE2 for 4 weeks or after 20 weeks of ACE2 mini-circle (MC) (10-30ug/mouse) administration. MC provides a form of gene delivery that is resistant to gene silencing and, in addition, greatly optimizes long-term in vivo overexpression of proteins of interest. ACE2MC resulted in a profound and sustained increase in serum ACE2 activity (2.4±0.3 vs. 497±135 RFU/ul/hr, $p<0.01$) but kidney ACE2 activity was unchanged (17.4±1.3 vs. 19.0±0.8 RFU/ug prot/hr). mACE2-treated mice injected with STZ developed diabetes similar to sham mice injected with STZ. Systolic BP was not different between non-diabetic mice, sham STZ-mice, and STZ-mice receiving mACE2 by either i.p. mrACE2 or ACE2MC. Urinary albumin was similarly increased in sham STZ-mice and in STZ-mice receiving mACE2. Glomerular mesangial score and glomerular cellularity were both increased to a similar extent in sham STZ-mice and in STZ-mice with mACE2 administration, as compared to non-diabetic controls.
In conclusion, profound and long-term augmentation of ACE2 activity confined to the circulation is not sufficient to attenuate glomerular pathology and albuminuria in STZ-induced diabetic kidney disease probably because of lack of kidney delivery of ACE2. Strategies to achieve over-expression of ACE2 at the kidney level are needed to demonstrate a beneficial effect of this enzyme on diabetic kidney disease.

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017

Ang-(1-7) Influences ET-1 Signaling Through MAS: ETBR Interactions: Implications in Pulmonary Hypertension

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ACE2 and Ang 1-7 have been shown to protect against pulmonary hypertension (PH). Mechanisms remain unclear. Considering the important role of ET-1 in PH pathophysiology and endothelial dysfunction, we asked whether

Ang 1-7 influences ET-1 signaling in endothelial cells and whether Ang 1-7 treatment influences the ET-1 system in PH. Human microvascular endothelial cells (HMEC) were stimulated with ET-1 in absence/presence of Ang 1-7 and showed that Ang 1-7 increased preproET-1 mRNA (250%), ET-1 release (125%) and ETBR protein (50%), $p<0.05$. ET-1 increases in e-selectin mRNA (400%), VCAM-1 protein (38%) and TNFα production (30%) were blocked by Ang 1-7, $p<0.05$. Pro-inflammatory effects were dependent on NO. Ang 1-7 increased NO production (257%) in a Mas and ETBR-dependent manner. Mas and ETBR interaction was observed by immunoprecipitation. To characterise physical interactions between Mas/ETBR, we utilised novel technology, employing a library of peptides scanning the MasR sequence, to define sites of ETBR binding. Substitution and truncation identified regions on MasR that confer specificity for ETBR binding. Peptide disruptors to prevent Mas/ETBR interaction were used for in vitro validation. We previously demonstrated in HMEC that Ang 1-7 stimulates Akt phosphorylation (180%), an effect inhibited by pre-incubation with peptide disruptors, $p<0.05$. To investigate pathophysiological significance of our findings, we investigated whether Ang 1-7 treatment ameliorates PH and whether this is associated with altered ET-1 status. Hypoxia was used to induce PH in mice: normoxic controls (NC), hypoxic PH (HP), normoxic (NA) and hypoxic PH (HA) treated with Ang 1-7 30µg/kg/day. In HP mice, RVSP (18.7 NC vs. 47.6mmHg HP, $p<0.05$) RVH (0.19 NC vs. 0.28 HP, $p<0.01$) and ET-1 levels (0.8 NC vs 2.4pg/ml HP, $p<0.05$) were increased and blocked by Ang 1-7. Hypercontractility and endothelial dysfunction in pulmonary arteries of HP mice was attenuated by Ang 1-7.
These findings indicate that vasoprotective effects of Ang 1-7 may be mediated through MAS:ETBR dimerization. In vivo studies support a relationship between Ang 1-7/MAS and ET-1 systems. In conclusion we have identified a novel link between Ang 1-7 and ET-1 through physical interactions between MAS and ETBR.

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018

Sox6 is a Critical Factor in Renin Cell Fate in Development and Pathology

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Introduction: the renin-angiotensin system (RAS) is an important component of blood pressure regulation in mammals. Renin, primarily expressed and secreted by kidney Juxtaglomerular (JG) cells, catalyzes the rate limiting step in RAS. However, the transcriptional mechanisms that govern the specification of renin expressing cells in development and under normal or pathophysiological conditions remain poorly understood. We sought to determine new regulators of renin cell fate during kidney development and JG recruitment.
Methods: Gene expression profiles of renal MSC and JG cells were determined by Affymetrix Mouse 430 2.0 array. JG cells and renal MSC were isolated from adult C57Bl6 Ren1c YFP mice by collagenase digestion of the kidney, followed by flow cytometry to select for the cells. Renin expression *in vitro* was induced by treatment with IBMX and Forskolin. Renal MSCs were transduced with lentivirus carrying vectors for Sox6, Sox6 shRNA or controls. *Ex vivo* analysis was performed in embryonic

kidneys (14.5 dpc) isolated and transduced with Sox6 shRNA or scrambled shRNA. The kidneys were then cultured for 4 days.
Results: Microarray data showed that the transcription factor Sox6 was highly expressed in FACS isolated JG cells compared to renal MSC (96-fold, n=3, P<0.05). This finding was validated by qPCR (100-fold, n=4, P<0.05). Sox6 was expressed in renin producing cells during kidney embryogenesis as determined by immunofluorescence and confocal microscopy (E14.5, N=4). Knockdown of Sox6 by shRNA in an *ex vivo* model of kidney development resulted in a 70% reduction of renin expression (N=4, P<0.01). Differentiation of adult renal MSCs to renin producing cells in vitro was enhanced by overexpression of Sox6 (20-fold, N=6, P<0.01). Knockdown of Sox6 by shRNA inhibited in vitro MSC differentiation (5-fold, N=6, P<0.001). In vivo, low salt and furosemide, which stimulates JG recruitment, increased Sox6 expression (5-fold, N=5, P<0.001) and colocalization with renin.
Conclusion: Our results support a novel and critical role for the transcription factor Sox6 in renin cell fate and in renal development and physiology. Further studies can provide an in-depth understanding of the role of Sox 6 in hypertension and its therapy.

J. Gomez: None.

019

Specific Knockdown of Renal Tubular Epithelial Ace Prevents Salt Sensitivity

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We previously showed that mice lacking renal angiotensin-converting enzyme (ACE) do not accumulate angiotensin (Ang) II in the kidney and are protected against different forms of experimental hypertension, including salt sensitivity. However, these studies did not identify the locus of renal Ang II generation. Since ACE expression is high in the renal epithelium, we hypothesize that tubular epithelial ACE is the main source of renal Ang II and, is responsible for salt sensitivity. To study this, we designed an inducible tubular ACE knockdown mouse (it-ACE) that has three transgenes: one encoding the transcription factor tTA (Tet-off transactivator) that is under the control of the KSP-cadherin promoter only expressed in renal tubular cells. The second and third transgenes are activated by tTA and encode a short-hairpin RNAs (sh-RNA) degrading ACE mRNA in renal tubules. We found a lower basal ACE expression in the kidney of it-ACE compared to WT mice (19 ± 4 vs. 100 ± 26 AU; p<0.01 by Western blot). Selective tubular ACE suppression was confirmed by immunohistochemistry. At baseline, it-ACE mice display normal systolic blood pressure (SBP; 108 ± 4 mmHg), GFR (1288 ± 24 µl/min/100g b.w.) and urine concentration (2516 ± 184 mOsm/Kg after 12h water deprivation) compared to WT mice. To test the response of it-ACE mice to salt-sensitivity, we used a post L-NAME salt sensitivity protocol: L-NAME (0.5 mg/mL; 4 weeks), washout (1 week), high salt diet (HS, NaCl 4%, 3 weeks). In WT mice, L-NAME increased SBP from 109 ± 5 to 139 ± 4 mmHg (p<0.01; n=5). SBP then returned to baseline during the washout and increased again in response to the HS diet (135 ± 3 mmHg;

p<0.01). Thus, WT mice, previously salt resistant, became salt sensitive. Remarkably, it-ACE mice, despite being as hypertensive as WT during the L-NAME phase, were still protected against salt sensitivity (110 ± 3 mmHg; p<0.01 vs. WT; n=6). Finally, we treated it-ACE mice with doxycycline (a tTA blocker) to restore tubular ACE specifically during the HS phase. Restoring tubular ACE in the mutant mice induced the development of salt sensitivity (134 ± 6 mmHg; p<0.01 vs. baseline; n=5). In conclusion, our data indicate that tubular epithelial ACE, and not ACE expression in endothelium or other locations, is essential for salt sensitivity.

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020

A Mitochondrial Renin-Angiotensin System: Internalization of Angiotensinogen

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There is compelling evidence for actions of an intracellular renin-angiotensin system (RAS) in various cell organelles including the endoplasmic reticulum, nucleus and the mitochondria (Mito). Indeed, angiotensin (Ang) AT1 and AT2 receptor subtypes were functionally linked to Mito respiration and nitric oxide production, respectively in a previous study. Since elucidation of mitochondrial pathways for expression of RAS protein components as well as Ang II or Ang-(1-7) is equivocal at this time, we undertook a biochemical analysis of the Mito RAS from adult male sheep kidney. Cortical Mito were isolated by differential centrifugation and a discontinuous Percoll gradient. Purified Mito were co-enriched in the voltage-dependent anion channel, an outer Mito membrane marker as well as ATP synthase, an inner membrane marker. Angiotensinogen (Aogen; 55 kDa) was detected in Mito extracts by an Aogen antibody to an internal sequence of the protein, but not with an antibody directed against the Ang I N-terminus. Two different renin antibodies identified a major 35 kDa protein band in the isolated Mito. Using the Ang I-directed Aogen antibody, active renin was confirmed by hydrolysis of Aogen that was abolished by aliskiren; however, trypsin exposure did not increase renin activity in the Mito. A pro-renin receptor (PRR) antibody failed to identify proteins in three Mito preparations, but revealed a prominent band in renal cortical membranes that corresponds to the size of PRR. Angiotensin peptides were quantified by three direct RIAs; the Mito content of Ang II and Ang-(1-7) were higher as compared to Ang I [23 ± 8 and 58 ± 17 vs. 2 ± 1 fmol/mg protein; p<0.01, n=3]. Additionally, both neprilysin and thimet oligopeptidase activities that processed Ang I to Ang-(1-7) were evident. Finally, cortical Mito internalized radiolabeled Aogen at a rate of 33 ± 9 fmol/min/mg protein (n=3) at 37°C. The subsequent analysis of the labeled Mito by SDS-gel fractionation revealed a predominant radioactive band of 55 kDa for Aogen. Collectively, our data suggest that the internalization of Aogen and subsequent processing by active renin may yield des-[Ang I]-Aogen and the active peptides Ang II and Ang-(1-7) that may potentially contribute to mitochondrial function within the kidney.

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021

Nephron Specific Deletion of the Prorenin Receptor Modulates Blood Pressure and Urinary Na+ Excretion

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The nephron prorenin receptor (PRR) may modulate blood pressure (BP) and Na+ balance. Since previous models of PRR knockout (KO) mice had early lethality and/or structural defects, we developed an inducible nephron-wide PRR KO using the Pax8/LC1 transgenes. Disruption of nephron PRR at 1 month of age caused no renal histological abnormalities. On a normal Na+ diet, wild-type (WT) and PRR KO mice had similar BP and Na+ excretion. However, PRR KO mice had elevated PRC (KO- 377 ± 77 vs WT- 127 ± 19 ng Ang-I/ml/hr) and a 50% decrease in renal ENaC-α protein. Protein levels of NHE3, NKCC2, NCC and ENaC-β/γ were similar between the two groups. Treatment with mouse prorenin (10 nM for 30 min) increased ENaC channel number by 2-fold, but not open probability, in isolated split-open cortical collecting ducts (CCD) from WT mice; this was prevented by Akt inhibition (A6730) but unaffected by blockade of AT-1 (losartan), ERK1/2 (U0126) or p38 MAPK (SB203580). Addition of prorenin (10 nM) did not change isolated CCD [Ca2+]i as assessed by Fura-2 loading (10 min exposure with readings every 3 sec). On a low Na+ diet, PRR KO mice had increased Na+ excretion (Day 2: KO - 66 ± 11 vs WT- 42 ± 6 µmol/day; Day 6: KO - 39 ± 4 vs ET- 23 ± 4 µmol/day) however, no differences in BP were observed. PRC was elevated in PRR KO mice on a low Na+ diet (KO- 384 ± 40 vs WT-174 ± 12 ng/ Ang-I/ml/hr). PRR KO mice had an attenuated hypertensive response to Angiotensin-II (Ang-II) infusion at 600 ng/Kg/min for 2 weeks (MAP: KO - 117 ± 4 vs WT - 133 ± 4 mm Hg over the course of Ang-II infusion). Urinary Na+ excretion was elevated in Ang-II treated PRR KO mice as compared to WT mice (KO-344 ± 14 vs WT-268 ±30 µmol/day). Taken together, these data indicate that nephron PRR, likely via direct prorenin/renin stimulation of an Akt-dependent pathway, stimulates CCD ENaC activity. Absence of nephron PRR promotes Na+ wasting and reduces the hypertensive response to Ang-II.

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022

A Short Open Reading Frame in the Angiotensin Type 1a Receptor 5' Leader Sequence Increases the Rate of Receptor Internalization

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Background: We recently found that a seven amino acid peptide (PEP7) encoded within a short open reading frame (sORF) in exon 2 of

the 5’ leader sequence (5'LS) of the angiotensin type 1a receptor (AT1aR) mRNA inhibits AT1aR-mediated activation of extracellular signal-regulated protein kinases 1 and 2 (Erk1/2) without having any effect on the AT1aR-inositol trisphosphate protein kinase C pathway. To investigate the mechanism by which PEP7 selectively inhibits the AT1aR-Erk1/2 signaling cascade, the start codon of the sORF was mutated at adenine -108 (A-108 to T-108) to create E1,2(-108T),3-AT1aR followed by cloning the E1,2,3-AT1aR and the mutant E1,2(-108T),3-AT1aR into the pEGFP-N2 plasmid. Methods: Human embryonic kidney 293 (HEK-293) cells were transfected with E1,2,3-AT1aR (intact sORF construct) or E1,2(-108T),3-AT1aR (disrupted sORF construct) by lipofectamine. Forty eight to seventy two hours later, live cell time lapse images were collected before and after treatment with Ang II (100 nM) using a TE300 Spinning disk laser scanning confocal microscope. Image analysis was performed using Velocity software. Results: Within 5 min of Ang II stimulation, punctae formed and moved throughout the cell membrane in cells transfected with both EGFP tagged receptors; however, even though the punctae represent the localized accumulation of identical AT1aR proteins, there were distinct differences in the intensity and time course of punctae. The rate of vesicle formation after Ang II treatment was markedly decreased by disrupting the PEP7 sORF [t1/2 (s): E1,2,3-AT1aR, 203s (N=21) vs E1,2(-108T),3-AT1aR, 328s (N=14); $p<0.0001$]. Conclusion: The PEP7 sORF facilitates Ang II-induced AT1R internalization. These findings suggest that we have uncovered a new mechanism governing agonist-induced AT1aR cellular trafficking that could have implications not only for regulation of AT1aR signaling cascades but also for other trafficking proteins that contain an upstream sORF within their 5'LS.

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023

Collecting Duct (Pro)Renin Receptor Mediates Angiotensin II-induced Hypertension

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Within the kidney, (pro)renin receptor (PRR) is predominantly expressed in the intercalated cells (IC) of collecting duct (CD) where its expression is induced by angiotensin II (AngII). Here we examined the function of PRR in the CD by analyzing mice with CD-specific deletion of PRR (CD PRR KO) using AQP2-Cre which has recently been shown to target both IC and principal cells (PC). Radiotelemetry demonstrated that the null mice were largely resistant to AngII-induced hypertension (MAP on day 7: Floxed/AngII 137.4 ± 3.5 vs. KO/AngII 121.2 ± 1.1 mmHg, $p<0.05$, n=4), accompanied with reduced urinary soluble PRR (sPRR) and aldosterone levels. Electrophysiology analysis demonstrated that within minutes activation of PRR by 10 nM prorenin induced a transient increase in amiloride-sensitive Na+ transport in

cultured mpkCCD cells (Ieq: 1.85 ± 0.17 vs. 1.30 ± 0.06 μA/cm2, p<0.05). Interestingly, this was followed by a second phase of ENaC activation after 6 h, which reached the plateau activation at 24 h, accompanied with increased aldosterone release as assessed by ELISA (14.41 ± 0.92 vs. 5.45 ± 0.28 pg/ ml/μg protein, p<0.05). The chronic but not acute phase of ENaC activation was abolished by eplerenone. Both phases of ENaC activation depended on Nox4-derived reactive oxygen species (ROS). Immunostaining using an antibody against sPRR (the N terminus) showed exclusive labeling in the principle cells (PC) whereas the labeling with the C-terminal antibody was exclusively found in IC. A recombinant histidine-tagged sPRR, termed sPRR-His, in the nanomolar range induced a similar dual effect on ENaC activation as prorenin. Intravenous infusion of sPRR-His in CD PRR KO mice for 5 days completely restored the hypertensive response to AngII (MAP: 135.5 ± 7.5 vs. 116.7 ± 5.7 mmHg, p<0.05). We conclude that: 1) CD PRR mediates AngII-induced hypertension; 2) PRR activation in the CD leads to increased ENaC activity acutely through the direct action of ROS and chronically through local generation of aldosterone; 3) sPRR derived from IC may act in a paracrine fashion to stimulate Na+ transport in PC.

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024

The Direct Renin Inhibitor Aliskiren Improves Vasodilation and Endothelial Function in Resistance Arteries from Diabetic and Hypertensive Patients

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We previously demonstrated that the direct renin inhibitor aliskiren (ALK) significantly reduced the remodeling of subcutaneous resistance arteries of hypertensive patients as compared to the angiotensin-converting enzyme inhibitor ramipril (RAM). Here we questioned whether endothelial function of resistance arteries would improve after 1 year of blood pressure (BP) control with ALK or RAM. Sixteen diabetic patients with mild essential hypertension were randomized to ALK (150-300 mg once daily, n=9) or RAM (5-10 mg once daily, n=7). Subcutaneous resistance arteries were dissected from gluteal biopsy and mounted on a pressurized micromyograph. Endothelium-dependent and -independent relaxations were assessed by concentration-response curves to acetylcholine (1 nM to 100 μM) and sodium nitroprusside (10 nM to 1 mM) respectively, in arteries pre-contracted with norepinephrine (10 μM). Carotid-femoral pulse wave velocity (PWV) was assessed by applantion tonometry. Forearm flow mediated dilation (FMD) was assessed by ultrasounds. The expression of P-eNOS/e-NOS and the markers of oxidative stress nitrotyrosine and LOX-1 were assessed by immunohistochemistry in resistance arteries.
Patients were similar for age, sex, BMI and

glycemic and metabolic control. Systolic BP was significantly and equally reduced by both ALK and RAM (153±8.9 mmHg reduced to 128±7.3 mmHg and 151±10.6 mmHg reduced to 121±12.1, respectively, P<0.01) whereas diastolic BP was significantly reduced only in ALK-treated but not in RAM-treated patients (94.2±7.17 mmHg reduced to 81.4±6.31 mmHg, P<0.01; and 84.7±12.22 reduced to 78.6±7.48, NS, respectively). PWV and FMD were similar in both groups before and after treatment. Endothelium-dependent vasodilation was improved only by ALK (max dilation 92.5±3.8% vs 50.5±14%, P<0.05) but not by RAM (max dilation 72.9±5.6% vs 61.8±14.5%, NS). Endothelium-independent vasodilation was similar in all the groups. Only ALK increased P-eNOS/eNOS expression (+44% vs before treatment, p<0.05). The markers of oxidative stress were similar in both groups before and after treatment.
In conclusion ALK improved endothelial function and induced vasodilation in resistance arteries from diabetic and hypertensive patients.

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025

Evidence for a Link between Gut Microbiota and Hypertension in the Dahl Rat Model

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Gut microbiota play a critical role in maintaining physiological homeostasis. This study was designed to evaluate whether gut microbial composition impacts hypertension. 16S rRNA genes obtained from cecal samples of Dahl salt-sensitive (S) and Dahl salt-resistant (R) rats, were sequenced. Bacteria of the phylum Bacteriodetes were higher in the S rats compared with the R rats. Further, the family S24-7 of the phylum Bacteriodetes and the family Veillonellaceae of the phylum Firmicutes were higher in the S rats compared to the R rats. Analyses of the various phylogenetic groups of cecal microbiota revealed significant differences between S and R rats. Both strains were maintained on a high-salt diet, administered antibiotics for ablation of microbiota, transplanted with S or R rat cecal contents and monitored for blood pressure (BP). Systolic BP of the R rats remained unaltered irrespective of S or R rat cecal transplantation. Surprisingly, compared to the S rats given S rat cecal content, systolic BP of the S rats given a single bolus of cecal content from R rats was consistently and significantly elevated during the rest of their life and had a shorter lifespan. Lower level of fecal bacteria of the family Veillonellaceae and increased plasma acetate and heptanoate were features associated with the increased BP observed in the S rats given R rat microbiota compared with the S rats given S rat microbiota. These data demonstrate a link between microbial content and BP regulation and because the S and R rats differ in their genomic composition, provide the necessary basis to further examine the

relationship between the host genome and microbiome in the context of BP regulation in the Dahl rats.

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026

Gut Bacteria Metabolite Attenuates Ang II-induced Cardiac Damage

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Increasing evidence suggests that the gut microbiota critically influence host health and immune homeostasis. Microbiome-host communication occurs via gut bacterial metabolites which are resorbed by the host and target various organs. Short-chain fatty acids (SCFA) are produced from bacterial fermentation, are highly abundant in the gut but can also be detected in the blood. Recently, the SCFA propionate has been shown to regulate T cell differentiation into effector and regulatory T cells in peripheral tissues. Since activation of the immune system is known to substantially contribute to hypertensive target organ damage and anti-inflammatory strategies have been shown to be beneficial in animal models, we hypothesized that treatment with propionate would be beneficial in angiotensin (AngII)-induced target organ damage. Male NMRI mice received AngII infusions for two weeks and propionate (P) or vehicle (C) in drinking water. To deplete endogenous SCFA production mice were fed a low-fibre diet. Body weight was similar among all groups. Propionate treatment significantly reduced albuminuria (C 1143 ± 193; P 302 ± 69 µg/d). Propionate significantly reduced cardiac hypertrophy as measured by heart-to-tibia ratio (C 10.1 ± 0.4; P 8.9 ± 0.4 mg/mm) and was confirmed by echocardiography. Propionate treatment significantly reduced interstitial (C 16.5 ± 0.8%; P 6.6 ± 0.2%) and perivascular cardiac fibrosis (C 1.5 ± 0.06; P 1.1 ± 0.03 µm/µm) as measured by fibronectin and collagen I immunofluorescence, respectively. In vivo cardiac electrophysiology studies showed a significantly reduced susceptibility to ventricular arrhythmias in propionate-treated mice (C 71 ± 14%; P 24 ± 16%), indicating the functional relevance of the improved cardiac morphology. Propionate reduced the expression of IL-17 in CD4+ T cells in spleen and lymph nodes as measured by flow cytometry. Our data indicate that propionate attenuates AngII-induced cardiac remodeling and reduces susceptibility to arrhythmias. The gut microbiome is a promising target for treatment of hypertensive heart disease.

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027

Intestinal Permeability and Dysbiosis are Linked to Hypertension

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Introduction: Emerging evidence implicates the involvement of intestinal microbiota in overall physiological homeostasis. Altered microbial composition is associated with metabolic, cardiovascular, and neurological diseases. However, the role of intestinal microbiota in blood pressure control and hypertension (HTN) remains unexplored. The present study was designed to evaluate the hypothesis that both intestinal dysbiosis and altered intestinal function are critical pathophysiological events in HTN. **Methods:** 16S ribosomal DNA from fecal samples from SHR and chronic angiotensin II (Ang II, 200ng/kg/min) rat models was utilized to compare gut microbial communities between normotensive and hypertensive animals. Gut permeability was assessed by accumulation of FITC-dextran (44mg/100g BW) in the plasma 4 hours following oral feeding. Ex vivo atomic force microscopy was used to determine small intestine and colon stiffness (a measure of permeability). Tight junction gene expression was quantified by qPCR. **Results:** We observed a significant decrease in microbial richness (20%), diversity (12%), and evenness (10%) in SHR vs WKY. This was associated with an increased Firmicutes (F)/Bacteroidetes (B) ratio (4±1 vs 24±5), a hallmark of gut dysbiosis. Additionally, we observed a 95% increase in plasma FITC-dextran in SHRs (1777±428 vs 3514±563 ng/ml, $p<0.05$), which correlated with decreased mRNA of several tight junction genes throughout the intestine, including *Ocln*, *Tjp1*, and *Cldn4*. In addition, we found increased stiffness of both small intestine and colonic tissue, as evidenced by increased elastic modulus in SHR (small intestine: 21.2±3 vs 53.7±8 kPa, colon: 15.7±2 vs 53.4±14 kPa, $p<0.05$). Similar decreased microbial richness and increased F/B ratio (~2 fold) were observed in the Ang II rat model at 4 weeks. Plasma FITC-dextran began to rise by day 14 (SBP=160 mmHg), and reached maximal increase of 65% by day 21 (SBP=185 mmHg). Furthermore, colon wall stiffness was significantly increased by day 21 of Ang II infusion. **Conclusions:** These observations show that increased gut permeability/leakiness and dysbiosis is associated with HTN. They are the first to demonstrate a profound intestinal pathophysiology and microbial dysbiosis in HTN.

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028

ACE2 Activator, Diminazene, Rebalances Gut Microbial Dysbiosis and Attenuates Pulmonary Hypertension

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Introduction: Our previous studies have established that increasing the levels of pulmonary Angiotensin converting enzyme2 (ACE2) either by genetic overexpression or by a small molecule activator, Diminazene aceturate (DIZE) provides protection against lung injury. In view of the mounting evidence of the involvement of the gut microbiota in inflammatory, metabolic and neurological diseases, we proposed the following hypothesis: gut dysfunction and microbial dysbiosis is associated with pulmonary hypertension (PH) and that the cardiopulmonary beneficial effect of DIZE is mediated, in part, by its influence on the gut

microbial composition.
Methods: PH was induced in male Sprague Dawley rats by a single injection of monocrotaline (MCT; 50mg/Kg s.c). A subset of MCT rats was treated daily with DIZE (15mg/Kg/day s.c) for 4-weeks, after which hemodynamic parameters were measured and fecal samples collected for bacterial 16S ribosomal DNA analysis. In addition, colon samples were isolated to determine tissue stiffness by ex vivo atomic force microscopy.
Results: MCT administration resulted in the development of PH as evidenced by increase in right ventricular systolic pressure (RVSP - Control: 30+2; MCT: 93+10 mmHg; $p<0.05$), which was associated with significant decreases in gut microbial richness (37%), diversity (22%), and evenness (16%). Furthermore, we observed a significant reduction in acetate- and butyrate-producing, and increases in lactate-producing bacterial population in PH animals. Elastic modulus and viscosity of the colon were increased by 61% and 86% respectively in MCT animals as compared with controls, indicating greater tissue stiffness (Elastic modulus - Control: 7.33+1.66; MCT: 11.84+3.41 kPa; Viscosity - Control: 145+19; MCT: 270+36 kPa*s; $p<0.05$). However, chronic treatment with DIZE attenuated all these parameters (RVSP - MCT+DIZE: 52+9 mmHg; Elastic modulus - MCT+DIZE: 8.97+3.84 kPa; Viscosity - MCT+DIZE: 180+77 kPa*s; $p<0.05$)
Conclusions: These observations demonstrate for the first time that a) gut microbial dysbiosis is associated with MCT-induced PH; b) DIZE attenuates PH pathophysiology and significantly rebalances dysbiosis. They suggest that the gut microbiota could be a potential target for PH therapy.

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029

Reduced Bone Marrow Adrenergic Receptor Signaling Modulates Inflammatory Factors and Alters Gut Microbiota

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Brain - bone marrow (BM) communication is implicated in regulation of blood pressure (BP) in neurogenic hypertension (HTN), and chronic elevation of sympathetic drive contributes to BM inflammatory cell (IC) activity and promotes vascular inflammation. Our recent study linked gut dysbiosis to human and animal HTN; thus, we hypothesize that blocking the effects of sympathetic drive in the BM will result in reduction of BM ICs and modify gut microbiota.
Methods: Whole BM cells were extracted from adrenergic receptor beta 1 and 2 knock out mice ($Adrb1^{tm1Bkk}Adrb2^{tm1Bkk}$/J KO) and reconstituted into lethally- irradiated C57BL/6J mice to generate C57-AdrB1.B2 KO chimera, characterized by reduced/diminished effect of sympathetic drive on the BM cells. Control mice (C57-C57) were generated by reconstitution of irradiated C57BL/6J with whole BM from C57BL/6J mice. All mice were recovered for 2-3 months prior to measurements. Specific primers were used to amplify variable region 4-5 of 16S rDNA from isolated fecal DNA. Purified amplicons were pooled to generate bacterial library, quantified by qPCR and subjected to Illumina Miseq sequencing. Reads were aligned with the Silva nonredundant 16S reference database. BP recordings were performed using

radiotelemetry (DSI). Blood was collected at Night (8-9pm, i.e. at highest activity) for analysis of circulating ICs. **Results**: We observed a significant reduction of circulating ICs ($CD4^+CD8^+$: 2.3±0.6% vs. 0.876±0.1%; $CD11b^+$: 15.4±4% vs. 8.5±0.8%; $CD184^+CD62L^+$: 25±4.3% vs. 6.6±0.8%; (% of mononuclear cells)), and increase in endothelial progenitors ($Tie2^+$: 21.3±1.5% vs. 32.2±5.7%) in chimera vs. control. A trend in BP reduction was observed in chimera (Day: 110±6 vs. 101±2 mm Hg; Night: 130±2 vs. 123±2 mm Hg). Expansion of *Bacteroidetes* (control: 34.2%±3.0 vs. chimera: 49.9%±3.3), and contraction of *Firmicutes* (control: 63.9%±3.0 vs. chimera: 48.9%±3.8) were observed in chimera, lowering the F/B ratio two fold. **Conclusion**: Diminished effect of BM sympathetic drive dampens BM-derived ICs and favors the bloom of Bacteroidetes phylum, suggesting the role of BM in gut homeostasis.

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030

Microbial Short Chain Fatty Acid (SCFA) Metabolites Lower Blood Pressure (BP) via Endothelial G-protein Coupled Receptor 41 (gpr41)

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Short chain fatty acid (SCFA) metabolites (acetate, propionate and butyrate) are byproducts of gut microbial metabolism that affect host physiology, and have been shown to dilate blood vessels *ex vivo*. We have previously shown that intravenous delivery of SCFAs to anesthetized mice decreases BP by activating Gpr41, which is expressed in blood vessels. Here, our aim was to identify the cellular localization of Gpr41 and to determine the role of Gpr41 in BP regulation. Using RT-PCR we observe that Gpr41 is readily detected in vessels with an intact endothelium, but is absent from vessels where the endothelium has been denuded. Thus Gpr41 is expressed in the endothelium. Since Gpr41 was previously found to mediate a hypotensive response to acute SCFA administration, we hypothesized that Gpr41 knockout (KO) mice would be hypertensive at baseline. Concordant with our hypothesis, we find that Gpr41 KO (n=4) have elevated systolic hypertension and pulse pressures compared to wild-type (WT, n=4) mice (Table 1); diastolic BP was not different between genotypes. In agreement with a phenotype of systolic hypertension, KO mice also exhibit elevated pulse wave velocity (Table 1). Administration of 200mM sodium propionate (a Gpr41 ligand) in the drinking water worsens the systolic hypertension in KO (Table 1), while administration of equimolar sodium chloride (control) does not affect BP in WT or KO. We hypothesize that the hypertensive effect of propionate in KO is mediated by another SCFA receptor, Olfactory receptor 78 (which we have previously shown to mediate increases in BP upon activation). In sum, these studies demonstrate that endothelial Gpr41 acts to lower baseline BP.

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031

Endoplasmic Reticulum (ER) Stress in the Subfornical Organ (SFO) Induces Peripheral Inflammation by Altering Autonomic Output

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Endoplasmic reticulum (ER) stress is a contributing factor in a variety of chronic diseases. We previously showed that ER stress in the SFO is involved in the activation of the adaptive immune system, which is causally linked with hypertension. However, the exact mechanism by which ER stress in the SFO activates peripheral T cells remains unclear. Recently it has been shown that the SFO is involved in modulating autonomic output to stimulate T cells. Here we tested if ER stress in the SFO increases autonomic drive to stimulate peripheral T cell proliferation. Five days of intracerebroventricular (ICV) delivery of the ER stress inducer thapsigargin (Tg, 1 µg/day) resulted in significant increases in peripheral T cells in aorta and blood as measured by flow cytometry (Aorta: Veh, 2.1 ± 0.7 vs. Tg, 10.1 ± 2.8 x 10^3 cells/aorta; Blood: Veh, 17.8 ± 1.3 vs. Tg, 34.8 ± 4.7 % of $CD45^+$ cells, n=8-13, $p<0.05$). ER stress in the brain resulted in a marked increase in plasma norepinephrine (Veh: 285 ± 28 vs. Tg: 406 ± 45 pg/mL, n=4-5, $p<0.05$). We also found that the effect of ICV Tg to increase peripheral T cells was blocked by hexamethonium (Hex, i.p., 30 µg/g) (Aorta: Tg, 10.1 ± 2.8 vs. Tg+Hex, 3.1 ± 0.4 x 10^3 cells/aorta; Blood: Tg, 34.8 ± 4.7 vs. Tg+Hex: 21.4 ± 1.9 % of $CD45^+$ cells, n=6, $p<0.05$). Consistent with earlier findings, ICV infusion of Tg increased ER stress markers in the SFO (*CHOP*: Veh, 1.15 ± 0.106 vs. Tg, 3.285 ±1.04; *grp78*: Veh, 1.032 ± 0.13 vs. Tg, 3.065 ± 1.102; *p58IPK*: Veh, 0.85 ± 0.213 vs. Tg, 7.065 ± 2.588, fold change, normalized to 18S, n=4-6, $p<0.05$), but did not alter the expression of these markers in aortas. This suggests that T cell activation is centrally mediated and not caused by peripheral vascular ER stress or inflammation. In conclusion, our data suggest that ER stress in the SFO modulates autonomic nervous system output and activates the peripheral immune system, a hallmark of the development of hypertension.

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032

Microglial TLR4 Mediate Angiotensin II-induced Reactive Oxygen Species Production within the Paraventricular Nucleus in Hypertension

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Angiotensin II (AngII) contribution to hypertension involves CNS inflammation that includes cytokine release and reactive oxygen species (ROS) production. The innate immune system, via TLR4 signaling, has been implicated in AngII-mediated inflammatory responses. Yet, whether microglia, the immune cells of the CNS, are key cell targets mediating these effects is still unknown. Thus, we studied here whether TLR4 is a molecular link connecting AngII mediated microglia activation and ROS production within the paraventricular nucleus (PVN) during hypertension. TLR4 and AngII type

1 receptor (AT1) mRNA expression was found in isolated PVN microglia, providing molecular evidence for AT1/TLR4 crosstalk. Isolated microglia TLR4 mRNA expression was 2.56*-fold higher in hypertension. TLR4 and microglia (IBA1) immunoreactivity density were increased in the PVN of SHR vs WKY (TLR4 1.8±0.02 vs 1.3±0.1*; IBA1 23±3.03 vs 13.6±0.6* Arbitrary units - AU). Altered density was attenuated in SHR treated with AT1 blocker Losartan (1.3±0.05 and 14.9±0.9 AU). Higher TLR4/IBA1 co-localization was found in SHR vs WKY (4.6±0.9 vs 1.3±0.2* AU), supporting microglia activation and TLR4 upregulation in hypertension. To study whether AngII-induced microglia activation and ROS production involved TLR4, we used TLR4 deficient (TLR4-D, C3H/HeJ) and sufficient (TLR4-S, C3H/OuJ) mice. Acute hypothalamic slices exposed to AngII (1μM, 60 min) showed increased IBA1 density in the PVN of TLR4-S (3.9±0.1 to 6.5±0.3*%) and WKY (9.8±1.1 to 16.2±1.4*%). This effect was blunted in TLR4-D (4.3±0.1 to 5.2±0.5%). In SHR, AngII failed to further promote microglia activation (17.3±1.8 vs 14.1±1.1 AU). AngII (1 μM) increased dihydroethidium (DHE) staining (an indirect measure of ROS) in the PVN of WKY (166±2*) and TLR4-S (129±2*) compared to vehicle (100±3%). This effect was blunted in TLR4-D (102±2%). AngII-induced ROS production was attenuated by the microglia inhibitor minocycline (100 μM, 131±2*%) and blunted by Losartan (2 μM, 111±6%), suggesting AngII-ROS production involves microglia and AT1. Our results support a major contribution of microglial TLR4 to AngII-mediated ROS and microglia activation within the PVN, actions that are upregulated in hypertension. *P<0.05.

V.C. Biancardi: None. **J.E. Stern:** None.

Neuronal Knock-out of AT1 Receptor Attenuates the Development of Doca-salt-induced Hypertension

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We previously reported that neurogenic hypertension is associated with an increase in A Disintegrin And Metalloprotease 17 (ADAM17) activity and a reduction of Angiotensin Converting Enzyme 2 (ACE2) activity in the hypothalamus. In addition, we showed that silencing ADAM17 or blocking Angiotensin (Ang)-II type 1 receptors (AT1R) in the central nervous system (CNS) prevented DOCA-salt hypertension, confirming the pivotal role of AT1R and ADAM17 in neurogenic hypertension. However, the interaction between AT1R, ADAM17, and ACE2 is still unclear. Since ADAM17 is known to be expressed in multiple cell types and can be activated by various receptors, we tested the hypothesis that neuronal AT1R are necessary for ADAM17-mediated ACE2 shedding in neurogenic hypertension. Male neuronal AT1R knockout (AT1R floxed crossed with Nefh-cre recombinase mice, 12-16 week-old, n=5) and littermate mice (n=8) were implanted with telemetry probes for continuous recording of blood pressure (BP) and heart rate (HR). Following DOCA-salt treatment, both strains developed hypertension, however mean arterial pressure (MAP; 123.9 ±6.4 vs. 138.4 ±4.3 mmHg) and HR (483.9 ±24.1 vs. 520.3 ±22.5 bmp) were significantly lower in neuronal AT1R knockout mice after 2 weeks of treatment, compared to controls. Western blot and enzyme activity assays from the hypothalamus of these DOCA-salt-treated mice revealed that the expression of pro-ADAM17 (inactive form)

was significantly increased (+17.0 ±5.3%, P<0.05), while the activity of ADAM17 was decreased (-37.4 ±10.5%) in neuronal AT1R knockout animals. Concomitant to the down-regulation of ADAM17, both the expression and activity of ACE2 were found to be significantly (P<0.05) up-regulated in the hypothalamus of neuronal AT1R knockout mice, by +20.7 ±8.5% and +32.3 ±10.1%, respectively. These results suggest that activation of neuronal AT1R is responsible for ADAM17-mediated ACE2 shedding and the maintenance of neurogenic hypertension.

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034

Opposite Effects of Hypertension and Training on Blood Brain Barrier Integrity in Autonomic Areas of the Spontaneously Hypertensive Rat

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It is well known that chronic hypertensive rats exhibit deficient blood brain barrier (BBB). We evaluate age-induced progression of BBB lesion in autonomic areas of the SHR and the possible effect of aerobic training on BBB integrity. SHR aged 1, 3 and 5 month were chronically cannulated for hemodynamic recordings in the conscious state (femoral), followed by anesthesia and dextrans' infusion (FITC-10kDA + RHO-70kDA, carotid artery). Twenty minutes later rats were sacrificed, brains were removed, post-fixed and cryoprotected. BBB permeability was evaluated in sequential 30 µm slices of the hypothalamic paraventricular nucleus (PVN), nucleus tractus solitarii (NTS) and rostroventrolateral medulla (RVLM) by the capability of FITC10 to leak into the brain parenchyma (in % area/area of interest, fluorescent microscope, ImageJ analysis). Other 3-months old SHR were submitted to treadmill training (T=55% of maximum capacity, 1h/day, 5 d/week) or kept sedentary for 8 weeks. Age-matched WKY served as control. Although SHR aged 1 month were normotensive and exhibited no BBB leakage (0.02±0.01 to 0.15±0.03% in all areas, values similar to WKY), leakage augmented sharply with the establishment of hypertension (average of 8.1±0.7% in SHR-3mo, 9.3±1.2% in SHR-5mo). In the WKY, there was only a small age-induced increase in BBB leakage (average of 0.63±0.05% in PVN, NTS and RVLM). Interestingly T promptly reduced dye leakage in the 3 autonomic areas of the SHR (~1.0±0.2% from 2 up to 8 weeks of training) without changing the leakage in PVN, NTS and RVLM of WKY rats and within the hypoglossus nucleus of the SHR (a non-autonomic area). T-induced improvement of BBB integrity in autonomic areas of the SHR were accompanied by significant reductions of HR (-10%) and MAP (-13%), increased HR variability (+2.1-fold), decreased pressure variability (-49%) and increased spontaneous baroreflex sensitivity (+2.3-fold). Data show that BBB lesion in SHR is caused by the establishment of hypertension and that T improves perfusion of autonomic brain areas in hypertensive individuals by preserving BBB integrity. This adaptive response

is crucial for a near normal neuronal activity, thus normalizing autonomic control of the circulation even in the presence of hypertension.

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035

Differential Sodium-evoked Activation of Pvn Magnocellular vs. Parvocellular Neurons in Rats Lacking Central GαI<SUB.2 Proteins Contributes to Sustained Elevations in Blood Pressure

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Aim: To determine the role of brain $G\alpha i_2$ proteins in mediating sodium-evoked PVN neuronal activation and blood pressure regulation in conscious rats.
Methods: 24-h intracerebroventricular scrambled (SCR) or $G\alpha i_2$ oligodeoxynucleotide (ODN; 25µg/5µl)-pretreated conscious Sprague-Dawley rats were monitored for changes in MAP in response to HS (IV 3M NaCl; 0.14 ml/100g). Rats were sacrificed at control (C), 10, 40, or 100-min post-HS for PVN cFos IHC and analysis of plasma AVP and NE. Separate groups received a V_{1a} receptor antagonist (IV; 10 µg/ml/kg) 5-min prior to HS.
Results: No difference was observed in sodium-evoked peak change in MAP and MAP remained elevated at 100-min in $G\alpha i_2$ but not SCR ODN rats (MAP 100-min post-HS [mmHg] SCR 134±2 *vs* $G\alpha i_2$ 146±3, P<0.05). Significant increases in the number of Fos^+ PVN magnocellular neurons were observed post-HS in SCR and $G\alpha i_2$ ODN groups ([Fos^+ cells] SCR C 3±1 *vs* 100-min 31±5; $G\alpha i_2$ C 2±0 *vs* 100-min 26±4, *P*<0.05). A rapid increase in circulating AVP was observed at 10-min in both SCR (plasma AVP [pg/mL] C 12.2±1.6 *vs* 10-min 62.8±6.9, *P*<0.05) and $G\alpha i_2$ ODN (plasma AVP [pg/mL] C 12.1±1.5 *vs* 10-min 67.7±7.7 *P*<0.05) groups and returned to control levels at 40- and 100-min. SCR ODN rats exhibited significant increases in the number of Fos^+ PVN parvocellular neurons ([Fos+ cells] C 15±1 *vs* 100-min 67±4, *P*<0.05) and a significant suppression in circulating NE post-HS (plasma NE [nmol/L] C 44.1±4.9 *vs* 10-min 17.4±3.9, *P*<0.05). $G\alpha i_2$ ODN rats exhibited significantly less Fos^+ parvocellular neurons compared to SCR ODN rats (100-min [Fos+ cells] SCR 67±4 *vs* $G\alpha i_2$ 30±2, *P*<0.05) and failed to suppress circulating NE (*P*>0.05). V_{1a} receptor blockade prevented a HS-evoked increase in MAP in SCR ODN rats while $G\alpha i_2$ ODN rats exhibited elevated MAP (MAP 100-min [mmHg] SCR 125±2 *vs* $G\alpha i_2$ 135±0, *P* <0.05).
Conclusion: Brain $G\alpha i_2$ proteins are required to mediate sodium-evoked parvocellular sympathetic, but not magnocellular vasopressinergic, responses to maintain physiological blood pressure regulation. A significant component of blood pressure control in this setting is regulated by the sympathetic nervous system, as supported by the attenuated activation of PVN parvocellular neurons and a failure to suppress circulating levels of NE in $G\alpha i_2$ ODN rats.

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036

Family History of Hypertension and Sympathetic Neural Reactivity to Mental Stress in Humans

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A number of recent studies have highlighted large inter-individual variability of muscle sympathetic nerve activity (MSNA) responsiveness to mental stress in humans. It remains unclear if family history of hypertension (FHH+) influences this variability. The purpose of this study was to examine blood pressure (BP) and MSNA responsiveness to mental stress in a large and generalizable cohort of young adults, and control for a variety of baseline factors that can influence MSNA reactivity. We hypothesized that subjects with FHH+ would demonstrate greater sympathoexcitation to mental stress than subjects without a family history of hypertension (FHH-). A total of 85 subjects from recently published (within 3 yrs; n=37) and ongoing (n=48) studies were examined. Data are presented on 45 subjects with complete MSNA recordings (15 FHH+ subjects and 30 FHH- subjects; 31 men and 14 women; age, 18 to 33 years). Heart rate (HR), BP, and MSNA were recorded during five min of supine rest and five min of mental stress (via mental arithmetic). Age, sex, body mass index, baseline mean arterial pressure (MAP), and baseline HR were identified as covariates. Baseline MSNA and HR were not statistically different between FHH+ and FHH- groups ($p>0.05$), whereas MAP was higher in FHH+ (84±2 and 79±2 mmHg; $p<0.05$). Mental stress increased MSNA in FHH+ subjects (Δ4.4±1.5 bursts/min), but not FHH- subjects (Δ1.0±0.2 bursts/min; time × group, $p<0.01$). Differences between FHH+ and FHH- groups remained when MSNA was normalized to heart rate (Δ2.8±1.0 bursts/100 heart beats vs. Δ-3.1±0.5 bursts/100 heart beats; time × group, $p<0.05$). Mental stress increased MAP (Δ11±1 mmHg and Δ10±1 mmHg; $p<0.001$) and HR (Δ18±4 beats/min and Δ17±2 beats/min; $p<0.001$) in FHH+ and FHH- subjects, but these increases were not different between groups (time × group, $p\geq0.05$). In conclusion, our findings demonstrate that FHH+ is associated with greater MSNA reactivity to acute mental stress when compared to FHH- individuals.

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037

A Role for IL-17 to Activate Cytolytic Natural Killer Cells in Response to Placental Ischemia

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Women with preeclampsia (PE), newly developed hypertension and renal dysfunction during pregnancy, have small-for-gestational-age babies and demonstrate an increase in the inflammatory cytokine IL-17, placental oxidative stress, and cytolytic natural killer (NK) cell activation. The stimulus of the cytolytic NK cell phenotype during PE is currently unknown. Moreover, the specific role of cytolytic NK cells in the pathophysiology of preeclampsia has not been clearly defined. The reduced uterine perfusion pressure (RUPP) model of placental ischemia exhibits many of the characteristics of preeclampsia including hypertension, renal dysfunction, chronic inflammation and intrauterine growth restriction (IUGR). In this study, we tested the hypothesis that placental ischemia results in cytolytic activation of NK cells, and examined a role for the increased IL-17, in response to placental ischemia, to activate cytolytic NK cells. In this study, blood pressure (MAP) and pup weight were measured, and PBMCs and placental

lymphocytes were examined via flow cytometry for surface makers of cytolytic NK cell activation. MAP significantly increased in response to placental ischemia from 103±4.1 mmHg in NP (n=6) to 129.1±3.1 mmHg (n=8) in RUPP rats ($p<0.001$). Neutralization of IL-17 with a soluble receptor attenuated the blood pressure response to 106.3±2.3 mmHg in RUPP+IL-17RC rats (n=3). Pup weight is significantly decreased in RUPP rats (2.52±0.18g in NP vs 2.03±0.05g in RUPP ($p<0.05$)), which increased to 2.54±0.36g in RUPP+IL-17RC. Cytolytic activation of circulating NK cells was not significantly changed among any of the groups (NP: 2.49±1.1%; RUPP: 7.74±3.2%; RUPP+IL-17RC:5.50±2.8%). However, cytolytic activation of placental NK cells increased in response to placental ischemia (NP: 3.4±1.1% vs RUPP 10.0±3.4%), and was completely attenuated after treatment with the soluble IL-17 receptor (RUPP+IL-17RC: 0.33±0.17%). These results suggest a role for placental ischemia and increased IL-17 to stimulate cytolytic NK cells. Furthermore, this study links the IL-17 pathway with cytolytic NK cell activation and IUGR in response to placental ischemia, potentially identifying new therapeutic targets to improve maternal and fetal outcomes of PE.

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Placental Growth Factor Supplementation Abolishes Placental Ischemia-induced Hypertension

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Preeclampsia (PE) is a pregnancy-specific disorder of new-onset hypertension. Unfortunately, the most effective treatment is early delivery of the fetus and placenta. Progress toward potential therapeutic targets has found that placental ischemia/hypoxia induced in animals by reduced uterine perfusion pressure (RUPP) or in human patients stimulates the release of hypertensive placental factors into the maternal circulation. For example, the antiangiogenic factor sFlt-1, which antagonizes and reduces bioavailable vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), is significantly elevated in PE patients and RUPP rats. It is clear that reductions in VEGF promote hypertension in RUPP rats as supplementation with recombinant VEGF at 180 ug/kg/day abolished the hypertension. However, it is unknown if reductions in PlGF levels also contribute to the hypertensive response. Thus, we tested the hypothesis that PlGF treatment would reduce placental ischemia-induced hypertension. On gestational day 14, Sprague Dawley rats were randomized to three groups: normal pregnant (NP, N=6), RUPP (N=5) and RUPP + 180 ug/kg/day PlGF (N=7). The rPlGF (AG31, a purified-recombinant human PlGF) was infused via i.p. osmotic minipump. Mean arterial blood pressure (MAP, carotid catheter) and pregnancy

weights were assessed on day 19. MAP was significantly higher in RUPP than NP rats (123±4 vs. 104±1, P<0.05). PlGF reversed these levels to NP values (105±3, P<0.05 vs. RUPP). Placental weights (NP: 0.5±0.02; RUPP: 0.51±0.04; and RUPP+rPlGF: 0.5±0.05) and fetal weights (NP: 2.30±0.07; RUPP: 2.00±0.15; and RUPP+rPlGF: 2.09±0.07) were similar among all groups. The number of live fetuses was reduced in RUPP than NP rats (5±2 vs. 12±1, P<0.05) with a slight increase in the RUPP+rPlGF group (8±1). The number of fetal absorptions was increased in RUPP than NP rats (9±2 vs. 2±1, P<0.05) with a slight increase in the RUPP+rPlGF group (5±1). In conclusion, these data indicate that the reductions in PlGF that occur as a result of placental ischemia contribute to the development of maternal hypertension. Our novel finding that rPlGF abolishes placental ischemia-induced hypertension suggests a strong role and therapeutic potential for this growth factor in PE.

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039

Normalization of Uterine Interleukin (IL)-15 in the BPH/5 Preeclamptic Mouse Improves Decidual Natural Killer Cell Activation at the Maternal-Fetal Interface

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Preeclampsia (PE) is a hypertensive disease that affects up to 10% of pregnancies worldwide. Although a leading cause of maternal and fetal morbidity/mortality, the cause is unknown. Inadequate remodeling of decidual vessels and reduced placental perfusion are hallmarks of PE. Decidual Natural Killer cells (dNKs) are critical in this process. Early in pregnancy, dNKs are activated by uterine IL-15 to promote angiogenesis at the maternal-fetal interface. Activated dNKs produce IFN-γ to facilitate vasodilation of decidual vessels. In the BPH/5 mouse model that spontaneously develops late gestation hypertension, proteinuria and placentopathies, we have demonstrated an early pregnancy reduction in dNK activation and IFN-γ mRNA at the maternal-fetal interface. We functionally linked uterine IL-15 overexpression to dNK loss in C57 mice. Here we tested the hypothesis that uterine IL-15 in BPH/5 mice contributes to dNK dysregulation at the maternal-fetal interface prior to placenta formation. BPH/5 e7.5 implantation sites show a 5-fold increase in IL-15 protein vs C57 (.7±.07vs.35±.17, n=4, p<.05). We have shown that Cox2 inhibition early in BPH/5 pregnancy improves fetoplacental development. Cox2-derived products influence expression of the pro-inflammatory cytokine, IL-15. To address if Cox2 inhibition alters IL-15 in BPH/5 implantation sites, celecoxib (a selective Cox2 inhibitor) was administered at e6.5 (10mg/kg orally). This normalized IL-15 in BPH/5 e7.5 implantation sites compared to C57 veh-treated (n=4, p<.05). Flow cytometry confirmed a 2-fold increase in DBA+/CD122+ (dNK) cell numbers in BPH/5 e7.5 implantation sites from celecoxib vs veh-treated mice (16.6 vs 7.95). Moreover, the dNKs from e7.5 BPH/5 celecoxib-treated implantation sites had a 2-fold increase in IFNγ mean fluorescent intensity vs BPH/5 veh-treated. This demonstrates that uterine IL-15 overexpression in BPH/5 contributes to dNK loss and that normalization improves dNK activation by increasing their expression of IFNγ at the maternal-fetal interface. This highlights the significance of uterine inflammation in dNK

regulation and suggests that aberrations in these cellular processes in early pregnancy may contribute to the placentopathies seen in PE pregnancies.

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040

Complement Activation in Placental Ischemia-induced Hypertension is not Dependent on Endothelin A Receptor Activation

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Early onset preeclampsia is characterized by hypertension, reduced placental perfusion, intrauterine growth restriction and increased activation of complement, part of innate immunity. We previously reported that inhibiting complement activation attenuates reduced uterine perfusion pressure (RUPP)-induced hypertension in pregnant rat and the concomitant increase in complement activation product C3a. Studies by other groups indicate endothelin A receptor (ET_A) activation plays a significant role in RUPP-induced hypertension. We hypothesized that ET_A was important in placental ischemia-induced complement activation leading to hypertension. Thus, the effect of ET_A antagonist atrasentan on hypertension and complement activation following placental ischemia in rat was determined. Dams received drinking water with or without 5 mg/kg/day atrasentan on gestation day (GD)13-19. On GD14, rats underwent Sham or RUPP surgery with clip placement on abdominal aorta and uterine arteries to decrease placental perfusion. On GD19, mean arterial pressure (MAP) via arterial catheter and serum C3a as indicator of complement activation were measured. RUPP surgery significantly increased MAP (109±5 mm Hg; n=7) compared to Sham (94±4 mm Hg; n=10) as well as C3a (0.36±0.08 to 0.69±0.12 units/ul). MAP was significantly reduced by atrasentan in RUPP (96±3 mm Hg; n=12) and unchanged by atrasentan in Sham (88±2 mm Hg; n=12). RUPP did not change preproendothelin mRNA in kidney cortex versus Sham, but did decrease fetal weight. In Sham, atrasentan significantly increased fetal weight (2.52±0.04 g; n=12) compared to no atrasentan (2.38±0.05 g; n=12). Importantly, atrasentan treatment did not significantly change RUPP-induced increase in C3a with 0.57±0.11 units/ul in RUPP atrasentan and 0.27±0.05 units/ul in Sham atrasentan. In contrast to other ET_A antagonists (A-127722, FR-139317), the present data indicate atrasentan may promote fetal growth. However, the ET_A receptor does not mediate increased complement activation and its subsequent involvement in placental ischemia-induced hypertension. Thus, our data suggest complement activation may precede involvement of endothelin in development and maintenance of high blood pressure due to placental ischemia.

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041

Elevated Circulating Copeptin in Mid-Gestation is Associated with Increased Aortic Stiffness and Vascular Endothelial Dysfunction in Pregnant Women at High Risk for Preeclampsia

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Circulating copeptin, a stable biomarker of vasopressin (AVP) secretion, is elevated throughout pregnancy in women who develop preeclampsia (PreE) and is a strong predictor of PreE as early as the 6th week gestation. Reduced vascular endothelial function and increased aortic stiffness occur in mid-gestation before clinical signs/symptoms of PreE manifest, suggesting that maternal vascular dysfunction may be an early event in the pathogenesis of PreE. However, it is unknown whether elevated copeptin/AVP in early/mid gestation contributes to vascular dysfunction in pregnant women who subsequently develop PreE. Therefore, we hypothesized that elevated copeptin would be associated with increased aortic stiffness and reduced vascular endothelial function in early/mid gestation of pregnant women at high risk for PreE. Pregnant women in the 1st trimester (n=72; age=30 ±1 yrs; BMI=34 ± 1 kg/m2) with at least 1 risk factor for PreE were enrolled. Aortic stiffness (carotid-femoral pulse wave velocity, CFPWV), vascular endothelial function (brachial artery flow-mediated dilation, FMD), blood pressure (BP) and plasma copeptin (ELISA) were assessed in both the 1st (11.7 ± 0.2 wks) and 2nd (18.8 ± 0.4 wks) trimesters. In the 1st trimester, CFPWV (7.3 ± 0.2 vs. 7.3 ± 0.5 m/sec, P=0.86), brachial artery FMD (12.9 ± 1.1 vs. 14.3 ± 2.0%, P=0.53), BP, BMI and age did not differ between women in the highest (1513 ± 221 pg/ml) vs. lowest (279 ± 12 pg/ml) quartile of copeptin (P<0.01). In contrast, 2nd trimester CFPWV was greater (7.2 ± 0.2 vs. 6.4 ± 0.2 m/sec, P<0.05) and brachial artery FMD was lower (10.2 ± 2.8 vs. 16.5 ± 1.3 %, P<0.05) among women in the highest (1714 ± 481 pg/ml) vs. the lowest (249 ± 13 pg/ml) quartile of copeptin (P<0.01), in the absence of differences in BP, BMI or age. For the entire cohort, (log)copeptin was significantly correlated with CFPWV (r=0.23, P=0.04) and tended to correlate with FMD (r=-0.23, P=0.06) in the 2nd but not in the 1st trimester. These data suggest that elevated copeptin in mid-gestation is associated with aortic stiffness and vascular endothelial dysfunction in pregnant women at high risk for PreE, but whether increased copeptin/AVP causes vascular dysfunction in pregnancies destined for PreE requires further studies using animal models.

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042

Cd74-dysregulation of Macrophage-trophoblastic Interactions in the Preeclamptic Placenta

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Objectives
Preeclamptic pregnancies feature placental anomalies. Villous trophoblast differentiation during placental development is regulated by feto-placental macrophages and disturbance of this well-balanced regulation can lead to pathological pregnancies. We hypothesized that Cluster of differentiation 74 (CD74) dysregulation of placental macrophages, leading to altered macrophage-trophoblast interaction, is involved in preeclampsia.
METHODS AND RESULTS
We performed microarray analysis of placental tissue. CD74 was one of the most down-regulated (-2.5 fold) genes in placentas from preeclamptic women. We confirmed this finding in early onset (<34 gestational week, n=26) and late onset (≥34 gestational week, n=24) samples from preeclamptic women, compared to healthy pregnant controls (n=28) by real-time RT-PCR and on protein level by Western blot and flow cytometry. We localized CD74 expression in placental macrophages by immunofluorescence, flow cytometry, and real-time RT-PCR. Number and mean fluorescence intensity (MFI) of CD74-positive macrophages were significantly lower in preeclamptic placentas (7692 MFI ± 4402), as compared to controls (16283 MFI ± 3047). In CD74-silenced macrophages, expression of adhesion molecules ALCAM (-2 fold), ICAM4 (-2.1 fold), and Syndecan-2 (-1.9 fold) was lower compared to control. Macrophage adhesion to a trophoblast layer were diminished (-1.3 fold). Naïve and activated macrophages lacking CD74 showed a shift towards a pro-inflammatory signature with an increased secretion of TNFalpha(21.8 pg/ml ± 13.2 vs. 8 pg/ml ± 4.3), CCL5 (1.9 ng/ml ± 0.4 vs. 0.8 ng/ml ± 0.2) and MCP-1 (3 ng/ml ± 2.6 vs. 1 ng/ml ± 0.5), when co-cultured with trophoblasts compared to control macrophages. CD74-knockout mice showed disturbed placental morphology, reduced junctional zone (1.6 mm2 ± 0.3 vs. 2.3 mm2 ± 0.5) and smaller placentas (0.09 g ± 0.01 vs. 0.11 g ± 0.02) with fetal growth restriction (0.7 g ± 0.1 vs. 0.9 g ± 0.2) when compared to WT mice.
CONCLUSIONS
We found that CD74 downregulation in placental macrophages is present in preeclampsia. CD74 downregulation led to altered macrophage activation towards a pro-inflammatory signature, a disturbed crosstalk with trophoblasts and an abnormal placental morphology.

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043

A Gwas Prioritized Candidate Gene,Nr2f2, Attenuates Salt-sensitive Hypertension and Associated Renal Function

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A haplotype-based re-analysis of the GWAS study by the Wellcome Trust Case Control consortium prioritized the transcription factor, Nuclear receptor2, family2 (NR2F2) also known as Chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII) as a gene associated with essential hypertension in humans. This gene is also prioritized as a differentially expressed positional BP QTL candidate gene on rat chromosome 1. To further examine Nr2f2 as a determinant of blood pressure, a mutant Dahl salt-sensitive (S) rat model was generated using the zinc-finger nuclease (ZFN) technology. As a result of this ZFN mediated disruption, a 15-bp deletion occurred within exon 2 of the Nr2f2 locus, resulting in the loss of 5 amino acids from the hinge region of the Nr2f2 protein. Systolic BP of the homozygous Nr2f2mutant rats was lower than that of the S rats (179±3 vs. 197±5 mmHg; $p<0.001$) as measured by the tail-cuff method and (165±2 vs. 201±3 mmHg; $p<0.001$) as monitored by the radio-telemetry method. Nr2f2mutant rats demonstrated lower proteinuria compared to S rats (87.97±7.07 vs. 122.63±7.07mg/day; $p<0.05$). Since Nr2f2 is reported to negatively regulate renin promoter activity, we tested renin activity in the Nr2f2mutant rats. However, there was no significant difference in either the plasma renin activity or renal renin protein expression when compared with S rats. Renal histological analysis showed that Nr2f2mutant rats have decreased collagen compared with the S rats. Furthermore, Nr2f2mutant rats showed significantly decreased TGF-β protein levels, when compared with S rat kidney ($p<0.05$). These data, taken together with our recently published data on the improved cardiac and vascular function in the Nr2f2mutant rats (Nat Commun. 2015 Feb 17;6:6252), lend support to Nr2f2 as a determinant of blood pressure and associated renal function. While the observed improvement in renal function could be a secondary consequence of lower hypertension, our data, combined with the important mechanistic finding that the enhanced transcription factor-transcription factor interaction of Nr2f2 with Fog2 (Friend of GATA4), suggest that the hinge region of Nr2f2 is important for regulating blood pressure and associated cardiac and renal functions.

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044

Pappa2 is Linked to Salt-sensitive Hypertension in Dahl SS Rats

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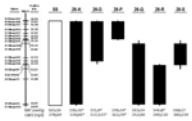
The goal of the present study was to narrow a 1.37 Mbp region containing five genes on chromosome 13 (positions 80.92-82.29 Mbp in the Rn5 genome assembly) that we have previously found to influence the mean arterial pressure (MAP) by at least 25 mmHg in SS rats fed a high salt diet (8.0% NaCl for 14 days). The creation of 6 overlapping subcongenic strains that cover this region identified a 0.71 Mbp

region (positions 81.01-81.72 Mbp) in which substitution of SS alleles with BN alleles reduced salt-induced hypertension in congenic SS rats by nearly 30 mmHg and significantly reduced urinary albumin excretion (UalbV) (see figure below; n=10-15/strain; * significant difference from SS; p<0.05). Analysis of the narrow candidate region revealed the presence of two protein-coding genes (*Pappa2* and *Astn1*) and a microRNA (*miR488*), none of which are known to be mechanistically involved in hypertension. *Pappa2* mRNA in these rat strains fed 0.4% NaCl diet was expressed at 6-10 fold higher levels in the renal cortex of the salt-resistant congenic strains with the BN allele (26-N, -O, and –P) compared to strains with the SS allele (26-Q, -R, and –S). A *Pappa2* coding sequence variant of unknown functional importance was identified. Immunohistochemistry and fluorescence *in situ* hybridization studies localized *Pappa2* to intercalated cells of the cortical and medullary collecting duct. *Astn1* and *miR-488* were not differentially expressed in renal tissue. Together, these findings point towards *Pappa2* as a candidate gene for salt-induced



hypertension in SS rats.

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045

Epigenetic Modification of Brain (pro)renin Receptor Contributes to the Development of Doca-salt Hypertension via Epithelial Sodium Channel

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We reported that elevated brain (pro)renin receptor (PRR) expression levels contributes to the development of salt-sensitive hypertension (SSH); however, the underlining mechanism on how PRR is regulated remains unknown. High-salt intake is a major life style epigenetic factors for the SSH. To test our hypothesis that epigenetic mechanism is involved in the regulation of PRR expression in SSH, we used deoxycorticosterone acetate (DOCA)-salt induced hypertension as a model of SSH. C57Bl/6J mice (n=5/group) were implanted with SHAM or DOCA (50mg) pellet and supplied with saline for 3 weeks. Histone modifications on PRR promoter were assessed in the hypothalamus using Chromatin Immunoprecipitation assays at the end of protocol. We found an increase (fold change) in histone 3 lysine 4 trimethylation (H3K4me3) on the PRR promoter in DOCA-salt (2.45±0.36) compared with SHAM treated mice (1.00±0.16, P<0.05). H3K4 methyltransferases (HMT) activity (OD/hour/mg protein), an enzyme complex responsible for H3K4 methylation is increased after DOCA-salt treatment (45±2, P<0.05) compared with SHAM (28±2). To examine the mechanisms of elevated HMT activity during DOCA-salt hypertension, we treated culture neurons (n=3 independent experiments/group) with control (155mM sodium), high salt (170 mM sodium), aldosterone (100 nM), or high salt+aldosterone with or without epithelial sodium channel (ENaC) blocker (amiloride, 100 nM) for 2 hours and test the HMT activity and PRR expression levels. High salt or aldosterone alone has minimal effects on HMT activity and PRR levels. High salt+aldosterone significantly increased HMT activity (41±3 vs. 24±2, P<0.05) and PRR

mRNA (1.6±0.1 vs. 1.0±0.1 fold change, P<0.05) levels compare to control. Amiloride attenuated HMT activity (25±4.1, P<0.05) and PRR mRNA levels (0.98±0.1, P<0.05) compared to high salt+aldosterone treatment. In summary, elevated PRR expression is associated with increased HMT activity and H3K4me3 modification on PRR promoter. ENaC blockade attenuates the elevation of HMT activity and PRR levels induced by high salt+aldosterone in culture neurons. We conclude that ENaC may be responsible for epigenetic up-regulation of brain PRR and thus the increase in BP during salt-sensitive hypertension.

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046

Deep Sequencing in mpkCCD Collecting Duct Cells Identifies Transcriptional Response to Vasopressin

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Blood pressure regulation depends in part on control of ion and water transport in the renal collecting duct by vasopressin and other hormones. To identify genes that are transcriptionally regulated by vasopressin in cultured mouse collecting duct cells (mpkCCD), we carried out both RNA-seq (n=9) and ChIP-seq for RNA polymerase II (Pol-II, n=3) after 24-hr treatment with vasopressin analog dDAVP or vehicle (Illumina HiSeq2000 sequencer). RNA-seq analysis showed significant changes in 33 transcripts out of 8393 quantified (Volcano plot thresholds: p<0.05 (Wilcoxon) and log2[ratio] outside of 99% confidence interval (CI) for vehicle-vs-vehicle controls). More transcripts increased (26) than decreased (7). The largest increases were seen for the transcriptional coactivator *Cited1* (log2[dDAVP/Vehicle]= 5.2) and the water channel *Aqp2* (log2[dDAVP/Vehicle]= 4.2). The ChIP-seq analysis showed significantly increased RNA-Pol-II binding in 209 genes versus only 9 with decreases. Plotting RNA-seq data versus ChIP-seq data for 3883 genes revealed 43 genes that exceeded the 95% CI for control-vs-control measurements for both variables, i.e., are transcriptionally regulated. The transcriptionally regulated genes included those involved in control of Na+/Cl- transport and/or blood pressure, viz. *Fxyd4* (aldosterone-regulated channel-inducing factor), *Tsc22d3* (the aldosterone-regulated leucine-zipper protein GILZ), *Atp1b1* (Na+-K+-ATPase beta subunit) and *Sik1* (salt-inducible kinase 1). Among transcriptionally upregulated genes were several transcription factors (*Elf3*, *Nfkbiz*, *Nr4a1*, *Myc*) that form the core of a proposed vasopressin/cAMP-activated transcriptional network. Conclusions: 1) Vasopressin signaling effects on transcription are biased with more genes increased than decreased. 2) Among the genes that are transcriptionally regulated in response to vasopressin are several that have been implicated in regulation of salt and water transport in the collecting duct.

P. Sandoval: None. **J. Lee:** None. **J. Claxton:** None. **J.D. Hoffert:** None. **M.A. Knepper:** None.

047

Genetics and Environmental Influences on Ambulatory Blood Pressure in Rats Compared With Circadian Blood Pressure Variability in Human

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Genetic susceptibility is an important factor in raising blood pressure (BP). Daily (circadian) rhythm characteristics are considered essential parameters for recognizing and treating increased risks in BP. To examine BP in genetics with environmental modifications, one-cell homozygous embryos were transferred into spontaneously hypertensive (SHR, pup:shr) or normotensive (WKY, pup:wky) rats' oviducts (embryos: s, w; oviduct-uterine: S, W) in a reciprocal fashion. Pups were cross-suckled at birth (nurses S, W) and weaned to normal diets at day-21. At Day-120, telemetered BPs were monitored for 5 consecutive days every 4 min and analyzed by the method of ANOVA. Ambulatory BP in 20 adolescents and adults were monitored automatically around the clock at 30-min to hourly intervals and analyzed by the least square rhythmometry method.
As expected, shr BPs were markedly reduced when they were transplanted to the W-uterine and/or the W-lactation milieu (sSS vs. sSW, sWS and sWW: 197 vs.178, 147 and 178 mm Hg). BP in wky was significantly altered only in the wSW group (wWW vs, wSW: 127 vs.131 mm Hg). All subjects showed significant circadian fluctuations with a peak in the late afternoon hours in most human subjects and rats as a nocturnal animal mostly close to midnight hours, while shr with W-uterine (sWS) a bit delayed peak hour (00:45) and with combined W-uterine/W-nursing (sWW) a bit earlier peak hour (20:12). Circadian double amplitudes (2A) in the human subjects varied from 8 to 26 mm Hg with higher 2A in elder adults, and 3-8 mm Hg in rats with significantly higher fluctuations in shr groups as compared to that of wWW (7.5±0.7 for sSS, 8.3±0.6 for sSW vs. 4.7±0.3 mm Hg for wWW).
The hypertensive-prone shr strain showed significantly lowered BPs in a normotensive WKY uterine environment and/or by WKY nursing mothers, indicating that environment influences can strongly modify genetic factors, yet the lowered shr MESORs by the WKY environments remained above the MESORs encountered in wky donors. Chronomes broader than circadian should be considered in interpreting BP responses as a gauge of vascular disease status.
Key Words: Ambulatory blood pressure monitoring, Embryo transfer; cross-suckling; spontaneously hypertensive and normotensive rat; vascular disease risk.

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048

Epigenetic Regulation of the Human Angiotensinogen Gene: Role of Individual SNPs and Implication for Hypertension

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Hypertension is a complex disease caused by a combination of genetic and environmental factors. Renin angiotensin system (RAS) dysfunction is a frequent accompaniment of essential hypertension. In this regard, polymorphisms in the angiotensinogen (AGT) gene increase RAS activity and cause blood pressure elevation. We have identified two distinct haplotypes of the human AGT gene constituted by a group of four SNPs in linkage disequilibrium. Variants -1670A, -1562C, and -1561T always occur with -6A and form the haplotype-I (Hap-I), while variants -1670G, -1562G, and -1561G always occur with -6G and constitute haplotype-II (Hap-II). We hypothesized that these SNPs, when present together as Hap-I or Hap-II in transgenic mice (TG) may lead to haplotype-dependent DNA methylation of CpG sites in the promoter of the hAGT gene. Methylation patterns alter gene transcription at an epigenetic level and this, in turn, could be dependent on individual polymorphisms. hAGT promoter CpG sites in the kidney are more methylated as compared to liver and fat. In the Kidney, the hAGT promoter CpG sites are methylated at -460, -434, -386, -346, -282, -261, -245, -229, -218, -185, -144, -18, -11, -7, +10, +42, +65 in Hap-II whereas, in Hap-I the promoter DNA methylation is observed at -460, -434, -401, -386 positions. Further, in both liver and fat the CpG sites were methylated at -11, +42, +65 positions in Hap-II TG mice, whereas in the Hap-I there were no methylation sites detected. The hAGT gene mRNA levels from these tissues were quantitated by qPCR and were 2.1 (kidney), 2.5 (liver) and 4.28 (fat) fold higher in haplotype-I vs. haplotype-II ($p<0.05$). Our results indicate that, in Hap-II TG mice liver, kidney and fat tissues having more CpG sites methylated in the promoter of the hAGT, and thus having less gene expression. On the other hand, Hap-I has either less or no methylation of CpG sites in the hAGT promoter and hence high gene expression. Thus, we show here for the first time that SNP blocks in the hAGT gene alter its methylation pattern in a tissue-dependent manner. This shifts the paradigm in favor of an interdependent system where epigenetic regulation is reliant on the gene haplotypes; together, these regulate protein expression and associated physiological outcomes.

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049

Preeclampsia in the Dahl Salt Sensitive Rat is Associated with Increased Uterine Artery Resistance and Reduced Placental Microvascular Density

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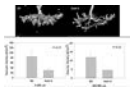
We showed that the Dahl salt sensitive (Dahl S) rat is a spontaneous model of preeclampsia superimposed on chronic kidney disease and hypertension. This model exhibits intrauterine growth restriction, decreased pup weight, increased fetal death, and placental hypoxia; however, the underlying mechanisms are unknown. We hypothesized that a pathological remodeling and rarefaction in the maternal placental bed drives the development of preeclampsia in this model. Pregnant Dahl S and Sprague-Dawley (SD) rats were used in this study. Uterine artery resistance index (UARI) was calculated via Doppler ultrasound (Vevo 770) on day 18 of pregnancy (UARI=(peak systolic flow velocity - end diastolic flow velocity)/ peak systolic flow velocity). On day 20 of pregnancy, placentas were perfused through the uterine circulation with a silicon-polymer contrast (Microfil MV122). Individual placentas were then excised, scanned using a micro-CT scanner (SkyScan 1076), and 3D reconstructed for analysis and quantification of the placental vasculature on the maternal side. Microvascular density was calculated for vessels of diameters in the 0-500 µm ranges. UARI was higher in the Dahl S compared to the SD (0.71±0.02 vs 0.51±0.02, n=4-12, $p<0.05$). Density of placental microvessels in the 200-500 µm range was significantly decreased in placentas from Dahl S rats, and we observed a strong trend towards a decrease in density of microvessels in the 0-200 µm range (n=3-5, Figure). These results suggest that the impaired fetal growth and placental hypoxia in the Dahl S rat may be mediated by insufficient placental vascularization and reduced blood flow to the feto-placental unit.



E.E. Gillis: None. **A.R. Chade:** None. **F.T. Spradley:** None. **M.R. Garrett:** None. **J.M. Sasser:** None.

050

Loss of Argininosuccinate Lyase Leads to Nitric Oxide Deficiency, Endothelial Dysfunction, Impaired Angiogenesis, and Hypertension

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Nitric oxide (NO) is an important mediator of vascular homeostasis and its deficiency in murine models results in hypertension. However, there are few monogenic causes of NO deficiency in humans and the effects of such genetic forms of NO deficiency on vasculature are not well-studied. We have recently shown that argininosuccinate lyase (ASL), a urea cycle enzyme, is necessary for synthesis of NO. ASL deficiency results in decreased production of NO and hypertension in humans and mice. To investigate whether loss of Asl-mediated NO synthesis in the vascular endothelium alone can cause hypertension, we generated a mouse model with endothelial-specific deletion of Asl (VE-Cadherin Cre(tg/+); Asl(flox/flox), or cKO). Asl cKO mice developed hypertension and had higher mean arterial pressure compared to control littermates (102.2± 2.9 vs. 89.8±3.6 mmHg, $p<0.05$). This hypertension was secondary to endothelial-specific NO deficiency as demonstrated by abnormal relaxation of

aortic rings and correction with treatment with an NOS-independent NO supplement (MAP in sodium nitrite treated Asl cKO 97.7±3.8 vs. 97.6±7.3 mmHg in untreated control mice). To evaluate the human relevance of these findings, we developed human cell-based models from patients with ASL deficiency. Human induced pluripotent stem cells (hiPSCs) were generated and differentiated into endothelial cells. Interestingly, ASL-deficient hiPSCs differentiated less efficiently into endothelial cells as compared to control hiPSCs (9.7±4.0 vs. 20.8±3.7 % of CD144+; CD31+ cells, $p<0.01$). Furthermore, ASL-deficient hiPSCs-derived endothelial cells had a significantly reduced capacity to form capillary-like structures on Matrigel. Our study using a novel mouse model and hiPSCs-derived endothelial cells from patients with a rare Mendelian form of hypertension supports the hypothesis that structural and functional abnormalities in endothelial cells contribute to pathogenesis of hypertension. Our study is the first to use hiPSC-derived endothelial cells as a model system to study hypertension and highlights the utility of this technology in exploring the pathogenesis of other vascular diseases.

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051

The Role of Immunological Memory in Hypertension

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Immunological memory provides protection to repeated antigen challenges and is a cardinal feature of adaptive immunity. We have previously shown that adaptive immunity contributes to hypertension and have observed memory T cells in several models. We hypothesized that memory T cells contribute to long-term renal damage in response to repeated hypertensive challenges. To impose repeated episodes of hypertension, we treated C57BL/6 mice with L-NAME (0.5mg/ml) in drinking water for two weeks, allowed a two-week normotensive interval and then fed high salt (4% NaCl) for three weeks. L-NAME followed by high salt increased SBP to 151 ± 14 mmHg and caused a two-fold increase in $CD4^+$ and $CD8^+$ memory T cells in the kidney and bone marrow, as identified by the surface marker CD44hi. Intracellular staining showed that memory T cells were predominant sources of the inflammatory cytokines IL-17A and IFN-γ. Development and reactivation of memory T cells require the interaction of CD27 on T cells with CD70 on antigen presenting cells. Flow cytometry revealed that L-NAME/High salt increased expression of CD70 on splenic macrophages by 5-fold and dendritic cells by 3-fold. Because memory T cells are a major source of IFN-γ, we examined the hypertensive response to the L-NAME/high salt protocol in IFN-$\gamma^{-/-}$ mice. The hypertension caused by L-NAME was identical between WT, CD70$^{-/-}$ and IFN-$\gamma^{-/-}$ mice. In contrast, the hypertension induced by subsequent salt administration was markedly attenuated in CD70$^{-/-}$ mice (123 ± 1.3 mmHg, $p<0.02$). Likewise, mice lacking IFN-γ developed blunted hypertension during the salt-feeding phase (127.6 ± 5.5 mmHg, $p<0.04$). Interestingly, CD70$^{-/-}$ and IFN-$\gamma^{-/-}$ mice failed to

develop memory T cell formation in the kidney. The L-NAME/high salt caused striking albuminuria and increased urinary N-gal in WT mice, and these were absent in CD70$^{-/-}$ and IFN-γ$^{-/-}$ mice. In contrast, L-NAME/high salt had no effect on renal angiotensinogen levels. Thus, repeated hypertensive stimuli lead to accumulation of long-lived effector memory T cells that are major sources of inflammatory cytokines, which in turn promote renal dysfunction, salt sensitivity and hypertension. These studies provide further insight into how the adaptive immune system promotes hypertension.

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052

Placental Growth Factor (PlGF) Reduces Blood Pressure and Improves Endothelin Type B Receptor (ETBR)-Mediated Microvascular Dilation in Hypertensive Pregnant Rats

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Preeclampsia is a pregnancy-related hypertensive disorder (HTN-Preg) with an imbalance between anti-angiogenic soluble fms-like tyrosine kinase-1 (sFlt-1) and angiogenic PlGF, but the vascular targets involved are unclear. We have shown downregulation of endothelial ET_BR in Preg rats with reduced uterine perfusion pressure (RUPP), and studies have shown increased plasma sFlt-1 in RUPP rats. We tested if raising PlGF/sFlt-1 ratio by infusing PlGF (10 μg/kg/day) in RUPP rats would improve BP and microvascular ET_BR signaling, and vice versa, if lowering PlGF/sFlt-1 ratio by infusing sFlt-1 (10 μg/kg/day) in Preg rats increases BP and reduces ET_BR signaling. On day 19, BP was recorded and mesenteric microvessels were isolated for measurement of diameter and $[Ca^{2+}]_i$ (fura-2 340/380 ratio). BP was in PlGF-RUPP 105±2 < RUPP 126±1 and in sFlt-Preg 125±4 > Norm-Preg 97±5 mmHg. ET-1 vasoconstriction was in PlGF-RUPP 62.6±3.0 < RUPP 83.4±5.3 and in sFlt-Preg 76.1±4.7 > Norm-Preg 52.1±3.2%. ET-1 caused parallel increases in microvascular $[Ca^{2+}]_i$ that was in PlGF-RUPP 0.87±0.02 < RUPP 0.92±0.01 and in sFlt-Preg 0.93±0.02 > Norm-Preg 0.85±0.01. Endothelium removal or microvessel treatment with ET_BR antagonist BQ-788 enhanced ET-1 vasoconstriction and $[Ca^{2+}]_i$ in Norm-Preg and PlGF-RUPP, but not RUPP or sFlt-Preg. The ET_BR agonists sarafotoxin 6c (S6c) and IRL-1620 caused relaxation that was in PlGF-RUPP 42.9±10.8, 38.0±11.2% > RUPP 4.7±3.4, 7.5±2.3% and in sFlt-Preg 3.1±1.0, 5.4±1.6% < Norm-Preg 29.9±7.8, 28.0±9.1%. L-NAME partially reduced ACh- and ET_BR-induced relaxation in Norm-Preg, PlGF-RUPP, but not RUPP or sFlt-Preg, suggesting that PlGF improves the decreased NO-dependent and ET_BR-mediated vasorelaxation in HTN-Preg. Basal, ACh-, S6c-, and IRL-1620-induced nitrate/nitrite production was enhanced in mesenteric arteries of PlGF-RUPP and Norm-Preg vs. RUPP rats. Western blots and immunohistochemistry revealed greater levels of endothelial ET_BR in PlGF-RUPP and Norm-Preg vs. RUPP and sFlt-Preg. Thus improving PlGF/sFlt-1 balance reduces BP and ET-1 vasoconstriction, and enhances ET_BR-mediated NO-dependent vasodilation in RUPP rats, and

could be a new approach in the management of HTN-Preg.

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053

Uncoupling the Metabolic and Cardiovascular Actions of Leptin through mTORC1 Signaling

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The adipocyte-derived hormone leptin has a well-established role in the regulation of energy homeostasis, acting in the brain to decrease food intake and promote energy expenditure. Additionally, leptin increases regional sympathetic nerve activity (SNA) and arterial pressure. Multiple intracellular signaling cascades are activated by leptin via its long form receptor (LRb), but the specific roles of these pathways in mediating leptin's various effects have not been fully understood. Recent evidences suggest that the mechanistic target of rapamycin complex 1 (mTORC1) plays an important role in mediating leptin action. To determine the contribution of mTORC1 to the metabolic and cardiovascular effects of leptin, we generated conditional knockout mice that lack the critical mTORC1 subunit, Raptor, specifically in LRb-expressing cells ($LRb^{Cre}/Raptor^{fl/fl}$). Interestingly, body weight was comparable between $LRb^{Cre}/Raptor^{fl/fl}$ mice and controls (29.6±0.8 g vs 31.0±0.8g at 14 weeks of age). Moreover, leptin treatment (1μg/g bw, intraperitoneally, twice daily for 4 days) led to a similar decrease in food intake (-1.6±0.8 g in $LRb^{Cre}/Raptor^{fl/fl}$ mice vs -1.1±1.7 g in controls) and body weight (-5.9±0.8% vs -5.7±0.7%) in both groups. Next, we measured arterial pressure using radiotelemetry at baseline and in response to 2 μg intracerebroventricular (ICV) leptin. Baseline mean arterial pressure (MAP) was comparable between $LRb^{Cre}/Raptor^{fl/fl}$ mice (108±9 mmHg) and controls (103±7 mmHg). However, ICV leptin significantly increased MAP in control mice (30±14 mmHg), but not in $LRb^{Cre}/Raptor^{fl/fl}$ mice (1±9 mmHg, $P<0.05$ vs controls). The same pattern was observed for systolic and diastolic arterial pressure. Consistent with leptin's action on MAP, we observed a significant increase in renal SNA in response to ICV leptin in control littermates (106±20%) that was absent in $LRb^{Cre}/Raptor^{fl/fl}$ mice (-28±11%, $P<0.05$ vs controls) as determined by multifiber sympathetic nerve recordings. Our data suggest a critical role for mTORC1 signaling in mediating the cardiovascular sympathetic but not the metabolic actions of leptin, a dissociation that may have important implications for obesity-associated hypertension.

B.B. Bell: None. **D.A. Morgan:** None. **M. Rouabhi:** None. **K. Rahmouni:** None.

054

Prorenin Stimulates Firing Activity in Hypothalamic Neurons via Angiotensin II-Dependent and -Independent Mechanisms

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The brain RAS plays a central role in cardiovascular homeostasis by modulating sympathetic nerve activity and hormone secretion. Still, the relevance of intrinsic brain RAS function is questioned because renin levels

are too low to have an impact on angiotensin II (AngII) formation. The discovery of the prorenin receptor (PRR) brought new support to the brain RAS involvement in neurohumoral regulation. However, the mechanisms underlying PRR-mediated actions remain unknown. We used whole cell patch-clamp electrophysiology in hypothalamic slices to examine the effects of prorenin (PR) on the excitability of magnocellular neurosecretory neurons (MCNs) of the supraoptic nucleus and parvocellular RVLM-projecting presympathetic neurons of the paraventricular nucleus (PVN-RVLM), which play integral roles in cardiovascular control, and have been implicated in the pathophysiology of neurogenic hypertension.
PR application (2.5nM) increased the firing activity of both MCNs (basal 1.3±0.4 Hz *vs* PR 3.3±0.7 Hz; p=0.0086) and parvocellular neurons (basal 0.5±0.1 Hz *vs* PR 2.1±0.4 Hz; p=0.0006), including a subset of PVN-RVLM neurons (basal 0.5±0.1 Hz *vs* PR 1.4±0.4 Hz; p=0.048). In MCNs, this effect was blocked by a PRR antagonist (*PRO20*; 250nM; basal 1.9±0.6 Hz *vs* PRO20 1.3±0.5 Hz; p=0.017), but persisted in the presence of the AT1 receptor blocker, losartan (50µM; basal 0.8±0.2 Hz *vs* losartan 2.3±0.5 Hz; p=0.0018). Conversely, PR effects on PVN neurons persisted with PRO20 (basal 0.9±0.2 Hz *vs* PRO20 1.6±0.3 Hz; p=0.013), but were largely (~75%) blunted with losartan (basal 0.2±0.04 Hz *vs* losartan 0.6±0.1 Hz; p=0.0193; Δ basal 1.6±0.4 Hz *vs* Δ losartan 0.4±0.1 Hz; p=0.0175). These results suggest that while PR actions are primarily PRR-mediated in MCNs, they are predominantly AngII-dependent in PVN cells. Lastly, PR effects were abolished in both cell types by dialyzing neurons with the Ca^{2+} chelator BAPTA (10mM; MCNs: basal 0.6 ± 0.1 Hz *vs* BAPTA 0.3 ± 0.1 Hz; p=0.0277; PVN: basal 0.4±0.05 Hz *vs* BAPTA 0.5±0.2 Hz; p=0.6468).
Our results show for the first time that PR stimulates firing activity in both hypothalamic neurosecretory and presympathetic neurons, and support distinct AngII-independent and dependent signaling mechanisms in each neuronal population.

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059

Loss of AT1 Receptors in the Podocyte Does Not Protect Against Renal Injury

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Chronic kidney disease (CKD) arising from hypertension or other etiologies often progresses to ESRD despite treatment. One of the mainstays of treatment for CKD is blockade of the renin-angiotensin system (RAS). RAS blockers - angiotensin converting enzyme inhibitors (ACE-Is) and angiotensin receptor blockers (ARBs) - have the unique ability to decrease urine albumin excretion independent of systemic blood pressure. Despite their clinical effectiveness, RAS blockers do not completely stop CKD progression, thus there exists a need for new therapies. The association between proteinuria and an over-active RAS is well described and is mediated by angiotensin II (ang II) via the AT1 angiotensin receptor. This suggests that ang II, via AT1 receptors, promotes proteinuria and that this may be an important pathway in the pathogenesis of CKD. Yet, the mechanism and cellular site of action

where ang II causes proteinuria have not been clearly demonstrated. To investigate the role of AT1 in podocytes we used cell-specific gene targeting to generate mice lacking all AT1 receptors in the podocyte (PodKOs). PodKOs were then crossed with mice expressing a renin transgene (RenTg) to generate PodKO-RenTg mice. RenTg mice are hypertensive and provide an effective genetic model in which to study RAS over-activation. We hypothesized that AT1 signaling is a critical mediator of albuminuria in hypertensive kidney disease and that actions of AT1 receptors in the podocyte mediate kidney injury. PodKO mice develop normally and there is no difference in kidney weight between Cre- and Cre+ mice (6.9 ± 0.5 mg/g BW vs 7.0 ± 0.3 mg/g BW). At baseline, there was no significant difference in albumin excretion between 24 week old RenTg and PodKO-RenTg mice (210 ± 70.9 µg vs 228.7 ± 37.6 µg). When the mice were subjected to uninephrectomy to induce CKD, both RenTg and PodKO-RenTg mice developed similar degrees of albuminuria (279.9 ± 105.8 µg vs 366.8 ± 105.9 µg). However, kidneys from PodKO-RenTg mice had higher levels of glomerulosclerosis and mesangial expansion. Taken together, these data suggest that AT1 receptors in podocytes do not promote albuminuria and may have protective effects.

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060

POLDIP-2/NOX-4 Generates Hydrogen Peroxide that Impairs Myogenic Response of Afferent Arterioles from Mice with the Reduced Renal Mass Model of Chronic Kidney Disease

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Background: Because we have found that myogenic contractions are stimulated by superoxide ($O_2^{\cdot-}$) but inhibited by hydrogen peroxide (H_2O_2), we tested the hypothesis that H_2O_2 is the cause of the impaired myogenic responses of afferent arterioles from mice with the reduced renal mass (RRM) model of chronic kidney disease (CKD). **Methods:** Mice were subjected to 5/6 surgical nephrectomy or sham operations and fed 6% salt for 3 months. Single afferent arterioles were perfused, their diameter measured directly and $O_2^{\cdot-}$ and H_2O_2 measured by fluorescence microscopy. **Results:** The perfusion pressure of isolated afferent arterioles was increased from 40 to 80 mmHg to study myogenic responses. Arterioles from mice with RRM (vs sham) had a greater increase in $O_2^{\cdot-}$ (21.2 ± 1.9 versus 11.3 ± 2.5%; $p < 0.01$) and especially H_2O_2 (28.7 ± 3.7 versus 4.2 ± 0.4%, $P<0.005$), but a reduction in myogenic contraction (-1.7 ± 4.3 versus -14.4 ± 3.6%; $p < 0.005$) . Myogenic contractions were paradoxically reversed in afferent arterioles from mice with RRM after reduction in $O_2^{\cdot-}$ by PEG-SOD (+3.3 ± 1.5 versus -1.7 ± 4.3%, $P<0.05$) or deletion of $p47^{phox}$ (+2.5 ± 1.4 versus -1.7 ± 4.3%, $P<0.05$). In contrast, myogenic responses were increased even above the levels of shams in arterioles from mice with RRM after reduction in H_2O_2 by PEG-catalase (-19.1 ± 1.6 versus -1.7 ± 4.3%, $P<0.005$) or transgenic overexpression of catalase in smooth muscle cells (-10.7 ± 1.3 versus -1.7 ± 4.3%, $P<0.01$). Gene expression for NOX-4 and POLDIP-2 (main

source of H_2O_2) was increased 40-50% ($P<0.05$) in individual afferent arterioles from mice with RRM. Moreover, the myogenic contractions in the arterioles from POLDIP-2+/- mice with RRM were similar to POLDIP-2+/- with sham operations (-7.7 ± 0.9 versus -8.0 ± 0.6, P=NS). **Conclusions:** Afferent arterioles from mice with RRM had severely impaired myogenic responses that were attributed to increased H_2O_2 generation from POLDIP-2/NOX-4 that may therefore be novel targets to maintain autoregulation and protect kidneys from barotrauma in CKD.

L. Li: None. **E. Lai:** None. **Z. Luo:** None. **G. Solis:** None. **K.K. Griendling:** None. **W. Taylor:** None. **W.J. Welch:** None. **W.S. Christopher:** None.

061

Kidney-specific Klotho Gene Deficiency Impairs Sodium Excretion and Causes Hypertension

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Background & Purpose. Klotho was originally discovered as an anti-senescence gene. Mutation of klotho gene accelerates aging and shortens lifespan while overexpression of klotho gene extends lifespan in mice. We recently demonstrated that global mutation of klotho gene (+/-) increased blood pressure (BP). However, the underlying mechanism is not fully understood. The purpose of this experiment is to investigate if renal klotho plays a role in hypertension. Specifically, we assessed if kidney-specific deletion of klotho gene affects BP and renal function in male mice. Methods & Results. Briefly, kidney-specific Cre mice and klotho floxed mice were bred for generating kidney-specific klotho heterozygous (KspKL+/-) mice. Daily food and water intakes and urine output were monitored in wild type and KspKL+/- mice (16 weeks) using metabolic cages. BP was by direct cannulation of the carotid artery. Deletion of renal klotho significantly increased BP by 25 mmHg in mice without affecting the heart rate. Food and water intakes were not different between KspKL+/- and WT mice. Interestingly, 24-hr urine flow (UF) was significantly decreased in KspKL+/- mice (WT: 5.140± 0.3, KspKL+/-: 4.052±0.2,µL/min/100 gm body wt). The decrease in UF was also associated with a decrease in urinary sodium excretion (UNaV) in KspKL+/- mice compared to wild type (WT: 0.18± 0.01, KspKL+/-: 0.13±0.01, µmol/µL) without altering urinary potassium excretion. The cumulative sodium balance remained positive during the observation period with no significant difference between KspKL+/- and WT mice WT. Kidney-specific klotho deficiency did not affect the glomerular filtration rate (GFR). Conclusions. This study demonstrates for the first time that kidney klotho deficiency is sufficient to cause hypertension. Klotho deficiency-induced hypertension may be due to impaired Na excretion. Klotho may be a therapeutic target for kidney dysfunction and hypertension. An additional study is warranted to investigate if klotho regulates tubular ENaC and Na, K-ATP synthase.

Q. Ali: None. **X. Wang:** None. **Z. Sun:** None.

062

Inhibition of Phosphodiesterase 5 Attenuates Angiotensin II Dependent Hypertension and Renal Vascular Dysfunction in C57BL/6 but Not in eNOS-KO Mice

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Angiotensin (Ang) II is a key player in the pathogenesis of hypertension by influencing the NO/cGMP signaling cascade. Thus, Ang II induces vascular dysfunction by activating phosphodiesterase (PDE) 1 and PDE5 mediated cGMP-degradation. PDE1 is activated by increased intracellular Ca2+ concentrations observed during Ang II mediated vasoconstriction. PDE5 is activated by a cGMP mediated negative feedback mechanism. The present study examines the role of PDE1 and PDE5 in regulation of renal hemodynamics and blood pressure (BP) during Ang II induced hypertension.
In unconscious C57BL/6 (WT) mice, changes in renal blood flow (RBF) and BP was measured in the presence of specific PDE5- (sildenafil) or PDE1- (vinpocetine) inhibitors. Secondly, in WT and eNOS-KO mice, a mouse model characterized by low NO/cGMP levels, hypertension was induced by chronic Ang II infusion (500 ng/kg/min) for 14 days. Vascular relaxation was tested in the presence of acute or chronic PDE1 or PDE5 inhibition in isolated perfused kidneys.
During acute Ang II infusion, sildenafil increased RBF and decreased BP more potently than under baseline conditions. In contrast, vinpocetin had no effect on RBF and BP during normotensive or hypertensive conditions, Moreover, sildenafil but not vinpocetine improved NO (GSNO) dependent vasodilation in isolated perfused kidneys of Ang II treated WT mice. Next, we tested the impact of chronic PDE5 inhibition on Ang II dependent hypertension. Sildenafil (100mg/kg/d) attenuated Ang II dependent hypertension in WT (156±4 vs. 139±7mmHg; $p<0.05$) but not in eNOS-KO mice (166±4 vs. 172±4mmHg). Respectively, chronic Sildenafil treatment improved NO dependent vasodilation in isolated perfused kidneys of Ang II treated WT but not in eNOS-KO mice. In addition, urinary nitrite excretion, a marker for NO generation was significantly increased during Ang II-infusion compared to baseline in WT but not in eNOS-KO mice.
In conclusion, PDE5 seems to be the predominant PDE regulating RBF and renal vascular function. Moreover, sildenafil ameliorates Ang II dependent hypertension and improves vascular dysfunction. No effect of PDE5 inhibiton was observed in eNOS-KO mice supporting the hypothesis that Ang II activates PDE5 initially by increased NO/cGMP generation.

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063

Impaired Myogenic Response of the Af-art Contributes to the Increased Susceptibility to Hypertension and Diabetic Induced Renal Disease in Milan Normotensive Rats

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Previous studies have indicated that Milan normotensive (MNS) rats are more susceptible to the development of hypertension and diabetic induced renal injury than Milan hypertensive (MHS) rats, but the genes and pathways involved are unknown. MNS also develop proteinuria and chronic kidney disease (CKD) as they age, whereas hypertensive MHS do not. We compared the myogenic response of isolated perfused Af-Art and autoregulation of RBF and glomerular capillary pressure in 6-9

week old MNS and MHS rats. The diameter of Af-Art of MNS rats increased from 14.0 ± 0.5 to 14.2 ± 0.6 µm (n=6) when elevation in perfusion pressure from 60 to 120 mmHg. In contrast, the diameter of the Af-Art decreased significantly from 14.3 ± 0.5 to 11.5 ± 0.6 µm (n=6) in MHS rats. In vivo, RBF increased by 26% when RPP was increased from 100 to 140 mmHg in MNS rats but it remained unchanged in MHS rats. Glomerular capillary pressure rose by 11 mmHg in MNS following the elevation in RPP from 100 to 140 mm Hg but not in MHS rats. Protein excretion increased from 8.9 ± 0.7 to 158.2 ± 23.1 mg/day in MNS rats as the increased in age from 3 to 9 months of age but it did not increase in MHS rats. In com-parison to other strains susceptible and resistant to CKD, we noticed that both MNS and Fawn Hooded hypertensive (FHH) rats that do not autoregulate RBF also share the same sequence variant in the Adducin 3 gene. We performed a genetic complementation study to test whether this mutation might be responsible for the impaired myogenic response in MNS. The diameter of the Af-Art isolated from an F1 cross of MNS &FHH rats increased from 17.2 ± 0.9 to 18.5 ± 0.9 µM (n=5) in response to increase in perfusion pressure and RBF was not efficiently autoregulated in these animals. These data indicate a mutation in Adducin 3 which impairs myogenic response of the Af-Art and increased transmission of pressure to the glomerular capillaries may contribute to the development of CKD in MNS rats similar to what is seen in FHH rats.

Y. Ge: None. **F. Fan:** None. **R.J. Roman:** None.

064

20-HETE Contributes to Vasoconstriction of Renal Microvessels and Sodium Retention in Cyp4a14-/- Mice, a Model of 20-HETE Dependent Hypertension

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20-HETE (20-Hydroxyeicosatetraenoic acid), is a cytochrome P450 (CYP) 4A-derived arachidonic acid metabolite. 20-HETE has been linked to both pro-hypertensive (via increased vasoconstriction, vascular remodeling and vascular injury of renal microvessels) and anti-hypertensive (inhibiting ion transport in the distal nephron) functions. In this study we examined the effect of 20-SOLA (2,5,8,11,14,17-hexaoxanonadecan-19-yl-20-hydroxyeicosa-6(Z),15(Z)-dienoate), a water soluble antagonist of the actions of 20-HETE on renal hemodynamics and sodium (Na) excretion in Cyp4a14 knockout (CYP4a14-/-) male mice. The CYP4a14-/- male mice display hypertension accompanied by increased vascular 20-HETE levels. Administration of 20-SOLA (10mg/kg/day in drinking water) normalized blood pressure (BP) in male Cyp4a14-/- mice at day 10 of treatment (124±1 vs. 153±2 mmHg in untreated male Cyp4a14-/- mice; $p<0.05$). The normalization of blood pressure was accompanied by transient increase in the urinary sodium excretion in the Cyp4a14-/- male mice (8.3±0.7 vs. 5.8±0.5 µmol/g body weight/day; $p<0.05$). Importantly, 20-SOLA increased glomerular filtration rate (GFR) of Cyp4a14-/- mice (2.38±0.05 vs. 1.88±0.18 µL/min/mg kidney weight, $p<0.05$) as opposed to no changes observed in the wild type (WT: (2.26±0.18 vs. 2.33±0.20µL/min/mg kidney

weight). Evaluation of the renal blood flow (RBF) by laser Doppler flowmetry showed that treatment with 20-SOLA increased the RBF in Cyp4a14-/- mice by 12.3±4%, which remained unaltered in the WT. Additionally, the pressure-induced myogenic tone of isolated preglomerular microvessels was significantly elevated in Cyp4a14-/- mice; 20-SOLA treatment prevented the increase in myogenic responses. The natriuretic response to an isotonic saline loading challenge (10% of body weight, IP) was significantly attenuated in the Cyp4a14-/- mice as compared to the WT (35.5±2.8 vs. 57.4±8.3 percentage of Na load, $p<0.05$); this was corrected by 20-SOLA (61.7±5.7 percentage of Na load, $p<0.05$). These results confirm that 20-SOLA normalizes blood pressure of Cyp4a14-/- male mice and demonstrates that this is associated with increases in GFR, RBF and natriuresis.

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065

Expression of a Dominant Negative Cullin 3 Mutant (Cul3Δ9) Impairs Endogenous Cullin 3 Activity and Causes Impaired Vasorelaxation of Aorta

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Patients with dominant loss-of-function mutations in Cullin 3 (Cul3) resulting in deletion of exon 9 (Cul3Δ9) exhibit severe early onset hypertension correlated with reduced degradation of Cul3 substrates. We have shown that knockout of Cul3 induced by clustered regularly interspersed short palindromic repeats (CRISPR)-Cas9 in HEK293T cells resulted in accumulation of the Cul3 substrate RhoA. To gain insight into the mechanism of attenuated Cul3Δ9-mediated protein degradation, we utilized CRISPR-Cas9 genome editing to generate Cul3Δ9-expressing cell lines. We hypothesized that expression of the Cul3Δ9 protein will impair endogenous Cul3 wildtype (WT) function and accumulate Cul3 substrates. CRISPR guide RNAs targeting endogenous Cul3 were cloned into a plasmid expressing humanized Cas9. HEK293T cells were transfected and single cell clones selected. Sequencing revealed that genome editing induced a 548 bp deletion spanning exon 9 (Chromosome 2:224503433-224503979). Sequencing of the mRNA transcript also revealed that exon 9 was deleted, and Cul3Δ9 mRNA and protein was expressed. Although RhoA protein levels were normal, Cul3 substrates WNK4 and PLK1 were significantly increased in Cul3Δ9-expressing cells (WNK4: 1.5±0.12 Cul3Δ9 vs 1.0±0.02 WT; PLK1: 1.4±0.06 Cul3Δ9 vs 1.0±0.03 WT, $P<0.001$). Interestingly, Cul3Δ9-expressing cells exhibited impaired Cul3 ubiquitin ligase activity compared to WT. We also generated a novel transgenic mouse model inducibly expressing Cul3Δ9 protein specifically in smooth muscle (termed S-Cul3Δ9) and assessed vascular responses in the aorta using a wire myograph. Aorta from S-Cul3Δ9 transgenic mice exhibited impaired ACh relaxation compared to WT (at 30 μM: 55±2% sCul3Δ9 vs 71±7% WT, $p<0.0001$). In contrast, vasodilation to the nitric oxide donor, nitroprusside (SNP) was normal, as was constriction to endothelin-1. There was a significant increase in RhoA protein expression in aorta of S-Cul3Δ9 transgenic mice compared to WT (1.6±0.2 sCul3Δ9 vs 1.0±0.1 WT, $P<0.05$). These findings suggest a mechanism whereby Cul3Δ9 protein may interfere with endogenous Cul3 and impair

the degradation of Cul3 substrates such as RhoA or WNK4, contributing, at least in part-, to hypertension.

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066

The Undescribed Protein Caskin2 Is a Novel Regulator of eNOS Phosphorylation and Systemic Blood Pressure

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Disruptions in the function of the quiescent endothelial cells (ECs) that line mature vessels can both result in and contribute to the progression of numerous cardiovascular diseases including hypertension, atherosclerosis, and disorders of vascular permeability. Despite recent attention, the signaling pathways that are active in quiescent ECs remain poorly characterized relative to those that regulate EC activation. In an effort to provide mechanistic insight into these pathways, we have characterized the previously undescribed protein Caskin2, which we hypothesize is a novel regulator of EC quiescence. Caskin2 is expressed in ECs throughout the vasculature, including the aorta, coronary arteries, and renal glomeruli. In vitro, Caskin2 promotes a quiescent EC phenotype characterized by decreased proliferation and increased resistance to apoptosis-inducing factors. Caskin2 knockout mice are viable and fertile. However, preliminary radiotelemetry measurements indicate that Caskin2 knockout (KO) mice have mildly elevated systemic blood pressure (BP). Compared to wild type (WT) littermates (n=8), Caskin2 KO mice (n=7) had increased mean arterial pressure (119+/-1 vs. 113+/-1, p=0.012), systolic BP (138+/-2 vs. 132+/-2, p=0.023), and diastolic BP (99+/-1 vs. 93+/-1, p=0.014) at baseline. To explore the molecular mechanisms of Caskin2's effects, we used mass spectrometry to identify interacting proteins. Among the 67 proteins identified were the Ser/Thr phosphatase protein phosphatase 1 (PP1) and eNOS. Using standard in vitro biochemical techniques, we demonstrated that Caskin2 acts as a PP1 regulatory subunit. Interestingly, homologous expression of Caskin2 in vitro resulted in a marked increase in phosphorylation of eNOS on S1177, which is known to promote eNOS activity, and a decrease in phosphorylation on T495, which is associated with eNOS inhibition. Finally, PP1 has been shown to dephosphorylate eNOS T495 in vitro, suggesting a molecular mechanism for our in vivo findings. Ongoing work aims to determine if the interaction of Caskin2 and PP1 is required for the Caskin2-induced increase in activating phosphorylation of eNOS and to characterize the physiological mechanisms responsible for Caskin2's effects on BP in more detail.

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067

Endothelial S1P/S1P1 Signaling is Critical Regulator of Blood Flow and Pressure

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Background and objectives. Sphingosine-1-phosphate (S1P) is emerging as a pivotal signaling molecule within the vasculature. S1P has been known as a potent endothelial nitric oxide synthase activator through G-protein coupled receptor S1P1 and less abundant S1P3 on endothelial cells (EC). Although it has been recently shown that S1P/S1P1 signaling mediates EC alignment in response to flow, the role of S1P1 signaling in the pathogenesis of hypertension remains unknown. FTY720, an S1P1 functional antagonist, recently approved by the FDA to treat autoimmune conditions, induces a modest and transient decrease in heart rate in both animals and humans, suggesting that drugs targeting sphingolipid signaling affect cardiovascular functions in vivo. Thus, the aim of this study is to investigate whether S1P-S1P1 signaling pathway is crucial in blood flow and pressure regulation. Methods. The BP was evaluated in conscious mice with tail cuff system, whereas the vascular reactivity was examined with pressure myograph system in mesenteric arteries isolated from age matched WT and EC-S1P1-/- mice. Results. Here we report for the first time that S1P1-mediated signaling protects from hypertension. Administration of SEW 2871, an agonist of S1P1, to angiotensin-II-induced hypertensive mice lowered blood pressure to normotensive levels (110.7±3.1 vs. 160.7±2.4 mmHg, SEW 2871 vs. vehicle respectively). Pharmacological inhibition of S1P1 with W146 (100 nM and 1 µM) markedly reduced flow-induced vasodilation (Emax 25.2±3.8 and 2.5±2.3 vs. 48.6±4.9 µm, W146 100 nM and 1µM respectively vs. WT). These data were corroborated by using a genetic approach: mice EC-S1P1-/- were hypertensive (124.7±1.9 vs. 109±1.7 mmHg, EC-S1P1-/- vs. S1P1f/f mice) and showed a reduced response to flow-induced vasodilation (Emax 15.2±3.0 vs. 53.8±5.0 µm, EC-S1P1-/- vs. S1P1f/f mice mesenteric artery). Conclusion. Our study identifies S1P-S1P1 signaling as a new regulatory pathway of blood flow and pressure homeostasis, providing a novel therapeutic target for the treatment of hypertension.

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068

Role for the Rho-GAP Graf3 in the Pathogenesis of Human Hypertension

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Activation of RhoA in vascular smooth muscle cells (SMC) has been linked to vasoconstrictor-induced hypertension (HTN), but the relevance of this pathway to human disease was undetermined. We identified GRAF3 as a RhoA-GAP expressed specifically in SMC in mice and humans and reported that global GRAF3-deficient mice exhibited significant basal HTN (+ 25 mm Hg) that was fully reversed by treatment with a Rho-kinase inhibitor (Nature Comm. 2013;4:2910). Importantly, we recently created a tamoxifen inducible SMC-GRAF3 re-expression model which resulted in a near complete reversal of MAP (from 123 mmHg to 95 mm Hg), indicating that GRAF3's ability to limit RhoA activity in SMC is required for the maintenance of normal BP. Given that a BP-associated locus within the GRAF3 gene was identified by Genome Wide Association, we next sought to characterize the mechanisms that control GRAF3 expression. Through the use of a series

of siRNA-dependent approaches in cultured SMC, we found that SMC-specific expression of GRAF3 is mediated by the RhoA/MRTF-A/SRF-signaling axis. Interestingly, RhoA is known to be activated by mechanical forces and we found that GRAF3 expression was significantly increased (8-fold) in vessels subjected to pathological stress. The finding that GRAF3 expression was significantly increased in two separate hypertensive animal models relative to their littermate controls provides further evidence that GRAF3 expression is regulated as part of an auto-regulatory negative feedback loop to inhibit RhoA activity and SMC tone. Interestingly, the BP associated locus maps to the 80Kb first intron of the GRAF3 gene and we found that the minor GRAF3 allele that decreases BP was associated with a significant increase in GRAF3 mRNA in human tibial artery samples (3-fold increase; N=123; p=1e-10). We have identified regulatory elements within the hypertensive locus that exhibit SMC-selective transcriptional activity and have shown that a minor allele variation within one of these elements significantly increased transcriptional activity. Our studies add significantly to our understanding of the development and pathophysiologic consequences of hypertension and may lead to novel and perhaps individualized approaches to its treatment.

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069

Intrarenal Arteries Sense Mitochondrial N-formyl Peptides via Formyl Peptide Receptor in Wistar and Spontaneously Hypertensive Rats (shr)

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It is well established that chronic immune system activation contributes to hypertension and kidney injury. Mitochondria carry hallmarks of their bacterial ancestry and thus have emerged as a significant source of inflammatogenic damage-associated molecular patterns (DAMPs). One of these hallmarks is that it still uses an N-formyl-methionyl-tRNA as an initiator of protein synthesis. Recently, we have observed that mitochondrial DAMPs are elevated in the circulation of SHR, and that mitochondrial N-formyl peptides (F-MIT) infusion in rats induces systemic inflammation and vascular dysfunction via formyl peptide receptor (FPR) activation. However, we do not know if FPR plays a role in kidney injury and hypertension. We hypothesized that F-MIT activate FPR and lead to intrarenal dysfunction in 12 week old male Wistar and SHR (n=4-8). Wistar rats were treated with F-MIT (0.02 mg/kg) or non-formylated peptide (control) for 6 h and intrarenal arteries (diameter >100 μm) were isolated. To exclude systemic effects of F-MIT, intrarenal arteries were also isolated from control rats and treated ex vivo with 100 nM F-MIT or non-formylated peptide. F-MIT treatment in vivo increased intrarenal arteries FPR protein expression (2.3-fold vs. control) and decreased β-arrestin 2 (protein that internalizes FPR upon activation) and phosphorylation of endothelial nitric oxide synthase (4-fold vs. control). These results were reproduced in isolated arteries incubated with F-MIT or control for 6 h ex vivo. Similarly, in intrarenal arteries from untreated SHR, we found that FPR protein expression was higher (1.5-fold vs. Wistar Kyoto, WKY) and β-arrestin 2 protein expression was decreased (2-fold vs. WKY). Interestingly, although treatment with

hydrochlorothiazide (10-55 mg/kg/day) and reserpine (0.6-4.5 mg/kg/day) for 7 weeks attenuated the increase in blood pressure in SHR, anti-hypertensive therapy did not change FPR and β-arrestin 2 protein expression. Additionally, it was observed that the co-localization of FPR and β-arrestin 2 was decreased in intrarenal arteries from SHR. Overall, these data suggest that intrarenal arteries sense F-MIT. Also, FPR activation parameters following F-MIT treatment of normotensive rats are similar to those observed in SHR.

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070

AntagomiR-762 Prevents Angiotensin II Induced Aortic Fibrosis and Stiffening

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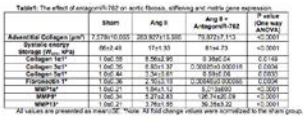
We and others have shown that hypertension (HTN) is associated with a striking deposition of collagen in the vascular adventitia. This causes vascular stiffening, which increases pulse wave velocity and contributes to end-organ damage. Through a screen of vascular microRNAs (miRNAs), we found that miR-762 is the most upregulated miRNA in mice with angiotensin II (Ang II)-induced HTN. qRT-PCR confirmed that miR-762 is upregulated 6.35±1.22 (p=0.03) fold in aortas of Ang II-infused mice compared with controls. This was a direct effect of Ang II, as miR-762 upregulation was not eliminated by lowering blood pressure with hydralazine and hydrochlorothiazide and was increased only 2-fold in DOCA salt HTN. To study the role of miR-762 in HTN, we administered a locked nucleic acid inhibitor of miR-762 (antagomiR-762). AntagomiR-762 administration did not alter the hypertensive response to Ang II, yet it normalized stress-strain relationships and aortic energy storage that occurs in systole (Table). Further studies showed that antagomiR-762 dramatically affected vascular matrix proteins, reducing mRNA for several collagens and fibronectin and dramatically upregulating collagenases MMP1a, 8 and 13 (Table). Thus, miR-762 has a major role in modulating vascular stiffening and its inhibition dramatically inhibits pathological fibrosis, enhances matrix degradation and normalizes aortic stiffness. AntagomiR-762 might represent a new approach to prevent aortic stiffening and its consequent end-organ damage.



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071

CX3CR1 Deficiency Aggravates Renal Damage in Angiotensin II Induced Hypertensive Kidney Injury

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A dense network of macrophages and dendritic cells (DCs) expressing the chemokine receptor CX3CR1 populates the kidney. We recently reported that CX3CR1 regulates the abundance of DCs selectively in the kidney and promotes renal inflammation in glomerulonephritis. Given that inflammation plays also an important role in hypertensive renal injury, we hypothesized that CX3CR1 deficiency should attenuate renal injury in hypertension by reducing the number of renal DCs.

Renal injury was induced by unilateral nephrectomy, angiotensin II (Ang II) infusion (1.5 ng/min/g) and salt (0.9% drinking water). Ang II induced heavy proteinuria (measured as albumin/creatinine ratio in mg/mg) compared to controls (0.1±0.0) and proteinuria was unexpectedly more severe in knockout mice than in wildtype (WT) mice (day 3 after of Ang II infusion: 19.2±5.4 vs. 2.5±0.6 $p<0.01$, day 14: 32.4±8.5 vs. 9.2±2.6 $p<0.05$ n=13 and 11 respectively). Histologic evaluation of kidney sections revealed also an increased number of proteinaceous casts and more glomerular injury in knockout mice (casts 1.1±0.3 vs. 0.1±0.1/high power field, glomerular injury score 0.95±0.06 vs. 0.57±0.06, $p<0.01$). Also plasma cholesterol levels were significantly higher in knockout mice than in WT mice (145±11 vs. 105±6 mg/dl, $p<0.01$). No difference was found for blood pressure and cardiac injury (hypertrophy, fibrosis, fetal gene expression) between both groups. Flow cytometric analysis revealed that Ang II infusion significantly increased the number of renal $CD11c^{+}/MHCII^{+}$ DCs in WT (38.5±1.4%, gated on $CD45^{+}$ cells) compared to controls (31.8±3.6%, $p<0.05$). However, a significant decrease of renal DCs was found in hypertensive CX3CR1 deficient mice (15.2±1.5%, $p<0.001$). In contrast, the number of $CD11b^{intermediate}/F4/80^{high}$ macrophages was 8 times higher in the kidney of CX3CR1 deficient mice compared to wildtype mice (5.5±1.5 vs.0.9±0.2%, $p<0.01$). These findings show that CX3CR1 deficiency reduces renal DC numbers and increases numbers of renal macrophages in hypertension. Surprisingly, despite reduced DC numbers, CX3CR1 deficiency increases proteinuria and glomerular injury, thus, CX3CR1 inhibition should be avoided in hypertension because it may promote proteinuria and renal injury.

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072

TRIF-Pathway of Innate Immune Responses Mediates Angiotensin II Hypertension

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We tested the role of two distinct adaptors of toll-like receptor (TLR) signaling on Ang II-induced hypertension and cardiac hypertrophy. These TLR adaptors, myeloid differentiation protein 88 (MyD88) and TIR domain-containing adaptor inducing interferon β (TRIF) facilitate distinct inflammatory signaling pathways. In an earlier study, we reported that MyD88-/- mice are protected from cardiac hypertrophy and pro-inflammatory gene expression after myocardial infarction. Our current results with 3 weeks infusion of Ang II (3000 ng/kg/min) vs. saline indicate that in MyD88-/- mice, the pressor response to Ang II and cardiac hypertrophy were increased more than in wild type (WT) mice. In Ang II-infused WT, systolic blood pressure (SBP) peaked at 147 ± 4 mmHg whereas in Ang II-infused MyD88-/- mice SBP reached a peak value of 163 ± 6 mmHg.

However, in mice with non-functional TRIF adaptor mutant (Trifmut), SBP did not increase during Ang II infusion and remained similar to the SBP in saline-infused mice (115 ± 3 mmHg). Baseline SBP was not different among WT, MyD88-/- and Trifmut mice. The increase in heart weight to body weight ratio (HW/BW) between saline and Ang II-infused mice was greater in MyD88-/- mice than WT mice (60% increase in MyD88-/- vs. 40% increase in WT), whereas it was less in Trifmut mice (22% increase). Accordingly, expression of several inflammatory genes (Tnfa, Nox4 and Agtr1a) was significantly greater ($P< 0.05$) in the heart and kidney of Ang II-infused MyD88-/- mice compared with Ang II-infused WT mice, whereas expression of these genes in Trifmut mice was either unchanged or reduced. We conclude that- (1) Ang II-induced hypertension, cardiac hypertrophy and inflammatory gene expression are mediated by activation of a TRIF-dependent pathway, but not by the MyD88-dependent pathways, and (2) Enhanced Ang II effects on SBP and hypertrophy in MyD88-/- mice suggest that MyD88 may serve as a negative regulator of the TRIF pathway in Ang II-induced hypertension. Selective targeting of these adaptor proteins may have significant therapeutic implications.

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073

Expression of Axl in Innate Immune Cells Contributes to Kidney Dysfunction and Onset of Hypertension

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Introduction: We previously reported that expression of the receptor tyrosine kinase Axl in hematopoietic cells is critical for kidney dysfunction in early hypertension. Here we investigated the role of Axl expression in innate immune cells in deoxycorticosterone acetate (DOCA)-salt induced hypertension.

Methods and Results: RAG1-/- mice lack adaptive immune cells and displayed the same (~25 mmHg) increase in systolic blood pressure (BP) as C57BL/6J mice after 1 week of DOCA-salt. While in metabolic cages RAG1-/- drank more (14.3$\pm$0.9 mL) than C57BL/6J mice (10.6$\pm$2.5 mL) per day after 1 week of DOCA-salt. Ultrasound imaging confirmed that RAG1-/- had ~20 % larger kidneys vs. C57BL/6J mice after DOCA-salt. RAG1-/- kidneys accumulated 2 times more fluid (2.8$\pm$0.1 %) compared to C57BL/6J mice (1.4$\pm$0.5 %) after DOCA-salt. Flow cytometry on kidneys from RAG1-/- confirmed absence of T and B lymphocytes, while DOCA-salt increased presence of macrophages (1.1$\pm$0.3 x10^9) compared to C57BL/6J mice (0.6$\pm$0.1 x10^9). We successfully generated Axl/RAG1 double knockout mice and subjected the littermates to 1 week of DOCA-salt. Increases in systolic BP were the same in Axl/RAG1+/+ and Axl/RAG1-/- littermate mice. No differences were found in kidney volumes between the Axl/RAG1 genotypes as well. However, 24 hrs excretion volumes increased in Axl/RAG1-/- (50$\pm$6 %) compared to Axl/RAG1+/+ (31$\pm$6 %) littermates. Finally, renal artery blood flow velocity (611$\pm$52 mm/s) and resistive index (0.62$\pm$0.03) were reduced in Axl/RAG1+/+ but not in Axl/RAG1-/- mice (665$\pm$45 mm/s and 0.68$\pm$0.01, respectively) when compared to their controls.

Conclusions: Our findings suggest that mice lacking lymphocytes compensate by increasing kidney macrophages that contribute to initial increase in BP. Depletion of Axl in innate

immune cells partially reverses kidney dysfunction by improving renal artery function in early hypertension.

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074

An Obligatory Role for B Cells in the Development of Angiotensin II-dependent Hypertension

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Background: Clinical hypertension is associated with raised serum antibody levels. However, no studies have examined whether B cells and antibodies play causative roles in the pathogenesis of hypertension. We investigated whether experimental hypertension is similarly associated with elevated IgG production, and if B cell/antibody deficiency affords protection against hypertension and associated vascular remodeling.
Methods and Results: Ang II-dependent hypertension in mice was associated with B cell activation/maturation as revealed by a: (1) a 25% increase in the proportion of splenic B cells that expressed the activation marker CD86; (2) an 80% increase in numbers of splenic plasma cells, (3) a 500% increase in serum IgG levels; and (4) marked accumulation of IgG deposits in the aortic adventitia. Indicative of a causative role for B cells, B cell activating factor-receptor-deficient (BAFF-R-/-) mice, which lack mature B cells, displayed blunted hypertensive responses to chronic angiotensin II infusion (Δ30±4 mmHg) compared to wild-type (WT) mice (Δ41±5 mmHg). Importantly, adoptive transfer of B cells into BAFF-R-/- mice restored the hypertensive response. BAFF-R-/- mice showed no evidence of Ang II-induced increases in aortic IgG accumulation. They also had 80% fewer F4/80+ macrophages, 60% fewer CD4+ T cells, and 70% reduction in TGF-β expression and collagen in their aortas compared to Ang II-treated wild-type mice. Ang II doubled aortic pulse-wave velocity, a measure of vessel stiffening, whereas BAFF-R-/- mice were largely protected (70% reduced) from this effect. Finally, pharmacological depletion of B cells with an anti-CD20 antibody blunted Ang II-induced hypertension (Δ37±9 mmHg vs Δ57±6 mmHg). Conclusions: We provide the first evidence that B cells are activated during development of hypertension and contribute to vessel remodeling and elevated blood pressure. These findings suggest B cells and/or the IgG antibodies they produce could be targeted for anti-hypertensive therapy.

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075

Proinflammatory T Helper 17 Cells as an Indicator of Hypertension

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Objectives: T cells and interleukin 17 (IL17) have been shown to be critical in the development of hypertension (HTN). This implicates a significant role of IL17 producing T helper 17 (Th17) cells in HTN. Thus, the objective of our study was to investigate if Th17 cell levels in the peripheral blood (PB) and the bone marrow (BM) are correlated with blood pressure. **Methods:** Saline or Ang II was chronically infused into C57BL6 mice (1000ng Ang II/kg/min using osmotic pumps) for 3 weeks. This resulted in an increase in MAP of 45±10 mmHg. BM and PB from saline and Ang II-treated mice were analyzed using FACS. A parallel human study was conducted using blood samples obtained from hypertensive patients (n = 8, systolic BP ≥125 mmHg) and normotensive subjects (n = 10, systolic BP <125 mmHg). FACS analysis was used to examine changes in the inflammatory cells levels in these patients. **Results:** We observed 87% and 36% increases in both Sca-1^+ c-Kit^+ Lin^- hematopoietic stem cell (HSC; 0.23±0.04% vs. 0.43±0.05%) and Sca-1^+ c-Kit^- Lin^- lymphoid progenitors (10.8±1.1% vs. 14.7±2.9%) in the BM of Ang II infused mice. These are upstream stem/progenitor cells for T cells. This was associated with 30% and 190% increases in $CD4^+$ $IL17^+$ Th17 cells in the BM and PB (1.20±0.09% vs. 1.61±0.19 % and 4.8±2.0 % vs. 14.7±3.8 % respectively) in the Ang II infused mice. Importantly, there were 58% and 206% increases of angiotensin II type 1 receptor (AT1R) expressing $CD4^+$ T cells in the BM and PB of Ang II HTN mice (0.40±0.04 % vs. 0.63±0.14 % and 1.1±0.2 % vs 3.4±1.1 % respectively) and ~ 85% of these cells were also positive for IL-17. Consistent with mouse data, analysis of PB showed a 470% increase of Th17 cells in HTN patients (0.48±0.18 % vs 2.72±1.2%). **Conclusions:** We observed (i) Increased hematopoietic stem/progenitor cells in Ang II HTN mice; (ii) increased Th17 cells in both HTN mice and humans and (iii) the majority of AT1R expressing $CD4^+$ T cells was Th17 cells. Taken together, these observations indicate that Th17 cells may be an important indicator of those destined to develop HTN and suggest that these cells may represent a novel therapeutic target.

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076

Early Administration of 17-hydroxyprogesterone Caproate Improves Fetal Growth Restriction Possibly by Reducing Sflt-1 and Placental Cytolytic Nk Cells in Response to Placental Ischemia During Pregnancy

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Preeclampsia (PE), new onset hypertension, is characterized by elevated anti-angiogenic factor soluble fms-like tyrosine kinase (sFlt-1), cytolytic natural killer (NK) cells and placental ischemia predicted with increased uterine artery resistance (UARI) which are likely culprits for decreased fetal weight during PE pregnancies. Cytolytic NK are an important arm of the immune system killing tumor and infected cells by perforin-granzyme-mediated cytolysis and have been shown to be increased in PE women compared to those with normal pregnancy. Currently, there is no effective treatment for PE except for early delivery,

making PE the leading cause for premature births worldwide. Administration of 17-hydroxyprogesterone caproate (17-OHPC) is used for prevention of spontaneous preterm labor, but is not included in the current management for PE. This study was designed to test the hypothesis that early administration of 17-OHPC could improve pregnancy outcomes in response to placental ischemia. To do so, 17-OHPC (3.32mg/kg) was administered intraperitoneally on gestation day 15 to reduced uterine perfusion pressure (RUPP) rats, UARI was measured using Doppler ultrasound and carotid catheters were inserted on day 18. Blood pressure (MAP), sFlt-1, and placental cytolytic NK cells were measured on GD 19. MAP in normal pregnant (NP) rats (n=12) was 94 $\pm$ 2, 126 $\pm$ 2 in RUPP (n=27) and 111$\pm$1 mmHg in RUPP+17-OHPC (n=15), $p < 0.05$. Pup weight was 2.3$\pm$0.09 in NP, 1.9$\pm$ 0.04 in RUPP rats, which improved to 2.1$\pm$0.06 grams in RUPP+17-OHPC $p < 0.05$. UARI was 0.6$\pm$0.01 in NP (n=3), 0.8$\pm$0.03 in RUPP rats (n=4), which improved to 0.6$\pm$0.04 in RUPP+17-OHPC (n=5), $p<0.05$. Total number of placental NK cells was 8.6 $\pm$ 3.1 in NP, 20.2 $\pm$2.4 in RUPP rats, which decreased to 1.6 $\pm$ 0.54 % in RUPP+17-OHPC, $p<0.05$. Activated placental NK cells was 3.8 $\pm$ 2.2 in NP, 11.9$\pm$2.01 in RUPP , which improved to 0.4 $\pm$ 0.2 % in RUPP+17-OHPC, $p < 0.05$. Plasma sFlt-1 was 36.1$\pm$7.5, 385.9$\pm$141 in RUPP rats (n=5), which was blunted to 110.2$\pm$11.1 pg/mL in RUPP+17-OHPC, $p<0.05$. In conclusion, early administration of 17-OHPC improves sFtl-1, UARI, activated cytolytic NK cells, pup weight and hypertension in response to placental ischemia. Therefore, 17-OHPC should be further considered for addition to the management of PE.

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077

In Vivo Evidence of AT1a Receptor-Mediated Uptake of Angiotensin II by the Proximal Tubule Visualized by Intravital Multiphoton Imaging

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The development of all forms of angiotensin II (ANG II)-dependent hypertension is associated with higher levels of intrarenal ANG II, which are greater than can be explained on the basis of circulating ANG II and suppressed cortical renin expression. In the present study, we used intravital multiphoton imaging to test the hypothesis that AT1 (AT1a) receptor-mediated uptake of ANG II by the proximal tubules of the kidney plays a major role in the underlying mechanisms. Adult male Munich-Wistar rats were anesthetized with Inactin and continuously infused with a pressor dose of Alexa 488-conjugated ANG II (50 ng/min, i.v.) for 2 hr. Time-dependent proximal tubular uptake responses of Alexa 488-ANG II were studied with mean arterial blood pressure maintained at ~150 mmHg throughout the experiment. After 30 min infusion, very low levels of Alexa 488-ANG II were visualized within proximal tubules and cortical collecting ducts (CCDs) ($p<0.05$). However, high levels of Alexa 488-ANG II were accumulated in the

lumen of CCDs, but not that of proximal tubules. After 1 hr infusion, moderate levels of Alexa 488-ANG II were visualized in the proximal tubules, but not in the glomeruli and CCDs. The most striking uptake responses were visualized in all segments of proximal tubules after 2 hr infusion. Internalized Alexa 488-ANG II was predominantly localized to the basolateral side, where it was colocalized with tetramethyl rhodamine methyl ester (TMRM), a mitochondrial membrane potential-dependent dye. TMRM is a lipophilic cationic dye that is primarily accumulated in the mitochondria of proximal tubules. Some internalized Alexa 488-ANG II was visualized around and over the nuclei. Furthermore, the uptake of Alexa 488-ANG II by proximal tubules was significantly attenuated in caveolin 1-knockout mice ($p<0.01$), and blocked in AT1a receptor-knockout mice ($p<0.01$). Our results provide strong intravital multiphoton microscopic evidence that circulating ANG II is primarily taken up by the proximal tubules of the kidney via an AT1a receptor-mediated mechanism, and that internalized ANG II may be transported to the mitochondria and the nucleus, where it may alter mitochondrial and nuclear function in the proximal tubules of the kidney.

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078

Functional Significance of the Chymase/Ang-(1-12) Pathway on Cardiomyocytes

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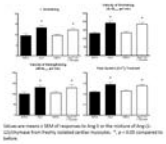
Angiotensin-(1-12) [Ang-(1-12)] functions in rodents and humans as a tissue substrate for the direct generation of Ang II via chymase. Since its direct cardiac effect have not been studied, the importance of this renin-independent mechanism for Ang II paracrine/intracrine actions in modulating cardiac contractility were determined in freshly isolated myocytes from 11 normal SD rats. Systolic amplitude (SA), peak velocity of shortening (dL/dtmax), the peak velocity of relengthening (dR/dtmax), and changes in the peak calcium transient ([Ca2+]i) were evaluated before and following exposure to Ang II (10-6 M), Ang-(1-12) delivered alone (range: 2x10-6 to 4x10-6 M) or after 1 h incubation with human recombinant chymase (10 µg protein/mL at 37°C). Both Ang II and the mixture of Ang-(1-12) with chymase elicited positive inotropic responses in freshly isolated cardiac myocytes associated with significant increases in peak systolic [Ca2+]i (Figure) while superfusion of Ang-(1-12) alone elicited an increase in dL/dtmax without significant changes in [Ca2+]i. The increases in contractility elicited by Ang II or the Ang-(1-12)/chymase mixture were abolished by prior exposure of the myocytes to losartan (10-5 M) or the chymase inhibitor chymostatin (8x10-5 M). We conclude that in single adult rat myocytes Ang-(1-12) stimulates contractile function through a chymase mediated action and by mechanisms that implicate a paracrine/intracrine activation of intracellular calcium.$$MISSING OR BAD IMAGE SPECIFICATION {63B45A2B-D11B-4637-A7A6-8109E33E2FDE}$$



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079

G Protein-Coupled Estrogen Receptor Deletion Exacerbates Pulse Pressure in Female but not Male Hypertensive Mice

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The protective cardiovascular effects of estrogen are mediated by both the classic steroid receptors (ERα/β) and the novel G protein-coupled estrogen receptor (GPER). Our previous work demonstrates a role for GPER in the beneficial effects of estrogen on blood pressure, vascular tone, end organ damage, and aortic remodeling in hypertensive females, due to both direct vasodilatory effects and local regulation of the renin-angiotensin system. Therefore, we hypothesized that genetic deletion of GPER in female mice exacerbates Ang II-induced hypertension and associated tissue damage. Male (M) and female (F) wt and GPER ko mice were implanted with radiotelemetry probes at 11-13 weeks of age to measure baseline blood pressure before infusing Ang II (700 ng/kg/min) for two weeks. MAP (in mmHg) was similar at baseline (F-wt: 108 ± 2, n=4, F-ko: 105 ± 2, n=3, M-wt: 106 ± 4, n=3, and M-ko: 105 ± 0.5, n=3; P = 0.62), and after Ang II (F-wt: 136 ± 11, F-ko: 131 ± 3, M-wt: 148 ± 7, M-ko: 135 ± 5; P=0.51). Pulse pressure was significantly higher in response to Ang II in both sexes (*P<0.0001), and GPER deletion exacerbated this increase in females (30 ± 1 vs 43 ± 4 mmHg, *P<0.05) but not males (37 ± 3 vs. 39 ± 4, P=0.61). Further evaluation of the circadian pattern revealed that pulse pressure was particularly higher in F-ko during the day (28 ± 1 vs. 40 ± 4 mmHg, *P<0.05). Moreover, F-ko mesenteric arteries exhibited enhanced contractility to both receptor-dependent (PGF2α, *P<0.05) and receptor-independent (KCl, *P<0.05) stimulation. Our findings indicate that in females with intact ERα/β signaling, GPER deletion does not alter the initial pressor response to Ang II but exacerbates pulse pressure and resistance artery contractility during hypertension. Pulse pressure is an indirect measure of arterial stiffness and an independent risk factor for adverse cardiovascular events. While pulse pressure increases with aging in both sexes, this increase is markedly exacerbated in women. Our results

indicate that loss of endogenous GPER activation due to low circulating estrogen may underlie increased pulse pressure in aging postmenopausal women.

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080

Role of High-mobility Group Box 1 in Angiotensin Converting Enzyme 2 -Mediated Cardioprotective Effects in a Mouse Model of Myocardial Ischemia

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Backgrounds: High-mobility group box 1 (HMGB1) activates inflammation process, and its elevation is associated with adverse clinical outcomes in patients with myocardial infarction (MI). Previous studies have demonstrated that angiotensin-converting enzyme 2 (ACE2), a key member of vasoprotective axis of the renin angiotensin system, provides protection against MI. However, the involvement of HMGB1 in ACE2-mediated protective effects in cardiovascular diseases has yet to be elucidated. Thus, we hypothesized that the cardioprotective effects of ACE2 are, in part, by inhibition of cardiac HMGB1 and inflammatory pathways.
Methods and results: Transgenic (TG) mice that ubiquitously overexpress ACE2 were used for the study. ACE2 levels in the hearts of TG mice were 58-times higher than wild type (WT). Mice were subjected to coronary artery ligation surgery and cardiac functions were evaluated by echocardiography 4 weeks post MI. MI resulted in a 53% decrease in ejection fraction (EF) and a 42% infarct area of left free wall. However, ACE2 TG mice showed 60% improvement of EF over WT mice. This was associated with a 55% decrease in the infarct area. Additionally, MI caused a three times increase in plasma HMGB1 levels which was associated with elevated macrophage infiltration and proinflammatory cytokine levels. In comparison to WT-MI mice, ACE2 overexpression attenuated the increase in plasma HMGB1 by 25% and reduced the proinflammatory cytokine levels to normal in the TG mice. To investigate the modulatory effects of ACE2 on HMGB1, adult cardiomyocytes incubated with an ACE2 activator (DIZE), a selective ACE2 inhibitor (C16), and a combination of DIZE and C16 were exposed to hypoxic condition for three hours. Hypoxia (H) caused 35% cell death and 44% increase in the HMGB1 level (H: 23.6± 0.8 vs normoxia: 16.4± 1.2, ng/ml). DIZE significantly attenuated the cell death (2% cell death) and 37% decrease in HMGB1 levels (DIZE+H: 14.8± 2.5 ng/ml), which was blocked by a selective ACE2 inhibitor (C16+DIZE+H: 20.8± 2.9ng/ml).
Conclusions: The protective effect of ACE2 against ischemia is correlated with decrease in HMGB1 and its downstream pro-inflammatory cascades. Thus, HMGB1 could be useful target for the development of novel treatment for MI.

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081

Intracerebroventricular (ICV) Infusion of Angiotensin-(1-7) Lowers Blood Pressure and Improves Autonomic Function in Antenatal Betamethasone Exposed Sheep

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We have previously shown that antenatal betamethasone exposure in sheep is associated with a deficiency in angiotensin (Ang)-(1-7) levels in the cerebrospinal fluid of these betamethasone exposed (BMX) sheep and impaired baroreflex sensitivity for control of heart rate (BRS) by 6 weeks of age followed by persistent suppression of the reflex and elevation in mean blood pressure (MAP) by 6 months of age. In this study we sought to determine if supplementation of Ang-(1-7) administered via continuous infusion into the lateral ventricle of 4 month old BMX lambs (1-7, n=4) at a dose of 1.25ug/kg/hr for 1 week would improve the BRS, heart rate variability (HRV) and lower MAP and blood pressure variability (BPV). The control group received ICV artificial cerebrospinal fluid (aCSF, n=4). MAP and heart rate were recorded continuously via femoral arterial catheters and digitized before and one week after the ICV infusion. Both blood pressure and heart rate were analyzed to obtain measures of spontaneous BRS, HRV and BPV in the frequency and time domain. There were no differences in any of the measures between the two groups at baseline and no changes in the aCSF group after 1 week of ICV aCSF administration. Meanwhile, Ang-(1-7) ICV treatment significantly improved all measures of BRS compared to baseline, (HFa, 5.3±0.9 vs. 2.4±0.5 ms/mm Hg, p=0.03) and sequence ALL (6.5±1.6 vs. 1.9±0.9 ms/mm Hg, p=0.01). Ang-(1-7) also improved HRV measured as rMSSD (11.6±1.6 vs. 7±1.2 ms at baseline, p=0.04). Ang-(1-7) administration lowered MAP by 11.5 mm Hg ($p < 0.05$) compared to a rise of 2.0 mm Hg in the control group with no significant changes in heart rate or BPV in either group. These data support the hypothesis that Ang-(1-7) deficiency in the CSF contributes to the impaired autonomic function and reduced parasympathetic tone in betamethasone exposed sheep and replacement of the peptide in the brain will help restore these measures to normal levels. Elevating Ang-(1-7) levels in humans exposed antenatally to betamethasone may have beneficial cardiovascular effects.

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082

High Fat Diet Promotes Haplotype Dependent Differential Transcriptional Regulation of the Human Angiotensinogen Gene

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Angiotensinogen (AGT) is the only known precursor to angiotensin II. Systemic renin angiotensin aldosterone system (RAAS) is activated in human and experimental models of obesity. RAAS activation in obesity is linked to

the development of cardiovascular pathophysiologies. We have identified polymorphisms in 2.5 Kb promoter of human angiotensinogen gene (hAGT) that forms two haplotype (Hap) blocks: -6A/G (-1670A/G, -1562C/T, -1561T/C) and -217A/G (-532T/C, -793A/G, -1074T/C,& -1178G/A). Hap -6A/-217A (Hap -6A) is associated with human hypertension whereas, Hap -6G/-217G (Hap -6G) reduces cardiovascular risk. Here, we examine high fat diet-mediated allele-specific transcriptional regulation of the hAGT gene in adipose tissue, in vivo, in transgenic (TG) mice engineered with either haplotype of the hAGT gene. Twelve-week-old male TG mice with Hap −6A or −6G were fed normal diet (10% kcal as fat) and high fat diet (60% kcal as fat) for 10 weeks. Using Q - RT PCR and western blot we show increased hAGT expression in adipose tissue of the Hap -6A-TG mice after high fat diet than control diet (Hap -6A-0.68±0.04 vs. Hap -6G- 0.33±0.03 A.U., $p<0.05$). ChIP assay shows greater chromatin binding of GR, MR, CEBPβ and STAT3 transcriptional factors (Hap -6A-0.80±0.04 vs. Hap -6G- 0.26±0.06 A.U., $p<0.05$) to the hAGT transgenes in Hap -6A TG mice after high fat diet. No significant change was observed in the endogenous mouse AGT gene. In addition, after high fat diet, change (Δ) in inflammatory and redox markers was significantly ($p<0.05$) greater in TG mice with Hap I including, IL1 (4.6±0.8 vs. 2.1±0.49 fold), IL6 (4.0±0.69 vs. 2.1±0.2 fold) and NOX1 (8.3±0.4 vs. 2.5±0.6 fold). This is accompanied by reduction in levels of antioxidant defenses (SOD1: 0.97±0.0 vs. 1.4±0.1 fold; HO1: 0.77±0.1 vs. 1.3±0.2 fold) & activation of MAPK14 and ERK1/2 signaling. Taken together, our results show that SNPs in the hAGT Hap -6A favor high fat diet induced binding of transcriptional factors GR, MR, CEBP-β and STAT3 that lead to elevated expression of the hAGT in expanded mass of the adipose tissue. This also activates the RAAS with pathophysiological implications including, phosphorylation of kinases such as MAPK14 and ERK2, increase in tissue pro-inflammatory and oxidative stress molecules.

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083

Metabolic and Cardiovascular Effects of BBS1 Ablation From the POMC-containing Neurons

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Bardet-Biedl syndrome (BBS) is a pleiotropic autosomal recessive disorder associated with several features including obesity and hypertension. Deletion of *Bbs* genes globally, in the nervous system or in the leptin receptor-expressing cells recapitulated many of the BBS phenotypes including obesity and hypertension. Here, we assessed the effect of ablating the *Bbs1* gene from the neurons expressing proopiomelanocortin (POMC) neurons. Breeding $Bbs1^{flox}$ mice with $POMC^{Cre}$ mice created mice deficient in *Bbs1* gene only in the POMC-positive neurons (visualized by tdTomato expression). Importantly, $POMC^{Cre}/Bbs1^{fl/fl}$ mice display an obesity phenotype as indicated by the increased ($P<0.05$) body weight (48.6±1.7 vs 34.9±0.8 g in controls) and fat pads (3.3±0.3 vs. 0.7±1.1 g for inguinal fat, 2.1±0.2 vs. 0.4±0.05 g for perirenal fat, 3.3±0.3 vs. 0.8±0.3 g for reproduction fat and 0.4±0.02 vs. 0.2±0.02 g for brow fat) associated with increased ($P<0.05$) food intake (3.79.±0.14 vs. 2.97±0.15 g in controls) in 25 weeks old mice. $POMC^{Cre}/Bbs1^{fl/fl}$ mice displayed decreased ($P<0.05$) O_2 consumption (2.6±0.03 vs. 3±0.04 mL

O_2/100g/min in controls) and heat production (8.0±0.09 vs. 9.2±0.13 kcal/kg/hr in controls). These results indicate that hyperphagia and decreased energy expenditure contribute to the development of obesity in $POMC^{Cre}/Bbs1^{fl/fl}$ mice. Next, we assessed the consequence on arterial pressure (AP) and sympathetic nerve activity (SNA) of ablating the *Bbs1* gene from POMC neurons. Interestingly, deletion of the *Bbs1* gene in POMC neurons did not recapitulate the hypertension phenotype of BBS as indicated by the slight, but not significant increase in mean AP (116±4.7 vs 109±6.1 mmHg in controls). However, conscious renal SNA was significantly higher in $POMC^{Cre}/Bbs1^{fl/fl}$ mice relative to controls (132.4±11 vs 74.3±4.2 spikes/sec, $P<0.05$). Finally, the depressor effect of ganglionic blockade (hexamethonium) was exaggerated in $POMC^{Cre}/Bbs1^{fl/fl}$ mice (-68.6±4.4 vs -45.2±9.4 mmHg in control, P=0.017). These findings demonstrate that *Bbs1* gene in the POMC neurons is critical for energy homeostasis, but not for arterial pressure regulation.

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084

Significant Roles of Hyperglycemia and Obesity in the Disruption of Blood Pressure Circadian Rhythm in Leptin-receptor Mutant Db/db Mice

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Blood pressure (BP) exhibits 24-hour rhythm. Loss of BP oscillation has been found in up to 75% diabetic patients and is associated with increased risks of target organ injuries. However, the mechanisms underlying the disruption of BP circadian rhythm in diabetes remain poorly understood. We and others have demonstrated that type 2 diabetic *db/db* mice in C57/KsJ background have hypertension and severely disrupted BP circadian rhythm. Since these *db/db* mice were severely hyperglycemic (>600 mg/dL) as well as obese, it is unclear which factor or both contribute to the disruption of BP oscillation. Moreover, it is unclear whether clock genes are involved in the diabetes associated disruption of BP circadian rhythm. To address these specific questions, we cross bred the leptin receptor mutated *db/db* mice in the C57BL/KsJ background with PERIOD2::LUCIFERASE knock in mice in C57BL/6J background. At 4-5 months old, the blood glucose in these *db/db*-Per2 mice was higher than controls (320.3 vs 153 mg/dL) but was significantly lower than the C57/KsJ -*db/db* mice (608.5 mg/dL). However, the body weight of these *db/db*-per2 mice was significantly higher than both the C57/KsJ-*db/db* (66.4 vs 44.8 g) as well as control mice (33.9 g). The metabolic flexibility, which is represented by respiratory exchange ratio and measured using TSE LabMaster Indirect Calorimetry System, was significantly compromised in the *db/db*-per2 mice when compared to controls. We then determined the BP in the *db/db*-per2 mice using radiotelemetry under 12: 12 light: dark cycle. The circadian parameters of BP, including period length, amplitude and acrophase were calculated using Chronos-fit software. The results demonstrated that *db/db*-per2 mice have normal BP value but disrupted BP circadian rhythm, with decreased power of 24h oscillation, diminished amplitude and shifted acrophase. However, the extent of the disruption was significantly less than that we have reported in the C57/KsJ-*db/db* mice. By using LumiCycle, we are currently investigating the clock gene functions in various tissues including SCN, aorta, liver, and etc isolated from

db/db-Per2 mice. In summary, we demonstrated that both hyperglycemia and obesity significantly contribute to the disruption in BP circadian rhythm in *db/db* mice.

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085

Role of Suppressor of Cytokines Signaling 3 (Socs3) in Modulating Chronic Metabolic and Cardiovascular Effects of Leptin

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Suppressor of cytokine signaling 3 (SOCS3) is a negative regulator of leptin signaling. Hypothalamic SOCS3 is upregulated in obese animals fed a high-fat diet and has been suggested to contribute to development of resistance to leptin's anorexic effects. In this study we determined whether deletion of SOCS3 in the entire central nervous system (CNS) amplifies the chronic anorexic and blood pressure (BP) effects of physiological increases in plasma leptin in mice fed a normal diet. SOCS3$^{flox/flox}$-Nestin-cre mice were generated by breeding SOCS3$^{flox/flox}$ with Nestin-cre mice. BP and heart rate (HR) were recorded by telemetry, and oxygen consumption (VO_2) was monitored by indirect calorimetry in 22-week-old SOCS3$^{flox/flox}$-Nestin-cre (n=4) and control mice (SOCS3$^{flox/flox}$, n=4). Compared to controls SOCS3$^{flox/flox}$-Nestin-cre mice were lighter (30±1 vs 33±1 g) and normoglycemic (124±7 vs 146±10 mg/dl), consumed less food (3.0±0.4 vs 3.6±0.2 g/day) and had similar VO_2 (77±6 vs 73±3 ml/kg/min). SOCS3$^{flox/flox}$-Nestin-cre mice had similar MAP (103±3 vs 107±3 mmHg) but higher HR (666±15 vs 602±17 bpm) compared to control mice. Chronic leptin infusion greatly reduced food in SOCS3$^{flox/flox}$-Nestin-cre (46±3 vs 35±4%) and increased MAP (15±3 vs 7±2 mmHg) and VO2 (18±3 vs 14±2%) compared to control mice. No significant changes were observed in HR in either group. Leptin infusion significantly reduced blood glucose levels in both groups (124±7 to 97±7 vs 146±10 to 105±7 mg/dl). These results indicate that SOCS3 deletion in the entire CNS reduces body weight and food intake, and amplifies leptin's effect on appetite and blood pressure and also suggest the SOCS3 signaling attenuates the chronic actions of leptin on blood pressure as well as appetite regulation even in non-obese mice fed a normal diet. (NHLBI-PO1HL51971, NIGMS P20GM104357 and AHA SDG5680016)

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086

Insulin Receptor Signaling in the Subfornical Organ Protects Against the Development of Metabolic Syndrome

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Metabolic syndrome encompasses a combination of conditions including obesity, diabetes, dyslipidemia and hypertension. Brain insulin resistance has emerged as a contributor to the development of metabolic syndrome,

although the neural regions involved remain unclear. While most studies have focused on hypothalamic areas, recent evidence suggests that the subfornical organ (SFO), a circumventricular organ well-known for cardiovascular control, is also involved in metabolic regulation. We therefore hypothesized that the SFO insulin receptor protects against the development of metabolic syndrome. Male mice (6.5 wks) harboring a conditional allele of the insulin receptor gene (*INSR)* underwent SFO-targeted delivery of an adenovirus encoding a control vector (AdLacZ, n=7) or Cre-recombinase (AdCre, n=11) for selective removal of the SFO insulin receptor. Both groups remained on normal chow for 10 wks. Removal of the SFO insulin receptor did not influence food intake, but resulted in an ~40% greater increase in body weight (AdLacZ vs AdCre: Δ2.2±0.3 vs. 3.8±0.4 g; $p<0.05$). Consistent with the increased body weight, SFO insulin receptor deletion was associated with overall elevations in adiposity (e.g., abdominal fat, AdLacZ vs AdCre: 0.19±0.04 vs. 0.33±0.05 g; $p<0.05$). Analysis of the liver revealed substantial hepatic triglyceride accumulation in SFO-targeted AdCre mice (AdLacZ vs. AdCre: 62 ± 16 vs. 209 ± 29 mg/dl; $p<0.05$), with histological examinations (Oil Red O) revealing large lipid droplet accumulation following removal of the SFO insulin receptor. Parallel elevations in circulating triglycerides were also found (AdLacZ vs. AdCre: 1.4 ± 0.2 vs. 3.3 ± 0.6 mg/dl; $p=0.05$). These data demonstrate that ablation of SFO insulin receptors resulted in an overall deleterious metabolic state including increases in body weight, elevations in adiposity, hepatic steatosis and hypertriglyceridemia. These findings suggest that impairments in insulin signaling within the SFO contribute to the development of metabolic syndrome. Studies are ongoing to investigate the effect of SFO insulin receptor removal on blood pressure.

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087

Human Stomach Cell Gastrin Inhibits Renal NHE3 and NaKATPase in Concert With the Renal D1R

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The digestive track secretes gastrin in response to sodium ingestion stimulating increased renal sodium excretion. We previously showed that gastrin secreted by human colon cancer cells (SW626) can bind to cell surface cholecystokinin receptors on renal proximal tubule cells (RPTC), modulating the natriuretic dopaminergic system. We tested the hypothesis that a similar gastro/renal axis exists in humans. Novel human stomach antrum G-cell lines from 6 separate individuals were isolated and each shown to express gastrin mRNA and protein, and bind Phaseolus vulgaris Leucoagglutinin (PHA-l) by fluorescence lectin affinity (marker for gastrin secreting cells). It was determined that the D1 receptor (D1R) is also found on G-Cells. In human stomach tissue, gastrin expression was increased by Fenofibrate treatment, (PPARα agonist, $p= 0.07$, in 3 live ex-vivo cultured human stomachs). Moreover, we tested the effect of gastrin on CCKB2 receptors in human RPTC. Gastrin (100 nM 15 min.) increased RPTC phospholipace-C (PLC) activity by 1.07± 0.01 fold, N=35, $p<0.0001$, using a PLC-

FRET biosensor, CYPHR, but did not increase cAMP levels using the specific cAMP-FRET biosensor, ICUE-YR. Both gastrin and SKF83822 (100 nM, a cAMP specific D1R agonist, 15 min) alone reduced sodium influx into RPTC via NHE3 (from VEH 100± 4.7% to 73.5± 6.2% or 74.1± 4.3% respectively N=6, $p<0.05$), but gastrin along with SKF83822 decreased sodium influx more than either alone (57.2 ± 5.8% N=6, $p<0.05$). Sodium efflux via NaKATPase was reduced by SKF83822 (from VEH 100± 3.9% to 84.2± 3.3%, N=6, $p<0.05$), but not gastrin alone, however SKF83822 along with gastrin reduced sodium efflux more than SKF83822 alone (72.3±5.1% vs N=6, $p<0.05$). Additionally we found that Angiotensin II (AngII, 10 nM, 15 min.) increased NHE3 activity (12.3± 3.6% N=6, $p<0.01$) and this increase was completely blocked by gastrin (N=6, $p<0.01$). The PLC inhibitor U73122 reversed the inhibitory effect of gastrin on NHE3 and NaKATPase. Gastrin was also found to decrease the amount of fluorescent AngII binding to RPTCs (27.4± 6.1% N=6, $p<0.01$) and this decrease was completely blocked by U73122. Thus, both stomach gastrin and the renal D1R inhibit NHE3 and NaKATPase, to increase sodium elimination from the body after salt is ingested.

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088

Exercise Training Restores miRNA-1 and -29c in Obese Preventing Pathological Cardiac Hypertrophy via Targets in the Collagen and Calcium- Signaling Pathway

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Introduction: Overweight and obesity are risk factors in several cardiovascular diseases that lead to the pathological cardiac hypertrophy (CH) phenotype. We previously reported that aerobic exercise training (AET) counteracts CH in obesity. Here, we evaluate the role of microRNAs (miRs) and target genes involved in the AET-induced prevention and improvement of ventricular compliance in CH of obese Zucker rats. **Methods and Results:** Zucker rats were assigned into four groups: 1) lean group (LZR), 2) obese (OZR), 3) trained lean group (LZR+TR) and 4) trained obese group (OZR+TR). The AET consisted of swimming training with 10 weeks duration sessions of 60 min, 1x/day, 5x/week with overload by 4% of body weight. Our results showed that there were no differences in the cardiomyocytes diameter, however; heart weight was higher in OZR (29% - 0,031 to 0,024mg/cm) compared to LZR group, due to increased cardiac intramuscular fat and

collagen. AET prevented pathological CH in OZR+TR group normalizing heart weight. Cardiac miR-29c expression was reduced (47% - 43 to 100% of LZR) in OZR compared to LZR group paralleled by an increase of collagen fraction. AET restored miR-29c expression in OZR-TR along with the expression of its target gene collagen. In addition, miR-1 targets NCX1 gene. MiR-1 was upregulated (63% - 163 to 100%) while their target gene NCX1 was reduced (51% - 49 to 100%) in OZR compared to LZR group. Corroborate with NCX1, $pPLB^{Ser16}$ was reduced in OZR group, indicating damage in calcium handling by obesity. Interestingly, AET normalized cardiac miR-1 and calcium signaling protein levels in OZR-TR group. **Conclusion:** Our findings show the AET as a non-pharmacological therapy for the prevention and even reversal of pathological CH and dysfunction in obesity. Together, the data suggests that miR-1 and -29c play key role in cardiac remodeling which could be used as potential therapeutic target for cardiac disorders.

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089

CD36 Genetic Variant Impacts Nitric Oxide Regulation of Endothelial Function: Response to Chronic Treatment with Phosphodiesterase 5 Inhibition

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CD36, a scavenger receptor expressed on endothelial cells, interacts with thrombospodin-1, a matrix protein that modulates nitric oxide-soluble guanylate cyclase (NO-sGC) signaling. CD36 genetic variants associate with endothelial dysfunction, atherosclerosis, hypertension and insulin resistance. A coding variant of CD36 (rs3211938, G/T genotype) that causes partial CD36 deficiency (50% reduction) is common (~18%) in African Americans (AA); however, it is unknown, if this genotype influences NO-dependent endothelial function. This study examined whether potentiating NO-sGC pathways with the phosphodiesterase 5 inhibitor, sildenafil citrate, improves endothelial function and insulin sensitivity in AA women with or without the G/T genotype. Forty-six AA women with metabolic syndrome (MetS) participated in a 4-week, parallel-arm, double-blind, and placebo-controlled study. Carefully matched subjects were randomly assigned to sildenafil citrate 20 mg TID versus placebo; sildenafil (n= 23, 42±10 years old, BMI 39±5 kg/m2, fasting insulin 15±8 uU/ml) and placebo (n=23, age 43±10, BMI 39±6 kg/m2, fasting insulin 14±10 uU/ml). Primary endpoints were insulin sensitivity and endothelial function measured by intravenous glucose tolerance test and flow mediated dilation, respectively. Treatment compliance was documented with plasma sildenafil levels (mean 57±50 ng/ml). There was no difference in insulin sensitivity (p=0.676) or flow-mediated dilation (p=0.649) between intervention groups. However, subgroup analyses showed a significant interaction between sildenafil citrate treatment and G/T genotype (p=0.018). Sildenafil citrate improved endothelial function in G/T carriers (the mean difference: 2.9, the 95% CI: -0.90 to 6.8, p = 0.126) and decreased endothelial function in T/T carriers (the mean difference: -2.6, the 95% CI: -5.1 to -0.1, p = 0.040). We

conclude that the rs3211938 common CD36 genetic variant influences NO-dependent endothelial function in response to chronic treatment with phosphodiesterase 5 inhibition. Further studies are needed to determine if rs3211938 and other common CD36 genotypes influence endothelial function and the inter-individual variability in response to the drug.

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090

Outcomes of Hypertensive Crises as Predicted by Red Cell Distribution Width

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Background:
Red cell distribution width (RDW) is a measure of the variability in size of erythrocytes. A high RDW value indicates greater variation in size between individual erythrocytes and has been shown to be an independent predictor of mortality in patients with coronary artery disease, heart failure and in patients undergoing percutaneous coronary intervention (PCI). The aim of this study was to evaluate the prognostic value of RDW in predicting clinical outcomes in patients with hypertensive crisis.
Methods: We performed a retrospective study of 465 consecutive patients from January 2007 to March 2010 who presented with hypertensive crisis. Hypertensive crisis was defined as systolic BP >180 and/or diastolic BP >110mmHg with impending or progressive end organ dysfunction requiring inpatient hospitalization. The study sample consisted of 465 patients (38.9% men (181 of 465); mean age 59.6 $\pm$ 15.9). Baseline levels of RDW were measured at time of admission and analyzed as continuous and categorical variables (elevated RDW was defined as >14.5%). Multivariable regression analysis was performed for development of all-cause mortality, myocardial infarction, new-onset heart failure (defined as first time hospital admission for heart failure), stroke and MACE (MI, new-onset heart failure and stroke) at 2 years.
Results: RDW > 14.5% was a strong independent predictor of all-cause mortality at 2 years (OR: 1.90, 95% CI: 1.1-3.3, p<0.05). Elevated
RDW was also found to be an independent predictor of new-onset heart failure at 2 years (OR: 1.97, 95% CI: 1.1-3.7, p<0.05). Elevated RDW was not a predictor of MI, PCI or stroke at 2 years.
Conclusions: Elevated RDW level in patients with hypertensive crisis was an independent predictor of all-cause mortality and new-onset heart failure in patients with hypertensive crisis.

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091

High Density Adrenal MRI Detects Clinically Relevant Aldosterone-producing Adenomas with Higher Precision than Computer Tomography

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Background
Primary hyperaldosteronism is the most common cause of secondary hypertension, and part of the noninvasive diagnostic studies used to confirm the diagnosis include biochemical and computed tomography (CT) of the adrenal glands. If no adenomas are seen on imaging, invasive adrenal vein sampling, to diagnose unilateral disease amenable to surgery, is limited to cases with high pre-test probability. We developed a dedicated MRI protocol and investigated whether surgically-treatable unilateral aldosterone-producing adenomas missed by CT could be detected using this protocol.
Methods
We developed a dedicated MRI protocol of the adrenal glands using thin single shot T2-weighted scans, noncontrast MR angiography, reduced field of view diffusion, and dynamic contrast enhancement through the kidneys and adrenal glands with gadolinium (or ferumoxytol if eGFR < 30 mL/min/1.73m2). We then enrolled patients with biochemical evidence of primary hyperaldosteronism without evidence of adrenal lesions by CT and performed MRI.
Results
Five subjects with primary hyperaldosteronism and negative CT underwent MRI. Each had adrenal nodules by MRI. In three cases, a single, unilateral adrenal nodule was detected and followed up with ipsilateral lateralization of aldosterone/cortisol production by adrenal vein sampling. These patients underwent laparoscopic adrenalectomy with histologic diagnosis of an aldosterone-producing adenoma with significant improvement in blood pressure and/or reduction in antihypertensive medications. In the other two cases, adrenal nodules were found but adrenal vein sampling detected no lateralization of aldosterone/cortisol ratio.
Conclusions
As proof of concept, MRI of the adrenal glands may be a superior imaging modality to diagnose subtypes of primary hyperaldosteronism. With identification of adrenal lesions, this may capture additional cases of secondary hypertension than are amenable to surgical cure improving outcomes and quality of life for patients with severe hypertension. Moreover, it may imply that some cases of primary hyperaldosteronism ascribed to bilateral adrenal hyperplasia may indeed be due to aldosterone-producing adenomas undetectable by CT.

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092

Low Birth Weight per se Is Not a Determinant of Vascular Dysfunction and Presumably Future Cardiovascular Risk in Humans

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Background: Epidemiological studies suggest that low birth weight (BW) is associated with systemic vascular dysfunction and premature cardiovascular morbidity and mortality later in life. However, this association could be due to confounding factors associated with low BW,

such as genetic factors, pathological events during intrauterine life, and maternal or early-life environmental insults. Studies of monozygotic twins born with significantly different BW provide a unique opportunity to control for these potential confounding factors, thereby allowing to directly study the effect of low BW per se on systemic vascular function later in life.
Methods: We, therefore, measured flow-mediated dilation (FMD) of the brachial artery, carotid-femoral pulse wave velocity (PWV) and carotid intima-media thickness (IMT) in 13 monozygotic, monochorionic healthy twins pairs (mean±SD age, 13.5±3.4 years) who were born with significantly different BW (defined as >20% BW difference within pairs, 2310±600 vs. 1800±520 g, P<0.0001) and in 26 healthy age- and sex-matched control subjects born at term with normal BW (3460±360 g). None of the participants had suffered from intrauterine or perinatal complications.
Results: The major new findings were two fold; 1) systemic vascular function was comparable in low and high BW twins (FMD, 9.0±1.8 vs. 8.7±2.0%; PWV, 6.5±1.3 vs. 6.6±0.9 m/s; IMT, 390±30 vs. 390±20µm, all P values >0.2, low vs. high BW) and 2) systemic vascular function in twins was similar to the one observed in control singletons (FMD, 8.8±1.3 vs.8.9±1.8%, P=0.95; PWV: 6.5±1.1 vs. 6.6±1.2 m/s, P=0.80; IMT: 390±25 vs. 385±20µm, P=0.33, singletons vs. twins). Finally, there existed no relationship between BW and vascular function in the whole study population (FMD: r=0.001, P=0.99).
Conclusions: This study provides the first direct evidence in humans that low BW per se is not a determinant of systemic vascular dysfunction (and presumably future cardiovascular risk). Moreover, it indicates that independent of BW, vascular function in twins is normal. We suggest that the association between low BW and increased cardiovascular risk reported in earlier epidemiological studies was related to confounding factors associated with low BW.

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093

Could Spironolactone Be More Effective Than Sympathetic Renal Denervation to Treat True Resistant Hypertension? Preliminary Results From the DENERVHTA Study.

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Aim
To compare the efficacy and safety between two therapeutic strategies to reduce 24h-SBP in patients with resistant hypertension: renal denervation or the addition of spironolactone.
Methods
Twenty-one patients with office-SBP ≥150 mmHg and 24h-SBP≥140 mmHg despite receiving ≥3 full-dose antihipertensive drugs, one a diuretic, but without aldosterone antagonists, were randomized to renal denervation (by Simplicity®) or to spironolactone (25-50 mg), as add-on therapy. Changes in both office- (3 averaged readings) and 24h- BP (by Spacelabs®-90207) were evaluated at 6 months. Comparisons between

treatment groups were performed using analysis of variance adjusted by age, gender and baseline values.

Results: mean age was 62.7 ± 7.6 yr; men: 61.9% (13 of 21); diabetes: 47,6% (10 of 21). Mean BMI: 32.3 ± 6.1 Kg/m^2. Duration of hypertension: 13.4 ± 7.2 yr. Number of antihypertensive drugs: 4,1 ± 0,7. Mean office-BP: 167,5 ± 20,1 / 91,7 ± 12,4 mmHg. Mean 24h-BP: 151,8 ± 9,1 / 81,7 ± 8,4 mmHg. Baseline characteristics were not different between groups (p=NS for all). Comparison between groups of main changes over time is shown in Table.

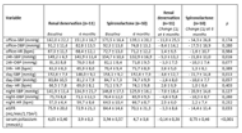
Reduction of 24h-SBP was higher in the spironolactone group after adjusting by age, sex and baseline 24h-SBP (p=0.016).

Reduction of eGFR was higher in the spironolactone group after adjusting by baseline eGFR (p=0.033).

Conclusions

1) As compared to renal denervation, spironolactone was more effective to reduce 24h-SBP after 6 months in patients with resistant hypertension. These differences were not significant as regards office-BP.

2) Spironolactone add-on treatment significantly decreased eGFR as compared to renal denervation treatment.



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094

Gain-of-function EPHX2 Variant rs41507953 Is Associated With Acute Kidney Injury Following Cardiac Surgery

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Acute kidney injury (AKI) affects 20-30% of patients following cardiac surgery and predicts increased mortality. Murine models suggest that epoxyeicosatrienoic acids (EETs) protect against renal ischemia/reperfusion injury, a contributor to AKI following cardiac surgery. Soluble epoxide hydrolase (sEH), encoded by *EPHX2,* hydrolyzes EETs to less active DHETs. **We tested the hypothesis that a gain-of-function *EPHX2* variant, rs41507953, is associated with AKI following cardiac surgery.** We first studied 371 cardiac surgery patients (67.3% male, mean age 65.6±12.9years and BMI 28.5±6.17kg/m^2) enrolled in a clinical trial in which DNA was collected. Ninety-eight patients (26.4%) developed AKI, defined by AKIN criteria. Rs41507953 genotypes were in Hardy-Weinberg equilibrium (AA:AG:GG=297:68:6). There was a significant association between the gain-of-function “G” allele and AKI, p=0.006. Adjusting for known risk factors for AKI including estimated glomerular filtration rate (eGFR), age, sex, race, history of diabetes, BMI, and use of cardiopulmonary bypass the rs41507953 “G” allele remained independently associated with higher rates of AKI [OR=2.09 (95%CI 1.132-3.848, p=0.018). We replicated this association between the rs41507953 “G” allele and AKI in another cohort of 800 cardiac surgery patients.

To assess the association between the rs41507953 “G” allele and sEH activity we measured 9,10 or 12,13-dihydroxyoctadecanoic acid/9,10 or 12,13-epoxyoctadecanoic acid (DiHOME/EpOME) ratios, measures of sEH activity, in plasma collected from 26 AA and 5 AG individuals in the original cohort. sEH activity was highest in plasma collected post-protamine administration. The 12,13 DiHOME/EpOME and the total DiHOME/EpOME ratios post-protamine were significantly greater in the AG vs. the AA genotype group, 11.66±12.91 vs. 2.32±3.03 (p=0.002) and 28.35±47.79 vs. 4.03±7.96 (p=0.014), respectively.
An *EPHX2* variant with increased sEH activity is associated with AKI following cardiac surgery. Pharmacologic inhibition of the sEH enzyme might protect patients from AKI following cardiac surgery.

M.M. Shuey: None. **F.T. Billings:** None. **S. Wei:** None. **G.L. Milne:** None. **N.J. Brown:** None.

095

Human (Pro)renin Receptor Activation Induces an Angiotensin II-Independent Pressor Response Mediated by NADPH Oxidase 4 Activity

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The binding of prorenin to the (pro)renin receptor (PRR) induces non-proteolytic activation of prorenin and generation of angiotensin II (Ang II). PRR activation can also induce Ang II-independent signaling pathways. However, whether Ang II-independent signaling pathways are critical for blood pressure (BP) regulation is not known. To address this question, we created transgenic mice that overexpress the human PRR (hPRR) selectively in neurons (Syn-hPRR). Activated human prorenin (hPRO) cannot cleave endogenous mouse angiotensinogen to generate Ang II. Therefore, administration of hPRO to Syn-hPRR mice can be used to examine Ang II-independent PRR signaling in BP regulation. Intracerebroventricular (ICV) infusion of hPRO increases BP in Syn-hPRR mice (ΔMAP 23 ± 4.6, n = 4) but has no effect on wildtype (WT) mice (ΔMAP 2 ± 0.8, n = 6). The hPRO-induced pressor response in Syn-hPRR mice is unaffected by co-infusion with the Ang II type 1 receptor blocker losartan (ΔMAP 19 ± 5.2, n = 8), suggesting that the response is independent of Ang II. Interestingly, co-infusion with an inhibitor of the reactive oxygen species-generating enzyme NADPH oxidase (NOX), diphenyleneiodonium, nearly abolishes the hPRO-induced pressor response in Syn-hPRR mice (ΔMAP 4.7 ± 1.0, n = 4), indicating that NOX activity is required. Additionally, we find that basal NOX activity is enhanced in the Syn-hPRR hypothalamus relative to WT mice (1.4 fold change). We next examined which NOX isoform is responsible for the hPRO-induced pressor response and enhanced activity. NOX4 mRNA levels are greater (2.7 ± 0.6 fold change), but NOX1 (1.2 ± 0.3 fold change) and NOX2 (1.2 ± 0.3 fold change) mRNA levels are not different, in the hypothalamus of Syn-hPRR compared to WT mice (n = 3). Adenovirus-mediated delivery of NOX2, NOX4, or a scrambled sequence shRNA was ICV injected in Syn-hPRR mice. After 7 days, we found that treatment with NOX2 (ΔMAP 20 ± 5.2) or scrambled (ΔMAP 23 ± 3.2) shRNA had no effect on the hPRO-induced pressor response (n = 5). However, the hPRO-induced increase in BP is

attenuated in Syn-hPRR mice injected with NOX4 shRNA (ΔMAP 5.9 ± 2.8). Together, these data indicate that NOX4 mediates the Ang II-independent pressor response to activation of the human (pro)renin receptor in Syn-hPRR mice.

M.N. Sullivan: None. **W. Li:** None. **C.D. Sigmund:** None. **Y. Feng:** None.

096

Sequential Activation of Nox1 and Gremlin1 Leads to Endothelial Proliferation in Human Pulmonary Arterial Hypertension

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Pulmonary arterial hypertension (PAH) is a rapidly degenerating and devastating disease of increased pulmonary vessel resistance leading to eventual right heart failure. Until now, palliative modalities have targeted the reduction of vascular tone with little success. Recent studies have delved into the mechanisms regulating increased pulmonary vascular resistance: aberrant vascular remodeling and occlusion. However, little is known of the molecular mechanisms responsible for endothelial proliferation, a root cause of PAH-associated vascular remodeling. We provide the first evidence to our knowledge of an upregulation of NADPH oxidase 1 (Nox1) at the transcript and protein (2.1±0.62 -fold, $P < 0.05$) level in resistance vessels from PAH vs. non-PAH subjects. This coincided with an increase in bone morphogenetic protein (BMP) antagonist Gremlin1 protein expression (2.3±0.47 –fold vs. non-PAH, $P < 0.05$) and reactive oxygen species (ROS) production (iodonium-inhibitable hydrogen peroxide production: 0.69±0.06 vs. 0.43±0.032 nmol/min/mg protein for PAH vs. non-PAH, respectively, $P < 0.05$). In vitro studies in human pulmonary artery endothelial cells (HPAEC) demonstrate that hypoxia (24 hr, 1 % O_2) drives Nox1 subunit expression (Nox1 protein: 1.4±0.075-fold vs. normoxia, $P < 0.05$), assembly and oxidase activity (superoxide production, nmol/min/mg protein: 14.0±1.9 vs. 6.00±0.94 for normoxia, $P < 0.01$) leading to elevation in sonic hedgehog (SHH; 1.5±0.011 fold, $P < 0.05$) and Gremlin1 (1.90±0.32-fold, $P < 0.01$) expression. Nox1 gene-silencing in hypoxia-exposed HPAEC abrogated this cascade. Moreover, hypoxia-induced endothelial cell proliferation (1.18±0.038-fold vs. normoxia, $P < 0.05$) was attenuated with loss of either Nox1 or Gremlin1. Finally, incubation of normoxic HPAEC with conditioned media from hypoxia-exposed HPAEC resulted in increased proliferation, which was abrogated by Nox1 suppression of donor cells. Together these data support a Nox1-Gremlin1 signaling axis in pulmonary vascular endothelium that is likely to contribute to pathophysiological endothelial proliferation and the progression of pulmonary hypertension. The findings also support targeting of Nox1 as a viable therapeutic option to combat PAH.

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Vascular Aging in Aldosterone Associated Hypertension: Role of NADPH Oxidase 1

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The vascular phenotype in hypertension is characterised by features typically observed in the ageing vasculature. Pathophysiological processes underlying premature vascular aging in hypertension remains unclear but aldosterone (aldo) and oxidative stress may be important. We postulated that physiological aging is amplified in hypertension due to increased aldo-induced Nox activation and redox signalling. We used arteries from adult WKY (18 weeks), aged WKY (52 weeks) and adult stroke-prone spontaneously hypertensive (SHRSP) rats. Blood pressure was measured by tail-cuff. Vascular function/structure was analysed by myography. Gene level was assessed by qPCR and protein by immunoblotting. BP was increased in SHRSP (180.7±2.5 vs. 127±2.7 mmHg, $p<0.05$). Endothelial dysfunction was observed in vessels from SHRSP. Increased vascular contraction in aged WKY rats was similar to SHRSP rats ($p<0.05$ vs WKY). Increased vascular stiffness was observed in arteries from aged WKY and SHRSP compared to WKY rats. Nox2 (0.82±0.4/2.4±0.9 vs 0.22±0.2), NoxA1 (4.9±2/9.5±5 vs 1±0.3) and NoxO1 (1.9±0.6/4.1±1 vs 1±0.4) mRNA was increased ($p<0.05$; SHRSP/aged WKY vs WKY). Nox1 mRNA (2.3±0.8 vs 1.1±0.4) was only increased in SHRSP rats ($p<0.05$; vs WKY). Similarly, mRNA levels of MCP-1 (2.3±0.5/3.9±1.9 vs 0.3±0.1) and RANTES (7.4±2/6.3±1.7 vs 1.1±0.2), aging-related inflammatory markers, and cell cycle inhibitors, p21 (3.2±1.1/3.1±0.7 vs 1±0.1) and p27 (2.2±0.7/2±0.8 vs 0.4±0.1), were increased in SHRSP and aged WKY rats ($p<0.05$; SHRSP/aged WKY vs WKY). ROS production (VSMC: 1.74±0.4 AU/protein), H2AX (DNA damage; 1.3±0.1) and aldosterone (plasma; 99.5±19 pg/mL) levels were increased in SHRSP rats ($p<0.05$; vs WKY). Aldo-induced Nox1 mRNA expression and p66SHC activation was exacerbated in VSMCs from SHRSP rats; an effect blocked by ML171 (a Nox1 inhibitor) and blunted in VSMCs from Nox1 KO mice. In conclusion, endothelial dysfunction and vascular remodelling in hypertension are associated with increased aldo-mediated activation of pro-inflammatory and redox-sensitive pathways. These processes involve Nox1. Our findings identify an important role for aldo/Nox1/ROS in molecular processes underlying vascular changes of ageing in hypertension.

A. Harvey: None. **A.C. Montezano:** None. **K.Y. Hood:** None. **R.A.M. Lopes:** None. **D. Graham:** None. **R.M. Touyz:** None.

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MicroRNA Regulation of NADPH Oxidase Mediates the Antioxidant Effect of Renal Paraoxonase 2

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Increased renal generation of reactive oxygen species (ROS) is important in the pathogenesis of hypertension caused by absent or dysfunctional dopamine receptor subtype. Germline deletion of the dopamine 2 receptor in mice increases renal NADPH oxidase (NOX) activity and decreases expression paraoxonase 2 (PON2) and results in ROS-dependent

hypertension. We determined if microRNA (miR) is involved in PON2-mediated regulation of NOX. Silencing PON2 in human renal proximal tubules cells decreased PON2 (-60±4%, n=3,*P<0.05) and increased NOX2 (110±15%, n=3,*P<0.05) and NOX4 (80±10%, n=3,*P<0.05) proteins, NOX activity (50±6%, n=3,*P<0.05), and ROS production (57±3%, n=4,*P<0.05). Inhibition of NOX activity by diphenyleneiodonium normalized the increase in ROS caused by PON2 silencing. Renal-selective silencing of Pon2 in mice by the renal subcapsular infusion of Pon2 siRNA decreased PON2 (~50%), and increased NOX2 (191±11%,n=3, P<0.05), NOX4 (60±4%,n=3, P<0.05), NOX activity (94±23%, n=3, P<0.05), and blood pressure (BP) (+41±6 mmHg, n=3, P<0.05). Pon2-/- mice also had higher BP than wild-type littermates (+15±2 mmHg,n=3/4,*P<0.05) but less than observed with renal-selective silencing indicating extrarenal compensation. Renal NOX2 (220±64%, n=3/4,*P<0.05) and NOX activity (195±77%, n=3,*P<0.05) were also increased in Pon2-/- mice. However, the renal expression of NOX4 was similar in Pon2-/- and wild-type littermates. The renal expressions of miR-23b, miR-34a and miR-155 (reported to regulate NOX expression) were also similar in Pon2-/- mice and wild-type littermates. However, renal miR-146a expression was decreased (-25±4%, n=3/4,*P<0.05) while miR-204 (150±12%, n=3/4,*P<0.05) and NFAT expressions (21±7%, n=3/4,*P<0.05) were increased in Pon2-/- mice. The increase in miR-204 could be a compensatory response because miR-204 has been shown to decrease NFAT expression. It is known that NFAT and NOX2 can positively regulate each other's expression while miR-146a negatively regulates NOX4 expression and inflammation. We conclude that PON2 by increasing miR-146a and decreasing NFAT expression negatively regulates NOX activity and reduce ROS production that would contribute to the maintenance of normal BP.

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099

Fetuin-A and Toll-like Receptor 4 Regulate Vascular Function: Role of Nox1

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Fetuin-A (FetA) regulates calcium and phosphate homeostasis. It is also an agonist to toll-like receptor 4 (TLR4) and is related to insulin resistance and inflammation. FetA has also been associated with endothelial dysfunction, which is regulated by oxidative stress. Mechanisms whereby FetA influences vascular function are unknown. We hypothesized that FetA through TLR4 and ROS production induces vascular dysfunction. Mesenteric arteries and vascular cells from WKY rats were studied. Vascular function was analysed by wire myography in the presence or absence of FetA (50 ng/mL) and/or CLI095 (CLI - 10-6M - TLR4 inhibitor). Levels of reactive oxygen species (ROS) were measured by chemiluminescence, Amplex Red (H_2O_2) and ELISA (nitrotyrosine) Protein oxidation and levels were measured by immunoblotting. WKY vessels exposed to FetA were less sensitive to acetylcholine (Ach)-induced and sodium nitroprusside (SNP)-induced relaxation, while sensitivity to phenylephrine was increased by

FetA; an effect blocked by N-acetylcysteine (antioxidant) and ML171 (Nox1 inhibitor). Inhibition of TLR4 blocked FetA effects on endothelial-dependent relaxation and contraction, but not on endothelial-independent relaxation. FetA increased ROS production (131±49.2%), but decreased H2O2 intracellular levels (63±14%) in endothelial cells (EC) (vs. veh, $p<0.05$); an effect blocked by CLI095. ROS production (66±12.2%), as well as, H2O2 (45±8%) and ONOO- (105±31.6%) levels, were increased by FetA in VSMCs (vs. veh, $p<0.05$). Protein oxidation was increased by FetA in VSMCs (103±26% vs. veh, $p<0.05$). In EC, eNOS inactivation (136±38%) and JNK activation (84±5%) were increased by FetA (vs. veh, $p<0.05$). In VSMCs, Rho kinase activity was increased (200±25% vs. veh, $p<0.05$) at 30 min; while myosin light chain (MLC) activation was only increased (25±3.56% vs. veh, $p<0.05$) at 15 min. In summary, FetA influences vascular function through Nox1-ROS dependent mechanisms. FetA-induced endothelial dysfunction and contractile responses involve TLR4. Our findings identify a novel system whereby FetA differentially influences vascular function through Nox1-ROS and TLR4. Vascular responses to FetA may depend on the specific pathway activated.

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Sorting Nexin 1: Molecular Mechanisms and Pharmacogenomics

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Sorting nexin 1 (SNX1) plays a pivotal role for the normal activity of renal dopamine D5 receptor (D5R). Kidney-restricted, Snx1-siRNA depletion of SNX1 results in impaired natriuretic response to salt load and hypertension in mice. Genetic ablation of the Snx1 gene (Snx1-/-) resulted in increased oxidative stress, impaired sodium excretion, and elevated systolic blood pressure (SBP, 131.3±6.4 mm Hg, n=5) in mice. The D5R has antioxidant properties by negatively regulating the expression of the NADPH oxidase (NOX). We found that NOX1, NOX2, and p47phox, as well as the antioxidant PON2, conceivably as compensation, were increased in Snx1-/- mice compared with wild-type littermates. Snx1-/- mice had higher ROS (218.6±7.7%), NOX activity (43.9±3.3 AU/mg protein/min vs. 25.98±3.5), and other markers of oxidative stress, e.g., malonyldialdehyde (32.5±3.4 pmol/mg protein vs. 17.2±2.1) and 3-nitrotyrosine (128.3±4%), which were all normalized by 10-day renal infusion of apocynin, a drug that prevents NOX assembly. The SBP in Snx1-/- mice was also normalized by apocynin (131.3±4.8 mm Hg to 105.7±0.3). Compared with human renal proximal tubule cells obtained from normotensive Caucasian males (NT cells), those from hypertensive subjects (HT cells) had reduced expression of SNX1 (160±2.1%, n=4=5/group), increased ROS (182±10.5%), and blunted cAMP response (110.8±35.3%) and sodium transport inhibition

(101.2±1.9%) in response to D1-like receptor stimulation. These observations were corroborated by results in siRNA-induced gene silencing in NT cells or "genetic rescue" in HT cells. We also evaluated 12 SNPs in the SNX1 gene as possible genetic predictors of BP response to monotherapy with HCTZ among hypertensive patients enrolled in the Pharmacogenomic Evaluation of Antihypertensive Responses study (n=768). Three of the 12 SNPs (rs12591947, rs11854249, and rs11635627) associated with poor BP response to thiazide (Δ SBP of -1.8 mm Hg vs. 113.5) among blacks. An SNX1 SNP (rs1802376) was associated with essential hypertension in a Caucasian population (n=502). Our data demonstrate the novelty and relevance of SNX1 in human pathology and pharmacogenomics of essential hypertension.

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Interleukin 17A Upregulates Both Renal Proximal and Distal Tubule Sodium Transporters in Angiotensin II-dependent Hypertension

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We have previously shown that angiotensin II (Ang II)-induced hypertension is associated with an increase in T cell production of interleukin 17A (IL17A), and that IL17A promotes hypertension and end-organ damage. However, the precise mechanism is unknown. Recently, we reported that Ang II infusion into C56Bl/6J wild type (WT) mice blunted the rate of natriuresis following an acute saline challenge, while the rate of salt and water excretion in IL17A-/- mice was unaffected by Ang II. Following 2 weeks of Ang II infusion (490 ng/kg/min), proximal tubule sodium hydrogen exchanger 3 (NHE3) abundance was depressed in IL17A-/- but not WT mice, suggesting enhanced pressure natriuresis in IL17A-/- mice. We then performed renal transporter profiling on mice deficient in IL17A, or the related isoform IL17F, after prolonged (4 weeks) of Ang II infusion (490 ng/kg/min), a time when the blood pressure reduction in IL17A-/- mice is most prominent. Interestingly, at this time, deficiency of IL17A, but not IL17F, blunted the activation of distal tubule transporters, specifically sodium-chloride cotransporter (NCC) and the epithelial sodium channel (ENaC). We hypothesized that IL17A directly modulates renal sodium transporters as a mechanism to regulate salt and water excretion and hypertension. To test this hypothesis, we treated cultured human renal proximal tubule cells and mouse distal convoluted tubule (mDCT15) cells with recombinant IL17A or IL17F. We found that IL17A, but not IL17F, increased NHE3 protein levels (1.4-fold, $p=0.003$) and SGK1 mRNA expression (3.9-fold, $p=0.01$). In mDCT15 cells, IL17A but not IL17F, increased NCC activity as measured by thiazide-inhibited sodium uptake (1.78 vs 1.62 µmol/mg/20min, $p<0.001$), and this increase was significantly blunted with an SGK1 inhibitor (GSK 650394) and in cells lacking Nedd4-2 (an

E3 ubiquitin ligase downstream of SGK1). Moreover, in mDCT15 cells, acute IL17A treatment caused phosphorylation of SGK1 on Ser78. These studies are the first to describe a mechanistic link by which IL17A modulates renal sodium transporters and suggests that targeting IL17A may improve renal function and slow the progression to renal failure in hypertension and other autoimmune disorders.

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Renal Mechanisms of Blood Pressure Homeostasis and Salt Sensitivity by Collectrin

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Collectrin (*Tmem27*), an ACE-2 homologue and a chaperone of amino acid transporters, is expressed in the endothelium and renal epithelia, including the proximal tubules and collecting duct. We previously reported that collectrin-deficient (KO) mice exhibit hypertension (HTN) at baseline and augmented salt-sensitivity associated with impaired endothelial-dependent relaxation and a rightward shift of the pressure-natriuresis relationship. To determine the effect of renal vs. extra-renal collectrin on blood pressure (BP) regulation, we performed renal cross-transplantation studies (at the Duke O'Brien Center), then treated with normal salt (NSD) and high salt (HSD) diets, in the following groups (n = 3-5/group): 1) wild-type (WT) mice with WT kidney (WT WT), 2) collectrin KO mice with WT kidney (WT KO), and 3) WT mice with collectrin KO kidney (KO WT). Collectrin KO WT group displayed a trend towards higher baseline systolic BP (SBP, mmHg); however, under HSD, collectrin KO WT had significantly higher SBP (WT WT 148.9, WT KO 151.6, KO WT 168.6, $p = 0.037$).

To determine the renal endothelial and epithelial contribution of collectrin in BP regulation, we assessed renal blood flow (RBF) by contrast-enhanced ultrasound and renal sodium transporters and channels by immunoblotting. KO mice displayed significantly reduced total, cortical and medullary RBF under both NSD and HSD ($n \geq 11$ each, $p \leq 0.01$). Moreover, at baseline, compared to WT mice (n = 4), KO mice (n = 5) displayed significantly elevated abundance of NHE3 ($p = 0.002$), activated NCC (NCC-pS71) ($p = 0.004$) and ENaC alpha ($p = 0.03$).

We next conducted a candidate gene association study in the HyperGen cohort (1,270 white participants, 50% men), using intronic SNPs in the *ACE2*, *TMEM27* and *CA5BP1* genes on the X chromosome. Only 2 SNPs, rs6629114 and rs41492646, both of *TMEM27* and in perfect linkage disequilibrium ($r^2=1.0$), were associated with diastolic BP (DBP). Meta-analysis of men and women showed this was statistically significant, ($p=0.04$ for both), with stronger effects in men ($p=0.028$).

In conclusion, deletion of collectrin alters renal

hemodynamics and epithelial sodium handling to favor sodium reabsorption. Collectrin is associated with DBP in human subjects.

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Increased Blood Pressure Drives Renal T-cell Infiltration in the Dahl Salt-Sensitive Rat

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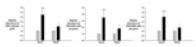
Previous studies have shown that renal T-cell infiltration is a key component of salt-sensitive hypertension in Dahl salt-sensitive (SS) rats. Here we used chronic servo control experiments to determine the contribution of renal perfusion pressure (RPP) to T-cell infiltration in the SS rat kidney. An aortic balloon occluder was placed around the aorta, between the renal arteries, and used to maintain blood pressure to the left kidney at control levels, ~128 mmHg, during 7-days of salt induced hypertension. During the same period, the right kidney was exposed to increased RPP, averaging 158 ± 4 mmHg by high salt (4% NaCl) day-7. The number of infiltrating T-cells was compared between the two kidneys. Renal T-cell infiltration was significantly blunted in the left-servo controlled kidney compared to the right-uncontrolled kidney. The number of mature (CD3+), helper (CD3+CD4+) and cytotoxic T-cells (CD3+CD8+) were all significantly lower in the servo controlled kidney than in the hypertensive kidney (Fig.1). This effect was not specific to T-cells, monocyte, macrophage and B-cell infiltration were all significantly exacerbated in the hypertensive kidney. Increased RPP was also associated with augmented renal injury, with increased protein casts and glomeruli damage in the hypertensive kidney. We conclude that during the development of salt-sensitive hypertension increased RPP contributes to renal T-cell infiltration in SS rats; when blood pressure is maintained at control levels T-cell infiltration is significantly attenuated in the servo-controlled kidney relative to the hypertensive kidney despite exposure to comparable sympathetic drive and circulating factors.



L.C. Evans: None. **G. Petrova:** None. **T. Kurth:** None. **D.L. Mattson:** None. **A.W. Cowley:** None.

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Targeting the Alms1 Gene in Rats Causes Hypertension, Obesity and Enhanced NKCC2 Trafficking

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Enhanced NaCl reabsorption by the thick ascending limb (TAL) is associated with salt sensitive hypertension in rodents and humans. NaCl absorption by the TAL depends on the apical Na/K/2Cl cotransporter - NKCC2. NKCC2 activity is regulated in part by protein-protein interactions with its carboxyl terminus that controls its trafficking to the apical membrane. We hypothesized that the proteins binding to this region of NKCC2 may be involved in NKCC2 regulation and trafficking. To identify new TAL

proteins that bind NKCC2, we performed a proteomics screen between the NKCC2 carboxyl terminus and TAL proteins. We identified ALMS1 (Alstrom syndrome 1) as a specific interacting partner for this NKCC2 region and confirmed that ALMS1 is expressed in TALs. Little is known about ALMS1 in renal function. Mutation of this gene causes severe metabolic syndrome in humans and is associated with hypertension. To study ALMS1 function we obtained ALMS1 knockout (KO) rats in collaboration with the rat genome editing consortium. We found that ALMS1 KO rats fed a normal salt diet have higher systolic blood pressure (151± 5 mmHg) compared to wild type littermates (125± 4 mmHg, p< 0.02). We also observed an increase in body weight in ALMS1 KO at all ages (WT: 344± 4 g vs KO: 429±11 g, at 11 weeks, p<0.05). ALMS1 KO rats showed large intra-abdominal and sub-cutaneous fat deposits. We then obtained TALs and measured surface and total NKCC2 expression. In TALs from ALMS1 KO, the percentage of total NKCC2 at the surface was higher compared to WT (27±4 vs 14±2 %, p<0.05, n=6). Total NKCC2 expression was not significantly different between ALMS1 KO and WT rats. We conclude that ALMS1 is important for blood pressure regulation. The mechanism for hypertension in ALMS1 KO is unclear but may involve an increase in NKCC2 trafficking to the apical membrane and activity. ALMS1 has been associated with poor kidney function, hypertension, and type 2 diabetes in humans. Understanding the function of ALMS1 could develop new avenues for research into these diseases.

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High Salt Activates Immune Cells to Promote Hypertension

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High salt intake and inflammation are implicated in the genesis of hypertension. We have previously shown that hypertension activates dendritic cells (DCs), by promoting the formation of immunogenic isoketal-protein adducts. Recently it has become clear that sodium can accumulate in the interstitial space and promote inflammation. We hypothesized that high salt activates antigen presenting cells including monocytes and DCs to produce immunogenic isoketals. Exposure of mouse splenic DCs to high salt (190 mM) for 24 hours led to an increase in superoxide production compared to regular RPMI media (146.3±9.5 vs. 100.0±5.0 % control, p<0.001). This was NADPH oxidase dependent because incubation with the gp91ds-tat peptide prevented this effect. High salt exposure also led to an increase in the activation markers CD80, CD86, and a 48% increase in DCs containing isoketal-protein adducts. Moreover, DCs exposed to high salt drove T cell proliferation (5198.2±2398.6 vs. 15.3±7.1 proliferated CD4+ cells and 25381.6±9495.6 vs. 9.8±4.1 proliferated CD8+ cells, p<0.05). This was not due to increased osmolality, as mannitol did not mimic these effects. Western blots of protein extracts from DCs indicated that all NADPH subunits (p47phox, p22phox, gp91phox and p67phox) were increased by exposure of cells to high salt, and that these effects were prevented by inhibition of the salt-sensing glucokinase (SGK1). In additional studies, we found that human monocytes, which are precursors of

myeloid DCs, from hypertensive subjects possess higher CD86 and isoketal-protein adducts compared to normotensives, and that these are further increased by exposure to 190 mM salt for 48 hours. Taken together, our data indicate that antigen presenting cells are activated by exposure to the high sodium environment that can occur in pro-hypertensive states, and that this likely involves increased expression and activation of the NADPH oxidase. Our data also describe a previously undefined role of the SGK1 in this process.

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Hypothalamic PVN GαI2 Protein-mediated Renal Nerve Dependent Sympathoinhibition Facilitates Suppression of NCC Activity and a Salt-resistant Phenotype

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Aim: We hypothesize hypothalamic paraventricular (PVN) specific Gαi2 proteins, which are up-regulated in the PVN during increased sodium intake, mediate global and renal sympathoinhibition to facilitate suppression of the activity of the sodium chloride cotransporter (NCC) and salt-resistance in the Sprague Dawley rat.
Methods: Groups of intact or bilateral renal denervated (RDNX) salt-resistant Sprague-Dawley rats received a bilateral PVN infusion of a scrambled (SCR) or Gαi2 oligodeoxynucleotide (ODN-300ng/side/day) and a normal 0.4% (NS) or high 8% NaCl (HS) diet for 7-days. On day-7 24h Na+ balance was assessed - in sub-groups MAP, plasma norepinephrine (NE) content, kidney NE content and NCC activity (peak natriuresis to iv bolus hydrochlorothiazide (HCTZ)) was assessed (N=6/gp/study).
Results: In SCR ODN infused rats HS-intake evoked a significant 3-fold site-specific increase in PVN Gαi2 proteins and suppressed plasma NE content (plasma NE [nmol/L] SCR NS 72±4 vs HS 40±5, P<0.05), endogenous kidney NE content (NE [pg/m] SCR NS 586±36 vs HS 496±41, P<0.05) and NCC activity (peak ΔUNaV to HCTZ [μeq/min] SCR NS 10.6±0.8 vs HS 7±1, P<0.05) without impacting sodium homeostasis or MAP. ODN-mediated PVN Gαi2 down-regulation caused renal nerve-dependent hypertension (MAP [mmHg] Gαi2 NS 126±2, Gαi2 HS 147±2, Gαi2 HS + RDNX 131±3 P<0.05) sodium retention (24h Na+ balance [meq] Gαi2 NS 0.6±0.3, Gαi2 HS 2.3±0.4, Gαi2 HS + RDNX 0.8±0.3 P<0.05), global and renal sympathoexcitation (plasma NE [nmol/L] Gαi2 NS 67±7, Gαi2 HS 105±11, Gαi2 HS + RDNX 74±6 P<0.05; kidney NE content [pg/mg] Gαi2 NS 614±48 vs HS 823±59, P<0.05) and a failure to suppress NCC activity (peak ΔUNaV to HCTZ [μeq/min] Gαi2 NS 10.2±2, Gαi2 HS 14.7±2, Gαi2 HS + RDNX 7.6±2, P<0.05).
Conclusion: PVN Gαi2 protein-gated pathways represent a sodium sensitive CNS mechanism acting to regulate renal nerve-dependent sodium excretion via, in part, actions upon the activity of the NCC. Based on recent studies by other groups we speculate the regulation of NCC in this setting is mediated by the actions of NE released from efferent renal nerves on renal adrenoceptors to impact the signal transduction network that regulates NCC expression.

R.D. Wainford: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; R01 HL107330, K02112718.

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Cytochrome B5 Reductase 3 Sensitizes Soluble Guanylate Cyclase to Nitric Oxide in Vascular Smooth Muscle

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The inability nitric oxide (NO) to stimulate soluble guanylate cyclase (sGC) has been linked to numerous cardiovascular diseases (CVD) including hypertension. While several studies have defined the importance of sGC expression in the cardiovascular system, the basic mechanisms that regulate sGC activity remain incompletely understood. Here, we report for the first time that sGC heme iron redox state, which is essential for NO-induced sGC activation, is regulated by cytochrome B5 reductase 3 (CyB5R3). Genetic knockdown and pharmacological inhibition of CyB5R3 in primary rat vascular smooth muscle cells resulted in a 60% loss in cGMP production. Conversely, the sGC activator Bay 58-2667, which activates oxidized or heme free sGC, reversed these effects. Consistent with our cell culture work, purified protein studies demonstrate that CyB5R3 can directly reduce oxidized sGC heme iron and sensitize sGC to NO. To test the functional importance of Cyb5R3 activity, we cultured mouse thoracodorsal arteries with a pharmacological inhibitor of Cyb5R3 (ZINC 747) and performed vascular reactivity studies using pressure myography. Arteries treated with ZINC 747 showed decreased responsiveness the NO donor DETA-NONOate but increase sensitivity to Bay 58-2667. We then treated mice with 10mg/kg/day of ZINC 747 using osmotic mini pumps, which caused an increase in mean arterial blood pressure (107.5±3.4 vs 131±13.16) measured via radio telemetry. Lastly, translational studies reveal that the CyB5R3 T116S polymorphism with allele frequency 0.23 only in African Americans is unable to reduce sGC and correlates with increased blood pressure. Considering the defining role of sGC in NO signaling and the fact that the oxidation state of sGC may predict responses to NO therapies and new classes of sGC activator medications, we anticipate that these studies may significantly impact our understanding of biology, precision therapeutics (right drug for the right patient) and pharmacogenetics (T117S SNP based drug selection).

A.C. Straub: None. **A.T. Nguyen:** None. **M. Rahaman:** None. **S.M. Mutchler:** None. **M. Miller:** None. **C. Watkins:** None. **M. Gladwin:** None. **V. Gordeuk:** None. **J. Prchal:** None. **E. Martin:** None.

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Role of Primary Endothelial Cilia in Hypertension

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Abstract

Primary cilia are mechanosensory organelles that are projected into the lumen of blood vessels. It has been demonstrated that vascular endothelia require primary cilia to sense and transmit external mechanical stimuli into internal biochemical reactions. One of these reactions includes the biosynthesis and release of nitric oxide, which is one of the most potent endogenous vasodilators. This idea has *only*

been investigated in cultured endothelial cells *in vitro*. Based on this finding, however, a very bold hypothesis is formed to test that abnormal cilia function results in vascular hypertension. Our laboratory has recently generated and obtained several conditional mouse models to specifically study the function and structure of primary cilia in vascular endothelia. These models include **1)** mice without cilia function (*Pkd1* or *Pkd2*); **2)** mice without cilia structure (*Tg737* or *Kif3a*). Our data indicate that mice with abnormal cilia function (*Pkd1*) or structure (*Tg737*) show significantly higher systolic (150±19 for *Pdgfbcre:Pkd1*$^{flox/flox}$ and 147±10 for *Tie2Cre:Tg737*$^{flox/flox}$ vs. 128±9 for wild-type) and diastolic (120±21 for *Pdgfbcre:Pkd1*$^{flox/flox}$ and 120±11 for *Tie2Cre:Tg737*$^{flox/flox}$ vs. 102±7 for wild-type) blood pressure than the corresponding wild-type mice. Because there is a positive and continuous correlation between blood pressure and cardiovascular diseases, satellite hypotheses are developed to look at the pathophysiological roles of endothelial cilia in cardiac functions and focal vascular diseases *in vivo*. Our data clearly point towards deteriorating phenotypes in the cardiac muscle, including cardiac fibrosis due to an increased cardiac workload. As a result, a heart-to-body weight ratio was significantly increased by 17 weeks old (0.008 *PdgfbCre;Pkd1*$^{f/f}$ vs. 0.006 *Pkd1*$^{f/f}$).The present study will very likely provide new insights for hypertension and offer advanced scientific understanding of vascular endothelial cilia in other cardiovascular diseases.

H. Saternos: None. **M. Hossain:** None. **W. AbouAlaiwi:** None.

Protective Effects of Nuclear Factor E2-related Factor 2 Activation on Angiotensin II Induced Microvascular Endothelial Dysfunction

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Background: Nuclear factor E2-related factor 2 (Nrf2) is a regulator of the cellular adaptive response to oxidative stress, but its effects on microvascular function are poorly characterized. Our previous studies in human glomerular endothelial cells found that tert-butylhydroquinone (tBHQ; a Nrf2 activator) reduced ROS, asymmetric dimethylarginine (ADMA, an endogenous NOS inhibitor) and increased eNOS. We hypothesized that tBHQ would prevent microvascular endothelial dysfunction via activation of Nrf2 / antioxidant responses in angiotensin II (ANG II) induced hypertension. Methods: Mesenteric resistance arterioles (MRAs) were isolated from mice infused for 14 days with ANG II (400 ng/kg/min) or vehicle and given oral tBHQ (0.1% of water) or vehicle (n=6 mice/group). Acetylcholine-induced endothelium dependent relaxation (EDR), endothelial derived relaxation factor (EDRF) and contracting factor (EDCF) of MRAs were assessed by myograph. Vascular nitric oxide (NO), and ROS were assessed by RatioMaster. ROS biomarkers (urinary excretion of malondialdehyde [MDA]) and 8-isoprotane [8-Iso]), ADMA and protein expressions of MRAs were measured. Results: tBHQ prevented the effects (all $P<0.05$) of ANG II infusion-induced the increases of urinary MDA (50±3 vs 83±11 nmol/mg creatine) and 8-Iso (1.4±0.1 vs 3.8±0.3 vs ng/mg creatinine) and decreases of EDR (75 ±

3 vs 53±5%), EDRF (23± 2 vs 17± 3%) and NO (0.3 ± 0.02 vs 0.2 ± 0.03 units), and enhanced EDCF (7 ± 1 vs 13 ± 2%) and associated ROS production (0.11 ± 0.03 vs 0.3 ± 0.08 units) and microvascular ADMA (18 ± 1 vs 13 ± 1 nmol/mg protein). ANG II increased the MRAs protein expressions (of β-actin, all P<0.05) for NOX1 (0.60 ± 0.03 vs 0.38± 0.04) and NOX2 (0.80 ± 0.04 vs 0.36± 0.05) and reduced phosphorylated eNOS (0.26 ±0.07 vs 0.53±0.09), whereas tBHQ increased Nrf2 expression (0.44 ±0.03 vs 0.73±0.04) and prevented these changes with ANG II infusion. Conclusions: tBHQ upregulates Nrf2 and thereby engages the antioxidant responses to protect microvessels of ANG II-infused mice from the adverse effectors of ROS, NO deficiency and ADMA. This is a novel potential therapeutic target that activates Nrf2/antioxidant response element to protect against vascular oxidative stress, endothelial dysfunction and CVD.

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Importance of the C-Terminal Transactivation Domain of STAT3 in Hypertension-Induced Cardiac Hypertrophy

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Signal transducer and activator of transcription 3 (STAT3) is known to have protective effects in the heart in acute oxidative stress such as myocardial infarction; however, the role of STAT3 in the heart in response to chronic stress, such as hypertension, is not defined. Here, we assessed the importance of STAT3 on cardiac remodeling post abdominal aortic constriction (AAC) using mice expressing a STAT3 protein lacking the transactivation domain (TAD; aa701-732) selectively in cardiomyocytes (cm-STAT3Δ). Loss of the TAD region impairs both the mitochondrial and transcriptional actions of STAT3. Both WT and cm-STAT3Δ mice developed hypertension to a comparable extent. However, cm-STAT3Δ mice exhibited a significant ($p < 0.01$) decline in ejection fraction (58.3 ± 4.7 to 24.2 ± 3.6, n=4) and fractional shortening (30.8 ± 3.4 to 11.2 ± 1.7, n=4) over 28 days following AAC, while WT mice exhibited a compensatory response in EF (58.6 ± 3.2 to 59.4 ± 6.0) and FS (30.7 ± 2.1 to 33.0 ± 2.8). Notably, hearts of cm-STAT3Δ exhibited a marked increase in diastolic left ventricular internal diameter (LVIDd), symptomatic of eccentric hypertrophy and dilated cardiomyopathy. In contrast, the pattern of change in LVIDd for WT mice was consistent with concentric remodeling. Banding caused comparable increases in heart to body weight ratios in WT (4.1 ± 0.1 to 6.4 ± 1.0) and cm-STAT3Δ (4.0 ± 0.1 to 7.2 ± 0.6) mice, although on average the increase was larger in cm-STAT3Δ mice. Interestingly, we also found that miR-199a-5p levels were only moderately higher (36%) after AAC in the ventricles of cm-STAT3Δ mice vs. wild type hearts as compared to cardiomyocyte-targeted STAT3 KO mice in which the DNA binding domain of STAT3 is deleted. miR-199a-5p is a known inhibitor of peroxisome proliferator-activated receptor delta (PPARδ) expression and mitochondrial

fatty acid oxidation in the heart. These new findings emphasize the distinctive and important role of the C-terminus of STAT3 in the hypertrophic response of the heart to hypertension and the progression to heart failure.

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Maternal Separation, a Model of Early Life Stress in Rats, Dysregulates the Renal Vasculature Gene Expression Patterns During Late Nephrogenic Period and Adult Life

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We have shown that maternal separation (MatSep) induces permanent alterations in the renal vascular structure, hemodynamics and sympathetic outflow. Environmental stressors are typically associated with abnormal organ development. Recently, we found that MatSep pups show higher levels of plasma corticosterone and renal norepinephrine during postnatal life ($p< 0.05$). The aim of this study was to investigate the short and long-term effects of MatSep on gene expression patterns of the renal vasculature. MatSep was performed during early postnatal life timeframe (3hr/day). Undisturbed littermates served as control (C). Upon weaning, rats were allowed to grow and develop for several months. Kidney vessels were isolated at postnatal day 10 (PND10, n=5) and 6 months of age (6Mo, n=5) and flash frozen. mRNA was used to perform genome-wide analysis using Affymetrix rat Gene2.0 ST. EBPfc* analysis was conducted using DAVID bioinformatics resources at NIH website. MatSep altered 1108 genes expression ($P<0.03$, $FDR<0.3$,Table 1). We found a set of genes upregulated by MatSep at both PND10 and 6Mo, showing long-lasting changes in expression. The uncoupling protein 1 (UCP1), located proximal to the major vascular and nerve conduits to the kidney, was the highest MatSep-induced upregulation (3.9-fold, PND10). UCP1 network of genes were mostly related to cellular organization and metabolic and cardiovascular disease (e.g. GH, AT1R, AT2R, P38 MAPK, IFNγR, VEGF, ApoE). Thus, MatSep influences timing and direction of key genes involved in the renal vascular maturation. Upregulated non-shivering thermogenesis function early in life may be linked to impaired renal function. NIH R00 HL111354
$$MISSING OR BAD IMAGE SPECIFICATION {97AD4A55-CFF3-4ABC-8B3C-78C7704F3ABC}$$

Table 1. Genes altered by MatSep (fold change ranged from +3.9 to -1.3)

Gene regulation			*EBPfc = Enriched Biological Pathway functional clusters
Up	Down	Age	
321	17	PND10	Extracellular structure organization; vasculature development/angiogenesis; embryonic development
386	345	6Mo	Defense/inflammatory response; immune system development/regulation
21#	-	Both	Regulation of signal transduction/cell communication
18##	-	Both	and induction of apoptosis

developmental life genes; ## adult life genes

K. Chen: None. **A.S. Loria:** None.

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Endothelial Mineralocorticoid Receptor Knock Out Protects the Endothelium From Aldosterone-Mediated Vascular Stiffness

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Background: There is accumulating evidence that increased levels of aldosterone (Aldo) and increased vascular mineralocorticoid receptor (MR) signaling increases vascular inflammation and oxidative stress leading to endothelial

dysfunction and associated vascular stiffness. However, the specific role of endothelial cell (EC) MR activation in promotion of end stiffness in female mice has not been explored. Accordingly, we hypothesized that knocking out MR from ECs would attenuate the Aldo-induced endothelial dysfunction and vascular stiffness. Methods and Results: Twenty six week-old female ECMR knockout (ECMR-/-) and wild type (ECMR+/+) mice were infused with 250 µg/Kg/day Aldo for 3 weeks. To assess endothelial-dependent vasodilation, endothelial and aortic stiffness and blood pressure we utilized wire myography, atomic force microscopy (AFM), pulse wave velocity (PWV) (before and after Aldo perfusion) and tail cuff procedures. Aldo infusion did not increase BP or PWV and this was not affected by the presence or absence of ECMR. Aldo impaired endothelial-dependent vasodilation and increased EC stiffness 8.6 fold and these effects were mitigated in ECMR-/-. Aldo did not alter peri-aortic fibrosis by picrosirius red staining as measured by average gray scale intensities, nor did it cause medial thickening or aortic remodeling evaluated by the lumen to aortic wall ratio, in either Aldo-infused group. Moreover, levels of the oxidative marker, 3-nitrotyrosine (3-NT) did not differ in different compartments of the aortic wall in either Aldo treated group.
Conclusion: ECMR protects the endothelium from aldo-mediated impaired vasodilation and endothelial cell stiffness, and this protection occurs without changes in BP, total aortic stiffness, or vascular remodeling.

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Renal Phenotype of Inwardly Rectifying Potassium Channel Kcnj16 (Kir 5.1) Knockout in the Dahl Salt-Sensitive Rats

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The inward-rectifying channels play an important role in the control of resting membrane potential and tubular homeostasis in the kidney. Kcnj16 (Kir 5.1) form a heteromeric channel with Kcnj10 (Kir 4.1) at the basolateral membranes of aldosterone-sensitive distal nephron (ASDN); mutations in the human KCNJ10 gene result in SeSAME)/EAST syndrome, a complex disorder that includes salt wasting and hypokalemic alkalosis. To illuminate the importance of Kcnj16 (Kir 5.1) in the context of a disease state in vivo, we generated a Kcnj16 knockout rat model in Dahl salt-sensitive (SS) background by using ZFN technology. ZFN against Kcnj16 caused a 18-bp in-frame deletion that occurred in the second protein

transmembrane domain. IHC analysis demonstrated highly specific expression of Kcnj16 on the basolateral membranes of ASDN in the control kidneys of SS rats, which was completely abolished in Kcnj16-/- rats. The electrophysiological recording of K+ channels in the CCD basolateral membrane revealed activity of only homomeric Kcnj10 channels (21 pS channel in Kcnj16-/- rats compared to both 41 and 21 pS channels in SS rats). Thus, these data provide evidence of successful knock out of this protein and consequent degradation of the channel in renal tubules. The Kcnj16-/- knockout in SS rat induces electrolyte imbalance, epileptic seizures and result in changes in development (37% reduction in body and 54% in kidney mass). The mean arterial pressure was significantly lower in Kcnj16-/- compared to SS rats (91.3±1.8 to 104.7±5.5 mmHg) when animals were fed a low salt (0.4%) diet. Knockout of Kcnj16 resulted in hypokalemia (4.25±0.09 vs 2.08±0.12 mmol/L in serum of control vs KO rats), hypermagnesemia (0.49±0.02 vs 0.63±0.01 mmol/L in serum of control vs KO rats), and FSGS. Urea electrolyte balance was also disturbed compared to control animals. Importantly, change of the diet to high salt (4%) caused mortality of KO rats within 1-2 days. These data demonstrate critical role of Kcnj16 channels in renal salt handling and in the development of salt-sensitive hypertension.

O. Palygin: None. **V. Levchenko:** None. **D.V. Ilatovskaya:** None. **J.L. Barnett:** None. **A.M. Geurts:** None. **H.J. Jacob:** None. **A. Staruschenko:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; NHLBI.

K+ Rich Diet During Angiotensin II Hypertension Reduces Renal Na-Cl Cotransporter and Phosphorylation, but Not Blood Pressure

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During Ang II hypertension distal tubule Na-Cl Cotransporter (NCC) abundance and its activating phosphorylation (NCCp), as well as Epithelial Na+ channels (ENaC) abundance and activating cleavage are increased 1.5-3 fold. Fasting plasma [K+] is significantly lower in Ang II hypertension (3.3 ± 0.1 mM) versus controls (4.0 ± 0.1 mM), likely secondary to ENaC stimulation driving K+ secretion. The aim of this study was to test the hypothesis that doubling dietary K+ intake during Ang II infusion will lower NCC and NCCp abundance to increase Na+ delivery to ENaC to drive K+ excretion and reduce blood pressure.
Methods. Male Sprague Dawley rats (225-250 g; n= 7-9/group) were treated over 2 weeks: 1) Control 1% K diet fed (C1K); 2) Ang II infused (400 ng/kg/min) 1% K diet fed (A1K); or 3) Ang II infused 2% K diet fed (A2K). Blood pressure (BP) was determined by tail cuff, electrolytes by flame photometry and transporters' abundance by immunoblot of cortical homogenates.
Results: As previously reported, Ang II infusion increased systolic BP (from 132 ± 5 to 197 ± 4 mmHg), urine volume (UV, 2.4 fold), urine Na+ (UNaV, 1.3 fold), heart /body weight ratio (1.23 fold) and clearance of endogenous Li+ (CLi, measures fluid volume leaving the proximal tubule, from 0.26 ± 0.02 to 0.51 ± 0.01 ml/min/kg) all evidence for pressure natriuresis. A2K rats exhibited normal plasma [K+] (4.6 ± 0.1 mM, unfasted), doubled urine K+ (UKV, from 0.20 to 0.44 mmol/hr), and increased CLi (to 0.8

± 0.1 ml/min/kg) but UV, UNaV, cardiac hypertrophy and BP were unchanged versus the A1K group. As expected, NCC, NCCpS71 and NCCpT53 abundance increased in the A1K group to 1.5 ± 0.1, 2.9 ± 0.5 and 2.8 ± 0.4 fold versus C1K, respectively. As predicted by our hypothesis, when dietary K+ was doubled (A2K), Ang II infusion did not activate NCC, NCCpS71 nor NCCpT53 (0.91 ± 0.04, 1.3 ± 0.1 and 1.6 ± 0.2 fold versus C1K, respectively). ENaC subunit abundance and cleavage increased 1.5 to 3 fold in both A1K and A2K groups; ROMK was unaffected by Ang II or dietary K.
In conclusion, evidence is presented that stimulation of NCC during Ang II hypertension is secondary to K+ deficiency driven by ENaC stimulation since doubling dietary K+ prevents the activation. The results also indicate that elevation in BP is independent of NCC activation

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Hydrochlorothiazide Lowers Systemic Blood Pressure Predominantly Through Vasodilation in Conditions Associated with Vasoconstriction

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Thiazide derivatives are specific inhibitors of the Na+_Cl- Co-transporter (NCC) in the kidney distal tubules, and the most commonly used diuretic for the treatment of mild hypertension. The mechanism by which thiazides reduce the blood pressure (i.e. renal vs. extra renal) is not well understood. The blood pressure response to thiazides requires an initial volume loss (averaging about 1.5 kg) since it is not observed in individuals who are ingesting a high-salt diet. The objective of this study was to elucidate the renal and extra renal effects of thiazides. Using NCC deficient mice, we demonstrate that hydrochlorothiazide (HCTZ), a commonly used thiazide derivative, reduces blood pressure in the presence of volume-depletion and augmentation of circulating angiotensin levels but not in mice with normal vascular volume (NCC KO at baseline = 131.6 ± 4.4 vs. NCC KO with HCTZ = 125.8 ± 3.7 mm Hg) vs. (volume depleted NCC KO at baseline = 118.2 ± 4.1 vs. volume depleted NCC KO with HCTZ= 96.37± 4.5 mm Hg; $p < 0.05$). The reduction in blood pressure by HCTZ occurs in the absence of increased salt excretion or urine output, indicating its extra-renal mechanism. Echocardiography demonstrated no significant changes in cardiac index in response to the drop in blood pressure by HCTZ (WT at baseline = 0.639 ± 0.05 vs. WT with HCTZ = 0.590 ± 0.01 ml/min/g) vs. (volume depleted NCC KO at baseline = 0.617± 0.07 vs. volume depleted NCC KO with HCTZ = 0.509 ± 0.04 ml/min/g). The antihypertensive effects of HCTZ were abrogated in the presence of paxilline, a specific blocker of large conductance calcium activated potassium channels (BK channels) in the vascular smooth muscle cell. Western blotting demonstrated enhanced expression of BK channels in vascular system of volume depleted NCC deficient mice vs. WT mice. Our results indicate that systemic vasoconstriction, as observed in volume depleted states, will amplify the extra-renal effects of HCTZ to lower blood pressure through vasodilation irrespective of the status of its renal target NCC. Patients with congestive heart failure who are on a combination of a loop diuretic and HCTZ are at increased risk of significant hypotension subsequent to the activation of extra-renal

effects of HCTZ, specifically when they become volume depleted.

S. Alshahrani: None. **J. Rubinstein:** None. **M. Jiang:** None. **S. Barone:** None. **J. Xu:** None. **K. Zahedi:** None. **M. Soleimani:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; principal investigator.

116

Characterization of a Sodium Responsive Human Sodium Bicarbonate Transporter NBCe2 in Human Proximal Tubule

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The electrogenic sodium bicarbonate cotransporter (NBCe2) is encoded by SLC4A5, variants of which have been associated with salt sensitivity of blood pressure (BP), which affects 25% of the adult population. NBCe2 is thought to mediate sodium bicarbonate cotransport primarily in the renal collecting duct, but NBCe2 mRNA is also found in the rodent renal proximal tubule (RPT), another site of bicarbonate transport. The protein expression or function of NBCe2 has not been demonstrated in the human RPT. We validated an NBCe2 antibody by siRNA and Western blot, as well as overexpression of an epitope-tagged NBCe2 construct in both RPT cells (RPTCs) and HEK293 cells. We immune-localized (peptide pre-absorbable) NBCe2 protein in the RPT of fresh and frozen human kidney slices, RPTCs isolated from human urine, and in isolated RPTC apical membrane. NBCe2 was primarily found in the Golgi while NBCe1, another electrogenic member of the same family, was primarily found at the basolateral membrane, under basal conditions. Following a short-term increase in intracellular sodium, NBCe2 expression increased at the apical membrane in cultured slices of human kidney (1.75±0.15 fold increase over basal, N=3, $p<0.05$) and polarized, immortalized RPTCs (0.08±0.015 vs 0.03±0.006 control, NBCe2/CD13 RFU, N=3, $p<0.05$), while the basolateral localization of NBCe1 was decreased (30.7±5.4% over basal, N=3, $p<0.05$). DIDS (electrogenic sodium bicarbonate inhibitor, 500 µM, 10 min) inhibitable bicarbonate dependent pH recovery in the presence of EIPA (NHE inhibitor,100 nM, 10 min) after ammonium chloride prepulse (20 mM, 5 min) was higher in the cells cultured under high salt conditions (26.7±7.2% over basal, N=3, $p<0.05$). Thus, NBCe2 appears to be important in apical sodium and bicarbonate cotransport under high salt conditions. Future studies will examine the role of NBCe2 in mediating increased renal sodium transport in human salt sensitivity of BP.

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NOS1β Mediates the Difference Between Juxtamedullary and Superficial Glomerular Injury with Aging

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Renal function measured by glomerular filtration rate (GFR) declines with aging. But the mechanism has not been clarified. Nitric oxide synthase 1 β (NOS1β) is highly expressed in the macula densa (MD) and modulates tubuloglomerular feedback (TGF), which is an important mechanism for GFR regulation. We hypothesized that expression of NOS1β in MD decreases with aging, which enhances TGF response and contributes to the decline of GFR. We further hypothesized that NOS1β expression in superficial (SF) MD is lower than that of juxtamedullary (JM) MD, which contribute to more severe renal injury in renal cortex with aging.
First, GFR was measured by plasma FITC-inulin clearance after a single bolus injection in conscious 12-week young mice and 20-month-old C57/BL6 mice. GFR was reduced by 36.4% in aged mice (236±9.7 vs 150±7.6 μl/min). Then we compared the glomerular injury in 20-month-old C57/BL6 mice with 12-week mice. The aged mice have significant severed injury compared to the young mice as indicated by injury scores (3.6) and expanded fractional mesangial area (FMA) per glomerulus (26.4%). In addition, injuries were more evident in SF glomeruli with larger FMA (29.3%) than that in JM glomeruli (16.9%). Next we measured NOS1β protein abundance and found that NOS1β protein in aged mice was reduced by 73% compared to young mice. In addition, we compared the NOS1β abundance between SF and JM glomeruli in aged and young mice. NOS1β protein level decreased by more than 90% in SF nephrons in aged mice compared with the young mice. In contrast, there was no significant changes in NOS1β expression in JM nephron between aged and young mice.
To determine whether NOS1β mediates the changes in GFR and renal injury, we repeated the experiments in age matched MD specific NOS1β knock out (MD-NOS1KO) mice. In MD-NOS1KO mice, GFR in aged mice was reduced by 29.5% (203±15.7 vs 143±13.2 μl/min) compared young mice. Aged MD-NOS1KO mice had more severer renal injury compared with age-match wild type mice with higher injury score (4.7 vs 3.6), increased FMA (40.9% vs 26.4%) and occurrence of glomerulosclerosis (GS 30.8%), but diminished difference between JM glomerulus (FMA37.4%, GS34.1%) and SF glomerulus (FMA42.2%, GS29.7%).

J. Wei: None. **S. Wang:** None. **J. Zhang:** None. **K. Jiang:** None. **K. Yip:** None. **R. Liu:** None.

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Role of Connecting Tubule Glomerular Feedback in Tubuloglomerular Feedback Resetting After Unilateral Nephrectomy

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Tubuloglomerular feedback (TGF) and connecting tubule glomerular feedback (CTGF) autoregulate nephronal afferent arteriolar resistance. In TGF, the macula densa signals the afferent arteriole to constrict when NaCl transport is enhanced by increased luminal NaCl, via sodium–potassium-2-chloride cotransporter-2 (NKCC2). CTGF is mediated by connecting tubule sodium transport via epithelial sodium channel (ENaC) and dilates the afferent arteriole. Attenuation or resetting of TGF occurs after unilateral nephrectomy (UNX), but the mechanism behind this resetting remains unclear. This TGF resetting after UNX has been implicated in progressive glomerular damage due to sustained increase in glomerular capillary pressure. Since TGF is attenuated after UNX, we sought to test the hypothesis that CTGF is enhanced and that it contributes to TGF resetting after UNX. To test this hypothesis, we performed right side UNX in Sprague Dawley (SD) rats. 24 hours after surgery, we performed micropuncture of individual rat nephrons while measuring stop-flow pressure (PSF), which is an index of glomerular capillary pressure and afferent arteriolar tone. PSF decreases with an increase in afferent arteriolar tone. TGF response was measured as a decrease in PSF induced by switching late proximal perfusion from 0,10,20,30 and 40nl/min. Maximal TGF response was 1.3 ± 1.7 mmHg in UNX rats while 8.2 ± 0.9 mmHg in sham-UNX rats indicating a TGF resetting in UNX rats. When CTGF was inhibited with the ENaC blocker Benzamil (1μM), TGF response was 10±1.2 mmHg in UNX rats and 14.8± 1.3 mmHg in sham-UNX rats, indicating the restoration of TGF responses in UNX. We conclude that enhanced CTGF contributes to the TGF resetting after 24 hours of UNX. Enhanced CTGF may be responsible for glomerular damage post UNX.

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The Imbalance of Mitochondrial Superoxide Dismutase Activity and Superoxide Production in Endothelial Dysfunction and Hypertension

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Clinical studies have shown that Sirt3 expression declines by 40% by age 65 paralleling the increased incidence of hypertension and metabolic conditions further inactivate Sirt3 due to increased NADH and Acetyl-CoA. Sirt3 activates a major mitochondrial antioxidant enzyme, superoxide dismutase 2 (SOD2) by deacetylation of specific lysine residues. We hypothesized that loss of Sirt3 activity increases vascular oxidative stress due to SOD2 hyperacetylation and this promotes endothelial dysfunction and hypertension. Indeed, Western blot analysis showed 3-fold increase in SOD2 acetylation and 1.5-fold decrease in Sirt3 level in hypertensive human subjects. Infusion of angiotensin II (0.7 mg/kg/day) in C57Bl/6J mice increased acetylation of vascular SOD2 by 2-fold, reduced SOD2 activity to 52% and raised blood pressure to 156 mm Hg. We have tested if Sirt3 depletion

exacerbates endothelial dysfunction and hypertension. Indeed, Western blot analysis of Sirt3-/- mice showed SOD2 hyperacetylation and reduced SOD2 activity, however, blood pressure and superoxide production was normal. Angiotensin II infusion in Sirt3-/- mice increased blood pressure to 182 mm Hg, increased superoxide, reduced endothelial nitric oxide and impaired acetylcholine-mediated vasodilatation compared with angiotensin II infused wild type mice. Treatment of hypertensive Sirt3-/- mice with SOD2 mimetic mitoTEMPO (1.4 mg/kg/day) after onset of angiotensin II-induced hypertension normalized blood pressure and restored endothelial dependent vasodilatation. We suggest that mitochondrial superoxide does not contribute to the basal vasodilation and blood pressure regulation however high salt, angiotensin II and inflammation increase mitochondrial superoxide. Indeed, superoxide production by complex I in submitochondrial particles was significantly increased in hypertensive mice and this was associated with complex I S-glutathionylation. Interestingly, SOD2 overexpression in transgenic mice prevents complex I S-glutathionylation, reduces superoxide production by complex I and attenuates hypertension. These data indicate that imbalance of SOD2 activity and mitochondrial superoxide production contributes to endothelial dysfunction and hypertension.

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Increased Mitochondrial Superoxide in the Brain, but not Periphery, Sensitizes Mice to Angiotensin II-Mediated Hypertension

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Mitochondrial superoxide ($O_2^{\bullet-}$) is a critical signaling intermediate in angiotensin II (AngII)-dependent hypertension. However, it remains unknown if increased mitochondrial $O_2^{\bullet-}$ flux in the absence of hypertensive stimuli is sufficient to affect blood pressure. We hypothesized that elevated levels of systemic mitochondrial $O_2^{\bullet-}$ leads to increased blood pressure and exacerbates AngII-induced hypertension. To test this, we utilized a conditional mouse model of manganese superoxide dismutase knock-out ($MnSOD^{lox/lox}$), which we have shown amplifies steady-state mitochondrial $O_2^{\bullet-}$ levels in all cell types examined. When combining the $MnSOD^{lox/lox}$ mouse with a tamoxifen-inducible cre recombinase expressed by the systemic ROSA26 promoter, MnSOD was knocked-down (30-98%, $p<0.05$) in peripheral organs after intraperitoneal tamoxifen administration. However, no changes in MnSOD protein levels were observed in the brain. Interestingly, mean arterial pressure (MAP) and heart rate were unaffected by the loss of MnSOD in these peripheral tissues, and moreover, upon subcutaneous infusion with AngII (400 ng/kg/min) both wild-type and MnSOD knock-down mice exhibited a similar increase in MAP. Due to these unexpected results, we examined the role of elevated mitochondrial $O_2^{\bullet-}$ levels specifically in the brain subfornical organ (SFO) by targeting the loss of MnSOD to this critical AngII-sensitive region. We observed a 60% decrease of MnSOD ($p<0.05$) with concomitant increase in mitochondrial $O_2^{\bullet-}$, as measured by MitoSox Red fluorescence, in the SFO following

adenovirus-mediated gene transfer of cre recombinase to the SFO in $MnSOD^{lox/lox}$ mice. Intriguingly, these mice demonstrated no change in baseline MAP (92.8 ± 0.4 mmHg in knock-down vs. 93.1 ± 0.4 mmHg in control mice), but did show a significant elevation in MAP upon peripheral AngII infusion (MAP_{max} = 137.8 ± 2.7 mmHg in knock-down vs. 128.3 ± 3.3 mmHg in control mice, $p<0.05$). Taken together, our data suggest that increased mitochondrial $O_2^{\bullet-}$ in the absence of hypertensive stimuli is not sufficient to alter baseline hemodynamics, but dysregulation of mitochondrial redox status in the SFO may be a predisposition to increased responsiveness to hypertensive stimuli, such as AngII.

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Gstm1 Deletion Exaggerates Hypertension, Oxidative Stress, and Kidney Pathology in Angiotensin II Hypertension Independent of Nox2 and Nox4

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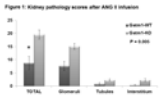
Gstm1 gene encodes an enzyme that belongs to a superfamily of Glutathione-S-transferases that metabolize a broad range of reactive oxygen species. In the African American Study of Kidney Disease Trial (AASK), we reported that participants with decreased expression of GSTM1 as carriers of the GSTM1 null allele have accelerated kidney disease progression. To establish cause and effect, we generated Gstm1-/- (KO) mice and determined their response in angiotensin II (AngII)-induced hypertension (at 1000 ng/kg/min for 4 weeks). By radiotelemetry, Gstm1 KO mice (n=6) displayed a modest but significantly higher baseline systolic blood pressure (SBP, mmHg) compared to their wild type (WT) littermates (n=6): KO 135.8 ± 1.4; WT: 129.9 ± 1.2; P = 0.01. AngII augmented the difference in SBP between WT and KO mice (KO 175.4 ± 6.2; WT 158.0 ± 2.6, p = 0.04). There were no differences in albumin/creatinine ratio (µg/mg) either at baseline (KO 29.1± 4.1; WT 27.3 ± 2.9) or after 4 weeks of Ang II (KO 4657 ± 631.6; WT 4697.7 ± 533.8). By quantitative real-time PCR, the renal expressions of Nox2 and Nox4 genes were almost identical and not statistically different between WT and KO mice. However, by luminescence, Gstm1 KO mice had significantly increased levels of superoxide radicals: [counts/min/1 mg of dry tissue] KO 128 ± 12 vs. WT 42 ± 8.5; P < 0.0001. Furthermore, there was significantly worse kidney pathology in Gstm1 KO mice (Figure 1), including focal segmental glomerulosclerosis (FSGS), fibrosis, chronic inflammation and epithelial cell reactivity. In conclusion, loss of GSTM1 enzyme increases Ang II induced hypertension, oxidative stress and kidney injury independent of Nox2 or Nox4.



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Activation of Nuclear Factor Erythroid 2-related Factor 2 (Nrf2) Enhances Cyclooxygenase 2 Expression via Promoter Antioxidant Response Element in Preglomerular Vascular Smooth Muscle Cells (PGVSMCs)

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Nrf2 is a key cytoprotective transcription factor driving many antioxidant and anti-inflammatory genes expression via binding to antioxidant response elements (AREs) in promoters of targeting genes. However, it was reported that Nrf2 also increased expression of pro-inflammatory and pro-oxidative genes IL-6 and NOX4 through the same mechanism. We detected 2 putative ARE consensus sequences within 1500 nucleotides (nts) located in the genomic region upstream of the transcription start site in rat cyclooxygenase 2 (COX2) gene. Therefore, we hypothesized that activation of Nrf2-dependent AREs upregulates COX2 expression in rat PGVSMCs. Incubation of the cells for 24 hours with Nrf2 activator, tert-butylhydroquinone (tBHQ), did not significantly alter Nrf2 expression but dose-dependently increased COX2 gene and protein in a dose response manner (5-20μM). Twenty μM of tBHQ treatment enhanced expression of COX2 gene and protein by 10.7±2.2 and 9.2±3.4 fold (both $p<0.01$, N=3), COX2 activity (from COX2-based product of fluorescent resorufin) by 229.9±28.8% ($p<0.005$, N=6) but reactive oxygen species (ROS) generated in the cells (from oxidized dihydroethidium-based fluorescent intensity) were reduced by 35.1±10.2% ($p<0.01$, N=4). tBHQ also significantly promoted Nrf2 nuclear translocation by 86±7.4% ($p<0.05$, N=3) and potentiated Nrf2 activity by 387.5±19.2% ($p<0.005$, N=4) determined with cell fraction immunoblotting and Nrf2-ARE binding-based enzyme-linked immunosorbent assay. Nrf2 expression was dramatically knocked down with markedly decreased COX2 expression and activity in the cells transfected with Nrf2 specific siRNA and treated with tBHQ compared to those transfected with the scramble control siRNA and treated with tBHQ. Chromatin immunoprecipitation (CHIP)-based PCR analysis revealed an enhanced recruitment of Nrf2 to the endogenous COX2 promoter spanning the ARE (-417 to -426 nts) after 24 hour-tBHQ treatment but knockdown of Nrf2 gene with siRNA significantly diminished the tBHQ-mediated role, suggesting that there is a functional ARE located in COX2 promoter. In conclusion, these results show a novel role of Nrf2 in inducing COX2 expression through binding to promoter ARE in the absence of increased ROS in rat PGVSMCs.

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Age-Related Endothelial Senescence is Driven by Thrombospondin-1-Activated NADPH Oxidase Nox1

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Organismal aging represents an independent risk factor underlying many vascular diseases, including systemic and pulmonary hypertension, and atherosclerosis. While the

mechanisms driving aging are largely elusive, a steady persistent increase in tissue oxidative stress has been associated with senescence. Previously we showed TSP1 elicits NADPH oxidase (Nox)-dependent vascular smooth muscle cell oxidative stress. However mechanisms by which TSP1 affects endothelial redox biology are unknown. Here, we tested the hypothesis that TSP1 induces endothelial oxidative stress-linked senescence in aging. Using rapid autopsy disease-free human pulmonary (PA) artery, we identified a significant positive correlation between age, protein levels of TSP1, Nox1 and the cell-cycle repressor p21cip ($p<0.05$). Age also positively associated with increased Amplex Red-detected PA hydrogen peroxide levels ($p<0.05$). Moreover, treatment of human PA endothelial cells (HPAEC) with TSP1 (2.2nM; 24h) increased expression (~1.9 fold; $p<0.05$) and activation of Nox1 (~1.7 fold; $p<0.05$) compared to control, as assessed by Western blot and SOD-inhibitable cytochrome c reduction. Western blotting and immunofluorescence showed a TSP1-mediated increase in p53 activation, indicative of the DNA damage response. Moreover, TSP1 significantly increased HPAEC senescence in a p53/p21cip/Rb-dependent manner, as assessed by immunofluorescent detection of subcellular localization and senescence-associated β-galactosidase staining. To explore this pathway in vivo, middle-aged (8-10 month) wild-type and TSP1-null mice were utilized. In the TSP1-null, reduced lung senescence, oxidative stress, Nox1 levels and p21cip expression were observed compared to wild-type supporting findings in human samples and cell experiments. Finally, prophylactic treatment with specific Nox1 inhibitor NoxA1ds (10μM) attenuated TSP1-induced HPAEC ROS, p53 activation, p21cip expression and senescence. Taken together, our results provide molecular insight into the functional interplay between TSP1 and Nox1 in the regulation of endothelial senescence, with implications for molecular control of the aging process.

D.N. Meijles: None. **I. Al Ghouleh:** None. **S. Sahoo:** None. **J.H. Amaral:** None. **H. Knupp:** None. **S. Attaran:** None. **R. Bienes-Martinez:** None. **J.C. Sembrat:** None. **M. Rojas:** None. **J.S. Isenberg:** None. **P.J. Pagano:** None.

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Luminal Flow Induces NADPH Oxidase 4 Translocation to the Nuclei of Thick Ascending Limbs

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Superoxide (O2-) exerts its physiological and pathophysiological actions in part by causing changes in gene transcription. In the thick ascending limb of the loop of Henle (TAL), luminal flow-induced O2- production is mediated by NADPH oxidase 4 (NOX4). Recently, polymerase (DNA-directed), delta interacting protein 2 (poldip2) has been shown to increase NOX4 activity. However, it is not known whether NOX4 translocates to the nucleus when activated or whether poldip2 participates in this process. We hypothesized that luminal flow causes translocation of NOX4 to the nucleus of the TAL facilitating its interaction with poldip2 in a protein kinase C (PKC)-dependent process. To test our hypothesis, we studied the subcellular localization of NOX4 and poldip2 using confocal microscopy and measured O2− production. Isolated TALs were studied in the absence and presence of luminal flow. To disrupt the cytoskeleton and inhibit PKC we used cytochalasin D (1uM) and staurosporine

(10nM), respectively. Luminal flow increased the amount of NOX4 in the nucleus from 4±1 arbitrary units (AU) to 31±1 AU ($p < 0.0001$; n=6). This redistribution was blocked by cytochalasin D (7±2, $p < 0.04$ vs. controls; n=6). Similarly, staurosporine decreased the intensity of NOX4 within the nuclear region of perfused TALs (3±2 AU vs controls, $p < 0.01$; n=5), Luminal flow increased poldip2 in the nucleus from 18±1 AU (n=3) to 29±1 AU ($p<0.01$; n=6). Net O2- production increased in response to luminal flow from 89±15 AU/S to 231±16 AU/S ($p < 0.001$; n=6). Inhibiting PKC decreased flow-induced O2− production in isolated TALs. However, disrupting the actin cytoskeleton with cytochalasin D did not block flow-induced O2− production (no flow + cytochalasin: 135± 19 vs. flow + cytochalasin: 285 ± 41 AU; $p <0.04$; n=7). We conclude that NOX4 translocates to the nucleus when activated by luminal flow, as does poldip2. Translocation is dependent on the actin cytoskeleton but O2− production is not. In contrast PKC mediates both processes. Translocation of NOX4 to the nucleus may facilitate changes in gene transcription caused by O2−.

F. Saez: None. **J.L. Garvin:** None.

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Overexpression of the Transcription Factor Dlx5 in the Placenta of Preeclamptic Patients Leads to Decreased Trophoblast Proliferation - A Novel Mechanism Involving Loss of Imprinting

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Objectives
Preeclampsia (PE) is one of the most-common, life-threatening pregnancy complication that affects maternal and offspring health, with short and long-term implications.
We hypothesize that epigenetic abnormalities and/or dysregulation of imprinted genes together with genes located in their proximity during placentation affect expression pattern of a broad spectrum of genes leading to preeclampsia. These genes can induce life-long epigenetic changes in the genome of the mother following preeclamptic pregnancy.
Methods and Results
Using large-scale bioinformatical analysis of human placenta gene expression in 25 preeclamptic patients and 23 controls we identified characteristic pattern for PE. It consists of three clusters of patients, suggesting that preeclampsia is a complex, heterologous disease. Moreover, the analysis identified distal-less homeobox 5 (DLX5), a maternally expressed gene, which is significantly upregulated in preeclamptic placenta (1.5 fold). We confirmed upregulation of DLX5 on mRNA level in the early onset (0.8 (n=6), indicating that LOI is involved in overexpression of DLX5 in PE. DLX5 is primarily known as a developmental transcription factor and is crucial for bone development. Although DLX5 is highly expressed in human placenta, its function and the target genes in trophoblasts as well as its

role during placentation still remains unknown. Using Sleeping Beauty (SB) transposon system, we stably overexpressed DLX5 in SGHPL-4 trophoblast cell line that significantly reduced trophoblast proliferation as indicated by high throughput sampler cell count (654.2 ± 395.5 vs. 1709 ± 1119) and MTT colorimetric assay (A570: 0.154 ± 0.097 vs. 0.29 ± 0.099).
Conclusions
We show that the imprinted transcription factor DLX5 is upregulated in preeclampsia and propose loss of imprinting as one underlying mechanism. Overexpression of DLX5 in first trimester trophoblasts reduces cell proliferation. Our data support the importance of dysregulated epigenetic mechanism as an important feature in preeclampsia.

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Systemic Administration of (Pyr1)-Apelin-13 at Late Pregnancy Reduces Blood Pressure, Proteinuria, and Improves Autonomic Function in Preeclamptic Rats

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Preeclampsia is associated with maternal perinatal morbidity and mortality and a high risk of premature birth and intrauterine growth restriction. The apelin system is a novel pleiotropic pathway with a potential for therapeutic targeting in preeclampsia. We have previously reported that total apelin content is lower in human preeclamptic chorionic villi. In this study, we determined whether (Pyr1)-apelin-13 improves hypertension, proteinuria, fetal characteristics, uteroplacental hemodynamics, and the autonomic function in preeclamptic rats (TgA, female transgenic for human angiotensinogen mated to male transgenic for human renin). (Pyr1)-apelin-13 (2 mg/kg/day) (n=7) or saline (n=5) was infused in TgA via osmotic minipumps starting at day 13 of gestation, when blood pressure begins to increase in these animals. Pregnant SD (n=6) rats were used as controls. At the 20th day of pregnancy, TgA rats had higher MAP (138±6 vs. 79±3 mmHg in SD, $p<0.001$) which was reduced by (Pyr1)-apelin treatment to 119±2 mmHg vs. TgA, $p<0.006$. TgA rats also had impaired heart rate variability measured as root of mean successive differences (rMSSD) compared with SD (2.7±0.4 vs. 3.8±0.3 ms in SD, $p<0.05$). Apelin treatment normalized rMSSD to 3.6±0.3, $p<0.05$. Similarly, baroreflex sensitivity measured in the sequence domain was lower in TgA (0.7±0.1 vs. 2.6±0.5 ms/mmHg in SD, $p<0.01$) and normalized with (Pyr1)-apelin-13 to 2.0 ± 0.4 ms/mmHg, $p<0.05$. Proteinuria was greater in TgA (53±9 vs. 10±2 mg/kg/day, $p<0.001$), and normalized by (Pyr1)-apelin-13 (18±6, $p<0.05$). Pup (3.0±0.1 vs. 3.7±0.1 g, $p<0.01$) and placental weight (0.41±0.01 vs. 0.45±0.01 g, $p<0.01$), and pup number (10.7±1.1 vs. 14.0±0.8, $p<0.01$) were lower in TgA vs. SD; however they were not changed by (Pyr1)-apelin-13. Uterine artery peak systolic velocity was not different between SD and TgA,

but increased with (Pyr1)-apelin-13 treatment (179.5±16.7 vs. 122.6±16.7 ml/min, p<0.05) with no change in resistance index. In conclusion, our findings suggest that (Pyr1)-apelin-13 may be beneficial for the treatment of preeclampsia due to its hemodynamic and renoprotective effects. We also report for the first time that these changes may involve central control of the cardiovascular system.

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Agonistic Autoantibodies to Angiotensin II Type I Receptor Contributes Partly to Placental Ischemia-induced Cerebrovascular Abnormalities

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Placental ischemia, a characteristic feature of preeclampsia, leads to impaired cerebral blood flow (CBF) autoregulation, cerebral edema, and increased blood-brain barrier (BBB) permeability; however, the placental factors that contribute to these cerebral abnormalities are not clear. Agonistic autoantibodies to the angiotensin II type 1 receptor (AT1-AA) are increased in preeclamptic patients as well as in a rat model of preeclampsia induced by placental ischemia. In this study, we tested the hypothesis that AT1-AA mediates placental ischemia-induced cerebrovascular abnormalities. To determine whether the AT1-AA contributes to impaired CBF autoregulation, we infused purified rat AT1-AA into normal pregnant rats from gestational day (GD) 12 to 19 via mini-osmotic pumps and measured CBF using laser Doppler flowmetry on GD 19. Autoregulatory index increased from 0.7 to 1.0±0.2 in the AT1-AA infused group over the range of 120 to 160 mmHg compared to pregnant controls (0.3 to 0.5±0.1 over the same range of pressures, p<0.05) suggesting impaired CBF autoregulation. However, AT1-AA infusion did not affect brain water content at baseline blood pressures (104±2 mmHg in normal pregnant rats vs. 113±2 mmHg in AT1-AA infused rats, p<0.01). To determine the role of endogenous AT1-AA in mediating placental ischemia-induced cerebrovascular abnormalities, losartan (5 mg/kg/day), an AT1 receptor antagonist, was administered in the drinking water from GD 14 to 19. Losartan reduced anterior brain water content from 79.6±0.2% in placental ischemic rats to 79.2±0.1% (compared to 79.1±0.1% in normal pregnant untreated rats) and BBB permeability from 0.06±0.01 in placental ischemic rats to 0.03±0.03 (compared to 0.03±0.004 in normal pregnant untreated rats). These results indicate that impaired CBF autoregulation in response to placental ischemia is due, at least in part, to increases in circulating AT1-AA. While AT1-AA infusion, by itself, did not alter brain water content at baseline blood pressures, the beneficial effects of losartan in placental ischemic rats suggests that the renin-angiotensin system may interact with other placental factors to promote cerebral vascular changes common to preeclampsia.

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Role of Hypoxia-dependent Autophagy in the Trophoblast of BPH/5, a Mouse Model of Pre-eclampsia (PE)

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Autophagy, a mechanism of cell survival, plays an important role in normal placentation and protects the fetus during stress. Previously, we showed that autophagy was increased in fetoplacental units (FPUs) from pregnant BPH/5 mice, a model that develops a PE-like syndrome including late-gestational hypertension, proteinuria, abnormal placentation and poor feto-placental outcomes. We also showed that increased autophagy led to reduced invasion capacity of BPH/5 trophoblasts. Evidence suggests that hypoxia increase autophagy, so we measured the invasion capacity of trophoblasts isolated from C57 mice in normoxic/hypoxic conditions. Trophoblasts cultured in hypoxia exhibited defective invasion, effect partially blocked by autophagy inhibitor chloroquine diphosphate (CQ; 1.7-fold decrease, n=4 $p<0.005$ hypoxia vs. normoxia; 1.35-fold increase $p<0.5$ CQ vs. hypoxia). We also tested the effect of conditioned media from BPH/5 or C57 placental explants on the invasion capacity of JAR cells, a human choriocarcinoma trophoblast cell line. Conditioned media from BPH5 explants decreased JAR invasion, but only for 24h after placental collection (1.8-fold decrease, n=4 $p<0.005$ vs. C571.33-fold $p<0.05$ vs. BPH/5). However, when the explants were kept in hypoxia, JAR invasion capacity decreased following treatment with BPH5 explants conditioned media, even after 48h; effect partially inhibited by CQ (1.6-fold decrease, n=4 $p<0.005$ vs. CTR; 1.7-fold, n=4 $p<0.05$ vs. BPH/5). As autophagy plays a key role in the invasion of trophoblasts and thus remodeling of uterine arteries, we performed a tube formation assay with endothelial cells treated with conditioned media from BPH/5 or C57 placental explants. Tube formation decreased after treatment with conditioned media from BPH/5 explants (1.7-fold decrease, n=3 $p<0.05$). Co-culture of endothelial and trophoblast cells under hypoxia resulted in less efficient tube formation. Treatment with CQ partially blocked this effect (1.55-fold decrease, n=3 $p<0.05$ vs normoxia; 1.2-fold, n=3 $p<0.05$ vs hypoxia). These data demonstrate that invasion capacity and tube formation are defective in trophoblasts from BPH/5 mice, suggesting that hypoxia-dependent autophagy may play a causal role in PE in the BPH/5 model.

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A Biopolymer-Stabilized VEGF Chimera Reverses Angiogenic Imbalance *in vitro* in the Reduced Uterine Perfusion Pressure Model of Preeclampsia

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Preeclampsia (PE) is a hypertensive disorder that complicates approximately 5-8% of all

pregnancies in the U.S. The unknown etiology of PE develops from molecular dysfunction at the maternal-placental interface, where inflammatory, oxidative stress, and anti-angiogenic pathways are initiated. The resulting hypoxic *in utero* environment contributes to placental ischemia from which the anti-angiogenic factor soluble fms-like tyrosine kinase-1 (sFlt-1) is released. A truncated isoform, sFlt-1 consists solely of the extracellular domain of the vascular endothelial growth factor (VEGF) receptor 1, and hence lacks both catalytic and regulatory activities. However, sFlt-1 retains a high affinity for VEGF and sequesters this ligand from binding with fully functional VEGF receptors, thus prevents the activation of angiogenic remodeling pathways. The rodent model of PE, reduced uterine perfusion pressure (RUPP), exhibits the sFlt-1 pathophysiology observed in human PE. In an effort to counteract excessive sFlt-1 production and restore angiogenic balance, we have constructed a VEGF chimera fused to an Elastin-like Polypeptide (ELP) carrier with increased in vivo half-life and stability which retains full VEGF signaling activity. When human umbilical vein vascular endothelial cells (HUVECs) are exposed to serum from normal pregnant or RUPP-treated rats, tube formation on extracellular matrix is inhibited 31% (± 2%) by the RUPP serum. This inhibition is reversed when ELP-VEGF, but not ELP control, is added to the culture medium (p = 0.0007, one-way ANOVA with Bonferroni multiple comparison), suggesting that ELP-VEGF counteracts the anti-angiogenic factors present in RUPP serum. We also characterized the pharmacokinetics, biodistribution, and placental transfer of ELP-VEGF in the pregnant Sprague Dawley rat. These
studies indicate that ELP-delivered VEGF has potential for counteracting the circulating anti-angiogenic factors in maternal plasma.

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Sphingosine-1-Phosphate Signaling Plays a Role in High Blood Pressure Programmed by Intrauterine Growth Restriction in Mouse

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Intrauterine growth restriction (IUGR) is a risk factor for hypertension and cardiovascular (CV) disease in later life, but the underlying mechanisms remain unclear. The bioactive sphingolipid metabolite sphingosine-1-phosphate (S1P) is critically involved in CV development in the fetus, and plays a significant role in the regulation of CV health in adulthood. S1P receptor (S1PR) type 1, 2 and 3 are widely expressed in CV system which S1PR1 has a protective role against kidney injury, while S1PR3 is involved in controlling BP. Yet, the contribution of S1P on BP in IUGR is unknown. In the present studies, we tested the hypothesis that IUGR alters renal S1P receptors expression during- and post-nephrogenesis, which contributes to high BP in male IUGR mouse. C57bl/6J mice underwent sham or reduced

uterine perfusion (RUP) at day 13 of gestation with delivery at full term. IUGR offspring (from RUP dam) had a lower birth weight than control ($p<0.05$). Kidneys were isolated from 2 day old male pups or adult 24 week old male control and IUGR. S1PR3 protein expression was increased in 2 day old IUGR kidneys (2.4 fold vs control, N=3, $p< 0.01$). At 24 weeks of age, S1PR3 mRNA levels were increased (1.2 fold vs control, N=4, $p< 0.05$) whereas S1PR3 protein levels were decreased (0.75 fold vs control, N=4, $p< 0.05$) in IUGR kidneys. mRNA and protein expression levels of S1PR1 and S1PR2 were not different between control and IUGR kidneys. Finally, we assessed the role of S1PRs agonist on BP of IUGR. Male IUGR offspring had a significantly higher BP compared to male control via carotid catheter in the conscious state (control: 112.1±2.1, IUGR: 125.0±3.7 mmHg; N=7, $P<0.05$). Acute administration of FTY720 (1 mg/kgBW i.p, Fingomod), a S1P receptor type 1, 3 agonist did not significantly alter BP in control (106.0 ± 5.7 mmHg) but significantly decreased BP in IUGR (105.7±2.3 mmHg, $p< 0.05$). A dose response to FTY720 (10 mg/kgBW) decreased BP in both control (94.0±2.0 mmHg, $p< 0.05$) and IUGR (99.3±2.3 mmHg, $p< 0.05$). Together our data suggest that IUGR programs an alteration of renal S1PR3 expression in both during- and post-nephrogenesis thereby contributing to an increase in sensitivity to S1PRs agonist. Thus, S1P signaling is a putative mechanism underlying the hypertension of IUGR offspring.

S. Intapad: None.

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Endothelial Cell Mineralocorticoid Receptor: A Critical Player in Aortic Stiffness and Cardiac Diastolic Dysfunction in Female Mice

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Enhanced activation of mineralocorticoid receptors (MRs) impairs insulin metabolic signaling, increases oxidative stress, and induces maladaptive immune responses with associated CV abnormalities. Emerging information suggests that obesity and insulin resistance predict CV stiffness in females. However, the specific role of endothelial cell MR (ECMR) has not been explored. Accordingly, we hypothesized that ECMR signaling modulated by a western diet (WD) impairs insulin signaling and increases inflammation, fibrosis and CV stiffness in females. Four week-old ECMR knockout ($ECMR^{-/-}$) and wild-type female mice were fed a mouse chow or WD containing fat (46%), sucrose (17.5%), and high fructose (17.5%) for 16 weeks. WD prompted MR to bind the hormone response element (nGnACAnnnTGTnCn) on the site of ENaC promoter and induced an increase in ENaC expression that was associated with increased aortic and EC stiffness as determined by in vivo pulse wave velocity and ex vivo atomic force microscopy techniques, respectively The elevated aortic stiffness was accompanied by increased expression of cytokines IL-17, MCP-1 and M1 markers CD 86, and CD11c. ENaC expression was reduced in the $ECMR^{-/-}$ vasculature with a decrease in WD-increase in aortic and EC stiffness.. $ECMR^{-/-}$ also improved aortic vasorelaxation to Ach, SNP (10^{-9}-10^{-4} mol/L), and insulin (0.1- 300 ng/ml), which were impaired by WD. Additionally, $ECMR^{-/-}$ restored WD-induced cardiac diastolic dysfunction assessed by cardiac MRI and echocardiography.

Diastolic dysfunction was related to cardiomyocyte hypertrophy, oxidative stress, and fibrosis and occurred with enhanced activation of S6 kinase-1, Erk 1/2, serine phosphorylation of IRS-1, inactivation of PI3K-AKT-eNOS signaling pathways and the pro-fibrotic TGF-β1/ Smad signaling pathway and increased macrophage pro-inflammatory polarization. $ECMR^{-/-}$ markedly attenuated the cardiac functional and changes signaling induced by WD. These findings suggest that increased ECMR signaling and associated ENaC activation plays a key role in WD induced insulin metabolic sinaling impairment, adaptive pro-inflammatory responses, macrophage polarization and associated aortic stiffness and cardiac diastolic dysfunction in females.

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Leptin-mediated Aldosterone Secretion Causes Hypertension in Obese Females

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Obesity causes hypertension (HTN) in males and females. While leptin contributes to obesity-induced HTN by increasing sympathetic activity, in males, it is unknown whether similar mechanisms trigger HTN in obese females. Females secrete 3 to 4 times more leptin than males, but do not exhibit high sympathetic tone with obesity. They however show inappropriately high aldosterone levels that positively correlate with adiposity and blood pressure (BP). Here we hypothesized that leptin induces HTN by increasing aldosterone production in obese females. Hypersensitivity to leptin, in lean mice deficient in protein tyrosine phosphatase 1B (PTP1B) or high leptin levels, in obese Agouti (Ay/a) mice induced HTN (WT: 115±2; KO: 124±2; a/a: 113±1; Ay/a: 128±7mmHg, $p<0.05$) but did not increase sympathetic control of BP (response to ganglionic blockade). Leptin sensitization and obesity however elevated plasma aldosterone levels and adrenal aldosterone synthase (CYP11B2) expression, in females. Chronic leptin (KO+AA: 115±5; Ay/a+AA: 114±5mmHg) or mineralocorticoid (KO+spiro:111±5; Ay/a+spiro: 121±6mmHg) receptors inhibition restored BP to baseline levels in females PTP1B KO and obese agouti mice. Leptin or leptin receptor deficiency in female ob/ob and db/db mice, abolished obesity-induced increases in adrenal CYP11B2 and plasma aldosterone while chronic leptin infusion in female mice triggered a dose-dependent increase in adrenal CYP11B2 and plasma aldosterone levels. Leptin-mediated aldosterone secretion was independent of changes in plasma angiotensin II, potassium and corticosterone (index of ACTH levels) and preserved in the presence of losartan or α and β-adrenergic receptors antagonists. Stimulation of human adrenocortical cells with leptin dose-dependently increased CYP11B2 expression and aldosterone production. While investigating the interaction between percentage of body fat, leptin and aldosterone levels in young healthy adult Caucasians we reported a positive correlation between adiposity and aldosterone, and between leptin and aldosterone in adult women only. Together these data suggest that leptin directly regulates aldosterone secretion and that leptin induces HTN via aldosterone dependent mechanisms in obese females.

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Role of Vascular and Myeloid Mineralocorticoid Receptor in Renal Ischemia/reperfusion

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Introduction: Renal ischemia/reperfusion (IR) is a major cause of acute kidney injury. It is associated with cardiac alterations and chronic kidney disease (CKD) development. We previously showed that mineralocorticoid receptor (MR) antagonism prevents the acute and chronic consequences of renal IR (Barrera-Chimal, Kidney Int, 2013 and JASN, 2015). However, whether the benefit of the MR antagonists is due to the blockade of the MR expressed in the vessels is unclear.
Objective: To study the specific contribution of endothelial and smooth muscle cells MR in acute and chronic consequences of renal IR.
Methods: To evaluate the contribution of vascular MR we generated two knockout (KO) mouse models. To allow MR inactivation in endothelial cells (MR^{endoKO} mice), floxed MR mice ($MR^{fl/fl}$) were crossed with mice expressing the inducible Cre recombinase under the VEcadh promoter. To allow MR inactivation in vascular smooth muscle cells (MR^{SMCKO} mice), $MR^{fl/fl}$ mice were crossed with mice expressing the inducible Cre recombinase under the SMA promoter. In these mice, sham surgery or bilateral renal IR for 20 min was performed in $MR^{fl/fl}$ and KO mice and the animals were studied at short term (24 h) and long term (30 days) after reperfusion.
Results: In $MR^{fl/fl}$ mice, IR induced renal dysfunction (plasma creatinine raised from 8.9±0.3 in sham to 33.8±4.8 umol/L in IR), tubular injury and increased mRNA levels of kim-1 (400-fold) and NGAL (220-fold). The MR^{endoKO} mice displayed similar alterations induced by IR as $MR^{fl/fl}$ mice. In contrast, after 24 h of renal IR, the MR^{SMCKO} mice presented normal renal function (plasma creatinine was 9.6±0.7 and 14.0±1.9 umol/L in sham and IR, respectively), absence of histological alterations and reduced kim-1 and NGAL levels.
After 30 days, the $MR^{fl/fl}$ mice developed CKD characterized by renal dysfunction (plasma creatinine from 10.5±0.1in sham to 15±0.8 umol/L in IR), tubule-interstitial fibrosis and increased mRNA levels of fibronectin and Galectin-3 (2-fold). The MR^{SMCKO} mice developed similar alterations.
Conclusion: We provide evidence that the deficiency of MR in the SMC protects against the development of acute kidney lesions induced by IR, however MR deficiency in SMC did not impact the appearance of CKD induced by IR.

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Activation of the Mineralocorticoid Receptor by O-glycosylation Through Hyperglycemia

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Introduction: Hypertension and diabetes are independent risk factors for cardiovascular disease, however why they frequently occur together is not clear. Inappropriate mineralocorticoid receptor (MR) activation is associated with diabetes and the metabolic syndrome, as well as hypertension and abnormal cardiovascular remodeling. MR antagonists are effective in reducing hypertension and delaying the onset of renal and cardiovascular complications in diabetes despite circulating aldosterone levels that are within normal limits. Glucose concentrations increase O-glycosylation of many proteins, thus alter their function. Hence, we hypothesized that increased O-GlcNac modification of the MR by high glucose enhances MR activation.
Methods: MR transcriptional activity was studied in a mouse cortical collecting duct (M1) cell line stably transfected with a cDNA construct including the MR and one with a hormone-response element driving a Gaussia luciferase reporter gene. The cells were incubated for 48 h with low (5mM) or high (25mM) glucose media with and without Thiamet-G (TMG), an O-GlcNAcase inhibitor to inhibit deglycosylation, and 6-diazo-5-oxonorleucine (DON), a glucosamine-fructose-6-phosphate amidotransferase inhibitor (GFAT) to reduce O-GlcNAc levels. Additionally, MR and GR antagonists were used to identify receptor specificity under low and high glucose conditions. O-GlcNac-modified MR was co-immunoprecipitated with an MR antibody and detected with an O-GlcNAc antibody.
Results: 1. Co-immunoprecipitation assays showed that high glucose and TMG increased O-GlcNac-MR by 3-fold. 2. Compared to low glucose, treatment with high glucose and with TMG increased the transcriptional activity of MR by 300%. 3. DON decreased MR-reporter activity by 75%. 4. High glucose alone had no significant basal effect but significantly increased MR activation by aldosterone. 5. MR reporter activity was increased similarly by aldosterone and corticosterone.
Conclusion: High glucose increased glycosylation of the MR, augmenting its transcriptional activity. Enhancement of MR activation by hyperglycemia may explain how MRs play a significant role in the cardiorenal pathology in Diabetes.

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Activated Intrarenal Renin Angiotensin System Contributes to the Development of Chronic Kidney Disease in Primary Aldosteronism

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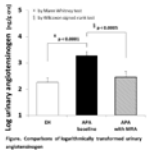
Background: Patients with primary aldosteronism (PA) have higher prevalence of albuminuria than those with essential hypertension (EH). We have shown that about 15% in PA patients had chronic kidney disease (CKD). Hence, the active screening of PA is important to prevent CKD. Urinary angiotensinogen (UAGT) has been reported as the earliest predictor of nephropathy in patients with type 1 diabetes (DM). UAGT indicates the intrarenal renin angiotensin systems (RAS) activation which plays an important role in the development of CKD. This study aimed to confirm the existence of activated intrarenal RAS by measuring UAGT in patients with PA.
Methods: Consecutive 58 patients of aldosteronoma (APA) participated. No patients

had CKD defined as eGFR <60 ml/min/1.73m² or urinary albumin (UA) ≥30 mg/g cre and DM defined as HbA1c ≥6.5% or taking treatments. As control subjects, 59 EH patients were included. All APA patients were treated by sufficient dose of mineralocorticoid receptor (MR) antagonists (MRA) until the day of adrenalectomy. Urine samples were collected on 10g salt diet between baseline and after MRA treatment. UAGT (ng/g cre) was measured and the value was transformed by logarithm.
Results: Age, sex distribution, blood pressure, duration of hypertension, eGFR, and UA were similar between two groups at baseline. However, UAGT was significantly higher in APA than in EH (Fig). In multivariate analysis, only plasma aldosterone was significantly related with UAGT in APA (t=3.572, p=0.0007). UAGT was significantly decreased after MRA treatment (Fig).
Conclusion: Intrarenal RAS is activated by aldosterone/MR in patients of PA, which might contribute to the development of CKD.



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Mineralocorticoid Receptor Activation In Macrophages Mediates High Fat/high Sucrose Induced Vascular Stiffness in Female Mice

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Enhanced mineralocorticoid receptor (MR) activation promotes vascular dysfunction and remodeling. We studied the role of MR activation in perivascular adipose tissue (PVAT) macrophages in the development of high-fat/high-fructose diet ("Western Diet", WD) induced vascular stiffness in female mice. Myeloid MR KO mice (MyMRKO) were fed a WD for 8 weeks (n=3). MyMRKO female mice (n=2) were infused with aldosterone (Aldo) for 3 weeks. Aortic stiffness was assessed in vivo by pulse wave velocity and ex vivo by atomic force microscopy. Vascular reactivity was studied in aortic rings.
MyMRKO mice were protected from Aldo and WD induced aortic stiffness and had greater endothelial-dependent and independent vasodilation compared to littermates (Fig. 1, 2). Immunohistochemistry suggested decreased peri-aortic fibrosis and macrophage infiltration in Aldo infused MyMRKO (Fig.1).
We conclude that MR KO in myeloid cells protects against WD and Aldo-induced vascular stiffness in female mice. Our data support a role for MR activation in PVAT macrophages in the pathogenesis of WD-induced vascular dysfunction.

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Conditional Knockout of Sphingosine-1-Phosphate Receptor 1 in Renal Collecting Ducts Promotes Sodium Retention in Mice

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Sphingosine-1-phosphate (S1P) is a bioactive sphingolipid metabolite formed by phosphorylation of sphingosine. We have recently shown that renal medullary infusion of S1P agonist increases sodium excretion possibly through inhibition of epithelial sodium channel (ENaC) activity via S1P receptor 1 (S1P1), which is prominently localized in the collecting ducts. The present study was to test the hypothesis that the S1P1 in collecting duct plays a pivotal role in the regulation of renal sodium handling. We generated conditional knocked-out (CKO) mice with collecting duct-specific ablation of S1P1 using loxP-Cre system. Functionally, urinary sodium excretion in S1P1 CKO mice was significantly decreased by about 60% compared with that in control mice after acute IV sodium loading (0.84±0.16 vs. 2.22±0.62 µmole/min/g kwt). Moreover, the pressure natriuresis was severely impaired in S1P1 CKO mice compared with that in the control mice (4.32±1.04 vs. 8.73±0.19 µmole/min/g kwt). Furthermore, chronic high salt-induced sodium retention was remarkably enhanced in S1P1 CKO mice comparing to the control animals (5.27±0.39 vs. 2.38±1.04 mmole/24h/100g bw). Our results suggested that S1P1 in collecting duct plays an important role in the regulation of renal sodium excretion and that deficiency of S1P1 in collecting duct impairs renal sodium handling and promotes sodium retention. Modulation of S1P1 function in the renal medulla could be a therapeutic approach for salt-sensitive hypertension.

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High Salt Intake Augments Excitability of Pre-sympathetic PVN Neurons Through Dysfunction of the Endoplasmic Reticulum Ca2+ ATPase

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High salt (HS) intake sensitizes pre-sympathetic neurons in the hypothalamic paraventricular nucleus (PVN) leading to augmented neuronal excitability. Recently, we reported that dysfunction of Ca^{2+} dependent K^+ channels in the PVN contributes to HS intake induced sympathoexcitation. The endoplasmic reticulum (ER) acts as a Ca^{2+} store and plays an important role in regulating intracellular Ca^{2+} homeostasis. The ER Ca^{2+} ATPase is responsible for maintaining the high level of ER Ca^{2+} and loss of function would deplete the Ca^{2+} store contributing to the reduced activity of Ca^{2+} dependent K^+ channels. We hypothesized that a 2% (NaCl) HS diet for 5 weeks would reduce function of the ER Ca^{2+} ATPase and augment excitability of PVN neurons with axon projections to the rostral ventrolateral medulla (PVN-RVLM) identified by retrograde label. In whole cell current-clamp recordings from PVN-RVLM neurons, graded current injections evoked graded increases in spike frequency. Maximum discharge was evoked by +200 pA injections and averaged 22±2 Hz (n=6) in normal salt (NS) control and was significantly augmented ($p<0.05$) by HS diet 34±5 Hz (n=8). Bath application of thapsigargin (TG) (0.5 µM),

the ER Ca^{2+}ATPase inhibitor, augmented excitability of PVN-RVLM neurons in NS (32±4 Hz, n=5, p<0.05), yet had no significant effect in HS rats (32±6 Hz, n=6). ER Ca^{2+} ATPase function was assessed in whole animal preparations by bilateral PVN microinjection of TG in anesthetized rats. PVN microinjection of TG (0.15, 0.3 0.75 and 1.5 nmol/100nl) increased sympathetic nerve activity (SNA) and mean arterial pressure (MAP) in a dose-dependent manner in NS rats. Maximum increases in splanchnic SNA (SSNA), renal SNA (RSNA) and MAP elicited by PVN TG (0.75 nmol/100nl; n=5) were 93±7%, 75±7%, and 11±2mmHg, respectively. In contrast, sympathoexcitatory responses to PVN TG (0.75 nmol/100nl; n=5) were attenuated in HS treated rats (SSNA 41±8%, RSNA 22±5%, p<0.05 vs. NS) while MAP responses demonstrated no significant difference (+8±2 mmHg, p>0.05 vs NS). Our data indicate that a HS diet reduces ER Ca^{2+} ATPase activity and augments excitability of PVN-RVLM neurons in vitro. Altered ER Ca^{2+} homeostasis may contribute to sympathoexcitation through loss of Ca^{2+} dependent K^+ channel activity in the PVN.

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Renal Afferent Nerve Sensitivity and the Pathophysiology of Salt-sensitive Hypertension

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Aim: Altered renal afferent nerve responsiveness contributes to hypertension in the Spontaneously Hypertensive rat. We hypothesized that increased salt-intake differentially impacts the renal afferent nerve sensitivity in salt-resistant vs salt-sensitive rats.
Methods: Groups of naïve Sprague-Dawley (SD), Dahl Salt-Resistant (DSR), Dahl Salt-Sensitive (DSS) or SD rats receiving a s.c. norepinephrine (NE:600ng/min) infusion were fed a 0.4% (NS) or 8% NaCl (HS) diet for 14 (SD) or 21 days (DSR & DSS). Following HS intake MAP and plasma NE content were determined. Via a renal pelvis preparation afferent nerve activity was assessed as NE-evoked (6250 pmol) substance P (SP) release (N=5/gp).
Results: Salt-resistant phenotypes (SD & DSR) remain normotensive and exhibit HS-evoked suppression of plasma NE and increased renal afferent nerve release of substance P. In contrast in salt-sensitive phenotypes (DSS and NE infused SD) a high salt diet evoked hypertension, increased plasma NE and abolished sodium evoked increased responsiveness of the renal afferent release of substance P.
Significance symbols: *p<0.05 vs. resp. NS gp; τ p<0.05 vs. resp. SD Naïve gp. value.
Conclusion: These data support a role of the afferent renal nerves in blood pressure regulation during high salt-intake. Increased responsiveness of the renal afferent nerves contributes to the maintenance of normotension in a salt-resistant rat phenotype. Conversely, our data suggest that in salt-sensitive phenotypes excess release of NE triggers impaired activation of the renal afferent nerves, a factor we postulate

contributes to the development of salt-sensitive hypertension.

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Deficiency of Serum Glucocorticoid Kinase 1 (SGK1) in T Lymphocytes Blunts Hypertension and Abrogates Renal/Vascular Inflammation

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We have previously shown that the T cell-derived pro-inflammatory cytokine, interleukin 17A (IL17A), is upregulated by and promotes angiotensin II-induced hypertension and contributes to renal and vascular dysfunction. It was recently demonstrated that an excess of 40 mM of sodium chloride enhances IL17A production from CD4+ T cells in an SGK1 dependent manner. We confirmed the effect of salt on CD4+ T cell differentiation and extended this finding to CD8+ T cells in which 40mM of excess salt increased the expression of IL17A (4.7 fold, p=0.0003), TonEBP (2.3 fold, p=0.002), and the salt-sensing kinase SGK1 (2.2 fold, p=0.001) in naive CD8+ cells cultured under Th17 polarizing conditions. As dietary salt intake is linked to hypertension, we hypothesized that T cell SGK1 is essential to the ability of T cells to mediate hypertension. To test this hypothesis, we crossed $SGK1^{fl/fl}$ mice with CD4cre mice to delete SGK1 in T lymphocytes. Interestingly, loss of T cell SGK1 resulted in a blunted blood pressure response to angiotensin II infusion (by 31.25 mmHg, p=0.01). Moreover, angiotensin II-induced renal and vascular inflammation was abolished in these mice compared to $SGK1^{fl/fl}$ control mice. Angiotensin II infusion increased total (CD45+) leukocytes in the kidney from 55.4 to $120.4x10^3$ ($p<0.01$) in $SGK1^{fl/fl}$ mice while there was no increase in mice with T cell deletion of SGK1 (48.1 to $47.5x10^3$, p=ns). Similarly, angiotensin II increased total (CD45+) leukocytes in the aorta from 5.7 to $52.4x10^3$ ($p<0.01$) in $SGK1^{fl/fl}$ mice compared to no increase in mice with T cell deletion of SGK1 (16.1 to $10.1x10^3$, p=ns). Further characterization of these mice will lead to a more thorough understanding of how T cells sense salt and promote hypertension. These studies demonstrate that T cell SGK1 may be a novel therapeutic target for hypertension and the associated end-organ inflammation.

A.E. Norlander: None. **M.A. Saleh:** None. **D.G. Harrison:** None. **M.S. Madhur:** None.

141

G-Protein Coupled Receptors GPR37 and GPR37L1 Regulate Sodium Reabsorption in Renal Proximal Tubule Cells

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GPR37 and GPR37L1 are closely related G-protein coupled receptors that are expressed mainly in brain glial cells and muscle-myenteric nerve layers in the GI tract. GPR37L1 transgenic mice have decreased systolic blood pressure (SBP) whereas GPR37L1 KO mice have increased SBP. However, there are no studies reporting kidney expression of GPR37 or GPR37L1 or their role in renal blood pressure regulation. Immunostaining and immunoblotting showed that GPR37 and GPR37L1 are expressed in the

apical membrane of proximal tubules of the mouse kidney; RT-PCR on proximal tubule and collecting duct cells obtained by laser capture micro-dissection of mouse kidney sections, confirmed these findings. In addition, chronic high salt diet increased the renal expression of prosaposin, a precursor for saposin C, a natural ligand for GPR37 and GPR37L1. Infusion of prosaptide, a synthetic ligand for GPR37 and GPR37L1, decreased SBP in mice by 10 mm Hg. To determine the roles of GPR37 and GPR37L1 in renal Na+ transport, we over-expressed separately the two proteins in human renal proximal tubule (RPT) cells (n=3). Intracellular Na+ was increased in GPR37- or GPR37L1-transfected RPT cells (3.1 ± 0.8 and 3.2 ± 0.6 fold respectively, $P<0.001$) compared with mock-transfected cells. Immunoblot analyses showed increased phosphorylation of Erk1/2 (1.33 ± 0.03 and 1.52 ± 0.06 fold, $P<0.05$), and ribosomal S6 protein (1.29 ± 0.03.5 and 1.39 ± 0.08 fold, $P<0.01$) in RPT cells over-expressing GPR37 or GPR37L1, respectively. Na+,K+-ATPase expression was decreased by 29% ± 3.5 ($P<0.05$) in GPR37L1 transfected RPT cells, but was unaltered in GPR37 transfected RPT cells. Taken together, these results show that both GPR37 and GPR37L1 are expressed in RPT cells and signal via the Erk1/2 pathway. GPR37L1 increases intracellular Na+ in RPT cells by decreasing the exit of Na+ due to a decrease in Na+,K+-ATPase expression and activity at the basolateral membrane while GPR37 increases intracellular Na+, by a mechanism independent of Na+,K+-ATPase. These results indicate that GPR37 and GPR37L1 may play a role in Na+ reabsorption in RPT cells and may be novel targets to designing drugs to treat patients with hypertension.

X. Zheng: None. **I. Armando:** None. **P.A. Jose:** None. **P. Konkalmatt:** None.

A Fructose-but Not a Glucose-enriched Diet, Induces a Salt-dependent Increase in Blood Pressure and Enhances NKCC2 Phosphorylation in Normal Rats

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Human consumption of fructose as a sweetener has increased in the past 30 years. High fructose intake has been implicated in the development of hypertension, diabetes, and obesity. In the US, the upper 10th percentile of the population consumes up to 40% of their caloric intake from added sugars, in which fructose represents half of these. Fructose metabolism is strikingly different from that of glucose. Yet, the effect of a fructose or glucose-enriched diet in salt handling by the kidney, affecting blood pressure, and its interaction with high salt intake has been poorly studied. In genetic models of salt-sensitive hypertension, the activity of the $Na^+/K^+/2Cl^-$ cotransporter (NKCC2) in the thick ascending limb (TAL) is abnormally enhanced. We hypothesized that chronic fructose in drinking water induces a salt-dependent increase in blood pressure and stimulates NKCC2 during high salt intake in normal rats. Sprague-Dawley rats were given 20% fructose or 20% glucose in drinking water for 1 week after which a high salt (HS) diet (4% Na^+ in chow) was started for 3 weeks. When we measured systolic blood pressure (SBP) by tail cuff plethysmography in fructose-fed and glucose-fed rats on a HS diet, only the fructose-fed rats had an increased SBP from 120±10 to 132±6 mmHg on day 7 of HS ($p<0.01$). SBP continued to increase up to 144±18 mmHg after 3 weeks ($p<0.01$ vs glucose). Fructose or glucose alone did not increase SBP after 4

weeks. We then repeated the protocol using radiotelemetry to monitor the blood pressure (BP). In rats fed fructose, by day 5 of HS the SBP increased by 12±3 mmHg ($p<0.02$) and SBP remained elevated for 3 weeks (delta: 10±2.5 mmHg, n=3). In rats fed glucose, a HS diet did not significantly change SBP for 3 weeks (n=5). Moreover, NKCC2 activity in the TAL is enhanced by phosphorylation at Thr96, 101. We found that NKCC2 phosphorylation was higher in rats fed fructose plus HS ($p<0.02$) but not in rats fed glucose plus HS for 3 weeks (HS: 100, fructose+HS: 250±40%, glucose+HS: 95±10%). Therefore, we conclude that a high fructose (but not a glucose) diet in normal rats induces a salt-dependent increase in BP independently from caloric intake. Thus, the increase in BP may in part be due to the stimulation of NKCC2 phosphorylation in the TAL by fructose.

K.N. King-Medina: None. **E. Henson:** None. **P. Ortiz:** None.

MP01

Vascular versus Tubular Renin: Role in Kidney Development

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Renin, the key regulated enzyme of the renin angiotensin system regulates blood pressure and fluid electrolyte homeostasis as well as morphogenesis of the kidney. Classically, renin is synthesized and released from juxtaglomerular cells located in the afferent arterioles at the entrance to the glomeruli. It has also been suggested that renin may also be synthesized by tubular cells. Interestingly, whole body deletion of the renin gene results in striking vascular and tubular abnormalities, hydronephrosis and a urine-concentrating defect. Given the complexity of such phenotype, it has not been possible to discriminate the relative contributions of vascular versus tubular renin. To investigate the relative contribution of renin in the vascular versus tubular compartments during kidney development we deleted renin independently in either compartment by crossing Ren1cfl/fl mice to Foxd1-cre or to Hoxb7-cre mice. We performed blood chemistries, histological analysis, immunostaining for specific cell markers, total kidney renin mRNA quantification and ELISA for renin in plasma. Vascular deletion of renin (Foxd1 lineage) resulted in neonatal mortality that could be rescued with daily injections of saline suggesting that this phenotype is due to an inability to concentrate the urine and improper medullary development. These mice showed absence of renal renin and negligible levels of plasma renin. Histologically, the kidneys had abnormal development of its arterioles, which became progressively thickened by disorganized, concentric hypertrophy of smooth muscle cells and marked hydronephrosis. On the other hand, lack of renin in the collecting ducts (Hoxb7 descendants) did not affect kidney morphology, intra-renal renin distribution, kidney renin mRNA expression or circulating renin during basal conditions or in response to a homeostatic stress such as low sodium diet. We conclude that renin generated in the renal vascular compartment is fundamental for the development and integrity of the kidney whereas the presence of renin in the collecting duct system is dispensable for normal kidney development and cannot compensate for the lack of renin in the vascular compartment.

Further, the main source of circulating renin is the kidney vasculature.

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MP02

Angiotensin II Mediates Microvascular Rarefaction *In Vivo* and Exacerbates Endothelial-To-Mesenchymal Transition *In Vitro*

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Cardiac fibrosis accompanies numerous cardiovascular diseases (CVD) such as hypertension and myocardial infarction and increases myocardial stiffness leading to contractile dysfunction. Recently, endothelial-to-mesenchymal transition (EndMT) has been shown to contribute to myocardial fibrosis. EndMT describes a process by which endothelial cells transform into mesenchymal cells such as fibroblasts and has been implicated in many fibrotic diseases. Angiotensin II (AngII) plays a key role in myocardial fibrosis and has been associated with the activation of fibroblasts to myofibroblasts and an increase in myocardial collagen deposition. Here, we assessed the role of AngII in capillary loss and EndMT *in vivo* and *in vitro*.

C57BL/6J mice were infused with H_2O (control) or 24µg/kg/hr AngII for 4 weeks. Mice infused with AngII developed significant cardiac fibrosis characterised by the deposition of collagen I (2.5-fold *vs.* control; $p<0.05$) and III (1.9-fold *vs.* control; $p<0.05$). Capillary density was assessed by CD31 immunohistochemistry and revealed significant vascular rarefaction (control 2161±111 *vs*. AngII 838±132 capillaries/mm^2; $p<0.05$). To investigate whether AngII can induce EndMT *in vitro*, human coronary artery endothelial cells were stimulated with 10ng/mL $TGF\beta_1$ alone or in combination with 1µM AngII for 10 days. AngII significantly enhanced $TGF\beta_1$-induced gene expression of α-smooth muscle actin ($TGF\beta_1$ 1.8-fold; $TGF\beta_1$±AngII 4.3-fold *vs*. control; $p<0.05$) and collagen I ($TGF\beta_1$ 9.2-fold; $TGF\beta_1$+AngII 30.2-fold *vs*. control; $p<0.05$). Concomitantly, AngII significantly increased α-smooth muscle actin protein expression ($TGF\beta_1$ 3.9-fold; $TGF\beta_1$+AngII 23.6-fold *vs*. control; $p<0.05$) and significantly decreased CD31 expression ($TGF\beta_1$ 0.9-fold; $TGF\beta_1$+AngII 0.7-fold *vs*. control; $p<0.05$), suggesting AngII acts in concert with $TGF\beta_1$ to enhance conversion of endothelial cells to myofibroblasts. Further studies investigating the underlying mechanism, including the role of the Smad pathway, are ongoing.

These results demonstrate that AngII induces vascular rarefaction *in vivo* and potentiates $TGF\beta_1$-induced EndMT *in vitro.* Understanding the molecular basis for these observations may help to identify new therapeutic options in CVD.

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MP03

Relationships between Serum Soluble (Pro)Renin Receptor Levels and Endocrine or Metabolic Factors in Patients with Essential Hypertension

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Background: (Pro)renin receptor[(P)RR] is a single trans-membrane receptor that binds to both renin and its precursor prorenin to activate tissue renin-angiotensin system (RAS). (P)RR is cleaved by furin to generate soluble (pro)renin receptor [s(P)RR], which is secreted into the extracellular space. Blood s(P)RR level is a candidate biomarker reflecting the status of the tissue RAS, and we have reported that serum s(P)RR levels are associated with renal function. Endocrine or metabolic factors contribute to progression of endorgan damages in hypertensive patients. Therefore, in the present study, we investigated the relationships between serum s(P)RR levels and endocrine or metabolic factors in patients with essential hypertension. Methods: We measured serum s(P)RR levels and assessed the relationships between serum s(P)RR levels and background factors including endocrine or metabolic factors such as age, body mass index (BMI), abdominal circumference, hemoglobin, uric acid, HbA1c, LDL-cholesterol (C), HDL-C, triglyceride (TG), high-sensitivity (hs) CRP, plasma renin activity, aldosterone concentration, noradrenaline, adrenaline, thyroid stimulating hormone, free T3, and free T4 in essential hypertensive patients. Results: A total of 302 patients (138 males) were enrolled. The mean value for age was 57±12 y.o., BMI was 24.9±5.1 Kg/m2, blood pressure was 140±16/85±12 mmHg, hemoglobin was 14.0±1.3 g/dl, estimated glomerular filtration rate (eGFR) was 75.2±20.6 ml/min/1.73 m2, HbA1c was 5.7±0.7%, and serum s(P)RR level was 21.0±5.8 ng/ml. Serum s(P)RR levels were significantly positively correlated with age ($r=0.209$, $p<0.0005$), abdominal circumference ($r=0.177$, $p<0.05$), HbA1c ($r=0.219$, $p<0.001$), TG ($r=0.180$, $p<0.01$), hs CRP ($r=0.162$, $p<0.05$), free T3 ($r=0.521$, $p<0.01$), and free T4 ($r=0.523$, $p<0.005$), and were significantly negatively correlated with hemoglobin ($r=-0.243$, $p<0.05$), eGFR ($r=-0.272$, $p<0.0001$), and HDL-C ($r=-0.199$, $p<0.005$). Conclusion: In patients with essential hypertension, serum s(P)RR levels were shown to be associated with endocrine or metabolic factors in addition to renal function, suggesting serum s(P)RR level as a useful biomarker that reflects the status of endocrine or metabolic disorders.

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MP04

Cardiac ACE2/Angiotensin-(1-7) in Rats Expressing Human Angiotensinogen Gene

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When compared to Sprague Dawley (SD) control rats, transgenic rats expressing the human angiotensinogen (AGT) gene [TGR(hAGT)L1623] exhibit hypertension associated with cardiac hypertrophy and higher cardiac tissue angiotensin (Ang) II. Whether the hypertension and cardiac hypertrophy in these rats expressing the human AGT are related to a non-canonical pathway for Ang II formation or suppression of the counter regulatory mechanism mediated by ACE2 and Ang-(1-7) has not been established. Consequently, cardiac peptides were determined by RIA in 9 [TGR(hAGT)L1623] and 11 SD male rats (17 weeks of age). ACE2 activities in homogenized heart tissues were determined by HPLC. Cardiac Ang II content was four times higher (37.05 ± 5.04 vs. 9.62 ± 0.93 fmol/mg protein; $p < 0.0001$) while the Ang-(1-7) level increased only 1.3 times (18.02 ± 1.62 vs 13.37 ± 1.74 fmol/mg protein; $p=0.06$) in TGR(hAGT)L1623 rats when compared with SD rats. Although, the Ang II/Ang-(1-7) ratio was higher in transgenic rats harboring the human AGT gene (2.10 ± 0.27 vs 0.90 ± 0.19; $p < 0.005$), ACE2 activities between these two strains of animals were not different (12.21 ± 0.76 vs. 10.80 ± 0.91 fmol/min/mg; $p > 0.05$). Since human AGT protein is not cleaved by rat renin, our data continues to support the view that hypertension and cardiac hypertrophy in this transgenic strain are induced by activation of a non-renin mechanism rather than a primary suppression of the compensatory Ang II degrading pathway mediated by ACE2. Further studies are necessary to determine the role of enzymes affecting Ang-(1-7) metabolism in the observed inadequate balance between Ang II and Ang-(1-7).

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MP05

Knockdown of Gamma-adducin Expression Impairs the Myogenic Response of the Cerebral and Renal Arterioles

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We recently identified a region on chromosome 1 containing 15 genes that rescues the impaired myogenic response in the afferent arteriole (Af-art) and the development of renal injury in Fawn Hood Hypertensive (FHH) rats. We also found an inactivating K572Q mutation in the gamma-Adducin (Add3) gene in FHH rats in this region that may play a causal role. To test this hypothesis, the present study examed the effect of knockdown of the expression of Add3 with DsiRNA on the myogenic response of renal and cerebral arterioles. A newly designed 27-mer Add3 DsiRNA blocked the expression of the Add3 protein in 293 cells and reduced Add3 mRNA expression in a dose-dependent manner up to 75% in cultured middle cerebral arteries (MCA). The inner diameter of MCA and renal Af-art 36 hours after co-transfection of Add3 DsiRNA dilated by 9 ± 2% and 4 ± 3%, respectively, in response to an elevation in transmural pressure from 60 to 140 mmHg. In contrast, these vessels still constricted normally by 11± 2% and 10 ± 1% in vessels transfected with scrambled siRNA. Add3 DsiRNA had no

effect on the vasoconstrictor response of the Af-art to NE (10-7 M). The large conductance calcium sensitive potassium (BK) current was 5-fold higher in vascular smooth muscle cells (VSMC) freshly isolated from the MCA that were transfected with Add3 DsiRNA as indicated by the appearance of SiGLO red fluorescence compared with that seen in non-transfected cells. Administration of IBTX normalized the elevated BK channel current recorded from VSMC transfected with Add3 DsiRNA, but it had little effect in non-transfected cells. These results indicate that knockdown of the expression of Add3 impairs the myogenic response of both MCA and Af-art and it is associated with an elevation in BK channel activity. It also supports the hypothesis that the K572Q mutation of Add3 found in FHH rats may play a causal role in the impaired myogenic response and autoregulation of renal and cerebral blood flow by elevating BK channel activity in VSMC.

F. Fan: None. **M.R. Pabbidi:** None. **Y. Ge:** None. **R.J. Roman:** None.

MP06

Mitochondrial Dynamics and Vascular Effects: Role of OPA1 in Hypertension

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Background
Defects in mitochondrial dynamics have been associated with various disorders, including cardiovascular diseases. OPA1 is essential for mitochondrial inner membrane fusion and mutation in OPA1 is associated with autosomal dominant optic atrophy. Since OPA1 has been reported to be associated with cell apoptosis, cell proliferation, mitochondrial ATP synthesis, calcium homeostasis and reactive oxygen species (ROS) production, we investigated its role in vascular function and/or structure in physiological and pathological condition like hypertension.
Methods and Results
We used the heterozygous Opa1 mouse model carrying the recurrent Opa1 delTTAG mutation and OPA1 silencing in artery smooth muscle and endothelial cells. Electron microscopy revealed altered mitochondrial cristae structure in vascular smooth muscle cells and endothelial cells of Opa1+/- mice, 6 month-old Opa1+/- mice had a normal baseline blood pressure and vascular function (contraction and dilation). A chronic treatment with L-NAME induced hypertension in mice. Systolic blood pressure was significantly greater in Opa1+/- than in wild type (WT) mice. This was associated with a stronger reduction in endothelium-dependent relaxation to acetylcholine of resistance arteries in Opa1+/- than in WT animal. On the other hand, hypertension-induced wall hypertrophy in the aorta was absent in Opa1+/- in association with reduced proliferation and increased apoptosis of vascular cells.
Conclusions
Thus mitochondria alteration due to OPA1 down-regulation did not affect baseline blood pressure and vascular tone but induced an excessive elevation in blood pressure in hypertension. These results suggest for the first time that OPA1 may play an important role in protection of the vasculature in pathological conditions such as hypertension.

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MP07

Endogenous Chemerin Amplifies Nerve-Dependent Arterial Contraction

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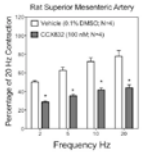
The adipokine chemerin causes contraction of isolated arteries and is implicated in blood pressure regulation, especially in the obese population that have elevated levels of circulating chemerin. Because chemerin is expressed in the perivascular adipose tissue (PVAT) that facilitates the sympathetic innervation of the blood vessel, we tested the hypothesis that chemerin (endogenous and exogenous) would amplify the effects of the sympathetic nervous system in mediating electrical field stimulated (EFS) contraction. The model was the superior mesenteric artery with PVAT, mounted into tissue baths for isometric contraction. Immunohistochemistry validated a robust expression of chemerin in the PVAT surrounding the superior mesenteric artery. EFS (0.3-20 Hz) caused a frequency-dependent, prazosin-sensitive contraction that was reduced (~40%) by the chemerin receptor ChemR23 antagonist CCX832 (100 nM; figure) but not by the inactive congener CCX826 (100 nM). Exogenous chemerin (1 µM) amplified EFS-induced contraction in a manner that was also blocked by CCX832. Chemerin did not directly modify contraction of the superior mesenteric artery (-PVAT) to cumulative concentrations of norepinephrine (1 nM - 10 µM), supporting that contractile amplification by chemerin was not at the level of smooth muscle. These studies raise the interesting possibilities that endogenous chemerin and/or ChemR23 modifies nerve-mediated contraction. This is significant because of the well appreciated role of the sympathetic nervous system in blood pressure



control.

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MP08

Syndecan-1 is Involved in Osteoprotegerin-induced Vascular Dysfunction

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Osteoprotegerin (OPG), an inhibitor of vascular calcification, has pleiotropic vascular effects independently of its actions on calcification. OPG has been associated with vascular inflammation and remodelling and may be important in cardiovascular disease where OPG levels may be elevated. Molecular mechanisms and functional consequences of OPG stimulation in the vasculature are unclear. We propose that syndecan-1, a membrane glycoprotein, may be important and that

reactive oxygen species (ROS) play a role in OPG signalling. Vascular reactivity of resistance arteries from WKY rats was studied by wire myography in the presence or absence of OPG (50 ng/mL) and/or synstatin (SSNT - 10-6M - syndecan-1 inhibitor). Rat endothelial cells (EC) and vascular smooth muscle cells (VSMC) were studied. Levels of ROS were measured by chemiluminescence, Amplex Red (H2O2) and ELISA (nitrotyrosine; peroxynitrite - ONOO-). Protein oxidation and levels were measured by immunoblotting. Exposure of resistance arteries to OPG induced endothelial (decreased relaxation to acetylcholine) and VSMC (decreased relaxation to sodium nitroprusside - SNP) dysfunction, as well as, increased contraction to phenylephrine. All responses were blocked by SSNT, N-acetylcisteine (antioxidant) and ML171 (Nox inhibitor). In EC, OPG-induced ROS production (240±46.1% increase vs. veh, $p<0.05$) was blocked by SSNT. OPG decreased H2O2 production/release (61±5.4% vs. veh) and increased eNOS Thr 495 phosphorylation (inhibitory site) (100±24% vs. veh, $p<0.05$). In VSMC, OPG increased H2O2 (69±3%) and ONOO- (43±12%) levels, protein oxidation (61±15%), Rho kinase (200±39%) and myosin light chain activation (55±3%) (all vs. veh, $p<0.05$). Increase in OPG-induced ONOO- levels was exacerbated by SNP (130±16% vs. veh, $p<0.05$), a nitric oxide donor. In conclusion, vascular dysfunction elicited by OPG is mediated by syndecan-1 and ROS. Whether syndecan-1 also impacts on OPG-sensitive calcification is unclear. Our data identify a novel molecular mechanism through syndecan- 1/ROS that may underlie injurious effects of OPG.

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MP09

Adrenal-specific Deletion of TASK Channels Evokes Normal-Renin Hypertension

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Objectives:

Dysregulation of aldosterone (Aldo) production is predicted to evoke major features of idiopathic primary hyperaldosteronism (IHA): low renin, elevated blood pressure and suppressed control by high Na. We have previously demonstrated in mice that global deletion of background TWIK-related acid-sensitive K (TASK) channels (TASK-1, TASK-3) effect a ~20mV decrease in the membrane potential of Zona Glomerulosa (ZG) cells to produce frank autonomous overproduction of Aldo, low renin, and hypertension (HT), mimicking the salient features of human IHA. In the current study, we ask if specific deletion of TASK channels in ZG cells is sufficient to produce hyperaldosteronism and the predicted sequela or if extra-adrenal deletion of TASK channels is required.

Design and Methods:

We generated a trigenic mouse-line (*AS*$^{+Cre}$::TASK-1^{ff}::TASK-3^{ff}, zT1T3KO) in which TASK-1 and TASK-3 subunits were specifically deleted in ZG cells. The renin-angiotensin-aldosterone system (RAAS) was evaluated in mice housed in metabolic cages and stabilized on various salt diets. Urinary Aldo concentration was measured and normalized to creatinine (ng Aldo/mg creatinine; 24 hr. urine collection).

Blood pressure was recorded in conscious, freely moving mice using radio telemetry, and plasma renin concentration was measured from tail vein sampling.

Results:

Overproduction of aldosterone on normal-salt diet (0.3% Na) was modest in zT1T3KO mice compared to littermate controls (WT; WT 9.4; KO 11.8 ng/mg, 1.25-fold). Suppression of Aldo production by high-salt (2% Na) was blunted, exaggerating the difference in Aldo production between genotypes (WT 3.0; KO 7.4 ng/mg, 2.43-fold). zT1T3KO mice were hypertensive (mean MAP: WT 103.5; KO 113.1 mmHg), yet renin levels remained normal. Neither hyperaldosteronism nor HT could be corrected by angiotensin II receptor blockade, suggesting overproduction of Aldo and HT are independent of RAAS.

Conclusions:

Limiting TASK deletion to ZG cells results in normal renin HT driven by modest autonomous hyperaldosteronism, a stark contrast to the phenotypic features of IHA recapitulated by global TASK deletion. Together these mouse models provide insight into the role of ZG- vs extra-adrenal-dysfunction in the pathology of IHA.

N.A. Guagliardo: None. **T.H. Le:** None. **D.A. Bayliss:** None. **D.T. Breault:** None. **P.Q. Barrett:** None.

MP10

Nobelty and Significance of the Rapid Measurement of Plasma Aldosterone Concentration and Active Renin Concentration

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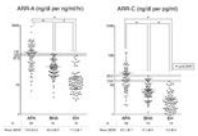
The plasma aldosterone/renin ratio (ARR) for the clinical diagnosis of primary aldosteronism (PA) is calculated with plasma aldosterone concentration (PAC) per plasma renin activity (PRA) or active renin concentration (ARC). It takes 3-4 days to get the results. Of late, we developed the novel rapid non-RIA assays of PAC and ARC, which are measurable in 10 minutes. Both were simultaneously measured by chemiluminescent enzyme immunoassay (CLEIA) system machine with their specific monoclonal antibodies and were automatically washed by the immobilized magnetic particles. We retrospectively compared RIA-assayed PAC,PRA and ARC with CLEIA-measured PAC and ARC in 243 patients with aldosterone producing adenoma (APA, n=86), bilateral hyperaldosteronism (BHA, n=78) and essential hypertension (EH, n=79), and investigated whether the calculation of ARR with ARC (ARR-C, ng/dL per pg/mL) instead of PRA (ARR-A, ng/dL per ng/m/hour) affects the diagnosing of the APA patients, which are potentially surgically curable. CLEIA-measured PAC were significantly correlated with RIA-assayed PAC ($y=0.9846 x + 2.5708$, Spearman's $r =0.9072$, $P<0.0001$). CLEIA-measured ARC (the lower detection limit = 0.25 pg/mL) were also

significantly correlated with RIA-assayed ARC (y=1.0103 x + 0.9156, Spearman's r =0.8166, P<0.0001). ARR-A and ARR-C of APA patients were 129 ± 4.3 and 25.1±2.7 (Mean ± SEM), respectively. ARR-A and ARR-C of BHA patients were 40.4 ± 2.9 and 6.7 ± 0.5, and those of EH patients 17.2 ± 2.1 and 3.2 ± 0.4, respectively. Our novel rapid CLEIA-assays of PAC and ARC will be able to be clinically more useful for detecting APA or BHA because it takes only ten minutes to get the results. 

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MP11

Striatin Heterozygous Knockout Mice Have Increased Aldosterone Sensitivity

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Background: We recently demonstrated that mice lacking one copy of the striatin gene (Strn+/-) have salt sensitivity of blood pressure (BP) as compared with WT mice. To determine whether Strn+/- mice have increased sensitivity to aldosterone (ALDO), we assessed the effect on blood pressure of an ALDO infusion in WT and Strn+/- mice fed a liberal sodium diet. Methods: In this study we used 12 week old WT and Strn+/- littermate male mice. For each genotype, mice were placed on HS diet and randomized to either: 1) placebo 2) ALDO (200 µg/Kg/day) or 3) ALDO plus 100 mg/kg/day eplerenone. BP was measured by tail cuff plethysmography at baseline and after treatment. After 21 days of treatment, animals were placed in metabolic cages for 24 hours. Finally, animals were sacrificed and organs excised. Primary endpoints were BP, renal immunohistochemistry, protein analysis by western blot and mRNA expression by RT-PCR. Results: BP increased significantly in Strn+/- mice treated with ALDO (ΔBP: 12 ± 4 mmHg, p=0.03) but not placebo (ΔBP 6± 6 mmHg); the BP effect of ALDO was blunted by eplerenone (Δ 6 ± 3 mmHg). In contrast, none of the treatments had a significant effect on BP in WT mice. Kidney weight was significantly increased after 3 weeks of ALDO treatment in both WT and Strn+/- mice and this increase in kidney weight was prevented by treatment with eplerenone, with no difference between genotypes. WT mice had an increase in glomerular volume (GV) in HS/ALDO treated that was blunted by eplerenone. Interestingly, Strn+/- mice had increased GV across all 3 treatment groups compared with WT mice. pAkt/Akt ratios were reduced in Strn+/- mice versus WT mice across all treatments. Classic genomic MR targets (ENaC and SGK1) and non-genomic targets (pAkt/Akt) were significantly modulated in kidney tissue of Strn+/- mice compared to WT mice with chronic ALDO. Conclusion: Strn+/- mice have an increased sensitivity to infused ALDO (increased BP response and increased rise in renal ENaC and SGK1 protein) as compared to WT mice. Since loss of striatin directly reduces nongenomic not genomic action of ALDO, this study demonstrates for the first time that modifying the nongenomic pathway may under chronic, in

vivo conditions led to increased sensitivity to the genomic actions of ALDO.

A.E. Garza: None. **L. Pojoga:** None. **R. Baudrand:** None. **B. Moize:** None. **T. Yao:** None. **G.K. Adler:** None. **G.H. Williams:** None.

MP12

The AA2-Ratio: A Novel Screening Test for Primary Aldosteronism in Hypertensive Patients

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Primary aldosteronism (PA) is severe form of hypertension characterized by a strongly increased aldosterone secretion mediated by adenomas or other forms of adrenal hyper-activity. Once detected, PA can be usually cured by either surgical intervention or by appropriate pharmacologic treatments. This is also reflected in clinical guidelines of Endocrine Societies in Europe and the US, suggesting extensive PA screening activities among resistant hypertensive patients. The incidence of PA among hypertensive patients varies strongly between different studies, which is in part caused by the complex state-of-the-art testing procedure that unfortunately is far away from being a versatile PA screening tool. Despite strong limitations regarding selectivity, sensitivity and the interference with multiple anti-hypertensive drugs, the aldosterone-renin-ratio (ARR) is widely used for PA case detection. However, there is still a strong demand for accurate and reliable and patient friendly PA case detection. The use of novel and more accurate technologies for quantification of aldosterone and renin activity might help to improve the power of the ARR as a diagnostic tool for PA. However, there is a big need for a versatile PA screening assay that doesn't interfere with anti-hypertensive treatments and therefore allows the clear identification of PA patients without complex corrections and adaptions being necessary and without increasing the patient's cardiovascular risk in the course of the diagnostic process. The Aldosterone-to-Angiotensin-II-Ratio (AA2-Ratio) is a novel mass-spectrometry based high-throughput test for PA that combines the plasma levels of aldosterone and physiologically active angiotensin II into a diagnostic ratio. The test performance is superior to the ARR in terms of the diagnostic window and method accuracy. The AA2-Ratio does not interfere with standard anti-hypertensive drugs including ACE inhibitors. First data obtained in a proof-of-concept study investigating PA positive and negative patients proved the AA2-Ratio to be a powerful and cost-effective diagnostic tool for the diagnosis of PA in clinical practice.

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MP13

Etamicastat Prevents Arterial Blood Pressure Increase in Mice Lacking Salt-inducible Kinase 1 (SIK1)

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Loss of salt-inducible kinase 1 (SIK1) triggers an increase in blood pressure (BP) upon a chronic high-salt intake in mice (Circ Res 2015;116:642-

52). Here, we address possible acute mechanisms that may relate to the observed high BP in mice lacking SIK1. SIK1 knockout (*sik1*$^{-/-}$) and wild-type (*sik1*$^{+/+}$) littermate mice were challenged for seven days with a normal- (0.3% NaCl) or high-salt (8% NaCl) diet. Systolic BP (SBP) was significantly increased in *sik1*$^{-/-}$ mice (137.0±17.2 mmHg) after seven days of high-salt intake, as compared to *sik1*$^{+/+}$ mice counterparts (120.6±4.5 mmHg). The renin-angiotensin system (RAS) and the sympathetic nervous system (SNS) were assayed in order to investigate the possible causes for the increase in SBP in *sik1*$^{-/-}$ mice fed a high-salt diet. No differences in renin (normal-salt: 463.4±17.9, high-salt: 462.9±28.9 pg/ml) and angiotensin II (normal-salt: 45.8±10.0, high-salt: 39.0±8.5 pg/ml) serum levels were observed. The activity of dopamine β-hydroxylase (DβH), the enzyme that converts dopamine (DA) to norepinephrine (NE), was significantly increased in the adrenal glands of *sik1*$^{-/-}$ mice fed a high-salt diet (356.7±32.8 nmol/mg protein) as compared to *sik1*$^{-/-}$ mice on a normal-salt diet (184.4±14.4 nmol/mg protein). Similarly, urinary catecholamines (DA, NE, epinephrine) and L-DOPA were significantly increased (3- to 7-fold increase) in *sik1*$^{-/-}$ mice fed a high-salt diet as compared to *sik1*$^{-/-}$ mice on a normal-salt intake. Altogether, this data supports the view that *sik1*$^{-/-}$ mice fed a high-salt diet develop SNS overactivity. Next, we addressed the question if reducing SNS activity in *sik1*$^{-/-}$ mice fed a high-salt diet would ameliorate hypertension. For that purpose, the effect of etamicastat, a peripheral reversible DβH inhibitor, was evaluated on the development of high BP upon high-salt diet. Etamicastat treatment (50 mg/kg/day), started prior to high-salt feeding, completely prevented SBP increase in *sik1*$^{-/-}$ mice fed a high-salt diet (116.8±4.7 mmHg). It is concluded that the SNS is involved in the development of salt-induced hypertension in *sik1*$^{-/-}$ mice and that the DβH inhibitor etamicastat is able to reduce SNS overactivity and high BP in this mouse model of hypertension.

N. Pires: A. Employment; Modest; BIAL - Portela & Cª, S.A. **B. Igreja:** A. Employment; Modest; BIAL - Portela & Cª, S.A. **E. Moura:** A. Employment; Modest; BIAL - Portela & Cª, S.A. **M. Bonifácio:** A. Employment; Modest; BIAL - Portela & Cª, S.A.. **P. Serrão:** None. **P. Soares-da-Silva:** None.

MP14

A K572Q Mutation in Gamma-adducin Is Responsible for the Impaired Myogenic Response and Autoregulation of Renal and Cerebral Blood Flow in FHH Rats

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The FHH rat is a genetic model of hypertension induced CKD, but the genes and pathways involved are unknown. We recently reported that the myogenic response of the renal and cerebral arteries is impaired in FHH rats and it was restored in a FHH.1BN congenic strain in which a small region of Chr. 1 containing 15 genes, including gamma-Adducin (Add3), from BN rats was transferred into the FHH

background. We further identified a K572Q mutation in Add3 in FHH versus FHH.1BN rat. The present study examined the contribution of Add3 to the impaired myogenic reactivity using Add3 transgenic and KO rats. The diameter of the middle cerebral artery (MCA) decreased by 20-30% in SD, FHH.1BN and FHH.Add3 transgenic rats when perfusion pressure was increased from 40 to 140 mmHg. In contrast, the MCA of FHH and SD.Add3 knockout rats did not constrict. The myogenic response of the MCA is also impaired in MNS rats that share the same mutation in Add3 as in FHH rat and this phenotype was complemented in a F1 cross of FHH and MNS strain. Autoregulation of CBF was impaired in FHH rats and rose by 99 ± 7% when MAP was increased from 100 to 190 mmHg and was restored in FHH.Add3 and FHH.1BN rats. Similarly, the diameter of Af-art of FHH, MNS and a F1 cross of FHH and MNS rat increased in response to increase in perfusion pressure but decreased in FHH.1BN rats that have wild type Add3. FHH rats exhibited impaired autoregulation of RBF in comparison with FHH.1BN rats. Pgc estimated from the stopflow pressure increased by 20 mmHg in FHH rats when RPP was increased from 100 to 140 mmHg versus only 4 mmHg in the congenic strain. FHH rats developed severe renal injury and proteinuria rose from 37 ± 2 to 260 ± 32 mg/day as they aged from 12 to 21 weeks, but rose by a significant lesser extent in FHH.1BN and FHH.Add3 rats. BK current in VSMC isolated from the MCA was 5-fold higher in FHH vs. FHH.1BN rats. Administration of IBTX normalized the elevated BK channel current and restored the myogenic response in FHH rats but it had little effect in FHH.1BN. These results indicate that the K572Q mutation in Add3 plays a causal role in the impaired myogenic response and autoregulation of renal and cerebral circulation in FHH rats and may contribute to the development of renal and cerebral end organ damage with aging and after the onset of hypertension.

F. Fan: None. **A.M. Geurts:** None. **M.R. Pabbidi:** None. **Y. Ge:** None. **D.R. Harder:** None. **R.J. Roman:** None.

MP15

Afferent Arteriolar Endothelial-dependent Dilation is Impaired Prior to the Development of Radiation-induced Nephropathy and Hypertension

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Chronic kidney disease (CKD) occurs in 15% of hematopoietic stem cell transplant (HSCT) patients, and has been clearly linked to irradiation at the time of the HSCT. CKD due to radiation also occurs after radionuclide therapy for cancer. Radiation nephropathy is expressed in rats and in humans as proteinuria, azotemia, and hypertension. There is a latent period of 6-8 weeks after irradiation to the development of proteinuria, azotemia, and hypertension in rats. This study tested the hypothesis that afferent arteriolar responses to the endothelial-dependent dilator acetylcholine are impaired prior to the development of proteinuria, azotemia, and hypertension in rats exposed to total body irradiation (TBI). Male WAG/RijCmcr rats were subjected to TBI (11 Gy) and afferent arteriolar responses to acetylcholine using the juxtamedullary nephron technique were determined at one, three, and six weeks. Systolic blood pressure (117 ± 6 vs. 119 ± 4 mmHg) and BUN (15.6 ± 1.4 vs. 15.8 ± 0.8 mg/dl) were not different between control and TBI groups at 6 weeks. Afferent arteriolar

diameters averaged 22.5 ± 0.8 μm (n=30) in controls and 21.7 ± 0.7 μm (n=27) in TBI rats and were not different between control and TBI groups at 1, 3, or 6 weeks. Acetylcholine dilator responses were progressively attenuated from one to six weeks in TBI compared to control rats. During exposure to acetylcholine (0.01, 0.1, 1, and 10 μmol/L), afferent diameter increased by 8 ± 2%, 18 ± 3%, 27 ± 2% and 31 ± 3% in control rats (n=6), and 3 ± 2%, 9 ± 3%, 12 ± 3%, and 16 ± 3% in kidneys of 3 week TBI rats (n=8, $p<0.05$). However, TBI did not change the norepinephrine-mediated vasoconstrictor responses or the nitroprusside vasodilator responses at 1, 3, or 6 weeks. Renal microvessels were isolated from control and TBI groups at 3 and 6 weeks for protein expression assessment of endothelial enzymatic pathways Cyp2C, COX, and NOS. We found that epoxygenase Cyp2C23 and Cyp2C11 protein expressions were significantly lower 3 and 6 weeks following TBI compared to control rats. Taken together, these results indicate that the impaired afferent arteriolar endothelial-dependent acetylcholine responses and decreased epoxygenase enzymes precede the development of proteinuria, azotemia, and hypertension in irradiated rats.

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MP16

ReninAAV Uninephrectomized db/db Mice as a Novel Diabetic Kidney Diesease Model for Testing New Therapeutics on Top of RAS Inhibition

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ReninAAV comprises a novel approach to induce persistent hypertension in diverse murine models. ReninAAV induced hypertension is persistent and facilitates the development of animal models of diseases accelerated or driven by hypertension such as diabetic kidney disease (DKD). A single injection of ReninAAV ($5x10^9$ GC) in uninephrectomized (uNx) *db/db* mice results in progressive DKD. Impaired renal function was detected as early as one week post injection with an 8.9±3.1-fold increase in ACR (albumin:creatinine) as compared to baseline values, and at 11 weeks post injection significantly ($p<0.01$) increased serum creatinine (0.20±0.03mg/dL) and ACR were detected (68,890±7,209μg/mg) versus LacZAAV uNx *db/db* controls (0.08±0.01mg/dL and 1,364±83μg/mg, respectively). To determine if treatment with the ACEi Linsinopril halted progression of renal disease uNx *db/db* were given ReninAAV and followed for 4 weeks until ACR reached approximately 15,000μg/mg. Mice were randomized into 4 treatment groups: untreated, Lisinopril low (30mg/L), Lisinopril

medium (100mg/L) and Lisinopril high (300mg/L) and followed for an additional 8 weeks. Untreated mice showed progressive increase in ACR developing a final ACR of 35,318±4,177µg/mg. Lisinopril dose dependently reduced ACR significantly compared to baseline ($p<0.001$, final ACR of 7,869±1632, 2,730±582 and 2,014±44µg/mg for the 30, 100 and 300 mg/L groups respectively), albeit still elevated compared to control LacZAAV uNx *db/db* mice (1,364±831µg/mg) that did not receive ReninAAV. Lisinopril treatment at 100 and 300mg/L also resulted in improvement in final serum creatinine values ($p<0.05$, 0.18±0.01 and 0.14±0.01mg/dL respectively vs untreated ReninAAV group 0.26±0.03mg/dL), yet residual increased serum creatinine were apparent compared to control LacZAAV uNx *db/db* mice (0.08±0.012mg/dL). This study establishes ReninAAV treated uNx *db/db* mice as a novel model of progressive DKD and demonstrates that suppression of RAS with Lisinopril improved, but only partially prevented progression of renal disease, thus enabling testing of new potential therapeutics on top of RAS inhibition.

S.M. Harlan: A. Employment; Significant; Eli Lilly and Company. **H.E. Baker:** A. Employment; Significant; Eli Lilly and Company. **C.R. Shrake:** A. Employment; Significant; Eli Lilly and Company. **M.D. Breyer:** A. Employment; Significant; Eli Lilly and Company. **J.G. Heuer:** A. Employment; Significant; Eli Lilly and Company.

P001

Comparative Study on Steroidgenic Activity in Aldosterone-Producing Adenoma With ATPase or CACNA1D Gene Mutations in Japanese Patients With Primary Aldosteronism

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Object: Our aim is to clarify the regulatory mechanism of aldosterone synthesis in patients with aldosterone-producing adenomas (APA) harboring ATPase or CACNA1D gene mutations.
Design and patients: We subjected 108 patients with unilateral APA, and tested somatic mutations by using each APA tissue. ATPase and CACNA1D genes were analyzed among 33 APAs without KCNJ5 gene mutations. We also evaluated pathological findings of steroidgenic enzymes and isolated cells prepared from 2 ATP2B3- and 1 CACNA1D-mutated APAs were incubated with various stimulants for clarifying steroidgenic activity.
Results: There were 1, 2, and 2 cases whose APAs possessed ATP1A1, ATP2B3, and CACNA1D mutations, respectively. Compared with the wild-type group without any somatic mutations of KCNJ5, ATPase or CACNA1D, the patients with ATPase mutations showed severe phenotype of hyperaldosteronemia even with smaller-sized tumors, although the CACNA1D-mutated APA patients showed similar characteristics. Pathological findings clearly demonstrated that the ATPase-mutated APA was mainly composed of compact eosinophilic tumor cells, while the CACNA1D-mutated APA mainly did of clear tumor cells with relatively weak 3βHSD2 immunoreactivity. In vitro incubation study with isolated APA cells demonstrated that aldosterone production of ATP2B3 mutated APA cells was more responsive to (Bt)2cAMP than that of the other types of cells (almost 2-fold in the wild group and the CACNA1D-mutated cells vs. 4-fold increase in the ATPase-mutated cells). On the other hand, CACNA1D-mutated APA cells showed greater

responsiveness to ACTH compared with the other types of cells (almost 2-fold in the wild group and ATPase-mutated cells vs. 4-fold increase in the CACNA1D-mutated cells). Conclusion: Responsiveness of aldosterone production stimulated by ACTH or cyclic AMP differed in each case with different cell types. The mutation of ATPase seems to promote accelerated intracellular Ca signaling systems, of which activation may be quantitatively differed in the case of CACNA1D mutation. Thus, our data suggested that the regulatory effect of ACTH on aldosterone synthesis might vary according to the basal intracellular conditions, such as upregulation of Ca signaling induced by each mutation.

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P002

Aldosterone Promotes the Release of miRNA-Containing Exosomes from Endothelial Cells, Leading to Uptake by Smooth Muscle Cells

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Background: Aldosterone increases exocytosis from endothelial cells, which may contribute to the detrimental actions of aldosterone in the vascular system. We propose a role for exocytosis of exosomes; these small extracellular particles contain host cell genetic material and once released they can circulate in the blood and transfer their contents (e.g. miRNAs) to recipient cells. We aimed to examine the effect of aldosterone on exosome release from endothelial cells, the miRNA content of exosomes and finally, exosome trafficking of miRNA from endothelial cells to smooth muscle cells.
Methods and Results: Exosomes were isolated via ultracentrifugation from the media of human coronary artery endothelial cells (HCAECs) following incubation with aldosterone (100nM) or vehicle (DMSO). Exosomes were analyzed using western blot analysis testing for exosome and cell markers, and by nanoparticle-tracking analysis (NanoSight); aldosterone increased exosome concentration by 2.65 fold (19.1x1010 particles/mL) compared to the vehicle control (7.2x1010 particles/mL). RNA was extracted from isolated exosomes and host HCAECs and levels of miR-24 measured by qRT-PCR. miR-24 levels were unaffected by aldosterone stimulation in HCAECs (0.58±0.19; p=0.07) but were significantly increased in exosomes isolated from the media (2.35±0.07 fold; p=0.03). To test exosome paracrine trafficking, HCAECs were transfected with a c.elegans miRNA (cel-miR-39) and incubated adjacent to human coronary artery smooth muscle cells (HCASMCs) separated by a 0.4 μm filter, with or without aldosterone. Levels of cel-miR-39 in HCASMCs increased significantly (2.42±0.52 fold; p=0.02) in aldosterone-stimulated cells, compared to control. Blocking transcription with Dactinomycin (2.07±0.61 fold; p>0.05) or blocking the mineralocorticoid receptor had no effect on aldosterone-mediated trafficking (3.43±1.49 fold; p>0.05). Conclusion: This study demonstrates that aldosterone: 1) increases exosome secretion from endothelial cells, 2) increases miR-24 content of exosomes and 3) contributes to an increased uptake of exosome-packaged microRNAs by smooth muscle cells. Together, this represents a novel mechanism by which aldosterone contributes to vascular disease.

S. Robertson: None. **A. Romano:** None. **E. Dababneh:** None. **C. Bursill:** None.

P003

Hyperactive Brain Renin Angiotensin System Induces Polydipsia Through mTORC1 Pathway

Kenjiro Muta, Donald A Morgan, Justin L Grobe, Curt D Sigmund, Kamal Rahmouni, The Univ of Iowa, Iowa City, IA

Mechanistic target of rapamycin complex 1 (mTORC1) is a molecular hub for signaling pathways mediating a wide range of cellular events involved in the regulation of various physiological and pathophysiological processes. We previously demonstrated that Angiotensin II (Ang II) activates mTORC1 and its downstream effector ribosomal protein S6 kinase in neurons *in vitro*. Here, we investigated the role of brain mTORC1 in hypertension and polydipsia induced by Ang II. In wild-type mice, acute stimulation of angiotensin type 1 receptor signaling by intracerebroventricular (ICV) injection of Ang II (1 μg, 30 min) activated mTORC1 signaling in the subfornical organ (SFO), a critical brain region in cardiovascular control and fluid balance, as indicated by the significant increase in the number of phosphorylated S6-positive cells (32±2 vs 13±3 in vehicle group). Similar upregulation of the mTORC1 pathway in the SFO was also found in the mice treated subcutaneously with Ang II (1000 ng/kg/min) using an osmotic minipump for 1 week (27±3 vs 11±2 in vehicle group). To verify functional roles of the Ang II activation of mTORC1 in the SFO, we utilized hypertensive and polydipsic transgenic mice (sRA) that have a hyperactive brain renin-angiotensin system, resulting in SFO-overproduction of Ang II. Interestingly, sRA mice exhibited substantially elevated phospho-S6 immunoreactivity only in the SFO (64±6 vs 36±8 in controls) but not in other cardiovascular regulatory regions including the paraventricular nucleus. ICV delivery of mTORC1 blocker, rapamycin (10 ng/day for 7 days) significantly ($p<0.05$) reduced daily water intake (-4.5±0.7 mL) compared to vehicle-treated sRA mice (-0.7±0.6 mL). In contrast, systolic blood pressure remained unchanged with rapamycin treatment (123±1 vs 125±4 mmHg in pre-treatment) and was consistently higher than the control group (110±4 mmHg). These results suggest that mTORC1 activity in the SFO is a critical determinant of the polydipsia evoked by Ang II.

K. Muta: None. **D.A. Morgan:** None. **J.L. Grobe:** None. **C.D. Sigmund:** None. **K. Rahmouni:** None.

P004

Telemonitoring of Blood Pressure in Low-Income African American Patients With Congestive Heart Failure

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Introduction: Uncontrolled blood pressure (BP) due to non-adherence to medical therapy or inadequate medical therapy in African American (AA) patients with congestive heart failure (CHF) increases the risk of CHF exacerbation. Use of telemonitoring (TM) to monitor BP in low-income AA patients with CHF has not been studied.
Aim: To identify patients with uncontrolled BP in low-income AA patients with CHF using a TM device.
Methods: We conducted a prospective study of 15 patients with CHF (Ejection Fraction <45%) randomly selected from a Cleveland Clinic

outpatient clinic serving a low income AA population. Patients were trained to use a tablet monitor & a BP machine at their home to record their BP everyday for a period of 28 days. Using a secure internet connection patients BP measurement was transmitted to a password protected website where it was studied daily by a nurse to identify abnormal parameters (BP equal to or greater than 140/90 mm of Hg). The nurse contacted patients with abnormal BP measurements to ensure medication adherence.
Results: 15 patients consented to participate in the study. During the study period 67% (10/15) patients used the TM device to record BP for more than 50% (14/28 days) of the days. Of these, 50% (5/10) patients had either systolic or diastolic blood pressure equal to or greater than 140/90 mm of Hg for more than 50% of the times they were monitored (Figure 1).
Conclusions: Use of TM can help identify patients with CHF whose blood pressure is not at goal and may increase patient's medication adherence. Whether TM is a cost effective tool to improve clinical outcomes in low income AA patients with CHF & uncontrolled BP needs to be proven in larger studies.
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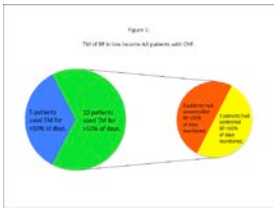

G.V. Shah: None. **M. Reali-Sorrell:** None. **B. Barzilai:** None. **A. Brateanu:** None.

P005

Mitochondrial Dysfunction in Heart of Coronary Artery Disease: Correlation with Telomerase Activity

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Rational:
Heart disease is the leading cause of death worldwide and abnormalities in mitochondrial function are increasingly recognized in association with cardiomyopathy, heart failure, endothelial dysfunction and coronary artery disease (CAD). However the direct contribution and mechanism of the mitochondrial dysfunction on the development of CAD is not fully determined. We have recently shown a critical role of TERT, the catalytic subunit of telomerase, as a regulator of mitochondrial integrity in the microcirculation. We observed increased expression of the dominant negative splice variant of TERT (**β**-del) in the Left Ventricle from subjects with CAD. Therefore, we hypothesize that TERT (**β**-del) decreases mitochondrial telomerase activity in the human heart resulting in mitochondrial damage that contributes to an environment that promotes the development of CAD.
Methods:
Fresh and frozen tissue samples of discarded heart tissue from subjects with and without CAD were used. Protein, cell lysate or

mitochondria were isolated using standard techniques. Mitochondrial DNA, levels of NAD+ and ATP as well as mitochondrial oxidative phosphorylation were evaluated.

Results:

PCR analysis revealed an increased frequency of mitochondrial common deletion, an established marker for mitochondrial abnormalities (0.9±0.2 in CAD; vs 1.5±0.2 in non-CAD; N=8; P<0.05). NAD+ and ATP levels were significantly decreased in CAD subjects compared to non-CAD (291±62 and 0.5±0.1 RLU/mg protein in CAD vs. 4203±336 and 84.1±24.8 pmol/mg protein in non-CAD respectively; N=15; P<0.005). Decrease respiration control index (RCI) in the presence of either complex I substrate K (+)-pyruvate/malate (PM) or complex II substrate K (+)-succinate (SUC) was observed in tissue form subjects with CAD (KPM-RCI: 2.9±1.3; SUC-RCI: 7.6± 1.9 in CAD vs KPM-RCI: 8.5±1.9; SUC-RCI: 19.1± 8.3 in non-CAD; N=3; P<0.05)

Conclusions:

Together these results point to significantly impaired mitochondrial function in subjects with CAD that are associated with decreased in mitochondrial telomerase activity.

K. Ait-Aissa: None. **J. Kim:** None. **G. Morgan:** None. **J.H. Santos:** None. **A.K.S. Camara:** None. **D.D. Gutterman:** None. **D.H. Betts:** None. **T. Donato:** None. **A.M. Beyer:** None.

P006

Liraglutide Attenuates Cardiac Fibrosis and Vascular Dysfunction in a Non-diabetic Angiotensin II-infusion Model via Anti-inflammatory and Anti-oxidant Mechanisms

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Glucagon-like peptide-1 (GLP-1) based therapies are used to treat type II diabetes via increasing insulin secretion and inhibiting glucagon production. Recent evidence suggests that activating the GLP-1 receptor may also mediate direct vaso-protective effects. Therefore the objective of the study was to determine whether GLP-1R stimulation conferred cardio- and vaso-protection in a non-diabetic setting using the angiotensin (Ang) II infusion model of hypertension and cardiovascular dysfunction. Male C57Bl/6J mice (4-6 months) were assigned to one of the following 4 week treatment protocols: 1) vehicle (saline), 2) Ang II (800ng/kg/day), 3) Ang II + liraglutide (30µg/kg/day), 4) Ang II + liraglutide (300µg/kg/day). All treatments were administered via osmotic mini-pumps (s.c). After 4 weeks the effect of liraglutide treatment on blood pressure, vascular function and cardiac remodelling was examined. Liraglutide (both doses) attenuated Ang II-induced increase in systolic blood pressure (Ang II: 175.3 ± 8.6mmHg vs Ang II+Lirag (30) 150.2 ± 6.4 mmHg or Ang II+Lirag (300): 145.4 ± 6.9 mmHg) without affecting blood glucose levels. Liraglutide (both doses) completely prevented Ang II-induced endothelial dysfunction (% maximum relaxation: Ang II=50.7 ± 7.8%; Ang II+Lirag (30)=82.7 ± 5.8; Ang II+Lirag (300)=81.5 ± 6.1%). In the heart, liraglutide prevented Ang II-induced cardiomyocyte hypertrophy (n=7-10; p<0.05) and reduced collagen deposition (% collagen expression: Ang II=4.4 ± 0.5 vs Ang II+Lirag(300)=2.9 ± 0.3; n=7-9; p<0.01). This anti-fibrotic effect was attributed to reduced fibroblast/myofibroblast expression as well as decreased inflammation with reduced NFκB and MCP-1 expression and decreased oxidative stress with a significant reduction in superoxide production using high dose of liraglutide. Overall, stimulation of GLP-1R in a non-diabetic

setting protected against Ang II-mediated cardiac hypertrophy, cardiac fibrosis and vascular dysfunction, indicating potential for use of GLP-1 based therapies in treatment of cardiovascular disease independent of diabetes.

H. Lee: None. **M. Brdar:** None. **R. Widdop:** None. **A. Dear:** None. **T. Gaspari:** None.

P007

Hypertension and Myocardial Mechanics in Patients with Hypertrophic Cardiomyopathy

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Introduction: Strain abnormalities in patients with Hypertrophic Cardiomyopathy (HCM) is associated with poor prognosis. Our aim was to assess whether the presence of hypertension in HCM altered myocardial mechanics using strain echocardiographic imaging.
Hypothesis: Hypertension is a risk factor for diminished deformation in patients with HCM.
Methods: A retrospective chart review of patients undergoing HCM evaluation at our facility between December 2008 and December 2011 was performed. Patients were divided into subsets based on the presence of hypertension as a documented diagnosis. All patients had comprehensive echocardiographic assessments with strain imaging. Clinical data was extracted from the electronic medical record. Comparisons in strain values were made between both subsets.
Results: A total of 200 patients was assessed. The average age was 56 years (60% male) and 42.5% of the patients had a diagnosis of hypertension. Global longitudinal strain, Mid strain, Basal strain, Anterior, Lateral, Inferior, Inferolateral strain had no relation to the presence of hypertension. Septal strain (mean without hypertension -14.306 vs with hypertension -16.071, p value: .0069) Inferoseptal strain (mean without hypertension -12.813 vs with hypertension -15, p value: .0018), and Anteroseptal deformation (mean without hypertension -15.301 vs with hypertension -16.784, p value: .0445) however, was increased in patients with hypertension compared to patients with no history of hypertension (See Table)
Conclusions: The presence of hypertension is associated with septal strain abnormalities. Thus, future exploration of hypertension control and hypertrophic cardiomyopathy prognosis and outcomes need to be explored.

K.K. Manocha: None. **D.F. Snipelisky:** None.

P008

Impact of Hydrogen Sulfide on the Epigenetics of Diabetic Cardiomyopathy.

Sudhakar Veeranki, Sourav Kundu, Srikanth Givvimnai, Suresh C Tyagi, Univ of Louisville, Louisville, KY

Although Diabetic cardiomyopathy results in enhanced risk for heart failure, epigenetic changes leading to diabetic heart failure are unclear. Hydrogen sulfide (H2S) has been implicated in the preservation of heart function owing to its anti-inflammatory and positive metabolic changes. In the current study, we investigated whether or not chronic H2S treatment (by giving NaHS) reverses diabetic cardiomyopathy using mouse model of type-1 diabetes: Akita mice. Regulators of mitochondrial biogenesis, calcium handling and molecules that regulate post-ischemic recovery were assayed by western blotting and Q-PCR. Further, we considered epigenetic modifications such as microRNA expression

changes and DNA methylation alterations to understand the causes of diabetic heart failure with and without NaHS treatment for 30 days. Our data indicated that chronic H2S treatment significantly reduced the mitochondrial fission inducers: Drp-1 (24%) and Fis-1 (17%) in the Akita mouse hearts. Further, there was enhancement (10%) in the SERCA2a expression after NaHS treatment in the diabetic hearts. Also, there was significant decrease (16%) in TNF-1α protein expression in diabetic hearts after NaHS treatment. In addition, there was significantly increased expression of post-ischemic recovery regulators such as: Notch3 (157%), C-JUN (160%), PGC-1α (173%), HIF-2α (72%) , and NRF-1 (149%) after NaHS treatment. These results suggest that the chronic NaHS treatment ameliorates diabetic cardiomyopathy through decreasing mitochondrial fission and inflammation and enhancing Ca2+ handling; as well as mitigating epigenetic changes leading to enhanced post-ischemic recovery potential.

S. Veeranki: None. **S. Kundu:** None. **S. Givvimnai:** None. **S.C. Tyagi:** None.

P009

Relationship Between Increased Systolic and Diastolic Pressure and Left Atrial Train Rate

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Background

Left atrial (LA) strain rate analysis by two-dimensional speckle tracking can represent a new tool to evaluate LA function. Office blood pressure is the most powerful non-invasive index of arterial stiffness which can determine LA function.

We hypothesized that increased systolic and diastolic pressure were associated with increased left atrial strain.

Methods

We consecutively examined 2D speckle-tracking in an asymptomatic cohort, with cardiac geometry, LA volume, LA ejection fraction, LA strain rate analyzed. The LA speckle tracking echocardiography curves were obtained using R-wave onset of the electrocardiogram as a reference point. The LA strain rate (SRs, SRe and SRa) were analyzed by commercialized software (GE EchoPAC; GE Vingmed, Norway).

Results

A total of 4052 volunteers were divided into quintiles by systolic or diastolic blood pressure (systolic pressure cut point 110, 118, 126, 136mmHg; diastolic pressure cur=t point 68, 71, 80, 85mmHg). A trend toward the greater systolic or diastolic pressure, the higher LA diameter, LA volume and LA strain rate across 5 groups were observed (Table1 and Table 2, all trend $p<0.001$ except LA SRs).

Conclusion

Increased in systolic and diastolic pressure were independently associated with increased LA strain rate, LA diameter and LA volume in asymptomatic populations.

T. Yang: None. **T. Hung:** None. **J. Kuo:** None. **J. Hou:** None. **H. Yeh:** None. **C. Hung:** None.

P010

Specific Renal Outer Medulla Upregulation of α-and β-Adrenergic Receptor Subtypes in Spontaneously Hypertensive African Green Monkeys

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The African Green Monkey (*Chlorocebus aethiops sabaeus*) is a highly translational model of spontaneous hypertension (HT). Within the cohort of measured colony animals, 33% (98 of 300) of adult African Green Monkeys exhibit systolic blood pressures (SBP)> 140 mmHg, assessed by forearm cuff plethysmography. Heart rate is elevated in HT vs. normotensive (NT) animals (121±2 bpm vs. 134±2 bpm, $p<0.05$) suggesting that sympathetic nerve activity is likely elevated in the HT African Green Monkey, similar to patients with essential hypertension. This study assessed mRNA expression of adrenergic receptor subtypes in the renal cortex, outer medulla, and inner medulla of male HT and NT African Green Monkeys. Primers were custom designed and evaluated for appropriate specificities and efficiencies (95-105%) for α_{1a}, α_{1d}, α_{2c}, β_1 and β_2 adrenoceptors. Gene expression was measured with qRT-PCR and normalized to RPS32 expression in the liver of each animal. The HT cohort of African Green Monkeys (n=18) assessed an averaged SBP of 168.24 ± 7.25mmHg compared with NT animals (n=18) that averaged 96.61 ± 3.20mmHg. Expression of α_{1a}, α_{1d} and α_{2c} genes were all increased in the renal outer medulla of HT animals (α_{1a}: NT RQ 0.24 ± 0.15 vs. HT RQ 0.89 ± 0.22, $p<0.05$; α_{1d}: NT RQ 0.43 ± 0.24 vs. HT RQ 1.99 ± 0.74, $p<0.05$; and α_{2c}: NT RQ 1.11 ± 0.53 vs HT RQ 1.41 ± 0.35, $p<0.05$). β_1 adrenergic receptor expression in the outer medulla was similar in NT and HT animals (β_1: NT RQ 0.75 ± 0.30 vs. HT RQ 1.82 ± 0.64, $p>0.05$), but β_2 adrenoceptor expression was upregulated in HT animals (β_2: NT RQ 0.36 ± 0.13 vs HT RQ 2.05 ± 0.82; $p<0.05$). Expression of each adrenoceptor subtype was similar in the renal cortex and inner medulla of HT animals ($p>0.05$, all groups compared with Mann-Whitney U Test). These data suggest that the renal outer medulla of HT African Green Monkey may be sensitized to sympathetic nerve activity via elevated adrenergic receptor gene expression. Thus, subtle changes in renal sympathetic outflow may elicit renal medullary vasoconstriction and contribute to the development of the spontaneous hypertensive phenotype in the African Green Monkey.

M.K. Rhoads: None. **J.L. Osborn:** F. Ownership Interest (includes any stock, stock option, partnership, membership or other equity position in an entity regardless of the form of the entity, or any option or right to acquire such position, and any rights in any patent or other intellectu; Significant; Biomedical Sciences Research Group, LLC.

P011

Source and Catabolism Sites of the Cardio-renal Protective Peptide N-Acetyl-Seryl-Aspartyl-Lysyl-Proline (Ac-SDKP)

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Ac-SDKP is a natural tetrapeptide with anti-fibrotic and anti-inflammatory properties in vascular, myocardial and kidney tissues. It is degraded by angiotensin converting enzyme (ACE) and treatment with ACE inhibitors increases its plasma levels. However the main sites of Ac-SDKP production and catabolism are unknown. Thus, the aim of this study was to determine the source and catabolism sites of Ac-SDKP. Ac-SDKP was measured in brain, thymus, lungs, heart, spleen, kidney, liver, intestine, and bone marrow from male 200-250 gr Sprague Dawley rats. Samples from arterial blood delivered to and venous blood exiting from kidney, spleen, intestine, liver, and lungs

were obtained, and the vein to arterial (V-A) plasma levels differences were calculated as an index of organ production or elimination. Additionally, urinary Ac-SDKP excretion over 24 hrs and Ac-SDKP renal clearance were measured. Inulin clearance was also determined. The Stop Flow technique was performed to evaluate the renal handling of Ac-SDKP in different segments of the nephron. The greatest amounts of Ac-SDKP were found in Thymus, Spleen and Bone marrow (111.3±9; 92.2±24; 70.3±17 pmoles/mg of protein respectively), while the lowest values were found in brain, large intestine and heart (10.7±6; 14.8±6 ;19.8±7; pmoles/mg of protein, respectively). Ac-SDKP is secreted by different organs into the circulating blood. The highest V-A difference was found in the spleen (15.2±7 nM), reflecting its production, while the lowest V-A differences was observed in the lungs (-1.2±3.7 nM), reflecting a high degradation of Ac-SDKP in this tissue. No V-A differences were found in kidney (0.98±7 nM). It was not possible to obtain venous samples from Thymus or Bone marrow. The renal clearance of Ac-SDKP was 0.15 ml/min/100g body weight, equivalent to a fractional excretion of 14.5%. The stop flow technique showed the highest Ac-SDKP levels in the distal part of the nephron, while the proximal segments showed the lowest (11 Vs. 4.2 Ac-SDKP/Inulin ratio). In conclusion, Ac-SDKP production occurs mainly in lymphoid organs. Expectedly, high Ac-SDKP degradation was observed in the lungs. In the kidney Ac-SDKP is filtered, probably degraded in the proximal tubule and secreted in the distal tubule.

C.A. Romero: None. **N. Kumar:** None. **N. Rhaleb:** None. **O.A. Carretero:** None.

Mycophenolate Mofetil Prevents Cerebrovascular Injury in Stroke-prone Spontaneously Hypertensive Rats

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The stroke-prone spontaneously hypertensive rat strain (SHR-A3) develops hypertensive end-organ injury, including strokes and progressive kidney disease, as a result of naturally occurring genetic variations. Our recent work identifies genetic variants in immune signaling pathways that contribute to susceptibility to end organ injury. These variants are present in SHR-A3 animals, but absent in SHR lines that resist end organ injury. To test the hypothesis that a dysregulated immune response promotes stroke susceptibility, 20-week old male SHR-A3 rats were salt loaded (1% NaCl in drinking water) and treated daily with the immunosuppressant mycophenolate mofetil (MMF, 25 mg/kg/day, p.o.) (n=8) or vehicle (saline) (n=9) for 8 weeks. Blood pressure was measured weekly by telemetry for the duration of the study. Compared to untreated controls, MMF-treated SHR-A3 had improved survival and significantly lower neurological deficit scores (1.44 vs 0.125; $p<0.02$). Salt loading resulted in a progressive increase in blood pressure, which was prevented in rats receiving MMF. At the time of euthanasia, mean systolic BP in the untreated group was 243.0 ± 5.6 mmHg compared to 213.6 ± 7.1 mmHg ($p<0.005$) in the MMF-treated SHR-A3 group. Gross morphology of the brain revealed cerebrovascular lesions including cerebral edema in 90% (8 out of 9) and microbleeds and hemorrhages in about 50% (5 out of 9) of the untreated SHR-A3 rats. These lesions were absent in the MMF-treated cohort. The expression of CD68, a marker of activated

microglia/macrophages, was up-regulated in the brain sections from vehicle-treated rats with microbleeds and hemorrhages, but was not detectable in the brains of rats receiving MMF. MMF also prevented renal injury in SHR-A3 rats, evidenced by reduced proteinuria (albumin to creatinine ratio) from 7.52 to 1.05 mg/mg ($p<0.03$) and lower tubulointerstitial injury scores (2.46 vs 1.43; $p<0.01$). Our findings provide evidence that suppression of immune activation prevents the occurrence of cerebrovascular and renal injury in salt-loaded SHR-A3 rats.

I.S. Dhande: None. **Y. Zhu:** None. **P.A. Doris:** None.

P013

Meta-analysis of Poor Outcomes and Permissive Hypertension after Acute Ischemic Stroke

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Background. Stroke is the leading cause of long term disability and second leading cause of death worldwide. The effectiveness of primary and secondary prevention of stroke by antihypertensive medications is well validated, however, support for permissive hypertension in the early course of acute ischemic stroke (AIS) has been questioned.

Materials and methods.

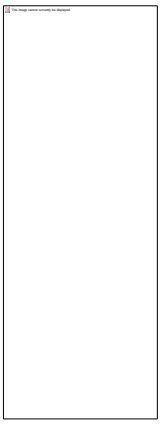
We searched Pubmed, Embase, and Cochrane databases to identify RCTs comparing different blood pressure reduction regimens with placebo in AIS patients within 48 hours after symptom onset and sample size of 100 or more patients. We excluded studies that do not report mortality or functional outcomes at the end of follow up. The main outcomes were all-cause mortality and death or severe morbidity which was defined as: modified Rankin Score >2 or Bartel ADL index<60, Glasgow outcome scale3, Mathew Impairment Scale <14. Relative risks (RR) and corresponding 95% confidence intervals (CI) were calculated using random-effect model.

Results.

In our analysis we included 20 trials involving 17,209 patients. There was no difference in all-cause mortality RR 1.04 (95% CI 0.95-1.13), p=0.4 nor in mortality or severe disability RR 1.03 (95% CI 0.99-1.08), p=0.16 between active blood pressure reduction and permissive hypertension strategy. There was no evidence of heterogeneity between studies for both outcomes I^2=2.6% and I^2=14.1% , p for heterogeneity =0.42 and =0.27, respectively.

Conclusion.

Use of antihypertensive therapy in acute period of ischemic stroke does not have an effect on disability or all-cause mortality.



A. Ivanov: None. **A. Mohamed:** None. **A. Korniyenko:** None.

P014

Poor Blood Pressure Control and Severe Coronary Artery Lesions Are Risks of Cerebral Microbleeds in Patients With Coronary Artery Disease During Antiplatelet Therapy

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Background and Purpose: Brain hemorrhage is a serious complication of antiplatelet therapy, particularly dual antiplatelet therapy (DAPT), in patients with coronary artery disease (CAD) who undergo percutaneous coronary intervention (PCI). Cerebral microbleeds (CMBs) detected on MRI is a surrogate of symptomatic brain hemorrhage. However, little is known regarding CMBs in CAD patients during long-term antiplatelet therapy. Therefore, we investigated the temporal change of CMBs during antiplatelet therapy in CAD patients after PCI.
Method: Brain MRI was performed at baseline and after 8-month follow-up in consecutive 14 patients who underwent PCI and antiplatelet therapy (DAPT in 13 patients).
Results: Baseline MRI revealed CMBs in 2 patients (14%). New CMBs were detected by follow-up MRI in 2 other patients (14%). Although blood pressure (BP) at baseline did not differ between the CMB-positive and CMB-negative patients, BP after 8 months was significantly higher in CMB-positive patients than CMB-negative ones (systolic BP: p=0.03, diastolic BP: p=0.02). Moreover, CMB-positive patients had greater number of coronary artery lesions and higher SYNTAX score at baseline than CMB-negative patients (Figure).
Conclusions: CAD patients with poor BP control and severe coronary artery lesions would be at higher risk for CMBs and eventually brain hemorrhage during antiplatelet therapy. Accordingly, strict coronary risk control, especially BP control, is necessary in CAD patients receiving long-term antiplatelet therapy. 

Y. Iwamoto: None.

P015

Bilateral Common Carotid Artery Stenosis in Hypertensive Rats Impairs Dilation in Penetrating Arterioles and Posterior Communicating Arteries

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Hypertension and chronic cerebral hypoperfusion (CCH) are leading risk factors for cognitive impairment. We induced hypoperfusion in stroke prone spontaneously hypertensive rats (SHRSP) by bilateral carotid artery stenosis (BCAS) to create a novel, physiologically relevant model of cognitive impairment. We hypothesized that BCAS in SHRSP would impair endothelium dependent dilation and lead to outward remodeling of the penetrating arterioles (PAs) and posterior cerebral arteries (PCAs). The PAs are critical for maintaining parenchymal perfusion and controlling blood flow to the neurovascular unit, and the PCAs are integral in regulating the severity of CCH. Both artery types were studied by pressure myography and data are shown as mean±SEM, SHAM vs BCAS (an n=4 to 12 in each group) with $p<0.05$. Short-term memory, evaluated by novel object recognition testing, was impaired 8 weeks after BCAS (novel exploration quotient: 0.60±0.1 vs 0.45±0.1). Endothelium-dependent dilation was assessed with carbachol (data shown as % dilation at 10^{-6}M). BCAS impaired endothelial function in PAs, as evidenced by the abolition of the dilation (14±3.3% vs -10±4.7%) but did not significantly alter sodium nitroprusside induced dilation (60 ± 9.7% vs. 49 ± 6.4%). Inhibition of nitric oxide and prostacyclin production with L-NAME (100

μM) and indomethacin (10 μM), respectively, did not change dilation in either group. This suggests that endothelium-derived hyperpolarizing factor plays a role in PA dilation in SHRSP. PAs from BCAS rats had a reduced wall thickness (at 60 mmHg: 7±0.5 vs 5±0.6 μm), and wall-to-lumen ratio (at 60 mmHg: 0.10±0.002 vs 0.07±0.0001). BCAS increased wall stress in PAs (at 60 mmHg: 306±30.2 vs 427±48.3 dynes/cm^2). Endothelial dysfunction also was evident in the PCAs after BCAS (50 ± 13.0% vs 3 ± 14.9%) but the pattern of remodeling in these larger arteries was different. We did not observe a change in the wall thickness or wall-to-lumen ratio in PCAs after BCAS, but the lumen diameter was increased (at 80 mmHg: 105±7.7 vs 116±5.7 μm). These data suggest that endothelial dysfunction in the PAs and PCAs may worsen the severity of cerebral hypoperfusion and cognitive decline in SHRSP. The thinner walls in the PAs may also increase the risk of hemorrhage in the BCAS rats.

N. Matin: None. **C. Fisher:** None. **W.F. Jackson:** None. **A.M. Dorrance:** None.

P016

GYY4137, a Hydrogen Sulfide Donor, Mitigates Ang II-induced Extracellular Matrix Remodeling in Glomerular Endothelial and Mesangial Cells

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Hypertensive nephropathy is associated with progressive alteration of extracellular matrix (ECM) components. Both mesangial and glomerular endothelial cells have the ability to synthesize and degrade ECM proteins such as collagens by changes in the activity of matrix metalloproteinases (MMPs) and their inhibitors, tissue inhibitors of metalloproteinases (TIMPs). Endo180 is an extracellular fibronectin type II domain involved in lysosomal degradation of collagen which has been shown to mitigate renal fibrosis. More recently, hydrogen sulfide (H2S) has also been shown to mitigate hypertensive renal remodeling, however, its mechanism remains unclear. In this study, our aim was to investigate whether Angiotensin-II (Ang II) treatment alters the expression of Endo180, MMPs and TIMPs leading to dysregulation of collagen metabolism and whether GYY4137 (H2S donor) restores their levels to achieve homeostasis. Mesangial and mouse glomerular endothelial cells (MCs and MGECs respectively) were treated without or with Ang II (200 nM) and GYY4137 (250 μM) for 48hrs. Cell lysates were analyzed for MMP-13, -14, TIMP-1, Endo180, and collagen IV by Western blot, RT-PCR, and immunohistochemistry. In MGECs, Ang II treatment compared to its control decreased MMP-13/TIMP-1 ratio (0.75±0.44 vs. 2.48 ±0.73), and upregulated MMP-14/TIMP-1 ratio (0.64±2.10 vs. 0.96±1.47), and collagen IV (0.77±0.07 vs. 0.58±0.06). GYY4137 treatment mitigated these changes. In contrast, Ang II treatment in MCs decreased Endo180 compared to control (0.72±0.04 vs. 1.07±0.23), but did not alter the expression of MMP-13/TIMP-1, MMP-14/TIMP-1 ratios, and collagen IV level compared to control or MGECs. Similarly, immunostaining showed downregulation of MMP-13 and Endo180 in Ang II treated MGECs which was normalized following GYY4137 treatment. Endo180 was also normalized in MCs following GYY4137 treatment however, there was no change in MMP-14/TIMP-1 ratio or collagen IV level. We conclude that Ang II treatment causes adverse ECM remodeling in MGECs via downregulation

of Endo180 and MMP-13 and upregulation of MMP-14 and TIMP-1 and in MCs by decreasing Endo180, and GYY4137 mitigates these changes in part, by modulating Endo180/MMP/TIMP pathway.

M. Amin: None. **S. Pushpakumar:** None. **S. Kundu:** None. **G. Tyagi:** None. **A. Tyagi:** None. **U. Sen:** None.

P017

Contribution of High Mobility Group Box 1 to Hyperhomocysteinemia-induced Podocyte Injury and Glomerular Sclerosis

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High mobility group box 1 protein (HMGB1), a nuclear DNA binding protein is released under pathological conditions and locally act as one of potent damage-associated molecular patterns (DAMPs) to produce tissue injury and chronic inflammation. However, it remains unknown so far whether HMGB1 is implicated in homocysteine (Hcys)-induced podocyte injury and glomerular sclerosis during hyperhomocysteinemia (hHcys). In the present study, we found that homocysteine (Hcys) dose-dependently increased the production of HMGB1 in cultured podocytes, and that HMGB1 binder and inhibitor, glycyrrhizin (Gly) completely blocked its release induced by Hcys. Furthermore, inhibition of HMGB1 preserved podocyte function by restoring Hcys-induced suppression of VEGF secretion, decrease in expression of podocin and elevation of desmin level (podocyte damage markers). In *in vivo* studies, C57BL/6J wild type mice were fed a folate free (FF) diet or normal chow for 8 weeks to produce hHcys and administrated with vehicle or glycyrrhizin (1mg/kg/day) locally into the renal cortex. Western blot analysis of renal tissue showed that the FF diet significantly increased HMGB-1 (2 folds) and desmin expression (1.8 folds) compared to control mice, which was blocked by HMGB 1 inhibitor Glycyrrhizin. The urinary protein and albumin excretion were significantly higher in mice on the FF diet compared to ND fed mice. In glycerrhizin treated mice, however, the hHcys-induced albuminuria was significantly lowered (urinary albumin excretion in vehicle- and glycerrhizin-treated hHcys was 50 ± 3 and 32 ± 2.5 μg/24h/g BW, respectively). Morphological examinations showed that hHcys-induced more profound injury in glomeruli of vehicle treated mice than in glycerrhizin treated mice (the glomerular damage index in vehicle and glycerrhizin-treated hHcys mice was: 2.8 ± 0.4 vs. 1.3 ± 0.3). Based on these results, it is concluded that HMGB1 is one of important mediators of hHcys-induced podocyte injury and glomerular sclerosis. HMGB1 may be a therapeutic target for treatment or prevention of glomerulosclerosis associated with hHcys.

K.M. Boini: None. **M. Xia:** None. **S.M. Conley:** None. **G. Li:** None. **S. Koka:** None. **T.W. Gehr:** None. **P. Li:** None.

P018

Periodic Water Intake Exacerbates Hypertension, Decreases Renal Function and Increases Renal Inflammation in Spontaneously Hypertensive Rats

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Water is essential for life. The kidneys are major regulators of fluid balance but also appear susceptible to adverse effects of periodic water intake since epidemiological studies have linked low water intake to chronic kidney disease. However, the impact of periodic water intake on renal health has largely been ignored. This study investigated the chronic effects of periodic drinking on blood pressure, kidney function, and immune cell infiltration in rats with pre-existing hypertension.
Mean arterial pressure (MAP) was measured continuously in spontaneously hypertensive rats (SHR) that were only given access to water for 2 hours per day versus control rats given free access to water over a 4-week period (n=>8 per group). Glomerular filtration rate (GFR) was measured via transcutaneous FITC-sinistrin clearance at baseline and at the end of the 4-week protocol. Renal immune cell infiltration and cytokine production was assessed by flow cytometry.
Basal MAP and renal function were similar between the control and water-restricted (WR) groups. Water restriction led to a significant increase in MAP from baseline (8.8±4.5mmHg versus 0.6±5.5mmHg in WR and control SHR, respectively; P<0.05). Moreover, GFR decreased from baseline by 29±6% in the WR SHR (P<0.001). In comparison, GFR did not change significantly over time in the control SHR (-5.3±2.6% from baseline; P>0.05). The increase in MAP and reduction in renal function in the WR-SHR was associated with periodic increases in urine osmolality (69±26% versus baseline, P<0.05), indicative of cycles of dehydration and replenishment. Renal immune cell infiltration was similar between the groups. However, cytokine analyses revealed a phenotypic shift towards a pro-inflammatory Th1 phenotype in kidney T cell infiltrate from WR versus control SHR. This was indicated by a greater proportion of T cells producing IFN-γ in SHR subjected to water restriction than control SHR (9.5±3.3% versus 2.9±0.9%, respectively; P<0.05).
In conclusion, recurrent dehydration associated with periodic drinking exacerbates hypertension, renal dysfunction and inflammation in male SHR. This highlights the importance of regular water intake for the maintenance of kidney health, particularly in populations with existing disease.

L.M. Hilliard: None. **L. Bulmer:** None. **K.M. Mirabito:** None. **A. Vinh:** None. **K.M. Denton:** None.

P019

Upregulation of Autophagy Enhances (pro)renin Receptor Expression via P62 -erk1/2 Signaling Pathway in Podocytes

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Abnormally up-regulated or down-regulated autophagy induces cell death. p62 is selectively degraded by autophagy, and was also recently reported to modulate extracellular signal-regulated kinases (ERK1/2) activity. However, the effect of autophagy on PRR expression is not known. We hypothesized that autophagy activation increases PRR expression via modulation of p62 -ERK1/2 signaling pathway. Cultured mouse podocytes were treated with the autophagy activators, rapamycin (100 nm) or Earles's balanced salt solution (EBSS), for 48 hours. Both rapamycin and EBSS significantly increased mRNA and protein expressions of PRR (see below attached figures), decreased p62 protein levels and increased ERK1/2 activation by phosphorylating pTpY185/187. Confocal microscopy studies showed significant increase in protein levels of microtubule-associated

protein-1 light chain 3B (LC3B) and PRR, and decrease of p62/SQSTM1 level in response to rapamycin or EBSS. Using autophagy related 5 (ATG5) cDNA or ATG7 cDNA transfection for 72 hours to enhance podocytes autophagy showed the same results as rapamycin and EBSS treatments. Inhibition of autophagy with bafilomycin A1 (10 nm) reversed the effects of rapamycin. ERK1/2 inhibitor U0126 (10 μM) significantly attenuated mRNA and protein expressions of PRR in podocytes treated with rapamycin, while has no effect on p62/SQSTM1 level. In conclusion, up-regulation of autophagy enhanced PRR expression through p62-ERK1/2 signaling pathway.

Key words: Podocytes; (Pro)renin receptor; autophagy; p62; ERK1/2

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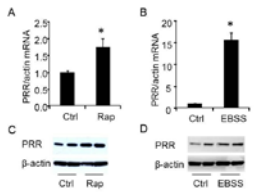

C. Li: None. **S. Quadri:** None. **H. Siragy:** None.

P020

Decreased Survival Rate in Female Obese Leptin Receptor Mutant Dahl Salt-Sensitive Rats that Develop Chronic Kidney Disease

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Obesity contributes to sex differences in the risk for chronic kidney disease (CKD) in which males tend to develop CKD earlier in life than females. Therefore, in the current study, we examined whether there were sex differences in the development of CKD in the obese leptin receptor mutant Dahl salt-sensitive (SS^{LepR}mutant) strain which was derived from Zinc-finger nucleases. We observed an increase in body weight in both female and male SS^{LepR}mutant rats when compared to SS rats throughout the study. Glucose tolerance was impaired significantly in female and male SS^{LepR}mutant rats versus SS rats by 18 weeks of age. The SS^{LepR}mutant strain also developed hyperinsulinemia in comparison to their lean SS counterparts (6.86±0.83 vs 0.72±0.04 ng/mL, respectively, n=6). However, blood glucose in the SS^{LepR}mutant strain remained within normal range throughout the course of the study regardless of sex. Female and male SS^{LepR}mutant rats developed severe systolic hypertension (via tail-cuff) by 18 weeks of age when compared to the values measured in SS rats (199±7 and 201±10 vs. 159±5 and 155±4 mmHg, respectively, n=6). Yet, the rise in arterial pressure occurred earlier in female SS^{LepR}mutant rats than males. Protein excretion was significantly higher in the SS^{LepR}mutant strain as opposed to the values observed in SS rats at 18 weeks of age regardless of sex (488±61 and 631±86 vs. 50±17 and 149±23 mg/day, respectively, n=6). At the end of study, kidneys from the SS^{LepR}mutant strain displayed increased glomerulosclerosis and interstitial fibrosis than SS rats. Female and male SS^{LepR}mutant rats had a significant increase in plasma creatinine levels and averaged 2.1±0.4 mg/dL (n=6) compared to the normal value of 0.5±0.1 mg/dL (n=6) observed in the SS strain suggesting the presence of severe CKD. While both, female and male, SS rats survived the length of study, the survival rate of female SS^{LepR}mutant rats was markedly reduced compared to their male counterparts (62%, 21

of 34 vs. 25%, 6 of 24, respectively). Overall, these data indicate that the SS^{LepR}mutant strain may be a useful model to study sex differences during the development of CKD associated with obesity.

K. McPherson: None. **D. Guillory:** None. **L. Taylor:** None. **D. Spires:** None. **A.C. Johnson:** None. **M.R. Garrett:** None. **J.M. Williams:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHA 12SDG9440034, NIGMS NIH P20GM104357.

P021

The Combined Effects of Elevated Intrarenal Ang Ii and Blood Pressure Causes Greater Renal Injury in the Non-clipped Kidneys in 2k1c Rats

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In 2-kidney 1-clip (2K1C) hypertension, intrarenal angiotensin II (Ang II) levels are increased in both kidneys, which are often implicated in the further augmentation of the intrarenal renin-angiotensin system and the development of hypertension and kidney injury. We recently reported that angiotensinogen (AGT) expression and secretion are increased only in the non-clipped kidneys (NCK). These findings provide the basis for the hypothesis that elevated Ang II levels, augmented AGT, and high arteriolar pressure in NCK synergistically cause greater kidney injury. Accordingly, we compared the degrees of kidney injury between clipped kidneys (CK) and NCK using a 2K1C model that maintains normal renal blood flow and GFR in the CK. Left kidneys of male rats were clipped for 21 days. Histological and immunohistological analyses were performed on both the CK and NCK. Twenty glomeruli and 20 microscope fields in the cortex and the medulla were examined for each group. The PAS-positive area in the glomeruli was higher in NCK compared with sham (33.9 ± 0.89 vs. 12.4 ± 0.51%) and CK (vs. 15.1 ± 0.58%). Similarly, the Masson's trichrome-positive area in the medullary region was greater in NCK compared with sham (2.21 ± 0.10 vs. 1.32 ± 0.05%) and CK (vs. 1.67 ± 0.10%), but the changes were not observed in the interstitial tissue of the cortex. Immunoreactivity for CD68, a marker of the macrophage and monocyte levels, was higher in NCK compared with sham (0.72 ± 0.07 vs. 0.36 ± 0.04%), but similar to that in CK. In contrast, accumulation of the immune cells in the glomeruli was greater in NCK compared with sham (8.99 ± 0.69 vs. 3.46 ± 0.46%) and CK (vs. 3.08 ± 0.24%). The proliferating cell nuclear antigen levels, a marker of cell proliferation, were greater in NCK (3.49 ± 0.09 %) but not in the CK. Levels of vimentin, a cell transformation and regeneration marker, were also higher in NCK compared with CK and sham. Increased vascular wall thickness (α-SMA) was observed in both kidneys. These results indicate that pathological factors associated with the high blood pressure are required for the development of renal injury in the 2K1C model including glomerular expansion, medullary fibrosis, immune cell infiltration in glomeruli and cell proliferation/transformation.

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P022

Association of Longitudinal Change in Kidney Function with Left Ventricular Structure and Function as Measured by Cardiac MRI: The Multi-Ethnic Study of Atherosclerosis (MESA)

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Background
This study examines the association of change in estimated glomerular filtration rate (eGFR) with change in left ventricular (LV) structure and function over 10 years of follow-up.
Method
MESA participants with cardiac magnetic resonance imaging (CMR) measures and eGFR measured at both baseline (2000-2002, year-0) and the 10 year follow-up (2010-2012, year-10) exam were studied. Participants with cardiovascular disease (CVD) were excluded. LV mass and volume indexed to body surface area, LV ejection fraction (LVEF), and LV mass volume ratio (MVR) were determined by CMR. We stratified participants by the presence of eGFR < 60 ml/min/m2 at baseline and at year 10 into 4 groups; eGFR ≥60 at both year-0 and year-10 (reference), ≥60 at year-0 and <60 at year-10, <60 at year-0 and ≥60 at year-10, and <60 at both year-0 and 10. Multiple linear regression models were used to evaluate the association between eGFR groups and change in LV parameters (year-10 - year-0) after adjusting for the demographics, baseline CVD risk factors, change in risk factors, and baseline LV parameters.
Results
2,722 participants (age 59 ± 9 years, 53% women, 42% White, 13% Chinese, 25% African American, 20% Hispanic) were included. Compared to the reference group, the group with eGFR ≥60 at year-0 and <60 at year-10 and the group with eGFR <60 at both year-0 and 10 group showed increasing MVR during 10 year follow-up. No difference in longitudinal change in LV mass or LVEF by eGFR groups were noted (Table).
Conclusion
In this multi-ethnic cohort of adults without clinical CVD, reduced eGFR at baseline or during follow-up was associated with the development of concentric remodeling represented by increased MVR.

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P023

Toll-like Receptor 4 Deficiency Reduces Macrophage Mediated Renal Injury in Ang-II Induced Hypertension

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Infiltration of macrophages has been amply demonstrated in the kidney of humans and

experimental models of hypertension however, its role in tissue injury and repair remains unclear. Furthermore, recent studies implicate upregulation of toll-like receptor 4 (TLR4) and inflammation in hypertension and vascular remodeling. Previously, we observed that C3H/HeJ mice lacking functional TLR4 were hyporesponsive to Ang-II induced hypertension and renal damage. In this study, we hypothesized that TLR4 deficiency inhibits macrophage mediated inflammation and TGF-β-induced fibroblast accumulation to reduce renovascular injury and fibrosis. Male C3H/HeOuJ (functional TLR4) and C3H/HeJ mice (dysfunctional TLR4) aged 8-10 weeks underwent subcutaneous osmotic pump insertion for infusion of Angiotensin-II (Ang-II) at 1000 ng/kg/min or vehicle (0.9% saline) for 4 weeks. Blood pressure (BP) was measured twice weekly by non-invasive tail-cuff plethysmography. Ang-II treatment increased mean BP in both groups but to a greater degree in C3H/HeOuJ mice compared to C3H/HeJ (138.6± 6.3 vs. 103.4±7.2 mm/Hg). Plasma creatinine in saline treated C3H/HeOuJ and C3H/HeJ mice was normal (0.37±0.026 and 0.32±0.023 mg/dL respectively) in contrast to ang-II treated mice (1.34±0.075 vs. 0.78±0.052 mg/dL). Flow cytometry revealed increased F4/80+ and CD40+ cells indicating pro-inflammatory M1 macrophage in Ang-II treated C3H/HeOuJ compared to C3H/HeJ mice. Similarly, Ang-II increased the mRNA and protein expression of CD40, MCP-1, TNF-α and TGF-β in C3H/HeOuJ compared to C3H/HeJ mice. Immunostaining revealed increased colocalization of CD45 and procollagen-1 in renal sections of C3H/HeOuJ mice suggesting increased accumulation of myeloid fibroblasts than in C3H/HeJ mice. The expression of α-smooth muscle actin an indicator of myofibroblast and extracellular matrix proteins, collagen type I and fibronectin (Western blot and immunostaining) was increased in C3H/HeOuJ compared to C3H/HeJ mice. In conclusion, our data suggests that in C3H/HeJ mice, deficiency of functional TLR4 reduces macrophage activation and inflammation which in turn decreases TGF-β-induced renal fibrotic response in Ang-II induced hypertension.

S. Pushpakumar: None. **M. Amin:** None. **U. Sen:** None.

P024

Pharmacological Treatment of Hypertension and Lv Dysfunction Predictors in ESRD Patients With AV Fistula

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Background: Effects of anti-hypertensive medications on left ventricular dimensions and systolic function in patients with arterio-venous (AV) fistulas have not been well investigated.
Material and Methods: Medical charts and echocardiograms of 346 patients with AV fistula were reviewed. Of 346, 149 patients had TTE prior to the AV fistula surgery, 197 had TTE after the AV fistula surgery, and 76 patients had TTE before and after the AV fistula surgery. Data on medication use was available in 314 patients. ANOVA, chi-square, and logistic regression tests were employed.
Results: In patients scheduled for AV fistula placement, 20% (31/149) patients had systolic dysfunction and 15% (22/142) had increased LV end-diastolic dimensions (LVEDD). Moderate systolic LV dysfunction was observed in 6% (9/149) and additional 8% (12/149) had severe LV dysfunction. Increased LVEDD with some LV dysfunction was noted in 27%

(38/142).Following the AV fistula placement, 18% (36/197) of patients had systolic dysfunction and 12% (22/187) had increased LV end-diastolic dimensions (LVEDD). Moderate or severe systolic LV dysfunction was observed in 6% (5/197). LV systolic dysfunction or dilatation was noted in 23% (43/187).
Of 314 patients, 63% were on beta-blockers (BB), 25% were on ACE inhibitor or an ARB , 43% on calcium-channel blocker , and 15% on alpha-antagonist . BB, ACEi-ARB, or AA were not associated with increased LVEDD or systolic dysfunction before or after the AV fistula placement. Prior to AV fistula, CCB treatment was not related to LV dilatation (36% in each group, p=0.981) **Post AV fistula, CCB treatment was associated with increased LV dimensions (71% vs. 46%, p=0.029) but not LV systolic dysfunction (49% in LV dysfunction vs. 38% in the rest, p=0.446)**. This association persisted after adjustment for co-morbidities and demographic parameters.
Conclusions: LV systolic dysfunction and/or dilatation are common in patients undergoing AV fistula surgery. Despite decreased use of Ca-channel blockers in patients with LV dysfunction prior to AV fistula, Ca-channel blockers are associated with increased LV dimensions post AV fistula, and probably should be avoided in this vulnerable patient population.

S. Tariq: None. **J. Anderson:** None. **R. Dhingra:** None. **M. Torosoff:** None.

P025

Clinical Experience on Combination Therapy With Olmesartan and Amlodipine in Treatment of Resistant Hypertension

Cyril James, Snezhana Tisheva, Diana Gaidarova, Nadia Stancheva, Dilyana Yakova, Martin Hristov, Shreya Ohri, Konstantin Gospodinov, Asiq Yanakeva, Tatqna Chakalova, Univ Hosp, Dr. Georgi Stranski, Pleven, Bulgaria; Soumya Jose, MOSC Medical Coll, Kolenchery, India

Objective: To analyse the effect of fixed dose combination therapy with Olmesartan/Amlodipine in the treatment of Resistant Hypertension.
Design: Questionnaire based cross sectional study. Method: The study was carried out among 364 patients admitted with history and 24 hour holter blood pressure monitor evidence for Resistant Hypertension in the Department of Cardiology between 1st January 2012 and 31st January 2015. Patients with history of Resistant Hypertension were screened with 24 hour holter blood pressure monitoring and those who fullfilled the criteria for Resistant Hypertension according to the ESC guideline for Management of Arterial Hypertension based on the treatment history and 24 hour holter blood pressure monitoring were included in the study after obtaining informed consent. Patients included in the study were started on therapy with Olmesartan/Amlodipine at fixed dose combination along with diuretic and Beta-Blocker. Patients were continued on this fixed dose combination therapy for 3 months. After the 3 month period patients were reassessed with control holter blood pressure monitoring to access the efficacy of the treatment and the circadian control of arterial blood pressure.
Result : From the study it was observed that 68.68% patients (250 of 364) had reached optimal control of arterial blood pressure by the fixed dose combination therapy with Olmesartan/Amlodipine. 17.04% patients (62 of 364) had non optimal control of arterial blood pressure and 8.79% patients (32 of 364) continued to be with resistant hypertension in-spite of the maximal dosage of the fixed dose

combination therapy with Olmesartan/ Amlodipine. 5.49%- patients (20 of 364) discontinued the treatment due to pedal edema. We observed from the screening holter blood pressure monitoring that 29.94% - patients (109 of 364) with resistant hypertension were non-dippers and after the fixed dose combination therapy with Olmesartan/Amlodipine only 10.44% patients (38 of 364) were non-dippers.
Conclusion: The study revealed that fixed dose combination therapy with Olmesartan/Amlodipine in patients with Resistant Hypertension has high efficacy and minimal side effects with good circadian control of arterial blood pressure.

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P026

Relationship Between Antihypertensive Adherence and Emergency Department Blood Pressure

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Background
Blood pressure is often uncontrolled in the Emergency Department (ED), but the contribution of medication non-adherence to this is not known.
Methods
Three hundred ED patients with hypertension were enrolled from June 2012 to April 2013 to evaluate the association of antihypertensive adherence (measured by serum assay and by survey) with systolic blood pressure (SBP) in the ED. Presence of anti-hypertensive medications in the blood was detected using a previously validated mass spectrometric assay. Adherence was defined as the ratio of detected to prescribed antihypertensive medications (1=completely adherent, 0=completely non-adherent, <1=partially non-adherent). Patients also completed the Adherence to Refills and Medications Scale (ARMS, where a score of 12 defines adherence and >12 indicates some degree of non-adherence). Spearman's rho was used to evaluate correlation of the assay and ARMS. A single BP was measured by research staff using an oscillometric cuff with the patient in a seated position. Multiple linear regression was used to assess the relationship between adherence and SBP, adjusting for age, sex, race, insurance, literacy/numeracy, education, and comorbid conditions. Missing numeracy for 18 subjects was imputed.
Results
Three participants failed to meet inclusion criteria. Remaining 297 patients were 59.1±11.2 (SD) years old, 53.9% female, 62.3% White, 37.7% diabetic, and 24.6% had chronic kidney disease. Patients were prescribed a median of 2 medications (interquartile range 1 to 3, maximum 5). Mean SBP was 136.7±24.2 mmHg. By the assay, 214 (72.1%) patients were adherent, 59 (19.9%) partially non-adherent, and 24 (8.0%) completely non-adherent. By ARMS, 65 (21.9%) were adherent, 232 (78.1%) were non-adherent (rho=-0.23, p<0.001). Adherence by assay was associated with 11.6mmHg lower SBP (95% CI -17.7 to -5.4 mmHg, p<0.001) compared to partial or complete non-adherence. Adherence (by ARMS) was associated with 5.9 mmHg lower SBP, but this was not statistically significant (95% CI -12.6 to 0.8 mmHg, p=0.08).
Conclusions

In this preliminary study, antihypertensive adherence measured by a mass spectrometry assay was associated with lower SBP among patients in the ED. The assay may be a useful tool for future research.

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P027

GCH1 and GULP1 Play a Role in Hypertension Associated With Ovarian Hormone Loss Independently of Body Weight Gain

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Background and Objective: Ovarian hormone loss is associated with an increased incidence of hypertension, body weight (BW) gain and inflammation. Since BW gain is also associated with an increased incidence of hypertension, it has been difficult to separate the role of the immune system in hypertension from BW gain. Dahl salt-sensitive (DS) and Dahl salt-resistant (DR) rats gain BW after ovariectomy; however, only the DS rat becomes hypertensive. In this study, we took advantage of the DS/ DR model to determine which T cell genes are associated with hypertension induced by ovarian hormone loss-independently of body weight gain. Methods and Analysis: DS and DR rats (1 month) were either ovariectomized (OVX) or subjected to sham surgery (Sham). At 4 months of age, a microarray analysis was conducted on isolated splenic T-cells from these 4 groups and differential expression was confirmed by real-time PCR. Results: In contrast to DR rats which remain normotensive, the mean arterial pressure (MAP) significantly increased by 14 mm Hg in DS rats after ovariectomy [MAP (mmHg): DS-OVX, 143 ± 3.4 vs DS-Sham, 129 ± 9.4; n=10-11/group; p=0.0002; DR-OVX, 123 ± 15 vs DR-Sham, 126 ± 4.8; n=11-12/group; ns]. Both rat strains, however, exhibited a similar 17-20% increase in BW after ovariectomy compared to sham treatment [BW (g): DS-OVX, 372 ± 34 vs. DS-Sham, 308 ± 13; n=6-7/group; $p<0.05$; DR-OVX, 323 ± 22 vs, DR-Sham, 276 ± 49; n=7/group; $p<0.05$]. Microarray analysis suggested that among many others, the genes GTP Cyclohydrolase 1 (GCH1) and Engulfment Adaptor PTB Domain Containing 1 (GULP1) were specifically associated with resistance or susceptibility to hypertension induced by ovarian hormone loss. Real-time PCR confirmed that GCH1 and GULP1 were selectively elevated by ovariectomy in DS but not DR splenic T cells. Conclusion: GCH1 and GULP1 play a role in hypertension associated with ovarian hormone loss independently of BW gain, which has implications for women with ovarian hormone deficiency due to premature ovarian failure or elective oophorectomy.

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P028

Intravenous Anti-hypertensives Used for Non-urgent Hypertension in Inpatients

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Background: Asymptomatic hypertension is common in hospitalized patients but there are no clear treatment guidelines. Due to the concern for harm from elevated blood pressures (BP) physicians often prescribe rapidly acting intravenous (IV) agents, which may cause dangerous BP drops. We evaluated if the use of IV hydralazine or labetalol were associated with adverse events.
Methods: We retrospectively identified patients >18 years of age admitted to Boston Medical Center over 1 year who had a recorded systolic BP > 160 mmHg or diastolic BP > 120mmHg during their hospitalization. Patients with hypertensive emergency, or admitted to intensive care or neurology services were excluded. We examined adverse events over the subsequent 24 hours comparing the use of IV labetalol and/or hydralazine versus use of neither IV anti-hypertensive in Cox proportional hazard models adjusted for age, gender, race, systolic and diastolic BP.
Results: 5,431 patients met inclusion criteria of which 203 patients received IV anti-hypertensives (78 labetalol only, 107 hydralazine only, 18 hydralazine and labetalol). In adjusted analyses, patients who received IV anti-hypertensives had increased risk of requiring IV fluids (hazard ratio [HR] 1.44; 95% confidence interval [CI] 1.03-2.01; P<0.03), and developing tachycardia (HR 1.82; 95% CI 1.18-2.82; P<0.007) compared to patients who did not receive IV agents. There was no difference in orders for computed tomography head, neurology or critical care consult, electrocardiogram, and troponin. The use of IV hydralazine compared with no IV drugs was associated with increased IV fluid use (HR 2.03; 95% CI 1.35-3.05; P<0.001) and tachycardia (HR 1.81; 95% CI 1.04-3.16; P<0.04), with no association of adverse events with IV labetalol use. There was no significant difference in 20% or 50% systolic blood pressure drop at 2 or 6 hours between IV hydralazine, IV labetalol or no IV anti-hypertensive groups.
Conclusions: In contrast to previous concerns, our data suggest that the use of IV hydralazine or labetalol was not associated with severe adverse outcomes. Our retrospective study at a single center has several limitations and did not include all possible confounders, and future studies need to replicate these findings in other centers.

F. Rahman: None. **J. Weinberg:** None. **S. Bernard:** None. **L. Arnold:** None.

P029

Vendor Specific Differences in the Sprague Dawley Rat Strain Alter Baseline Blood Pressure and Body Composition and Influence the Impact of Slow Fetal Growth on Later Cardiovascular Risk

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Low birth weight is associated with increased risk for cardiovascular (CV) disease including hypertension in later life. We previously reported that reduced uterine perfusion (RUP) in timed pregnant Sprague Dawley (SD) dams purchased from Harlan induced intrauterine growth restriction (IUGR) associated with hypertension and enhanced sensitivity to angiotensin II (Ang II) in male rats at 4 months of age. In addition, male IUGR exhibited a two-fold increase in testosterone relative to control. Upon castration, hypertension and sensitivity to Ang II were abolished suggesting that programmed CV risk is testosterone dependent in the male IUGR. The aim of this study was to

determine if vendor differences in the SD strain impact CV risk following a developmental insult. Timed pregnant SD rats were purchased from Charles River and underwent either RUP or sham surgery at day 14 of gestation. Birth weight was significantly reduced in IUGR relative to control ($P<0.05$). At 4 months of age, body weight remained significantly decreased in male IUGR relative to control, blood pressure did not differ when measured in conscious, chronically instrumented animals, and male control and IUGR from Charles River also exhibited a similar blood pressure response to acute Ang II. However, baseline blood pressures were higher in male control from Charles River versus blood pressure previously noted for male control from Harlan. Thus, these results suggest that vendor specific differences in the SD rat strain influence baseline CV risk and effect the developmental programming of CV risk following slow fetal growth.

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P030

Early Cognitive Deficits Induced by Diabetes Are Masked by an Increase in Exploratory Behavior: Evidence From TLR2 Knock-out Mice

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Toll-like receptor 2 (TLR2) has been shown to contribute to cardiovascular complications of diabetes such as nephropathy. However, the role of TLR2 in cerebrovascular inflammation and dysfunction leading to cognitive impairment, an emerging cerebrovascular complication of diabetes and hypertension, remains unknown. We reported that streptozotocin (STZ)-induced type-1 diabetic TLR2 knockout mice were protected from decreased cerebral blood flow (CBF) at 4 and 6 weeks post induction. Given that decreased CBF precedes cognitive impairment, we hypothesized that these TLR2-KO STZ mice would subsequently be protected from cognitive impairment. 10 week old male C57Bl:6 and TLR2-KO mice were injected with 50mg STZ/kg body weight daily for 5 days and observed alongside control mice. Fasting blood glucose was measured, with levels > 240 mg/dL considered to confirm diabetes. Cognitive function was assessed via Y-maze testing. There was no difference in total arm entries between WT and WT-STZ mice or between WT and TLR2-KO. There was a significant decrease in total entries between TLR2-KO and TLR2-KO STZ (40.33 ± 1.88 vs.29.67 ± 2.09, $p\geq 0.05$, $n=12$/group). However, percent novel arm entries was statistically non-significant amongst all groups. Paradoxically, both diabetic groups had a non-significant increase in percent of time spent in the novel arm vs. their respective controls (WT-STZ: 35.7 ± 5.3 seconds vs. WT: 33.2 ± 4.4 seconds; TLR2-KO STZ: 41.6 ± 5.8 seconds vs. TLR2-KO: 37.9 ± 5.5 seconds). The lack of difference in percent novel arm entries is likely a result of the relative increase in exploratory behavior in the STZ groups. Additional experiments with open-field testing and novel object recognition test are being performed to determine if the apparent lack of change in short term hippocampal memory is due to a confounding artifact of the exploration time and whether these cognitive tests are accompanied by changes in CBF in this cohort. If indeed TLR2 KO mice are protected from early mild cognitive deficits, TLR2 antagonism may be

a novel target in the prevention/treatment of cerebrovascular complications of diabetes.

T. Hardigan: None. **C. Hernandez:** None. **A. Ergul:** None.

P031

Cyclooxygenase Inhibition Does Not Augment Reno-protective Effect of Soluble Epoxide Hydrolase Inhibitor in Streptozotocin-induced Diabetic Rats

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Cyclooxygenase (COX)-derived prostaglandins and cytochrome P450 epoxygenase/soluble epoxide hydrolase (sEH) derived epoxyeicosatrienoic acids (EETs) play important role in the regulation of vascular tone. The aim of this work is to examine whether COX inhibition exacerbates the reno-protective effects of increased EETs levels, via inhibiting EETs degradation by sEH using sEH inhibitor, in streptozotocin-induced type 1 diabetes. Diabetes was induced in 12 week old Sprague Dawley rats using streptozotocin (50 mg/kg, i.v). Rats were divided into 5 groups (n=6); control non diabetic, diabetic, diabetic treated with the sEH inhibitor trans-4-[4-(3-adamantan-1-yl-ureido)-cyclohexyloxy]-benzoic acid (t-AUCB, 10 mg/L in drinking water), diabetic treated with COX inhibitor meloxicam (5 mg/kg/day in drinking water) and diabetic treated with both inhibitors for two months. Inhibition of sEH reduced the elevation in proteinuria and creatinine clearance in diabetic rats (P less than 0.05) and meloxicam did not exacerbate t-AUCB effects. Glomerular permeability to albumin and albuminuria were elevated in diabetic rats and were reduced with t-AUCB treatment; however, meloxicam did not augment t-AUCB effects on reducing glomerular injury. Inhibition of sEH with t-AUCB also restored the decrease in glomerular integrin and nephrin expression levels in diabetic rats without synergestic effect from meloxicam. Renal collagen deposition and the inflammatory marker monocyte chemoattractant protein-1 (MCP-1) excretion levels were significantly elevated in diabetic rats and were reduced with t-AUCB treatment without further significant protective effects from meloxicam treatment. The number of TUNEL positive cells, a marker of apoptosis, was higher in the kidney sections from diabetic rats versus control (P less than 0.05), an effect that was significantly mitigated by t-AUCB treatment but not with meloxicam treatment. In conclusion, increasing EETs levels in type 1 diabetic rats, using sEH inhibition, reduced renal apoptosis, inflammation and injury whereas inhibition of COX failed to provide renal protection or to augment reno-protective effect of sEH inhibition in diabetic rats.

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P032

High Glucose Increases Prorenin Receptor (PRR) at the Cell Plasma Membrane in the Collecting Duct Which Promotes the Induction of Downstream Fibrotic Factors

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In streptozotocin (STZ)-induced Type-1 diabetic (T1D) rats, the collecting duct is the main source of intrarenal prorenin. The fact that binding of prorenin to the PRR triggers intracellular signals linked to tissue fibrosis has raised the expectation that prorenin might be directly

responsible for diabetic nephropathy (DN). We showed that STZ-induced T1D rats exhibit greater apical distribution of PRR in the collecting duct. Therefore, we hypothesize that high glucose increases PRR abundance at the cell plasma membrane (PM) of collecting duct cells, which contributes to the stimulation of downstream fibrotic factors. To test this, we used male Sprague-Dawley rats (14w of age) with STZ-induced hyperglycemia for 7d (ip, single injection; 60 ng/kg), and cultures of collecting duct M-1 cells treated for 0, 5 min, and 1, 6, 12 and 24h with normal glucose (NG; 5mM glucose + 20mM mannitol) and high glucose (HG; 25mM). After 7d, STZ-induced rats (N=9) showed plasma levels of glucose as 428±13 vs.138±9 gr/dL and insulin as 0.07±0.02 vs. 2.4±0.01 ng/mL; compared to controls (N=7). Although PRR mRNA levels did not differ between groups; PRR protein levels and its downstream target TGF-β mRNA levels were augmented in the renal medulla of STZ-induced rats (0.55±0.03 vs.0.44±0.02 PRR/β-actin protein ratio; P<0.01; 1.22±0.06 vs.0.97±0.03 TGF-β/β-actin mRNA ratio; P<0.01). Interestingly, PRR protein levels were maximum elevated at 1h in PM extracts from M-1 cells treated with HG (0.95±0.04 vs. 0.33±0.2 PRR/E-cadherin protein ratio) but not in NG-treated cells. HG also unregulated downstream fibrotic factor fibronectin (1.9±0.2 vs.0.46±0.05 fold change) compared with NG-treated cells. Immunofluorescence studies of M-1 cells treated with NG for 1h showed that PRR is mainly localized in the perinuclear areas, whereas in HG-treated cells it was predominantly localized toward the cell surface, as it was more clearly observed in de-convoluted images. These data indicate that hyperglycemia leads to PRR trafficking alterations by inducing PRR translocation towards PM in the collecting duct cells and suggest that the activation of PRR during hyperglycemia conditions might be a novel mechanism underlying the development of DN, particularly tubulointerstitial fibrosis in DM.

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P033

Acute Intravenous Glucose Injection Inhibits Tubuloglomerular Feedback and Increase Glomerular Filtration Rate

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Hyperfiltration is considered a risk factor for diabetic nephropathy, but the mechanism for alterations in glomerular filtration rate (GFR) in diabetes has not been clarified. Nitric oxide synthase 1 (NOS1) highly expresses in the macula densa (MD) and NO inhibits tubuloglomerular feedback (TGF). TGF is one of the major mechanisms for glomerular filtration rate (GFR) regulation. It is not clear whether glucose itself filtered into the tubules has any effect on GFR. We hypothesized the glucose enhances NOS1 activity in the MD, which blunts TGF and increases GFR.
First, we measured GRF in conscious mice by plasma FITC-inulin clearance. Three min after an intravenous infusion of 50µl of 4M glucose in wild type mice, GFR was increased by 19.1% from 236±9.7 to 281±13.4 µl/min. Intravenous saline infusion had no effect on GFR.
NO generation by the MD was then measured in isolated perfused juxtaglomerular apparatus with fluorescent probe DAF-2DA. In response to an increase in tubular glucose concentration from 0 to 300 mg/dl, NO production was increased by 78.4%. TGF, assessed in *vivo* by

measuring the change in proximal tubular stop flow pressure (Δ) induced by an increase of perfusion rate in late proximal tubules from 0 to 40 nl/min, was significantly blunted from 6.5±0.9 to 5.0±1.6 mmHg when tubular perfusate glucose concentration was increased 0 to 300 mg/dl.
To determine whether the glucose-induced changes were mediated by NOS1 in MD, we repeated the experiments in MD specific NOS1 KO (MD-NOS1KO) mice. Intravenous glucose infusion did not significantly increase GRF (from 203±15.7 to 220±6.9 µl/min) in the KO mice. Elevating glucose content in tubular perfusate did not alter the TGF response (Δ was from 7.8±1.3 to 7.3±2.1 mmHg) in MD-NOS1KO mice. We concluded that increase of tubular glucose concentration increases GFR by inhibiting TGF response, which is mediated by NO production from NOS1 in the MD.

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P034

Endothelin-1 Overexpression Exaggerates Diabetes-induced Endothelial Dysfunction by Altering Oxidative Stress Balance

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Objective:Increased endothelin (ET)-1 expression has been shown to cause endothelial dysfunction.Plasma ET-1 is increased in patients with diabetes.Since endothelial dysfunction often precedes vascular complications in diabetes, we sought to determine whether ET-1 contributes to diabetes-induced endothelial dysfunction. We hypothesized that overexpression of ET-1 in the endotheliumwill exaggerate diabetes-induced endothelial dysfunction.
Method:Diabetes was induced by streptozotocin treatment (STZ, 55 mg/kg/day, ip) for 5 days in 6weekold male wild-type (WT) mice and in mice overexpressinghuman ET-1 restricted to the endothelium (eET-1). Mice were studied 14 weeks later. Blood glucose,plasma ET-1 levels, mesenteric artery (MA) reactivity, mitochondrial superoxide production in aorta and endothelial nitric oxide synthase (Nos3), superoxide dismutase 1 (Sod1) and 2 (Sod2) mRNA expression in MA were determined.
Results:STZ-induced diabetes was confirmed by increased glycemia in WT(27.6±1.5 vs 10.7±1.1 µM, P<0.001)and eET-1 (23.2±1.0 vs 8.4±0.3 µM, P<0.001).Plasma ET-1 was increased in vehicle- (15.9±4.6 vs 0.6±0.04pg/mL, P<0.05) and STZ-treatedeET-1 (4.9±0.6 vs 0.8±0.1 pg/mL, P<0.05) compared to respective WT controls.Diabetes caused a reduction in vasodilatory responses to acetylcholine in WT (60.9±6.4% vs 83.9±3.4%,P<0.05), which was exaggerated in eET-1(48.6±5.1% vs 81.5±5.2% P<0.05). Mitochondrial superoxide production was increased by diabetes in WT (38.0±4.3 vs 21.6±2.3 RFU/µm2,P<0.05)and further augmented in eET-1 (49.8±1.7 RFU/µm2P<0.05).Nos3 expression was increased in vehicle-treatedeET-1 compared to WT(1.43±0.19 vs 1.00±0.10, P<0.05).Diabetes reduced Nos3 expression in eET-1 (0.75±0.08, P<0.05) but not in WT(1.08±0.09).Diabetes caused an increase in Sod1(1.52±0.17 vs 1.00±0.03, P<0.05) and Sod2 (1.32±0.17 vs 1.00±0.02, P<0.05) expression in WT (P<0.05) but not in eET-1.
Conclusions:Increased levels of ET-1 exaggerate diabetes-induced endothelial dysfunction. This

may be caused by a decrease in Nos3 expression, an increase in mitochondrial oxidative stress and a decrease in antioxidant capacity.

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P035

Endothelin-1 Overexpression Preserves Endothelial Function in Mice with Vascular Smooth Muscle Cell-specific Deletion of Ppar-gamma

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Objective:Peroxisome proliferator-activated receptor γ (PPARγ) agonists reduce blood pressure (BP) and vascular injury in hypertensive rodents and humans. Pparγ inactivation in vascular smooth muscle cells (VSMC)using a tamoxifen inducible Cre-Lox system enhanced angiotensin II-induced vascular injury. Transgenic mice overexpressing endothelin (ET)-1selectively in the endothelium (eET-1) exhibit endothelial dysfunction, increased oxidative stress and inflammation. We hypothesized that inactivation of Pparγ in VSMC(smPparγ-/-)will exaggerate ET-1-induced vascular damage.
Methods and Results:Elevenweek-old male control, eET-1, smPparγ-/-and eET-1/smPparγ-/-mice weretreated with tamoxifen (1 mg/kg/day, s.c.) for 5 days and sacrificed 4 weeks later. Systolic BP was higher in eET-1compared to control (123±5 vs 109±2 mmHg,P<0.05)and unaffected by Pparγ inactivation.Mesenteric artery (MA) vasodilatory responses to acetylcholine were impaired only in smPparγ-/-(P<0.05) compared to control (Emax: 52.0±6.7% vs 82.2±4.9%).Reactive oxygen species levels were increased in eET-1, smPparγ-/-and eET-1/smPparγ-/-compared to control (1.7±0.2, 2.2±0.2 and 2.8±0.4 vs 1.0±0.2, P<0.05).MA monocyte chemoattractant protein-1 expression was higher in smPparγ-/-compared to control (31.0±4.4 vs 18.7±2.5, P<0.05), and unaffected by ET-1 overexpression.Perivascular fat monocyte/macrophage infiltration was higher in eET-1 and smPparγ-/- compared to control (331±34 and 326±49 vs 140±8 cells/mm2, P<0.05), and further increased in eET-1/smPparγ-/- (557±77, P<0.05). Nitric oxide synthase (Nos)3 mRNA expression was increased only in eET-1 compared to WT (1.21±0.07 vs 1.00±0.02, P<0.05). Nos2 expression was increased in eET-1 and smPparγ-/-compared to WT (3.69±0.72 and 1.96±0.16 vs 1.00±0.13, P<0.05). The Ednra/EdnrbmRNA ratio was decreased in eET-1/smPparγ-/-compared to smPparγ-/-(0.76±0.04 vs 1.07±0.10, P<0.05).
Conclusion:Increased ET-1 paradoxically preserves endothelial function in mice with smPparγinactivation, despite enhancedoxidative stress and inflammation.

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P038

Testosterone Supplements Have Differential Effects on Blood Pressure in Old and Young Male Spontaneously Hypertensive Rats

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Testosterone ("T") supplements are widely used by men to improve their quality of life, libido, and protect against osteoporosis. In clinical studies, both high and low "T" levels were found to be associated with hypertension and cardiovascular risk. Endogenous "T" levels are reduced in obese men and rats. We have shown previously that "T" supplements in middle-aged (6 mos) obese Zucker rats improved symptoms of the metabolic syndrome and caused weight loss, but increased their blood pressure. How "T" supplements affect hypertensive men and rats is unknown. We hypothesized that "T" supplements would further increase blood pressure (BP) in both old and young male spontaneously hypertensive rats (SHR). Old (O=20-22 mos) and young (Y=10 wks) male SHR were treated with "T" (testosterone propionate 8 mg/10 mm silastic pellet; OT and YT, implanted sc) or placebo (empty pellets; OP and YP, sc). Pellets were changed every 3 weeks for 8 weeks. Mean arterial pressure (MAP) was measured by telemetry for 2 weeks. MAP in OP was higher than in YP (OP: 166±7 vs YP: 148±0.5 mmHg, $p<0.001$). As we predicted, "T" increased MAP in YT (YP: 148±1 vs YT: 157±1 mmHg, $p<0.001$). In contrast, "T" decreased MAP in OT (OP: 166±1 vs OT: 155±1 mmHg, $p<0.001$). These data suggest that in younger men, especially men who are already hypertensive, blood pressure should be monitored closely during "T" supplementation in order to prevent further cardiovascular disease. Since "T" reduced MAP in older male SHR, these data suggest that "T" supplements may not be as detrimental in older hypertensive men as in young men. Future studies will need to be done to determine the mechanisms by which "T" increases BP in young males and the mechanisms by which "T" reduces BP in old males. Supported by NIH-R01HL66072, PO1HL51971 (JFR), 14POST18640015 (ROM), EFF Endocrine Res Grant (LLY).

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P039

Testosterone Increases BP in Male SHR by Activating the Renin-angiotensin System: A Cautionary Tale for "Low T" Supplements

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Testosterone supplements are widely prescribed for men in the US to improve overall quality of life by increasing libido and sexual performance and protecting against osteoporosis. Whether testosterone supplements affect CVD either positively or negatively is not clear since study results are controversial. Previously, we showed that chronic testosterone supplements cause hypertension in obese male Zucker rats but improves inflammation and metabolic syndrome symptoms. We also found that testosterone supplements in young male SHR further increase their BP, and that castration attenuates the hypertension. In the present study we followed up on these previous studies

and tested the hypothesis that, because the SHR are lean, chronic testosterone supplements will increase lean body mass but increase the BP via a renin angiotensin system (RAS) mechanism. Male SHR were treated with testosterone (8 mg/10 mm silastic pellet implanted sc) or placebo (empty pellets sc), beginning at 12 weeks of age for 8 weeks. Fat mass and lean mass were measured by ECHO MRI. Contrary to our hypothesis, testosterone supplements had no effect on lean mass, but reduced fat mass by 49% (4.9±0.6 vs 2.5±0.4 %BW, $p<0.05$). Baseline mean arterial pressure (MAP by telemetry) was higher in testosterone supplemented rats than placebo controls (157±0.4 vs. 147±0.7 mmHg, $p<0.05$). Enalapril (200 mg/L) reduced MAP by 25% in testosterone group (157±0.4 vs 118±6 mmHg, $p<0.05$) and by 14% placebo rats (148±0.5 vs 127±5 mmHg, $p<0.05$). MAPs after enalapril were similar between placebo and testosterone-treated rats. These data suggest that the RAS contributes to the elevated BP in testosterone supplemented male SHR. The data also suggest that caution should be taken in prescribing hypertensive men with testosterone supplements. Supported by NIH-R01HL66072, PO1HL51971 (JFR), 14POST18640015 (ROM), EFF Endocrine Res Grant (LLY).

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P040

Role of 20-HETE in Elevated Blood Pressure in Hyperandrogenemic Female Rats, a Model of Polycystic Ovarian Syndrome

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Polycystic ovary syndrome (PCOS) is the most common reproductive disorder in premenopausal women, is characterized by hyperandrogenemia, metabolic syndrome and inflammation. They also exhibit elevated blood pressure (BP) but may not be treated since they do not meet the criteria for hypertension (BP>130/90 mm Hg). We have characterized a female rat model of hyperandrogenemia (HAF) using dihydrotestosterone (DHT) that mimics many characteristics of women with PCOS. In the present study we tested the hypothesis that androgen-induced upregulation of the cytochrome P450 4A2 isoform (CYP4A2) and the formation of 20-hydroxyeicosatetraenoic acid (20-HETE) in renal microvasculature contributes to the elevated BP in HAF rats. Female rats of SS.5BN consomic strain (wild type) rats and CYP4A2-/- rats on this same background were implanted with DHT (7.5mg/90d) or placebo pellets (n=5-8/grp) beginning at 6 wks of age; pellets were changed every 85 d. At 14 wks of age, rats were implanted with radiotelemetry transmitters, and mean arterial pressure (MAP) was measured for 10 days. Endogenous 20-HETE levels were measured using LC-MS in renal microvessels isolated using an Evans Blue sieving technique. DHT-treated HAF-SS.5BN rats had significantly higher MAP compared to placebo-SS.5BN (128±6 vs. 104±1 mmHg, $p<0.004$). In contrast, HAF-CYP4A2-/- rats had no change in MAP compared to placebo-CYP4A2-/- controls (120±4 vs 118±3 mmHg, p=NS). Endogenous 20-HETE levels in renal microvessels of HAF-SS.5BN rats were significantly increased compared to Placebo-SS.5BN (2.27±0.91 vs. 0.32±0.037 pmol/mg, $p<0.01$). The 20-HETE levels were lower in CYP4A2-/- than SS.5BN but DHT in HAF-CYP4A2-

/- had no effect on 20-HETE levels compared to Placebo- CYP4A2-/-. These results suggest that androgen-mediated upregulation of the expression of CYP4A2 and the production of 20-HETE in renal microvessels contribute to elevated BP in HAF rats. These data also suggest that methods to attenuate 20-HETE may provide a novel therapeutic to reduce BP in women with PCOS. Work supported by NIH RO1HL66072 and PO1HL51971.

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P041

Inhibition of MicroRNA-221 by Estradiol Contributes to its Differential Effects on Smooth Muscle Cell Growth and Endothelial Cell Capillary Formation

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MicroRNAs play a key role in vascular remodeling associated with cardiovascular disease. MiR-221 actively contributes to injury-induced neointima formation by inhibiting endothelial cell (EC) growth and promoting smooth muscle cell (SMC) growth. Since estradiol (E2) prevents neointimal thickening, we hypothesize that E2 mediates its vasoprotective actions by downregulating miR-221 expression and abrogating its effects on SMC and EC growth. RT-qPCR confirmed that both Human Umbilical Vein ECs (HUVECs) and Human Coronary Artery SMCs (HCASMCs) produce miR-221. Treatment of HCASMCs with PDGF-BB (20ng/ml) induced miR-221 levels from 100±8% to 189±9% ($p<.05$) and treatment with E2 (100nM) inhibited this to 126±4% ($p<.05$ vs PDGF). PDGF-BB stimulated DNA synthesis (BrdU incorporation), CyclinD1 expression (Western Blot) and migration (Scratch assay) in HCASMCs and these effects were mimicked by miR-221 (25nM) overexpression; and abrogated in HCASMCs transfected with miR-221 antimiR (25nM). E2 inhibited PDGF-induced HCASMC numbers by 30±4% and these effects were reversed by miR-221 ($p<0.05$). Inhibitory effects of E2 on PDGF-induced miR-221 production in HCASMCs were mimicked by estrogen receptor (ER) α agonist PPT, but not by ERβ and GPER agonists; and blocked by ERα antagonist MPP, suggesting these effects are ERα mediated. In contrast to SMCs, transfection of HUVECs with miR-221 mimic inhibited capillary formation and wound healing by 39±8% and 27±6%, respectively ($p<.05$). Neutralization of miR-221 with antimiR induced capillary formation and wound closure by 26±3 % and 51±15%, respectively ($p<.05$). E2 (10nM) inhibited miR-221 levels in HUVECs from 100 to 73±6% ($p<.05$). Moreover, transfection of HUVECs with miR-221 mimic inhibited E2-induced capillary formation (from137±9% to 85±13%; $p<.05$ vs E2) and wound closure (from 125±5% to 82±12%; $p<.05$ vs E2). Our findings provide the first evidence that E2 inhibits miR-221 production in HCASMCs and HUVECs and these effects contribute to its antimitogenic effects on HCASMCs and capillary promoting effects in HUVECs. Modulation of miR-221 by E2 represents a novel mechanism by which E2 may mediate its differential effects on SMC and EC growth, and confer vascular protection.

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P042

Development of a Novel Gper-1 Knock Out Rat Model Using a Modified Crispr/cas9 Technology

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The G-protein coupled estrogen receptor (Gper-1 or Gpr 30) is a newly recognized estrogen receptor that is widely expressed in various tissues including heart and blood vessels. Rat Gper-1 is a single exonic gene located on chromosome 12. Gper-1 is implicated in the regulation of blood pressure in female mice potentially through its function as a receptor for estrogen. These studies conducted using mice that are without a genetic background permissive for the development of hypertension are not particularly useful for evaluating the function of Gper-1 in the context of hypertension. To understand the function of Gper-1 in the context of a genetically permissive background, we attempted to knock-out the Gper-1 gene on the genome of the Dahl-salt sensitive (S) rat using an modified Clustered regularly interspaced short palindromic repeats/CRISPR associated protein 9 (CRISPR/Cas9) method. To ensure complete knock-out, instead of the traditional method of using a single gRNA, two gRNAs, each targeting one end of the 1128bp Gper-1 gene were developed. The gRNAs were injected into the Dahl S rat embryos. The embryos were then implanted into ten pseudo-pregnant females. Among the 125 pups born, 5 homozygous founders, 21 heterozygotes and 3 partial Gper-1 deletion founders were identified. In conclusion, using an advanced CRISPR/Cas9 technique a panel of Gper-1 rat knock-outs and targeted mutants were generated, which will serve as novel models for studying the structure-function relationships of the Gper-1 gene.

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P043

Estradiol Treatment Duration Significantly Impacts Renal Health in Midlife Ovariectomized Female Long Evans Rats

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Current clinical recommendations are that hormone therapy for the treatment of menopausal symptoms be limited to a few years. Our lab previously reported that both transient (40 days) and sustained (80 days) estradiol (E2) treatment initiated immediately after midlife ovariectomy (OVX) in Long Evans rats similarly attenuated OVX-induced increases in blood pressure (BP). This study indicated that the benefits of E2 could be maintained long after treatment cessation. Therefore, the goal of the current study was to determine whether E2 duration similarly protected against renal damage. We hypothesized that both transient and sustained E2 treatment exert positive

effects on renal health in midlife OVX Long Evans female rats. Female retired breeders underwent OVX at 11 months of age and received an implant of E2 or vehicle (veh). After 40 days, implants were replaced comprising the following groups: veh>veh (n=9), E2>E2 (n=7), E2>veh (n=10). Animals were placed in metabolic cages at both the 40 and 80 day treatment time points to measure proteinuria, urinary peptides, and creatinine excretion. At the end of the 80 day treatment tissue weights were obtained. Interestingly, kidney hypertrophy was significantly increased in the E2>E2 when analyzed as raw wet weight (veh: 1.0 ± 0.05 g; E2>veh: 0.95 ± 0.02; E2>E2: 1.2 ± 0.04; P<0.0001), or normalized to body weight (veh: 2.5 ± 0.1 mg/gBW; E2>veh: 2.5 ± 0.03; E2>E2: 2.9 ± 0.11; P<0.005). Creatinine excretion was not different between groups (veh: 7.0 ± 0.6 mg/day; E2>veh: 6.4 ± 0.4; E2>E2: 7.6 ± 0.7; P=0.29). However, there was a significant increase in proteinuria measured by the Bradford assay in the E2>E2 group (veh: 2.0 ± 0.8 mg/mgCr; E2>veh: 0.9 ± 0.3; E2>E2: 6.8 ± 2.6; P<0.05). The E2>E2 group also had a significantly lower percentage of urinary peptides (veh: 96 ± 0.8%; E2>veh: 97 ± 0.7%; E2>E2: 79 ± 8.1%; P<0.05), indicating impaired renal function. These results indicate that in midlife OVX Long Evans rats, sustained E2 lowered BP but exerted detrimental effects on kidney function. In contrast, transient E2 treatment lowered BP without renal impairment. Overall, our results indicate that the period of exposure to estradiol may be important for renal health in aging postmenopausal women.

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Pleiotropic Effect of a <42.5 kb QTL Region on Rat Chromosome 10 Regulating Blood Pressure and Tumorigenesis

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This study is focused on a translationally significant, GWAS locus for cardiovascular disease (QT-interval) on human chromosome 17. We have already validated and high resolution mapped the homologous genomic segment of this human locus to <42.5 kb on rat chromosome 10. The locus in rats regulates both QT-interval and blood pressure and contains a single protein-coding gene, rififylin (Rffl). While there are no exonic variants, the expression of Rffl is differential between Dahl S and S.LEW congenic rats, which are strains used for mapping this locus. A previous study points to altered rate of endocytic recycling as the underlying mechanism, through which Rffl operates to control both cardiac QT-intervals and blood pressure. Rffl also contributes to tumorigenesis by negatively regulating caspases and tumor suppressor genes. Moreover, the expression of Methyl-CpG Binding Domain Protein 2 (Mbd2) is also differential between Dahl S and S.LEW congenic rats and Mbd2 can mediate the repression of methylated tumor suppressor genes. Therefore, we hypothesized that the higher expression of Rffl and Mbd2 render the S.LEW congenic strain more susceptible to tumor development. To compare the tumor susceptibility between S and S.LEW congenic strain, azoxymethane (AOM)-induced colon tumorigenesis was assessed. The number

of colon tumors was significantly higher in the congenic strain compared with the S rat ($p<0.01$). Interestingly, the incidence of another phenotype, polyarteris nodosa (PAN), was also higher in the congenic strain. However, the identity of the quantitative trait nucleotides that regulates the expression of Rffl and Mbd2 is unknown. A novel long non-coding RNA (lncRNA), which we refer to as Rffl-lnc1, was identified within the rat Rffl 5'UTR intron locus. Compared to the S rat, the Lewis alleles of Rffl-lnc1 are polymorphic, with a large 19bp deletion. To further assess the role of Rffl-lnc1, the CRISPR/Cas9 system has been successfully applied to construct a Rffl-lnc1 knock-out model of the S rat and a 19bp knock-in rescue model of the S.LEW congenic strain. Functional evaluation of these targeted disruption and rescue models is underway to test the physiological role of Rffl-lnc1 and the 19bp polymorphism in regulating blood pressure and tumorigenesis.

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P045

High Methionine, Low Folate and Vitamin B6/B12 Containing Diet Leads to Cognitive Functions Impairment by Epigenetic Silencing of Netrin Gene

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Hypermethylation of the genes silence the transcriptions of functional genes associated to cognitive functions. Loss of memory due to epigenetic modifications, called memory-epigenetics was studied. Netrin is a glycoprotein involved in neurogenesis, axonal guidance and maintenance of synaptic plasticity. We hypothesized that high methionine-low vitamins containing diet leads to memory impairment by increasing global DNA methylation and therefore, silencing the netrin gene. Wild type (C57BL/6J) mice were fed diet containing excess met (1.2%), low-folate (0.08 mg/kg), vitamin B6 (0.01 mg/kg), and B12 (10.4 µg/kg) for 6 weeks. Mice were weekly examined for cognitive functions. Our results using a passive avoidance test confirmed a loss in fear motivated long-term memory starting from 4th week that continued up to 6th week of diet ($F=37.14$, $p<0.001$). The loss of memory was negatively associated with an increase in global DNA methylation in mice brains fed with diet ($R2=-0.99$, $p<0.001$). We found a time-dependent decrease in netrin protein expression ($F=27.63$, $p<0.001$) and an increase in methylation of netrin gene promoter, defined by restriction digestion-PCR analysis, in mice fed with diet. The increase in methylation of netrin gene promoter was further validated by high resolution melting and sequencing analysis. In addition, the association of netrin with memory impairment was confirmed by delivering netrin protein to diet-fed mice brains through intracerebral route. The data suggest that netrin introduction helped in considerable memory regain (~50%) in mice on diet. Taken together, these results suggest that high met, low folate and vitamin B6/B12 containing diet can induce defects in learning and memory. Furthermore, the data indicates that decrease in netrin level due to hypermethylation of its gene promoter can be associated with memory loss.

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P046

Interplay of Intronic and Promoter Region Polymorphisms Up-regulate Human Angiotensinogen and Cause Hypertension in Transgenic Mice

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Angiotensinogen (AGT) is the substrate for the RAS-cascade and polymorphisms leading to its overexpression are linked to hypertension. We have shown that SNPs in linkage disequilibrium with -6A of the hAGT gene cause increased AGT expression and hypertension. Recently, two genome wide association studies (GWAS) have linked SNP at the +1164 in intron-I with increased blood pressure. The +1164A allele has stronger homology with HNF-3 binding site as compared to +1164G. HNF3 belongs to the family of "pioneer" transcription factors, which enable chromatin access for other tissue-specific transcription factors. Thus, we propose that a haplotype, where multiple SNP-groups occur together, is a better model to predict and study disease correlation with genetic variations than individual SNPs alone. Intronic SNPs could increase chromatin access thus modulating promoter-transcription factor (TF) interactions. To study these possible interactions between the intronic SNPs in the promoter and intron I of the hAGT gene, we have divided this gene in two major haplotypes: haplotype-I (containing -6A & +1164A) and II (containing -6G & +1164G). Reporter construct containing haplotype-I has increased promoter activity as compared to haplotype-II on transient transfection in human liver cells, without (hap-1: 73±2 vs. hap-II: 23.64±3.21 A.U; $p<0.05$) and with dexamethasone treatment (hap-1: 1174±68vs. hap-II: 333.3±37 A.U; $p<0.05$). Double transgenic mice (TG) containing human renin gene (hREN) and haplotype-I of the hAGT gene (containing 2.0 Kb of the promoter, all the introns, exons and 3'UTR of the hAGT gene at the HPRT locus) have increased basal and GR induced expression of the hAGT gene in liver, kidney and fat as compared to haplotype-II. Complementary CHIP analysis shows increased TF-chromatin binding in the liver of haplotype-I TG mice for HNF3β (4.35 folds), C/EBPβ (2.1 folds), STAT-3 (3.6 folds) and GR (4.2 folds). Crucially, haplotype-I TG mice have increased ($p<0.05$) SBP (137±4 mm Hg) when compared to haplotype-II mice (126±3 mm Hg). In conclusion, intronic SNPs regulate hAGT gene expression via complex interplay with SNPs in the gene-promoter and this underscores the need for a haplotype-based approach to genetic variability and disease correlation.

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P047

Epigenetic Mechanisms of Sodium Butyrate- and Retinoic Acid-dependent Attenuation of Renal Fibrosis and Inflammation in Npr1 Gene-targeted Mice

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The objective of the present study was to determine the combined effect of sodium butyrate (NaBu), a histone deacetylase (HDAC) inhibitor and all-trans retinoic acid (ATRA) on attenuation of renal fibrosis and inflammation in Npr1 (coding for natriuretic peptide receptor-A) gene-disrupted mutant mice. Adult (18-20 week old) male Npr1 gene-disrupted

heterozygous (1-copy; Npr1+/-) wild-type (2-copy; Npr1+/+), and gene-duplicated (3-copy; Npr1++/+) mice were treated by injecting ATRA-NaBu hybrid drug (1.0 mg/kg/day) intraperitoneally for 2-weeks. A marked attenuation in tubulo-interstitial fibrosis was observed in Npr1+/- mice after treatment with ATRA-NaBu (50%, $p < 0.001$). Western blot analyses exhibited the reduction in renal expression of collagen type I alpha 2 and transforming growth factor-beta (55%, $p < 0.001$, 67%, $p < 0.001$, respectively) in ATRA-NaBu-treated Npr1+/- mice compared with vehicle-treated mice. A significant decrease in systolic blood pressure was observed in ATRA-NaBu-treated Npr1+/- mice (treated 110.3 ± 2.6 vs. Npr1 +/- control mice, 126.3 ± 2.7, $p < 0.01$). The ATRA-NaBu also enhanced plasma cGMP levels (pmol/ml) in Npr1+/- (treated, 22.7 ± 3.3 vs. control, 6.2 ± 1.6; $p < 0.05$), Npr1+/+ (treated, 50.9 ± 3.5 vs. control, 20.8 ± 3.6; $p < 0.05$), and Npr1++/+ (treated, 71.4 ± 6.4 vs. control, 34.5 ± 2.9; $p < 0.05$) mice. Treatment with ATRA-NaBu significantly lowered renal levels (pg/mg protein) of monocyte chemoattractant protein-1 (MCP-1) (treated, 109.5 ± 9.9 vs. control, 24.3 ± 1.7; $p < 0.01$) in Npr1+/- mice compared with vehicle-treated mice. Western blot analyses confirmed the reduction in renal expression of MCP-1 (72%, $p < 0.001$) in ATRA-NaBu-treated Npr1+/- mice compared with control mice. Moreover, the increased HDAC activity in Npr1+/- mice was significantly reduced by ATRA-NaBu treatment compared with untreated Npr1+/- control mice. The present results provide direct evidence that ATRA-NaBu acts as a potent antifibrotic agent and repairs the renal pathology in Npr1+/- mice, which will have important implications in prevention of hypertension-related renal pathophysiological conditions.

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P048

Blood-Pressure Associated Variants in Natriuretic Peptide Receptor C Affect Human Vascular Smooth Muscle Cells Proliferation and Calcium Flux in Response to Angiotensin II

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Introduction: A recent genome-wide association study revealed a significant association between variation at natriuretic peptide receptor C (NPR3) gene locus and blood pressure (BP).
Objective: To functionally characterise the effect of BP- associated SNPs (single nucleotide polymorphisms) at the NPR3 gene locus in the context of BP regulatory pathways.
Methods: A collection of primary human umbilical smooth muscle (HUASMCs) and endothelial (HUVECs) cells were genotyped for NPR3 gene SNP rs1173743, rs1173747, rs1173756 and rs1173771. Endogenous mRNA and protein expression levels were assessed by qRT-PCR and western blotting. Open chromatin regions were assayed using formaldehyde-assisted isolation of regulatory elements. Cell proliferation and migration were detected by cell counting and scratch assays. Angiotensin II (AngII)-induced Calcium flux was investigated using an intracellular fluorescent probe

(Calcium 6).
Results: The NPR3 gene risk allele (major allele) of intronic SNP rs1173747 was associated with lower endogenous mRNA ($p<0.001$) and protein levels in HUASMCs. This was consistent with its minor allele being located within an open chromatin state ($p<0.05$). Furthermore, cells carrying the major-allele of rs1173747 had increased proliferation ($p<0.05$) and Calcium flux in response to AngII stimulation ($p<0.05$). No difference in migration rates were detected. No such genotype-dependent characteristics were observed in HUVECs.
Conclusions: This study has identified potential mechanisms for BP-associated SNPs in NPR3 gene locus to influence BP predominantly via effect on vascular smooth muscle cell behaviours

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P049

Relationship between Serum Ghrelin levels and Primary Hypertension: A Meta Analysis

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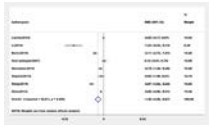
Background: Ghrelin is a novel gut derived peptide hormone suggested to play a role in the etiopathogenesis of Primary Hypertension (HTN). It is hypothesized that ghrelin regulates arterial blood pressure via modulation of central sympathetic activity and peripheral nitric oxide dependent vasodilatory mechanisms. The aim of the present study is to conduct a meta-analysis to evaluate the relationship between serum ghrelin levels and HTN.
Methods: We searched MEDLINE, CINHAL and COCHRANE databases for studies reporting serum ghrelin levels in the HTN and non HTN study population. We included case controls, cohort and cross-sectional studies. We calculated the weighted standardized mean difference (SMD) in serum ghrelin levels between the HTN and control groups.
Results: Our search strategy yielded 309 articles and we included 8 studies enrolling 1659 participants. The median age of the HTN group was 52 yrs. (IQR 47-56) compared to 49 yrs.(IQR 39-54) in the control group. The median body mass index in the HTN group was 28 kg/m2 (IQR 27-34) compared to 25 kg/m2 (IQR 23-27) in the control group. The median percentage of female population in the HTN group was 48 % (IQR 37-61) compared to 51 %(IQR 32-57) in the control group. The unweighted median serum ghrelin levels in the HTN group were 733 pg/ml (IQR 571-5607) compared to 857 pg/ml (IQR 592-7387) in the control group. The SMD of serum ghrelin level was -1.381 (95% CI -2.089,-0.673) $p<0.001$ comparing those in the HTN group and control group.
Conclusion: Serum ghrelin levels are significantly and negatively associated with HTN. Further studies are needed to address potential confounding factors and confirm this association.



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P050

Association Between Circulating Selenium Levels and Primary Hypertension: A Meta-analysis

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Background: Increasing evidence support the role of oxidative stress in the development of Primary hypertension (HTN). Selenium is an essential micronutrient with antioxidant properties mediated via selenoenzymes like glutathione peroxidases. It is hypothesized that selenium plays a role in blood pressure regulation and HTN prevention. We conducted a meta-analysis to evaluate the relationship between circulating selenium levels and HTN.
Methods: We searched MEDLINE, CINAHL and COCHRANE databases for studies reporting serum selenium levels in the patients with HTN and healthy controls. We calculated the weighted standardized mean difference (SMD) in the serum selenium levels between the HTN and control groups.
Results: Our search strategy yielded 313 articles and we included 10 studies enrolling 10420 participants. The median age of the HTN group was 57 yrs. (IQR 56-58) compared to 46 yrs. (IQR 42-50) in the control group. The median body mass index (BMI) in the HTN group was 28 kg/m2 (IQR 26-29) compared to 25 kg/m2 (IQR 25-27) in the control group. The median percentage of female population in the HTN group was 51 % (IQR 46-53) compared to 51 % (IQR 51-54) in the control group. The unweighted median serum selenium levels in the HTN group were 88 µg/l (IQR 83-113) compared to 95 µg/l (IQR 86-128) in the control group. The SMD of serum selenium level was -1.52 (95% CI -2.36, -0.67) P<0.001 comparing those in the HTN group and control group.
Conclusion: Serum selenium levels are significantly and inversely associated with HTN and this association was not explained by age, sex or BMI. Further studies are needed to confirm this association by adjusting for potential confounders.

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P051

Renal Denervation Normalizes Arterial Pressure but Has No Effect on Renal Inflammation or Glucose Metabolism in Obese Hypertensive C57bl6j Mice

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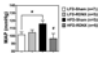
Clinical studies have shown that renal denervation (RDNX) decreases arterial pressure (AP) and improves glucose metabolism in drug resistant obese hypertensive patients. In the present study, we used a murine model of obesity-induced hypertension (HTN) to test the hypothesis that RDNX lowers AP and improves glucose metabolism via an interaction of renal nerves with inflammatory mediators in the kidney. 8-week old C57Bl/6J mice were fed either a low fat diet (LFD; 10 KCal% from fat) or a high fat diet (HFD; 45 KCal% from fat) for 10 weeks. Two parallel protocols were conducted.

In a metabolic protocol, body weight, food intake, body-composition and glucose metabolism were measured. In a cardiovascular protocol, radiotelemeters were implanted for measurement of AP. C57Bl6J mice on a HFD exhibited an inflammatory and metabolic syndrome phenotype including increase splenic and renal total T cells, increased fat mass, high blood pressure, hyperglycemia and glucose intolerance as compared to LFD mice. RDNX but not Sham surgery after 12 weeks of HFD normalized AP (116 ± 4 in sham vs. 97± 6 mmHg in RDX HFD mice). RDX had no effect on AP in LFD diet mice. RDX in obese hypertensive mice had no effect on renal T cells or cytokines. Finally, RDX had no effect on glucose metabolism in HFD mice as determined by the glucose tolerance test at 2 weeks after RDX. We conclude that the antihypertensive effect of RDX in obesity-induced hypertension is not associated with improvement in glucose metabolism or renal inflammation in the mouse. 

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P052

Homocysteine Increases Macrophage-Derived Paraoxonase -1 Expression Independent of CD68

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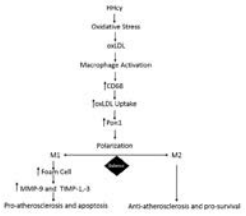
Although atherosclerotic plaque rupture is the leading cause of myocardial infarction, the mechanisms are unclear. Macrophages burdened with oxidized LDL (oxLDL) become foam cells: hallmarks of plaque progression and instability. One of the main macrophage-specific receptors for oxLDL is CD68. Paraoxonase-1 (PON1) is a high-density lipoprotein (HDL)-associated lactonase capable of retarding/inhibiting LDL oxidation. Elevated levels of homocysteine (Hcy), an amino acid homologue and independent cardiovascular risk factor, is metabolized by Pon1.
Given the literature connections of oxLDL, Pon1, Hcy, and macrophages to atherosclerosis, we hypothesized that Pon1 is produced by murine macrophages and its expression is increased by Hcy via CD68.
Murine J774a.1 macrophages were treated with LDL, oxLDL, Hcy, or oxLDL+Hcy. Also, separate treatment groups included macrophages that had CD68 silenced by CD68 siRNA transfection. Cell lysates were analyzed for CD68 and Pon1 expression via Western blotting.
Pon1 is present in macrophages. Hcy along with oxLDL significantly increases Pon1 (51%, 1.51 vs 0.97) expression compared to controls than oxLDL alone. Pon1 expression is significantly decreased (33%, 0.67 vs 1) with silencing of CD68. Pon1 expression is significantly decreased more with oxLDL (82% 0.17 vs 1) in presence of CD68 silencing but is significantly increased with oxLDL+Hcy (24% 1.24 vs 1). CD68 expression tends to increase more with oxLDL+Hcy than oxLDL alone when compared to control and the tendency follows with silencing of CD68. Our results conclude that Hcy increases macrophage-derived Pon1 expression

independent of CD68.



I. Chernyavskiy: None. **L. Winchester:** None. **S. Veeranki:** None. **S. Tyagi:** None.

P053

Nicotine-induced *Renal Inflammation* in Genetic Hypertension is *Not Dependent* on *Renal Sympathetic Nerve Activity*

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We recently demonstrated that cholinergic stimulation with nicotine in vivo leads to renal inflammation and premature development of hypertension in Spontaneously Hypertensive Rats (SHR), but not in Wistar Kyoto (WKY) controls. Nicotine can stimulate immune cells directly and influence inflammation indirectly by increasing sympathetic nerve activity (SNA). We hypothesized that increased renal SNA contributes to nicotine-induced renal inflammation in SHR. We tested this hypothesis by measuring the number of CD68+ macrophages in kidneys of prehypertensive SHR (3-5 weeks old, n=7) and age-matched WKY rats (n=3) after subcutaneous infusion of nicotine via osmotic mini-pump (625 mcg/kg/hr) for 24 hours. Each rat was subjected to unilateral renal nerve denervation (RND) and sham surgery on the contralateral kidney 1 week before implanting the mini-pump. RND failed to abrogate nicotine-induced renal inflammation, actually increasing CD68+ macrophage infiltration in renal tubulointerstitium of SHR (110 ± 3 vs. 89 ± 1 cells/unit area in denervated vs. contralateral intact kidneys, respectively, $p<0.001$). RND had no effect on the number of infiltrating CD68+ macrophages in the tubulointerstitium of WKY controls (83 ± 3 vs. 79 ± 2 cells/unit area in denervated vs. intact kidneys, respectively, $p>0.05$). Renal norepinephrine content was measured by ELISA to confirm RND. We conclude that nicotine-induced renal macrophage infiltration in SHR is not dependent on renal SNA, and speculate that renal SNA may be anti-inflammatory in this model.

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P054

Inhibition of Endosomal MyD88-dependent Signaling with Chloroquine Improves Endothelial Function and Lowers Blood Pressure in Spontaneously Hypertensive Rats

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Uncontrolled inflammation and chronic immune system activation are common in hypertension. However, the exact mechanisms by which they occur are not well understood. Innate immune system recognition and response to damage-associated molecular patterns (DAMPs) is becoming an increasingly accepted mechanism. Mitochondrial DNA (mtDNA) is a DAMP that is recognized by Toll-like receptor (TLR)9 and is elevated in the circulation of spontaneously

hypertensive rats (SHR). Therefore, we sought to determine the contribution of TLR9 in hypertension. We hypothesized that TLR9 inhibition with lysosomotropic agent chloroquine would improve endothelial function and lower blood pressure in SHR. We treated adult SHR and control Wistar-Kyoto rats (12 weeks old), as well as a group of young SHR (5 weeks old) with chloroquine (40 mg/kg/day, i.p.) for 21 days. Chloroquine lowered adult SHR blood pressure (Veh: 201±2 vs. CQ: 182±5 mmHg, $p<0.05$) and inhibited cyclooxygenase-dependent contraction to acetylcholine (ACh) in isolated mesenteric resistance arteries (MRA) from adult SHR [%NE (ACh 1 µmol/L), Veh: -31±8 vs. CQ: 70±10, $p<0.05$]. Chloroquine also inhibited co-localization of not only TLR9, but also TLR7 and TLR8 (MyD88-dependent endolysosomotropic TLRs) with MyD88 in adult SHR MRA, as well as expression of MyD88 in young SHR MRA. Finally, we observed that prophylactic treatment with chloroquine, during the critical pre-hypertensive stage, ameliorated the development of high blood pressure (Veh: 190±4 vs. CQ: 174±5 mmHg, $p<0.05$) and components of the adaptive immune system (decreased aortic-derived CD45+ leukocytes, circulating CD3+ T lymphocytes, and expression of CD44 on circulating and aortic-derived CD3+ T lymphocytes) upon maturation to adulthood in SHR. In conclusion, endosomal MyD88-dependent signaling from TLR7, 8, and 9 contribute to high blood pressure, endothelial dysfunction, and recruitment of the adaptive immune system in SHR. These findings support the involvement of the innate immune system in the pathogenesis and maintenance of hypertension.

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P055

Systemic Arterial Hypertension Induces Pulmonary Injury Beyond Protein Degradation and Atrophy of Diaphragm

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Background: Systemic arterial hypertension (SAH) is a chronic disease associated with systemic inflammation. Although cardiovascular adaptations resulting from SAH are more evident, little is known about the respiratory alterations. The purinergic receptor P2X7 plays a key role in the immune modulation, beyond to control the vascular tone and the development of inflammation and fibrosis. Aims: Evaluate the effects of SAH on the pulmonary inflammation and remodeling and on the diaphragm, and the involvement of purinergic receptor P2X7 and of ubiquitin-proteasome system (UPS) in this response. Methods: Spontaneously hypertensive rats (SHR) and normotensive Wistar (N) with 18 weeks-of-age were evaluated for: inflammation and remodeling of airways and pulmonary vessels and for P2X7 receptor expression. The morphology and biochemistryin diaphragm

muscle for myosin ATPase reaction and ubiquitin-proteasome system (UPS), respectively. Results: The SHR showed a higher wall/lumen of the pulmonary arteries, as well as increased collagen deposition on the wall of these arteries. Increase in P2X7 receptor expression in pulmonary vascular wall (SHR:4.3%±0.7% vs. N:0.3%±0.2%) and bronchial epithelial (SHR:29±0% vs 2.8% N:10.5%±1.3%). The diaphragm was increased cross-sectional area (CSA) of type I fibers (16%) and reduction in CSA of type II (41%), increased the UPS activity and lipid peroxidation. SHR did not change in the analysis of ubiquitinated proteins and misfolded proteins. Conclusion: SAH induces important pulmonary disorders such pulmonary vascular remodeling, with increased expression of the purinergic receptor P2X7 associated with atrophy and protein degradation on diaphragm.

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P056

MHC CLass II-associated Invariant Peptide (CLIP) Antagonism During Chronic Lipopolysaccharide Treatment Preserves Afferent Arteriolar Autoregulatory Behavior

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Lipopolysaccharide (LPS) is a cell wall component of gram-negative bacteria that can activate toll-like receptor 4, which in turn, activates the innate immune system. Chronic immune system activation is linked to blunted afferent arteriolar autoregulatory behavior and kidney injury. MHC Class II-associated invariant peptide (CLIP) provides a critical step in antigen processing, presentation, and adaptive immune system activation. Accordingly, we postulated that treatment with a competitive CLIP antagonist (CAP) during chronic low-dose LPS exposure would preserve afferent arteriolar autoregulatory behavior. Rats were implanted with osmotic minipumps (day 0) for infusion of LPS (0.01mg/kg/day) or saline (0.9% NaCl; 0.5µl/hr) for 8 days and then the kidneys were harvested for juxtamedullary nephron studies. Four groups (n=6/group) were studied: Control, LPS, LPS + CAP and Sham + CAP. Both LPS + CAP and Sham + CAP groups were treated with CAP (3mg/kg/day; i.p.) on days 1-7. Autoregulatory behavior was assessed in these groups by increasing perfusion pressure in 15 mmHg increments from 65 to 170 mmHg. Starting baseline diameters were similar across the control (15.2 ± 1.2 µm), LPS (14.1 ± 1.0 µm), LPS + CAP (15.1 ± 1.0), and Sham + CAP (15.7 ± 0.6) groups. When perfusion pressure was increased from 65 to 170 mmHg, control and sham + CAP afferent arteriolar diameter decreased significantly by 26 ± 4% and 25 ± 2% ($P<0.05$), respectively. In contrast, afferent diameters from LPS treated kidneys decreased by just 5 ± 2% over the same pressure range indicating impaired afferent arteriolar autoregulatory behavior. In LPS + CAP treated kidneys, afferent arteriolar diameter decreased by 17 ± 2% ($P<0.05$) over the same pressure range signifying preservation of autoregulatory behavior. These data support the hypothesis that CLIP antagonism during chronic low dose LPS treatment preserves afferent arteriolar autoregulatory behavior. Inhibiting CLIP may

open novel therapeutic targets for inflammatory kidney disease.

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P057

Is Hypertension a Disease of the Bone Marrow?

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Decades of evidence have implicated involvement of inflammation in the development and establishment of hypertension (HTN); however, the central mechanisms remain elusive. We propose a hypothesis that dysfunctional bone marrow (BM) activity is critical in HTN, in view of the fact that BM is the predominant source of inflammatory and angiogenic cells. We provide the following evidence in support of this hypothesis: (1) BM from animal models of HTN is proinflammatory. Ablation of the spontaneously hypertensive rat (SHR) BM, and reconstitution with BM from the normotensive Wistar Kyoto (WKY), results in significant reduction in mean arterial pressure (MAP), as well as the decrease in proinflammatory and increase in angiogenic cells in the chimeric SHR; (2) Oral minocycline treatment attenuates MAP, restores autonomic balance, and decreases inflammation in both the SHR and Ang II rat HTN models; (3) Sympathetic nerve activity (SNA) and norepinephrine levels in the BM of the SHR and Ang II rat HTN models are elevated compared to normotensive rats; (4) C57-AdrB1.B2 knock-out (KO) chimera, generated by reconstitution of irradiated C57BL/6J mice with the BM cells of the adrenergic receptor beta 1/2 KO mice (Adrb1tm1Bkk Adrb2tm1Bkk/J), exhibits reduced peripheral inflammatory cell counts. Furthermore, transcriptomics analysis of the BM cells from these chimeric mice revealed significant changes in 67 signaling pathways, thirty-five of which were directly related to modulation of different immune system responses ($P<0.01$). Most notable were changes in the pathways involved in activation and migration of monocytes and T lymphocytes. These observations demonstrate that BM plays a critical role in regulation of the inflammatory status, and support our hypothesis that dysfunctional BM activity may be an important aspect in the development and establishment of HTN. AHA14SDG18300010.

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P058

Regional Sympathetic Activation Induced by Obstructive Apnea

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Obstructive sleep apnea increases muscle sympathetic nerve activity (SNA) in humans that contributes to cardiovascular dysfunction including hypertension. The purpose of the present study was to determine if there are regional differences in apnea-induced sympathetic activation in rodents. Male Sprague Dawley rats (n: 8, 270-350 g) were anesthetized with urethane (1.2 g/kg i.v.) and prepared for recordings of renal, splanchnic, and lumbar SNA, phrenic nerve activity (PNA), mean arterial pressure (MAP) and heart rate (HR). Animals were exposed to 10 apneas performed by clamping a tracheal tube during 20 s each 2 min in room air. Changes in rectified and integrated (20 ms time constant) SNA were calculated by subtracting background noise after death. Basal MAP and HR were 89 ± 3 mmHg and 402 ± 14 bpm. Apneas in room air produced similar increases in SNA across various nerves (Δ renal: 96 ± 16%; Δ splanchnic: 109 ± 30%, and Δ lumbar: 93 ± 7%). These bursts increase in amplitude and the maximum response occurred in the end of 20 s of obstruction. Apnea also induced hypertension (Δ: 10 ± 2 mmHg), bradycardia (Δ: - 51 ± 25 bpm) and increased in PNA (Δ: 791 ± 194%). To test the contribution of chemoreceptors, obstructive apnea was repeated when animals were breathing 100% oxygen. Basal MAP and HR were 89 ± 3 mmHg and 402 ± 14 bpm. Hyperoxia reduced the sympathetic responses induced by apnea in renal, splanchnic and lumbar SNA (Δ renal: 40 ± 7%, Δ splanchnic: 68 ± 32%, and Δ lumbar: 52 ± 8%). The cardiovascular and respiratory response were also reduced (ΔMAP: 6±1; ΔHR: -22±8 and ΔPNA: 586 ± 149%). Therefore, obstructive apnea induces similar changes in SNA across various end-organs that depend in part of hypoxia stimulus.

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P059

A A Role of Aminopeptidase A in the Brain on Cardiovascular Regulation of Conscious Rat

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Objective: Aminopeptidase A (APA) have important role in conversion of Ang II to Ang III. Intravenous APA administration lowers blood pressure in hypertensive rats. In contrast, APA inhibition in the brain lowers blood pressure in hypertensive rats. Therefore APA might have different role on cardiovascular regulation. However, a role of APA and Ang III on cardiovascular regulation especially in the brain has not been fully understood. Our purpose of present study was to investigate a role of APA and Ang III in the brain on cardiovascular regulation in conscious state.
Method: 12-13 weeks old Wistar Kyoto rat (WKY) and 12-16 weeks old spontaneously hypertensive rat (SHR) were used. i) APA distribution in the brain was evaluated by immunohistochemistry. Protein expression of APA was evaluated by Western blotting.

Enzymatic activity of APA was evaluated using L-glutamic acid γ-(4-nitroanilide) as a substrate. ii) WKY received icv administration of Ang II 25ng/2µL and Ang III 25ng/2µL. We recorded change in mean arterial pressure (MAP) in conscious and unrestraied state and measured induced drinking time. iii) SHR received icv administeration of recombinant APA 400ng/4µL. We recorded change in MAP in conscious and unrestraied state and measured induced drinking time.
Result: i) APA was diffusely immunostained in the cells of brain stem including cardiovascular regulatory area such as rostral ventrolateral medulla. Protein expression and APA activity in the brain were similar between WKY (n=3) and SHR (n=3).ii) Icv administration of Ang II increased MAP by 33.8±3.8 mmHg and induced drinking behavior for 405±90 seconds (n=4). Icv administration of Ang III also increased MAP by 24.7±2.4 mmHg and induced drinking behavior for 258±62 seconds (n=3). These vasopressor activity and induced drinking behavior was completely blocked by pretretment of angiotensin receptor type 1 blocker.iii) Icv administration of APA increased MAP by 10.0±1.7 mmHg (n=3).
Conclusion:These results suggested that Ang III in the brain increase blood pressure by Angiotensin type 1 receptor dependent mechanism and APA in the brain may involved in blood pressure regulation as a vasopressor enzyme.

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P060

Angiotensin AT1A Receptors on Vasopressin-Expressing Cells are Dispensable for DOCA-salt Hypertension

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Increased blood pressure in the deoxycorticosterone acetate (DOCA)-salt model of low-renin hypertension is correlated with increased vasopressin (AVP) secretion, and is sensitive to inhibition of the brain renin-angiotensin system (RAS). Further, AVP-deficient Brattleboro rats are largely resistant to DOCA-salt hypertension. These findings lead us to hypothesize a role for AT1A receptors localized to AVP-expressing neurons in the control of AVP secretion, specifically in low-renin hypertension. Blood pressure was assessed via tail-cuff plesthysmography and total daily AVP secretion assessed via urine copeptin in mice with specific disruption of the AT1A gene in AVP-expressing cells (AVP-Cre x AT1Aflox/flox mice, “KO”) under both baseline and DOCA-salt treatment conditions. Specific activity of Cre-recombinase within the paraventricular and supraoptic nuclei of AVP-Cre transgenic mice was confirmed by fluorescent microscopy in brain sections from mice expressing a conditional fluorescent reporter (AVP-Cre x ROSA-stopflox-tdTomato mice). At baseline, AVP secretion (via urine copeptin) trended downward with large variation (control n=17, 363±182 vs KO n=5, 33±11 pg/day; p>0.05) but there was no significant difference in blood pressure (control n=27, 107±1.3 vs KO n=12, 111±2.2 mmHg; p>0.05) compared to littermate controls. In response to DOCA-salt, blood pressure (control n=23, +10.35±2.1 vs KO n=8, +12.91±2.0; p>0.05), urine output (control n=23, +12.65±0.8 vs KO n=9, +12.73±1.5 g/day; p>0.05), and fluid intake (control n=23, +16.17±1.3 vs KO n=9, +14.83±2.5 mL/day; p>0.05) increased normally

in KO mice. Preliminary findings indicate normal or possibly exaggerated urine copeptin levels in KO mice following DOCA-salt, and an exaggerated AVP release in response to increasing serum osmolality. Collectively, these data suggest that AT1A receptors on AVP expressing cells are required to mediate baseline secretion of AVP, but that these receptors are dispensable for DOCA-salt mediated increases in circulating AVP and blood pressure.

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P061

Selective Deletion of Intracellular Renin in the Brain Causes Hypertension and Elevated Sympathetic Nervous System Activity

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Renin expression is regulated by two distinct promoter-1st exon combinations that target renin either for secretion (exon 1a for secreted renin) or cytoplasmic retention (exon 1b for intracellular renin, icREN). We developed icREN knockout (KO) mice by selectively deleting exon 1b. icREN KO mice are essentially brain-specific knockouts of icREN because icREN is predominantly expressed in the brain. Notably, systolic blood pressure measured by telemetry was increased in icREN KO mice (130±2 mmHg, n=8 vs 122±2 mmHg in controls, n=7, P<0.01). The low- to high-frequency ratio (LF/HF) derived from power spectral analysis of heart rate variability, a parameter of sympathetic nerve activity (SNA), was increased in icREN KO mice (KO: 1.24±0.21, n=7 vs control: 0.70±0.11, n=7, P<0.05). Body weight (BW) was normal in icREN KO mice compared to controls, but the BW gain and fat accumulation induced by high fat diet (HFD) were attenuated in male icREN KO mice (BW at 16 wks of HFD- KO: 36.8±1.2 g, n=8 vs control: 41.9±1.4 g, n=9; relative fat mass at 14 wks of HFD- KO: 27.7±1.7%, n=8 vs control: 34.4±2.3%, n=9, both P<0.05). The resting metabolic rate measured by respirometry was increased in icREN KO mice (0.156±0.005 kcal/h, n=46, P<0.05) vs controls (0.145±0.003 kcal/h, n=53), whereas food consumption and absorbed calories were not different. We previously reported that the brain renin-angiotensin system facilitates renal SNA (RSNA) response to acute intracerebroventricular (ICV) injection of leptin. Interestingly, the RSNA response to ICV leptin was greater in icREN KO mice (KO: 214±40 % baseline, n=5 vs control: 114±18 % baseline, n=10, P<0.01). AT1a receptor mRNA was upregulated in the paraventricular nucleus of icREN KO mice (P<0.05). Chronic ICV injection of losartan not only abolished the elevated blood pressure in icREN KO mice, but reduced it to below baseline

in controls (systolic blood pressure, 111±3 mmHg in KO, n=5; 124±4 mmHg in controls, n=6). These data suggest that icREN deletion increases the activity of brain renin-angiotensin system and elevates blood pressure and metabolic rate through sympathetic activation. We conclude that this novel icREN isoform contributes to cardiovascular and metabolic control possibly as part of an inhibitory neural circuit.

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P062

Long-term Exposure to Endothelin-1 Overexpression Increases Blood Pressure and Causes Small Artery Injury

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Background: The mechanisms of blood pressure (BP) regulation by endothelin (ET)-1 produced by endothelial cells are complex and remain unclear. Recently, we developed a transgenic mouse with tamoxifen-inducible endothelium-restricted human ET-1 overexpression (ieET-1) using Cre/loxP technology. ieET-1 mice exhibited BP rise after three weeks of induction in an ET type A receptor-dependent manner, in absence of vascular and kidney injury. It is unknown whether long-term exposure to ET-1 overexpression results in elevated BP elevation and vascular injury.
Methods: Nine to 12-week old male ieET-1 mice and control ieCre mice expressing a tamoxifen-inducible Cre recombinase (CreERT2) under the control of EC-specific Tie2 promoter, were treated with tamoxifen (1 mg/kg/day, s.c.) for 5 days and studied 3 months later. Metabolic cages were used to collect 24-hour urine for sodium, potassium and protein measurements. Renal artery flow (RAF) was assessed by ultrasonography. BP was determined by telemetry, plasma aldosterone by ELISA, and small mesenteric artery (MA) endothelial function and vascular remodeling by pressurized myography.
Results: Systolic BP was increased in ieET-1 compared with ieCre mice (144±5 vs 117±3 mmHg, P<0.001). RAF was decreased in ieET-1 compared with control (1.9±0.2 vs 3.0±0.3 mL/min, P<0.01). The excretion of urinary sodium, potassium and protein was similar in both groups. Plasma aldosterone levels were increased in ieET-1 compared with ieCre mice (1.99±0.20 vs 1.29±0.12 ng/mL, P<0.05). Endothelium-dependent relaxation responses to acetylcholine were impaired in ieET-1 compared to ieCre mice (36.3±4.7 vs 71.4±9.7%,

P<0.01), whereas endothelium-independent relaxation responses to sodium nitroprusside were unchanged. MA media/lumen and media cross-sectional area were similar in both groups, but stiffness was increased in ieET-1 compared to ieCre mice, as indicated by leftward displacement of the stress-strain curves (strain at 140mmHg: 0.61±0.04 vs 0.71±0.02, P<0.05).
Conclusions: The results demonstrate that long-term exposure to endothelial ET-1 overexpression caused sustained BP rise and small artery stiffening.

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P063

Pitavastatin Has Antihypertensive and Renoprotective Effects With Upregulation of No System and Down-regulation of Oxidative Stress in the Kidney of Spontaneously Hypertensive Rats

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Clinical trials have demonstrated renoprotective effects of atorvastatin (ATV) and pitavastatin (PTV), which belong to the strong statins, are more potent than other statins. We reported previously that ATV attenuated the development of hypertension in SHR with increasing the endothelial and neuronal NO synthases (eNOS, nNOS) expressions in the kidney, whereas ATV inhibited the eNOS phosphorylation at serin1177 (J Hypertes 28: 2278-2288, 2010). To clarify the mechanisms of renoprotective effects of PTV, the present study examined the effects of PTV on blood pressure, renal functions, NOS and oxidative stress in the kidney of SHR. Five-week-old, male SHR were given orally PTV (2mg/kg/day) or vehicle for 8 weeks. The systolic blood pressure (SBP) was measured. The NOS expression and eNOS phosphorylation were analyzed by Western blot. The NADPH oxidase activity was measured by the lucigenin-enhanced chemiluminescence method. PTV attenuated the progression of hypertension (220 ± 8 vs. 177± 4 mmHg, P<0.01) and albuminuria (684 ± 66 vs. 398 ± 42 mg/day, P<0.01) without changing plasma total cholesterol or creatinine. PTV increased the eNOS and nNOS expressions in the outer and inner medulla of the kidney (eNOS; by 182% and 186%, nNOS; by 315% and 194%, P<0.01). PTV significantly stimulated the eNOS phosphorylation at serin1177 in the inner medulla and inhibited the eNOS phosphorylation at threonine495 in the outer and inner medulla. PTV decreased hydrogen peroxide (13.4 ± 2.1 vs. 6.1 ± 1.2 nmol/day, P<0.05) and thiobarbituric acid reactive substances (TBARS) (236.6 ± 12.4 vs. 198.3 ± 10.6 nmol/day, P<0.05) in the urine and the NADPH oxidase activity (42681± 2515 vs. 32381 ± 1995 c.p.m/mg protein, P<0.01) in the renal cortex. These results indicate that PTV attenuates the development of hypertension and albuminuria in SHR with increasing the eNOS and nNOS expressions, changing the eNOS phosphorylation to an active form and mitigating oxidative stress in the kidney. The antihypertensive and renoprotective effects of PTV may be mediated in part by an upregulation of NO system and down-regulation of oxidative stress in the kidney.

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P064

Case Report of Mals Surgery in Postural Orthostatic Tachycardia Syndrome

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BACKGROUND : Postural Orthostatic Tachycardia Syndrome (POTS) is a form of dysautonomia associated with variety of symptoms like Headache, Abdominal discomfort, Dizziness/presyncope, Nausea, Fatigue, Lightheadedness, Sweating Sleep disorder, Tremor, Anxiety, Palpitations, Exercise intolerance. Median arcuate ligament syndrome (MALS, also known as celiac artery compression syndrome) is a condition characterized by abdominal pain, delayed gastric emptying, nausea, weight loss and other symptoms of autonomic dysfunction attributed to compression of the celiac artery and possibly the celiac ganglia by the median arcuate ligament. The researchers suggest that MALS should be considered in POTS patients who have persistent gastrointestinal symptoms. The purpose of this research is to study the celiac artery velocity in POTS patients. That led us to think about an operative procedure that can reduce POTS symptoms through MALS surgery.
PATIENTS AND METHOD: We present the case of a 17 year old male with POTS symptoms not improving with medical management and celiac artery ultrasound positive for MALS.His celiac artery velocities was higher in Inspiration as well as Expiration and MALS is confirmed with CT angiogram.He underwent laparoscopic MALS surgery where the Median arcuate ligament is flipped thereby decreasing the extrinsic compression on the celiac artery.
RESULTS: There appears to be tremendous improvement in the patient's symptoms after the surgery for MALS.His nausea had almost disappeared.Before surgery he had vomiting twice a week, after surgery he had vomiting four times in three months. His lightheadedness had disappeared. Bluish discoloration of arms and feet when he used to stand had disappeared in the hospital after the procedure. Sensations of hands had improved. Heat intolerance had improved. His Sleep and constipation improved. There was no much improvement in fatigue. Autonomic tilt table test is repeated after surgery to see if there is definite objective change, Heart rate in the first 10 minutes of tilt test before the surgery was >120bpm,Heart rate after the surgery was <100 bpm.
CONCLUSIONS: Laparoscopic MALS surgery was found to be extremely helpful in relieving POTS symptoms with immediate results.

C. Ashangari: None. **A. Suleman:** None.

P065

Pacemaker Tapping Maneuver in the Treatment of Orthostatic Hypotension

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A 77 year old man was referred to us with fainting spells especially after standing/walking few steps. Initially he went to a neurologist and ENT specialist where the cause could not be determined and symptoms continued. During annual physical, he noticed that his blood pressue dropped when he stood up suddenly. He had severe fainting spells and was placed

pacemaker in him.However,pacemaker did not make a huge difference.Blood Pressure monitoring was done and noticed 50 mmHg drop upon standing. Pacemaker was changed to constant pacing where his dizziness continued but he had no falls. He had periodic vision problems, numbness in fingers and legs, digestive problems, stomach issues and balance problems. His tilt table test showed orthostatic hypotension.
Medical management (florinef, mestinone, midodrine) did not relieve his symptoms .Pacemaker sensor was turned on and made more sensitive that after he taps on the pacemaker, heart rate increases for 10 minutes. We told him to tap on pacemaker whenever he is dizzy and see if there is any improvement in orthostatic symptoms. The later treatment had made a huge difference; he is practically asymptomatic and was very much satisfied with his current health. He is using the tapping maneuver 4-5 times a day and his fainting disappeared and no syncope.
First time our case report on Pacemaker tapping demonstrated significant improvement in treating symptoms of orthostatic hypotension.

C. Ashangari: None. **A. Suleman:** None.

P066

Case Report on IV Albumin in Postural Orthostatic Tachycardia Syndrome with Meniere's Disease

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Postural orthostatic tachycardia syndrome (POTS) is characterized by an increase in a patient's heart rate when they move from a supine to an upright position. This syndrome is often treated with a high sodium diet. Meniere's disease is a disorder of the inner ear generally treated with diuretics and a low sodium diet. This poses a challenge when treating a patient suffering from both Meniere's and POTS because by treating the Meniere's with diuretics and a low salt diet it will exacerbate the symptoms of POTS. We hypothesize that the administration of IV albumin will result in an increase in the plasma oncotic pressure thus intravascular volume while simultaneously decreasing extra cellular fluid pressure therefore leading to an improvement in the patient's symptoms.
This case involves a 35-year-old female suffering from low blood pressure, debilitating dizziness with fainting, shortness of breath with exercise intolerance, and chest pain that was sent to us for further evaluation and treatment of her symptoms. Growing up she had several recurring ear infections, and by the age of 30, she started developing symptoms of vertigo and dizziness that had no relationship to posture. She was diagnosed with Meniere's disease. The patient appeared to have primarily vestibular-type symptoms. Her tilt table was indicative of POTS. Her clinical symptoms were partly reproduced during the tilt table test. We were not able to completely alleviate the patient's symptoms by the administration of Midodrine. Intravenous treatment with Albumin and saline was started which was changed to treatment with IV Albumin alone. The patient reports improvement of her symptoms.
We hereby suggest the use of IV albumin in the treatment of POTS in a patient with Meniere's disease.

C. Ashangari: None. **S.E. Hamid:** None. **A. Suleman:** None.

P067

Novel Antagonists of Dopamine-β-hydroxylase Identified and Validated Through Structure Based Approach to Combat Hypertension

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Human dopamine β-hydroxylase (hDBH), expressed in noradrenergic nerve terminals of nervous system and in chromaffin cells of adrenal medulla, is a key constituent of catecholamine biosynthetic pathway. DBH inhibition has been shown to help the treatment of hypertension, cardiac hypertrophy and cardiac heart failure, which are major causes of mortality and morbidity worldwide. Existing hDBH inhibitors are too few, often result in side effects and are frequently non-responsive to specific population. Since no three-dimensional structure existed for full-length hDBH, structure based rational drug design was elusive till date, an issue to which we provided solution lately by building an experimentally validated in silico model for hDBH. The model was used in Autodock, Glide SP and XP software for structure based virtual screening against small molecule databases from NCI, USA. The docked structures were scored using Autodock, X-Score and Prime MMGBSA. Thus, 69 compounds were identified as prospective inhibitors of DBH, which were then tested in vitro against human serum DBH and its nearly identical homologue, bovine DBH (586 of 617 amino acids homologous), with known inhibitors nepicastat and disulfiram as positive controls. Three lead molecules UDSC171, UDSC180 and UDSC142 were discovered in the process as potent inhibitors of DBH with IC50s of 1μM, 5.5μM and 18μM, respectively. The binding of the inhibitors to the enzyme were validated using fluorescence and CD spectroscopy as well as ITC, revealing KD values in the range of 100nm to 1μM. In silico pharmacokinetic analysis indicated the molecules to be latest generation of DBH inhibitors having very high cell permeability and inability to cross the blood brain barrier. High doses (up to 50μM) of the lead compounds showed acceptable cellular tolerance against HEK 293 cell line and insignificant hemo-toxicities against human RBCs. Hence, in vivo evaluation of the lead molecules were done in small model systems like C. elegans and D. melanogaster reconfirming their nontoxic properties up to 15μM doses. These three leads are now being tested in cardiac hypertrophy and hypertension rat models with exciting preliminary results.

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P068

Comparison Effects of Leptin and Phentolamine on Cardiovascular System

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Background: Leptin participates not only in negative feedback control of body adiposity but

in cardiovascular and sympathetic regulations. The aim of this research is to study the acute effects of leptin on cardiovascular system. Rabbits were used as experimental animals.
Materials and Methods: Blood pressure and EKG have been recorded at basal and after injection of these medications. Intravenous bolus of 500μg/kg of leptin has been injected into marginal vein in first group (15 rabbits), whereas second group (15 rabbits), has been injected α-adrenergic receptor antagonist phentolamine (0.01 mg/kg).
Results: First group of animals received IV leptin 500 microgram/kg, there was significant increase in mean arterial blood pressure from 75.60±0.61 mmHg to 94.63±0.51 mmHg (P<0.001). Moreover, heart rate has been increased after IV leptin, from 225±3.04 beat/min to 291±4.42 beat/min (P<0.001). Second group of animals received phentolamine significantly decreased response of cardiovascular action of leptin. Leptin increased blood pressure nonsignificantly. Mean arterial blood pressure was before treatment 74.70±0.51 mmHg and became after treatment with phentolamine and leptin in sequences 77.63±0.41 mmHg (P>0.05). However, phentolamine did not impair effects of leptin on heart rate. Heart rate was 229±2.41 beat/min and significantly increased after treated with phentolamine and leptin in sequences 344±3.52 beat/min (P<0.001). This study elucidated significant positive relationship between blood pressure and heart rate in group which is treated with leptin (p<0.001), but this relationship was inversely significant related in group two which is treated with phentolamine (p<0.001).
Conclusions: These results suggest that intravenous injection of leptin causes increases in arterial blood pressure and heart rate in anesthetic rabbits, and these effects could be opposed by adrenergic blockade. This may be partially explained by the effect of leptin on cardiovascular system could be mediated by sympathetic pathway.
Keywords: Leptin, α-adrenergic receptor antagonist, blood pressure, heart rate.

K.M. Hasan: None.

P069

1H-Magnetic Resonance Spectroscopy Reveals Elevation of MyoInositol and Other Markers of Inflammation in the Dorsal Medulla of Children with Orthostatic Intolerance

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Children with orthostatic intolerance (OI) have exaggerated decreases in heart rate variability (HRV) and suppression of baroreflex sensitivity (BRS) with standing. Inflammation is proposed as a possible factor contributing to impaired HRV in cardiometabolic disorders; whether systemic or brain inflammation better predicts impaired HRV is arguable. We used 1H magnetic resonance spectroscopy (MRS) to quantify markers of neuronal and glial integrity in children with OI compared with asymptomatic controls. Fifteen subjects ages 10-18 years were evaluated for blood pressure, HR, and autonomic function in supine and upright positions and 7 tested positive for OI. An average of 2 weeks following OI testing all subjects underwent 1H-MRS scans of dorsal medulla on a clinical 3T magnet while supine. OI subjects had higher myoinositol (mIns) as a marker of glial inflammation than asymptomatic

controls (7.8 ± 0.4 vs 5.6 ± 0.9 mmol/L, p = 0.03). Trends were observed for higher glycerophosphocholine (higher GPC, reduced myelination and axonal integrity) (2.3 ± 0.2 vs 1.8 ± 0.2 mmol/L, p = 0.08) and lower N-acetyl aspartate (lower NAA, reduced neuronal integrity) (2.8 ± 0.3 vs. 3.7 ± 0.4 mmol/L, p = 0.1) in OI subjects vs controls (mean ± SEM). mIns concentrations did not correlate with indices of autonomic function measured in the supine position. However, supine measures of mIns correlated with autonomic measures taken in the upright position: negatively correlated with spontaneous BRS (R = -0.64, p = 0.01), parasympathetic tone measured by high frequency alpha index (HFα, R= -0.547, p=0.04) and HRV measured by root of mean square of successive differences (rMSSD, R = -0.45, p = 0.09); there was a positive correlation with HR (R = 0.53, p < 0.05). In summary, children with OI have higher mIns in dorsal medulla while supine that is predictive of impairment in BRS, HRV and parasympathetic tone upon upright posture. This first report that OI in children is associated with elevated mIns, a marker of glial inflammation in a variety of neuropathies, raises the intriguing possibility that brain inflammation plays a role in the autonomic dysfunction observed while standing in these subjects. Support: Centers for Integrative Medicine and Hypertension & Vascular Research; AHA12CRP9420029

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P070

Exercise Prevents Cardiac Diastolic Dysfunction in a Female Model of Over-Nutrition Induced Obesity by Reducing Oxidant Stress and Fibrosis without Lowering Bodyweight

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Obesity is being classified as a global epidemic by both the WHO and the CDC. Sedentary lifestyle and consumption of a high-fat/high-fructose Western diet (WD) are implicated in this epidemic. Women are particularly vulnerable to obesity related cardiovascular disease. Obese women suffer higher rates of hypertension, insulin resistance and heart failure, especially diastolic heart failure early in their lives. There are no evidence based treatments for diastolic heart failure. We hypothesized that voluntary daily exercise would prevent WD induced diastolic dysfunction by reducing oxidant stress, fibrosis and inflammation.

To test this hypothesis, we developed a diastolic heart failure model by subjecting C57BL6/J female mice to a solely WD fed regimen for 16 weeks. We treated a parallel cohort with daily exercise, via voluntary wheel running, for the entire 16 weeks of WD feeding alongside control diet (CD) groups (n=7 for each group). We monitored food consumption, running activity and body weight. After 16 weeks, we assessed diastolic function by cardiac MRI and echocardiography. Detailed myocardial staining and Western blotting were done for cardiac oxidant stress, fibrosis and inflammatory markers.

Both imaging modalities revealed diastolic dysfunction with WD feeding that was normalized by voluntary exercise. Mice ran similarly high intensities on both CD (6.5 km/d) and WD (7.1km/d). While WD feeding increased bodyweight, there was no reduction in weight with exercise. Body composition analysis showed WD fed mice treated with voluntary

exercise had increases in visceral fat weight similar to sedentary WD fed mice. There was a notable increase in lean body mass with exercise. WD feeding resulted in insulin resistance that was prevented by exercise. Finally, while WD feeding markedly increased oxidant stress and fibrosis in sedentary mice, exercise prevented myocardial oxidant stress and fibrosis.
Our work provides seminal evidence that diastolic dysfunction of over-nutrition induced obesity can be prevented by exercise. Surprisingly, our study suggests that the mechanisms behind the amelioration of diastolic dysfunction are predominantly through reductions in oxidant stress and fibrosis without reductions in body weight or visceral fat.

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P071

Mineralocorticoid Receptor Overexpression in Adipose Tissue Leads to Metabolic Syndrome and Induction of Prostaglandin D2 Synthase PTGDS and lipocalin-2 NGAL

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Objectives. Metabolic Syndrome (MetS) is a cluster of metabolic risk factors associated with a higher risk for cardiovascular events. Adipose tissue (AT) plays a central role in the obesity-related metabolic abnormalities and it's known that Mineralocorticoid Receptor (MR) activation affects adipocyte differentiation and function. The purposes of this study were to evaluate the molecular and metabolic consequences of adipocyte-targeted increase in MR expression in mice.
Results. In our study we showed that MR expression is increased in visceral adipose tissue (VAT) in a preclinical mouse model of MetS. Thus, we generated a double transgenic mouse model with a conditional and inducible overexpression of MR in AT (Adipo-MORE), demonstrating that increased expression of MR in AT contributes to MetS development with multiple metabolic abnormalities, including visceral obesity (VAT mass, control-MR 5.0±0.6 adipo-MORE 10.4±1.8, $p<0.05$), insulin resistance (HOMA index, control-MR 8.9±0.8 adipo-MORE 14.5±2.2, $p<0.05$) as well as dyslipidemia (total cholesterol, mg/dL control-MR 86.0±6.0 adipo-MR 109±6, $p<0.05$). We also identified prostaglandin D2 synthase (PTGDS) as a novel mediator of adipogenic effects of MR activation in adipocytes. Since adipokines play pivotal roles in the regulation of insulin sensitivity in obesity, we also focused on the role of lipocalin-2 NGAL, recently identified in

our laboratory as a target of aldosterone action in cardiovascular system, as new adipocyte MR target. It's known that NGALKO mice fed a high fat diet are protected from obesity-induced cardiovascular dysfunctions. In our model we observed an increase of NGAL mRNA levels (3 fold increase, $p<0.05$) in VAT. Moreover, NGAL was induced (2-8 fold increase, $p<0.05$) in primary cultures of adipocytes differentiated ex vivo from adipo-MORE mice in presence of aldosterone stimulation compared to controls. Conclusions. We demonstrated that PTGDS is a novel direct MR target in rodent adipocytes mediating adipogenic effects of MR activation. We also found that NGAL is induced in presence of MR overexpression in adipocytes in vivo and ex vivo. In consideration of our results, other analyses are necessary to better understand the role of NGAL as adipocyte MR target.

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P072

Adipocyte AT_2 Receptor Suppresses UCP1 Transcription and Thereby Resting Metabolic Rate

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The renin-angiotensin system (RAS) contributes to energy balance through opposing actions in the brain and periphery. We hypothesize that tissue- and receptor-specific RAS modulation may represent a novel therapeutic approach to obesity. Transgenic "sRA" mice exhibit brain-specific RAS hyperactivity through expression of human renin in neurons (synapsin promoter) and human angiotensinogen via its own promoter. Previously we documented that sRA mice exhibit a suppressed circulating RAS, and an elevated resting metabolic rate (RMR) that is sensitive to replacement of circulating angiotensin II or the AT_2 receptor (AT_2R) agonist, CGP-42112a (CGP, 100 ng/kg/min, s.c.). sRA mice exhibit a robust and specific elevation in uncoupling protein-1 (UCP1) expression and glucose uptake in inguinal white adipose tissue (iWAT). Chronic infusion of the AT_2R agonist, CGP-42112a (CGP) into sRA mice increased weight gain and normalized RMR and inguinal fat expression of UCP1, but had no effect on food intake or digestive efficiency. RMR suppression by CGP was not mediated through attenuation of adipose sympathetic nervous activity (SNA, measured via multifiber nerve recordings), leading to the hypothesis that AT_2R action on the adipocyte suppresses adipose sensitivity to SNA. Cultured primary mouse adipocytes were isolated from the iWAT, and norepinephrine (NE, 1 nM) treatment induced UCP1 mRNA (597 -fold of vehicle, $P<0.05$), however this UCP1 induction was attenuated with CGP co-treatment (10 nM, 0.2 -fold of NE alone, $P<0.05$). In human adipocytes, activation of AT_2R also prevented the NE-induced increase in UCP1 mRNA. NE additionally significantly increased lipolysis by adipocytes (measured via free glycerol in the media; $P<0.05$), but AT_2R activation had no effect (vehicle= 15.9 ug/mL; NE= 70.8 ug/mL; NE + CGP=64.7 ug/mL). These data support a suppressive action of AT_2R upon RMR that is mediated specifically through the suppression of UCP1 expression, but not its activation by lipolysis, within subcutaneous adipocytes. Ongoing studies are examining the transcriptional regulation of UCP1 by adipose AT_2R activation.

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P073

Chemerin Receptor Antagonism Improves Vascular Insulin Signaling in Diabetic Mice

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Adipose tissue releases many adipokines, including chemerin, which produces its effects through activation of chemokine-like receptor 1 (ChemR23). Chemerin influences major aspects of the metabolic syndrome, including adipogenesis and insulin sensitivity in adipocytes and skeletal muscle cells. Considering that chemerin impairs vascular reactivity and that amongst its many actions, insulin also influences vascular function, we postulate that chemerin decreases insulin-induced vasodilatation, by reducing PI3K/Akt signaling, and impairs glucose uptake by vascular smooth muscle cells (VSMC). Mesenteric arteries, endothelial cells (EC) or VSMC from C57Bl6 mice were incubated with chemerin (0.5 ng/mL, 1 h) or vehicle (veh). Chemerin decreased insulin-induced relaxation (0.1 - 3000 ng/mL, pD2: chemerin: 94.5±0.1 vs. veh: 23.6±0,1), which was prevented by the ChemR23 antagonist CCX 832 (pD2: veh= 16.9±0.1; chem= 174.1±0.1, CCX+chem= 37.4±0.1) and a PI3K activator (YS-49) (pD2: veh= 23.0±0.1; chem= 108.5±0.1, YS-49+chem= 28.5±0.1). Chemerin also decreased PI3K (0.8±0.1 vs. veh 1.1±0.1), Akt (0.8±0.1 vs. veh 1.2±0.1) and AMPK [0.7±0.2 vs. veh 1.0 ±0.1] phosphorylation in VSMC. IRS1 and IRS2 gene expression was decreased in VSMC stimulated with chemerin. In addition, chemerin decreased insulin-stimulated NO production in EC through activation of ChemR23 and PI3K and inhibited insulin-stimulated glucose uptake by VSMC (counts per minute: 5668±729.0 vs. veh 9923±662.7), which was mediated by ChemR23, AMPK and PI3K. Importantly, ChemR23 antagonism in diabetic (db/db) mice partially reversed decreased insulin-induced vasodilatation (mesenteric resistance arteries; pD2: db/m: 6.1±1.0; db/db: 7.3±0.2; db/db+CCX832: 6.9±0.3). In conclusion, chemerin decreases vascular insulin responses by reducing PI3K/Akt and AMPK signaling. Moreover, chemerin/ChemR23 system plays a crucial role in impaired vascular insulin signaling in diabetes, suggesting its involvement in the

pathogenesis of vascular insulin resistance. Our study may contribute to a better understanding of the role of chemerin in vascular insulin dysfunction in diabetes- and obesity-associated diseases. Financial Support: FAPESP, Brazil.

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P074

Role of Peroxisome Proliferator-Activated Receptor-γ (PPAR) in the Pro-opiomelanocortin (POMC) Neuron-Mediated Regulation of Energy Balance

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PPARγ, a master regulator of adipogenesis, was recently shown to affect energy homeostasis through its actions in the brain. Deletion of PPARγ in mouse brain, and specifically in the POMC neurons, results in resistance to diet-induced obesity. To study the mechanisms by which PPARγ in POMC neurons controls energy balance, we generated a transgenic mouse model in which a dominant-negative mutant (P467L) form of PPARγ is conditionally expressed in POMC neurons. The transgene will express both human PPARγ-P467L and the tdTomato reporter gene after it is selectively activated by a Cre-recombinase driver. Here, POMC-Cre was used to direct POMC neuron-specific expression of the transgene. Co-localization of tdTomato and ACTH staining in the Arcuate nucleus of POMC-Cre X PPARγ-P467L double transgenic (DTg) mice demonstrates successful transgene activation in POMC neurons. Interestingly, 25 week treatment with 60% high fat diet resulted in no difference in body weight in DTg mice compared to littermate controls (55.7±1.0 g vs 57.1±1.2 g, n>11). However, feeding these mice a 10% fat isocaloric-matched control diet led to a significant increase in the body weight of DTg mice compared to littermate controls (36.0±0.9g vs. 31.7±0.5g, $p<0.0001$, n=10). The increased body weight in DTg was due to increased adipose mass (DTg: 23.9±2.4% vs. control: 16.8±2.2%, p=0.04, n=10) and decreased lean mass (DTg: 60.5±2.0% vs. control: 66.5±1.7%, p=0.04) respectively, as demonstrated by nuclear magnetic resonance spectroscopy. We observed no difference between genotypes in food intake (DTg: 2.1±0.1g vs. controls: 2.2±0.1g, n>9) and cumulative body weight change (DTg: -4.5±0.7% vs. controls: -4.4±1.0%, n>9) in response to 1mg/kg (i.p.) leptin. Since interference with PPARγ in POMC neurons does not impede sensitivity to leptin, we speculate that increased weight gain in DTg mice on low fat diet may be due to differences in food intake, decreased sympathetic drive to adipose tissues, and/or decreased energy expenditure, mechanisms which are currently under investigation. We conclude that PPARγ in POMC neurons may play a role in energy balance under certain dietary conditions.

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P075

Stroke Volume and Visceral Fat Drive Differences in Blood Pressure Between Two Generations: A Population-Based Study of Adolescents and Their Parents

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Excess total body fat (TBF) and visceral fat (VF) are major risk factors of hypertension. Blood pressure (BP) increases with age, as do TBF and VF. Here we investigated whether TBF and VF contribute to BP differences between adolescents and adults. A population-based sample of adolescents (n=933, 12-18 years) and their parents (n=429, 38-65 years) was studied as part of the Saguenay Youth Study. In all participants, beat-by-beat values of SBP, DBP and underlying hemodynamic parameters (heart rate, stroke volume [SV] and total peripheral resistance) were obtained with a Finometer during a 52-minute protocol mimicking daily life activities and including posture and math-stress tests. TBF was assessed by bioelectrical impedance and VF was examined by magnetic resonance imaging. SBP was higher in parents than adolescents by an average of 10.2±0.3 mmHg in males and 9.1±0.3 mmHg in females ($p<0.0001$ for both sexes). DBP differed minimally throughout the protocol ($p=0.3$ and 0.1, respectively). In males and females, respectively, the 'generation' differences in SBP were reduced to 6.0±0.1 and 4.3±0.1 mmHg when adjusted for height and TBF ($p<0.0001$ for both), and were further reduced to 1.9±0.1 and 2.5±0.2 mmHg when additionally adjusted for VF ($p=0.1$ and 0.02). Of the underlying hemodynamic parameters, only SV was higher in parents than adolescents (by 46±2 ml in males and 39±2 ml in females, $p<0.0001$ for both). Again, the 'generation' differences in SV were reduced to 23±1 (males) and 20±1 ml (females) when adjusted for height and TBF ($p<0.0001$ for both sexes), and were further reduced to 11.8±0.6 and 15.7±0.8 ml when additionally adjusted for VF ($p<0.0001$ for both sexes). These results suggest that the transition from adolescence to middle-aged adulthood is associated with an increase in SBP (but not DBP), which is driven mainly by SV augmentation. They also suggest that VF contributes to the generational differences in both SBP and SV above and beyond the contribution of TBF, despite VF being a relatively small fraction of TBF. The co-occurrence of these differences in VF, SV and SBP may be related to sympathoactivation and renal handling of sodium and water reabsorption; further research is required to confirm this possibility.

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P076

Suppressor of Cytokine Signaling 3 (SOCS3) in POMC Neurons and Its Role in Regulating Blood Pressure, Body Weight and Glucose in Obesity

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Suppressor of cytokine signaling 3 (SOCS3), a negative regulator of leptin signaling, may be involved in development of obesity-induced leptin resistance. Although we previously showed that activation of proopiomelanocortin (POMC) neurons mediates the chronic effects of leptin on blood pressure (BP), the role of SOCS3 in modulating BP in obesity is still unclear. In this study, we investigated the role of SOCS3 specifically in POMC neurons in regulating body weight, glucose handling and BP in mice fed a normal or high fat (45%, HFD) chow. Male and female SOCS3flox/flox-POMC/cre mice in which SOCS3 was selectively deleted in POMC neurons and control SOCS3flox/flox mice were used. Food intake and body weight were measured from 8 to 16 weeks of age, and a glucose tolerance test (GTT) was performed at 20 wks of age. At 22 wks of age, mice were implanted with telemetry probe to measure BP and heart rate (HR) and fed a HFD for 6 weeks. Compared to control mice, both male and female SOCS3flox/flox-POMC/cre mice were lighter at 16 wks (29.1 ± 3.5 vs 31.9 ± 3.6 g in male and 21.5 ± 2.2 vs 26.1 ± 5.7 in female, n=9-11, $p<0.05$) but food intake was similar in both groups. Only male SOCS3flox/flox-POMC/cre mice exhibited improved glucose handling (AUC: 1059 ± 52 vs 1283 ± 54 mg/dL x 120 min, n=7-10, $p<0.05$) and no differences were observed in female mice. When fed normal chow, BP was similar in SOCS3flox/flox-POMC/cre and control mice (116 ± 7 vs 113 ± 5 mmHg) at 23 wks. After a HFD for 6 weeks, SOCS3flox/flox-POMC/cre mice had a greater BP increase compared to control mice (7.2 ± 1.9 vs 0.9 ± 1.8 mmHg, n=4-9, $P<0.05$) but no significant differences were observed in food intake or body weight between two groups. These results suggest that SOCS3 deletion specifically in POMC neurons reduced body weight in male and female mice, and improved glucose handling only in male mice. HFD increased BP in SOCS3flox/flox-POMC/cre but not in control mice, suggesting that SOCS3 in POMC neurons may modulate BP response to HFD.

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P077

Atorvastatin Ameliorates Cardiac Injury and Inflammation via Adiponectin-independent Activation of AMP-activated Protein Kinase and Inhibition of the Nf-κB Pathway in Rats With Metabolic Syndrome

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Background: Statins have been implicated in inhibition of inflammation and preventing new onset type 2 diabetes. However, the mechanism underlying the pleiotropic effects of statins remains controversial. We investigated the effects of atorvastatin on cardiac and adipose tissue pathology as well as glucose and lipid metabolism in rats with metabolic syndrome (MetS).
Methods and Results: We used DahlS.Z-Leprfa/Leprfa (DS/obese) rats, derived from a cross between Dahl salt-sensitive and Zucker rats, as a new animal model of MetS. DS/obese rats were treated with atorvastatin (6 or 20 mg/kg/day, p.o.) from 9 to 13 weeks of age. Treatment with atorvastatin ameliorated LV fibrosis and diastolic dysfunction as well as LV oxidative stress and inflammation without affecting hypertension or LV hypertrophy in DS/obese rats. Atorvastatin did not affect visceral or subcutaneous fat mass but alleviated adipocyte hypertrophy (high-dose) and inflammation (both high- and low-dose) in visceral adipose tissue in these rats. Both high- and low-dose atorvastatin reduced serum adiponectin concentration in DS/obese rats. However, both doses of atorvastatin similarly attenuated the decrease in phosphorylation of AMP-activated protein kinase (AMPK) as well as the increase in phosphorylation of NF-kB in the heart. Insulin resistance was similarly improved by both high- and low-dose atorvastatin. Glucose intolerance was partially ameliorated by low-dose atorvastatin and completely prevented by high-dose atorvastatin. Both doses of atorvastatin similarly attenuated the increases in serum levels of total cholesterol, LDL-cholesterol, and triglycerides in DS/obese rats.
Conclusions: Treatment of DS/obese rats with atorvastatin attenuated adipose tissue inflammation, without affecting obesity, as well as ameliorated LV inflammation, fibrosis and diastolic dysfunction and abnormal metabolism. The beneficial cardiac effects of atorvastatin are likely attributable, at least in part, to adiponectin-independent activation of AMPK and subsequent inhibition of the NF-κB pathway.

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P078

Cardiovascular Autonomic Impairment Triggers Cardiometabolic Dysfunction in an Experimental of Metabolic Syndrome

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The aim was evaluate on time-course of changes in cardiometabolic dysfunction model. Male Wistar and SHR rats were divided: Control

(C), hypertensive (H), hypertensive + fructose (HF). The fructose overload (100g/L) was initiated at 30 days (d) of life. All evaluations were at 37, 45, 60 and 90 d of age. Arterial pressure (AP) signals were directly recorded. The baroreflex sensitivity was evaluated by the tachycardic (TR) and bradycardic (BR) responses to AP changes. Cardiovascular autonomic modulation was evaluated by spectral analysis. Inflammatory markers were evaluated in adipose tissue. No differences were observed in glycemia and triglycerides between the groups. The SHR groups showed increased systolic (SAP), diastolic (DAP) and mean arterial pressure (MAP) compared to control groups in all times of evaluation. Additionally, SHR groups progressively increased AP during the time course evaluation. The HF group showed additionally increased in DAP and MAP (90: MAP: C: 114±1, H: 165±3, HF: 179±3 mmHg) vs. H group. The cardiac parasympathetic modulation (HF-IP) was decreased H group in 37 d and HF group in 37, 90 d vs. C group. The VAR-PAS was higher in SHR groups vs. C group in all times evaluated. The HF group showed additionally increase in VAR-PAS during the time course evaluation (45, 90 vs. 37 d). The vascular sympathetic modulation (LF-PAS) was higher H group in 60, 90 d vs. C group. The HF group showed increased LF-PAS in 45, 60, 90 d vs. C group, and vs. 37 d in the same group; and showed additionally increased in 90 d vs. H group (90: C: 3.7±0.6 H: 6.2±0.9 HF: 8.7±0.5 mmHg2). The spontaneous baroreflex sensitivity was lower in SHR groups all the times evaluated (90: C: 0.80±0.1 H: 0.51±0.05 HF: 0.32±0.02 ms/mmHg). The H group showed decreased BR in 60, 90 d vs. C group, the HF group showed decreased in 37, 45, 60, 90 d vs. C group (90: C: 1.15±0.1 H: 0.80±0.05 HF: 0.77±0.07 bpm/mmHg). The HF group showed decreased TR in 60, 90 d vs. C group. Increased TNF alpha and IL-6 were observed only in 45 and 60 d in HF group. The results showed that cardiometabolic dysfunctions are time-dependent in SHR, and fructose overload induces additional dysfunctions in this model. Furthermore, the baroreflex impairment seems to precede hemodynamic, metabolic and inflammatory changes in this model.

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P079

Impact of Combined Exercise Training in an Experimental Model of Hypertension Associated With Menopause

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In this study we tested the hypothesis that the cardiovascular autonomic dysfunction plays an important role on the management of inflammation and oxidative stress, and that these dysfunctions may in turn be modulated by combined exercise training in an experimental model of hypertension and menopause. Female rats were divided into (n=7/group): control (C) and hypertensive (H), hypertensive ovariectomized (HO) and hypertensive ovariectomized undergoing combined (aerobic+resistance) training (THO). We observed an additional increase in HO group (176±4 mmHg) in relation to H group (165±3 mmHg). However, the THO group (155±3 mmHg) showed a reduction of arterial pressure associated with resting bradycardia. The HO group (50.78±4.61 mmHg2) presented

an additional impairment in systolic arterial pressure variability when compared to C and H groups (23.69±0.45 and 34.09±2.37 mmHg2); this dysfunction was not observed in THO group (30.09±2.03 mmHg2). Additionally, an attenuation on vascular sympathetic modulation and an improvement in baroreflex sensitivity were found in the THO when compared to HO group. There was an increase in TNF-α in sedentary hypertensive groups (H and HO vs. C), which was not observed in THO group. Ovariectomy induced an additional increase in cardiac and renal oxidative stress, which were reduced in THO group. The THO group presented an increase in total antioxidant capacity when compared to the other groups. In conclusion, combined exercise training was able to reduce AP associated with improvement on cardiovascular autonomic control, probably reducing cardiac and renal inflammation and oxidative stress, in an experimental model of hypertension and menopause.

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P080

New Strategy, Old Remedy: Elucidating the Effect of Hibiscus Sabdariffa in a Neuronal Model of Oxidative Stress

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Introduction: Neurogenic hypertension is characterized by increased blood pressure, neuroinflammation, and neuronal oxidative stress. Thus, its pathophysiology dictates a unique therapeutic strategy. It is established that Hibiscus sabdariffa (HS) plays a beneficial role in decreasing blood pressure, yet the role of HS as an anti-oxidant defense system in neurogenic hypertension has not been investigated. Our objective was to generate an in vitro model of neuronal oxidative stress to test the hypothesis that HS creates a neuroprotective antioxidant defense system.
Methods: To create a model of oxidative stress, SY5Y, neuroblastoma cells, were treated with 32uM hydrogen peroxide. To determine the effect of methanol-extracted HSE (50 and 100ug $n \geq 8$) in this model of oxidative stress, SY5Y cells were treated with vehicle-PBS, HSE (24hrs), pretreated with HSE (24hrs) and then treated with H2O2 (8hrs). Using molecular techniques markers of oxidative stress were measured and percentages were out of 100. Data was normalized to the control and analyzed by one way ANOVA (p value < .05 was considered significant).
Results: HSE significantly increases cell viability (50 or 100ug > 99%) compared to H2O2 alone (43%). HSE significantly decreases ROS generation (113%-50ug versus 89%-100ug) compared to H2O2 (120%). HSE significantly decreases lipid peroxidation (11.3%-50ug versus 10.2%-100ug) compared to H2O2 (135%). HSE significantly increases GSH content (72%-50ug versus 91%-100ug) compared to H2O2 (58%). HSE significantly increases catalase activity (31%-50ug versus 51%,-100ug) compared to H2O2 (23%). HSE significantly increases mitochondrial complex 1 activity (125%-50ug versus 229%-100ug) compared to H2O2 (26.2%). There was no significant change in superoxide dismutase activity amongst groups.
Conclusion: These observations suggest that HSE creates an antioxidant defense system that

provides cytoprotection against H2O2-induced neuronal oxidative stress. Thus, HS, a known anti-hypertensive should be revisited to investigate its in vivo role in neurogenic hypertension.

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P081

Tempol Decreases Blood Pressure in Aged Female SHR When Treated With Acetazolamide

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Oxidative stress contributes to the development and maintenance of hypertension in male rodents, but studies in females have rarely shown a reduction in blood pressure (BP) with antioxidants. Tempol, a superoxide dismutase mimetic, decreases BP in young male SHR, but fails to reduce BP in either young or old female SHR, despite the fact that females have similar or higher levels of oxidative stress markers. The reason for the sex difference in the response to tempol remains unclear. Acetazolamide inhibits carbonic anhydrase in the proximal tubule thus increasing sodium delivery to the distal nephron, and thereby should increase distal oxidative stress. Acetazolamide was used to test the hypothesis that with increased sodium delivery to the distal nephron, tempol would reduce the BP in aging female SHR. Female SHR, 20-22 mos old, were divided into three groups (n=4-6/grp): Control (C), Acetazolamide (A), and Acetazolamide+Tempol (A+T). After baseline mean arterial pressure (MAP; telemetry), rats received vehicle (C) or acetazolamide (A and A+T). On day 8, rats in C and A+T groups were given tempol (30 mg/kg) for 11 days. Baseline MAP was similar (C: 170±7; A: 182±4; A+T: 172±6 mm Hg, p=NS). Tempol had no effect on MAP in C+T, but reduced MAP in A+T group (C+T: 169±1; A: 171±1; A+T: 151±5 mm Hg; $p<0.005$ A+T vs A, C+T). Basal renal oxidative stress measured by lucigenin chemiluminescence was not different in the groups; NADPH-stimulated oxidative stress was decreased in A+T compared to A and C+T (C+T: 641.8±72.2; A: 499.3±18.3; A+T: 406.2±56.3 RLU/mg/min; $p<0.05$, A+T vs A, C+T). Plasma total antioxidant capacity was increased by tempol only in A+T rats (C+T: 59.07±9.67; A: 69.01±4.66; A+T: 118.24±18.38 nmol/µl; $p<0.05$, A+T vs A, C+T). Thus tempol is capable of modestly reducing MAP in aging female SHR when proximal sodium reabsorption is blocked. The data suggest that oxidative stress-mediated BP control is dependent on increased sodium delivery to the distal nephron. Because hypertension in male SHR is attenuated with tempol alone, but not in females, taken together, the data suggest sex differences in sodium handling and thus localization of oxidative stress production in the kidneys of SHR. Supported by NIH-R01HL66072, PO1HL51971 (JFR), 14POST18640015 (ROM).

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P082

Mitochondrial Gene Expression is Decreased in Renal Inner Medulla of Non-human Primates with Spontaneous Hypertension

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Mitochondrial gene expression may influence renal function and consequently, long-term blood pressure control. The African Green Monkey (Chlorocebus aethiops sabaeus; AGM) exhibits heritable, spontaneous hypertension and thus is a translational model for the study of human essential hypertension. We hypothesized that renal mitochondrial gene expression in hypertensive AGMs is decreased and may contribute to renal mitochondrial dysfunction in specific kidney regions. AGMs were phenotyped as normotensive (NT, systolic blood pressure; SBP <120 mmHg) or hypertensive (HT, SBP > 140 mmHg) by forearm plethysmography. Gene expression was determined using qRT-PCR with RNA extracted from renal cortex, outer medulla (OM), inner medulla (IM), and liver of 18 HT (mean SBP 166±7 mmHg) and 18 NT (mean SBP 98±3 mmHg) animals. In renal cortical and OM tissue of HT vervets, COX 3 (Complex IV), Cyt B (Complex III), NADH4 (Complex I) and ATP8 (Complex V) expression were similar in NT and HT AGMs. In IM of HT AGMs, COX3, NADH4, ATP8, and CYTB were downregulated by 4.7-fold (p=0.007), 4-fold (p=0.002), 4.1-fold (p=0.006), and 3-fold (p=0.018), respectively. In the liver, COX 3 expression was decreased 1.9-fold (p=0.04), 1.6-fold for ATP8 (p=0.03), and 2.0-fold for CYTB (p=0.01). Expression of SDH (Complex II) and COX4 (Complex IV), nuclear encoded subunits of the OXPHOS chain, was also assessed. SDH expression was up-regulated 8-fold in renal OM (p=0.005) but unchanged in liver, IM, and cortex. COX4 expression however, was down-regulated in OM by 8-fold (p=0.05), in IM by 5-fold (p=0.011) but unchanged in cortex and liver. Gene expression of the mitochondrial transcription factor TFAM was downregulated by 4-fold in renal OM, and unchanged in renal cortex and liver. Citrate synthase activity showed no difference in mitochondrial number between NT and HT AGMs (p=0.73). We suggest that reduced expression of mitochondrially encoded OXPHOS subunits in the renal IM may contribute to the development of hypertension in the AGM. Mitochondrial gene down-regulation in the liver may be a consequence of the systemic hypertension. We conclude that altered mitochondrial gene expression may be a cellular response to increased oxidative stress in the renal inner medulla of HT AGM.

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P083

Gender Specific Effects in Nox4-/- Mice in Hypoxia Induced Pulmonary Hypertension and Pulmonary Vascular Resistance

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Oxidative stress and Noxs have been implicated in the pathogenesis of experimental and human pulmonary arterial hypertension (PH). Gender differences in PH may be, in part, due to

increased formation of NADPH oxidase (Nox) derived reactive oxygen species (ROS). A large body of evidence implicates E2 in the pathogenesis of PAH and an interaction between E2 and Noxs has been suggested. We hypothesised that i) Estrogen (E2) leads to Nox-induced oxidative stress, which promotes PASMC damage, ii) E2-induced Nox activation may account for gender differences in PH. Cultured human PASMC were stimulated with E2 (1nM). ROS production was assessed by chemiluminescence (O2-) and proliferation by BrdU assay. E2 increased superoxide (219%; $p<0.05$) and proliferation in PASMC, effects blocked by GKT137831 (Nox4/1 inhibitor). In vivo studies were conducted to assess the role of Nox4 in hypoxia-induced pulmonary hypertension in male and female mice. Hypobaric hypoxia was used to induce PH in mice, which were divided into 8 groups: normoxic WT (NWT), hypoxic WT (HWT), normoxic Nox4-/- (NKO) and hypoxic Nox4-/- (HKO) for both male and female mice. In male HWT mice, RVSP (20.5 NWT vs. 45.2mmHg HWT, $p<0.001$) and RVH by Fulton Index (0.20 NWT vs. 0.395 HWT, $p<0.01$) were increased, an effect that was significantly reduced in male Nox4-/- mice (RVSP: 36.4mmHg HKO; RVH: 0.030 HKO; $p<0.05$). In female HWT mice, RVSP (21.4 NWT vs. 39.8mmHg HWT, $p<0.05$) and RVH (0.20 NWT vs. 0.31 HWT, $p<0.01$) were elevated in hypoxia, yet female Nox4-/- mice were not protected against hypoxia-induced PAH (RVSP: 34.2mmHg HKO; RVH: 0.33 HKO). Hypoxia-induced endothelial dysfunction in both male and female WT pulmonary arteries was improved in male HKO, however, endothelial dysfunction remained in HKO females. In female Nox4-/- mice, increased spleen and uterine weight (which has been associated with altered ovarian hormone biogenesis) suggests a role for inflammatory and fibrotic processes.
In conclusion, genetic ablation of Nox4 in males, but not females, protects against the development of hypoxic PH. The effects of E2 on oxidative stress is present in PASMCs where Nox4-derived ROS may be an important regulator of, and impact on, molecular processes contributing to vascular remodelling in PAH.

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P084

Oxidative Stress Preconditioning Is Required for Leptin to Stimulate Renin Release From Mouse Juxtaglomerular Cells

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Leptin is enhanced in animal models of obesity. The role of leptin in hypertension is unclear with some studies showing antihypertensive actions and others showing a pro-hypertensive role. In normotensive, non-obese, animals, infusion of leptin induces natriuresis. However, in animal models of enhanced oxidative stress, obesity, or activated renin-angiotensin system, infusion of leptin tends to increase blood pressure. Renin is a critical mediator of angiotensin-II formation, and thus blood pressure control. However, the direct effect of leptin on the release of renin from juxtaglomerular (JG) cells has not been studied. We hypothesize that under normal conditions leptin does not affect renin release whereas during high oxidative stress leptin stimulates renin release. We isolated primary cultures of mouse juxtaglomerular (JG) cells. After treatment with different agonists, renin released to the supernatant was measured by

radioimmunoassay. We first tested the direct effect of murine leptin on renin release. We found that 1 hr treatment with leptin (0.1-1 uM) decreased basal renin by 20 ± 5% ($p<0.05$; n=8). Treatment of JG cells for longer periods (4 and 24 hrs) did not affect renin release (n=6) or total renin expression (n=4). We then tested whether oxidative stress modified the effect of leptin on renin release. For this we pre-incubated JG cells with 10 uM hydrogen peroxide (H_2O_2) for 1 hr. After this, the medium was removed and H_2O_2 was completely washed out from JG cells followed by treatment with vehicle (cont) or 10 uM leptin. We found that, in cells pre-treated with H_2O_2, leptin increased renin release by 49.2 ±16 %($P<0.05$ vs vehicle). By Western blot, we detected the expression of the leptin receptor in lysates from JG cells. We concluded that under normal conditions leptin inhibits renin release from JG cells. However, after exposure to H_2O_2, leptin stimulates renin release. Our data suggest the hypothesis that oxidative stress reverses the inhibitory effect of leptin on renin release and supports a pro-hypertensive role for leptin during chronic inflammatory conditions that induces oxidative stress.

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P085

Angiotensin II Type 2-receptor Agonist C21 Reduces Proteinuria and Oxidative Stress in Kidney of High-salt Fed Obese Zucker Rats

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Oxidative and nitrosative stress have been implicated in hypertension and kidney diseases. Recently we reported blood pressure reducing effect of AT2-receptor agonist C21 in high sodium-diet (HSD)-fed obese Zucker rats. In the present study we investigated C21-mediated reno-protection against HSD-related oxidative and nitrosative stress in obese Zucker rats. Obese rats (13 week) were fed normal sodium-diet (NSD) or HSD 4%, for 14 days, with/without C21, delivered subcutaneously via osmotic pump, 1 mg/kg/day. Compared with NSD controls, HSD-fed rats exhibited reduced eGFR (15.2±2.3 vs. 43.5±9.9 µL/min) and increased proteinuria (0.18±0.02 vs. 0.12±0.02 µg/min) and albuminuria (0.19±0.03 vs. 0.12±0.02 µg/min), which were associated with decreased cortical expression of the protein recycling receptors, megalin (0.25±0.02 vs. 0.47±0.07) and cubilin (0.77±0.16 vs. 1.27±0.14). C21 improved eGFR (27.4±4.9 µL/min) and reduced proteinuria (0.11±0.02 µg/min) and albuminuria (0.08±0.01 µg/min) without any effects on megalin or cubilin. Cortical NADPH oxidase (NOX) activity in HSD-fed rats was increased by 3-fold (0.37±0.07 vs. 0.13±0.04 ΔRLU/µg protein/min), while enzymatic defense (catalase/superoxide dismutase activity ratio) was unchanged. Urinary excretion of H_2O_2 (41.7±4.7 vs. 23.0±2.1 µM/mg creatinine) and 8-isoprostanes (7.9±1.6 vs. 1.4±0.2 pg/µg creatinine), indices of oxidative stress, were elevated in HSD-fed rats. C21 reduced NOX activity (0.11±0.04 ΔRLU/µg protein/min) as well as the excretion of H_2O_2 (30.5±3.0 µM/mg creatinine) and 8-Isoprostanes (4.1±1.0 pg/µg creatinine) in HSD-fed rats. Similarly, C21 also reduced carbonyls (5.5±1.4 vs. 10.5±0.9 µM/mg protein), an indicator of protein modification caused by oxidative stress. 3-nitrotyrosine, an indicator of nitrosative stress, remained unaltered in HSD and/or C21 groups. C21-

treatment of HSD-fed rats improved plasma nitrites (22.1±2.2 vs. 15.3±0.5 µM/L), which could have been due to reduced oxidative stress in this group. Together results indicate that AT2R agonist exerts antioxidant effects, possibly by reducing NOX activity and by rescuing NO levels, which potentially contributes to the reduction in salt-induced proteinuria/nephropathy in obesity.

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P086

Superoxide but Not Hydrogen Peroxide Increases Nuclear Translocation of Transcription Factor Sp3 and AT1 Receptor Expression in the Renal Cells

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Age-associated oxidative stress causes up-regulation of renal AT1 receptor function (AT1R) and hypertension in aging Fischer Brown Norway (FBN) rats. Here we studied the mechanism of up-regulation of renal AT1R, and further tested superoxide Vs hydrogen peroxide (H2O2) specificity in this phenomenon. We found that transcription factor Sp3 plasmid increased (Control vs Sp3: 0.1165 ± 0.01 vs 0.3810 ± 0.03) while Sp3 siRNA decreased (Control siRNA vs Sp3 siRNA: 1.11 ± 0.25 vs 0.64 ± 0.06) the levels of AT1 receptor protein in human kidney (HK2) cells. Whereas transcription factor NF-kB p-65 plasmid did not affect AT1 receptor protein levels in these cells (Control vs NF-kB: 0.25 ± 0.025 vs 0.31 ± 0.035). DDC, a superoxide prodrug, but not H_2O_2 treatments increased nuclear levels of Sp3 and NF-kB proteins in HK2 cells [(Control vs DDC, vs H_2O_2, vs H_2O_2 + tempol): Sp3 (0.50 ± 0.08 vs 1.28 ± 0.21 vs 0.68 ± 0.14 vs 1.04 ± 0.30 densities); NF-kB (0.4025 ± 0.13 vs 1.808 ± 0.54 vs 0.4950 ± 0.15 vs 0.6375 vs ± 0.18 densities)]. Tempol treatment, a superoxide scavenger, attenuated DDC-mediated nuclear accumulation of Sp3 (DDC vs DDC + tempol: 1.28 ± 0.21 vs 0.52 ± 0.12 densities) and NF-kB (DDC vs DDC + tempol: 1.808 ± .54 vs 0.43 ± 0.18 densities]. In addition, DDC but not H_2O_2 increased AT1 receptor mRNA expression, measured by RT-qPCR [(Control vs DDC, vs H_2O_2): (1.000 ± 0.0 vs 3.323 ± 0.79, vs 1.218 ± 0.49)]. This effect was attenuated by tempol treatment [(DDC vs DDC + tempol) (3.323 ± 0.79 vs 0.7225 ± 0.16 densities)]. Similarly, DDC treatment increased AT1 receptor protein expression that was attenuated by tempol treatment, measured by immunoblotting [(Control vs DDC vs DDC + Tempol) (0.3535 ± 0.99 vs 0.9975 ± 0.19 vs 0.3250 ± 0.02 densities)]. These results suggest that superoxide but not H_2O_2 regulates both Sp-3 and NF-kB in the renal cells. However, it is Sp3 but not NF-kB that up-regulates renal AT1 receptor expression. Taken together, this phenomenon may represent a mechanism for hypertension in aging FBNs.

M. Saleem: None.

P087

PDI via Keap1/Nrf2 Pathway Regulates Renal AT1 Receptors and Blood Pressure in Aging Rats

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Renal angiotensin AT1 receptor (AT1R) plays pivotal role in the regulation of blood pressure

(BP). We recently reported that age-associated oxidative stress causes hyper-activation of AT1R and hypertension in the aging Fischer Brown Norway (FBN) rats. Our objective was to understand the mechanism of higher oxidative stress contributing to the impaired AT1R function in the aged FBNs. Nrf2 transcription factor protects kidney from oxidative stress by activating the transcription of diverse antioxidant and detoxifying enzymes. Kelch-like ECH-associated protein 1 (keap1) inhibit Nrf2 activity by sequestering Nrf2 in cytosol. We hypothesize that protein disulfide isomerase (PDI), an enzyme involved in isomerization reaction, regulates keap1 and Nrf2. Nrf2, keap1 and PDI were determined by Western blotting and/or immunohistochemistry in the kidney of FBNs and human kidney 2 (HK2) cells. We found that the aging kidney had low levels of PDI (adult vs old: 5.38±2.60 vs 3.59±1.76, 33.3% decrease), which was associated with low nuclear levels of Nrf2 (adult vs old: 1.86±0.69 vs 0.87±0.18, 53.2% decrease) and high cytosolic levels of keap1 (adult vs old: 1.80±0.46 vs 2.40±0.49, 33.3% increase) in the aging FBNs. Moreover, PDI inhibition by bacitracin in HK2 cells significantly reduced Nrf2 accumulation (control vs treated: 1.25±0.26 vs 0.50±0.14, 60% decrease) in the nuclei. PDI siRNA treatment in HK2 cells decreased the level of PDI (scrambled vs PDI siRNA: 0.64±0.07 vs 0.52±0.08) while increased the level of AT1R (scramble vs PDI siRNA: 0.11 vs 0.20). These data suggest that PDI by regulating keap1/Nrf2 redox-pathway regulates AT1R, and may be involved in BP regulation in the aging FBNs.

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P088

Pravastatin Protects Against Glucose-induced Anti-proliferative, Anti-invasive and Anti-angiogenic Milieu in Cytotrophoblasts

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Objective: An increasing level of evidence supports the utility of pravastatin in prevention against preeclampsia (preE). We previously demonstrated that hyperglycemia induces cytotrophoblasts (CTBs) dysfunction characteristic of a preE-like phenotype. We sought to demonstrate the utility of pravastatin in rescuing CTBs from hyperglycemia induced dysfunction. **Methods:** Human CTBs were treated with 100, 150, 200, 300, or 400 mg/dL glucose for 48h. Some cells were pretreated with pravastatin (1ug/mL) for 2h, while others were co-treated with pravastatin (1ug/mL) prior to glucose treatment. Some cells were treated with D-Mannitol. Cell migration was performed by Matrigel migration assay kit according to manufacturer protocol. Cell lysates were utilized to evaluate the expression of urokinase plasminogen activator (uPA), plasminogen activator inhibitor 1 (PAI-1), proliferating cell nuclear antigen (PCNA) and p38 MAPK phosphorylation by western blot. Levels of vascular endothelial growth factor (VEGF), placental growth factor (PlGF), soluble fms-like tyrosine kinase-1 (sFlt-1), soluble endoglin (sEng) and interleukin 6 (IL-6) were measured in

culture media using ELISA kits. Statistical comparisons were performed using analysis of variance with Duncan's post hoc test. **Results:** Hyperglycemia inhibited CTBs migration, down-regulated uPA, PAI-1, PCNA and up-regulated p38 phosphorylation in CTBs treated with ≥150 mg/dL glucose compared to basal (100 mg/dL) (*$p < 0.05$ for each). Secretion of sFlt-1, sEng and IL-6 were increased while VEGF and PIGF were decreased in CTBs treated ≥150 mg/dl of glucose (*$p < 0.05$ for each). Both pravastatin pretreatment and co-treatment significantly rescued CTBs migration, up-regulated uPA, PAI-1, PCNA, down-regulated p38 phosphorylation, and corrected the angiogenic profile of CTBs ($p < 0.05$ for each). D-Mannitol had no effect on CTBs. **Conclusions:** Pravastatin mitigates the hyperglycemia-induced dysfunction of CTBs by attenuating the glucose-induced anti-proliferative, anti-invasive and anti-angiogenic phenotype. These data should alleviate critical concerns regarding pravastatin use on CTBs development early in pregnancy and support the current research to use of pravastatin in preE prevention.

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P089

Inhibition of AT1-AAs by Direct Binding Reduces Blood Pressure and Natural Killer Cell Activation in Response to Placental Ischemia of Pregnancy; Emphasizing the Importance of Novel Drug Development in the Treatment of Preeclampsia

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Preeclampsia (PE), hypertension with proteinuria during pregnancy, is associated with activating antibodies to the angiotensin II receptor (AT1-AA) and activation of cytolytic natural killer (NK) cells. The objective of our study was to determine if AT1-AA inhibition inhibits cytolytic NK cell activation in the reduced uterine perfusion pressure (RUPP) rat model of PE. We utilized a novel approach of specific epitope binding to inhibit AT1-AAs: AT1-AA inhibitory peptide (2ug/ml saline) was infused via miniosmotic pumps into RUPP rats beginning on day 14 of gestation. On day 19 of gestation, blood pressure (MAP) was measured and flow cytometry was performed to measure total and cytolytic NK cells in renal and placental tissues from all groups of rats.
MAP significantly increased from 87.1±6.2 in NP (n=5) to 125±2.3 in RUPP (n=6). Inhibition of AT1-AA by direct binding attenuated the hypertensive response to 74.9±19.02 mmHg in RUPP + AT1-AA inhibitor (n=3). Total renal NK cells measured at 10.21±3.69% in NP, 24.19±6.63% in RUPP, and 14.25±7.52% in RUPP + AT1-AA inhibitor. Total placental NK cells were 6.87±2.84% in NP (n=6), 27.62±10.97% in RUPP (N=6), and 6.35±3.90% in RUPP + AT1-AA inhibitor (N=5) rats. Importantly, activated cytolytic placental NK cells were significantly increased in NP (0.44±.24%) compared to RUPP (11.87±2.06%), and was blunted after epitope binding of AT1-AA in RUPP + AT1-AA inhibitor (2.33±1.02%, p<.0001 vs RUPP).
These studies indicate that AT1-AA inhibition improves maternal blood pressure and attenuates cytolytic activation of NK cells in response to placental ischemia, thereby emphasizing the importance of drug discovery for AT1-AA inhibition to improve pregnancy outcomes in preeclamptic patients.

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P090

Agonistic Autoantibodies to the Angiotensin II Type 1 Receptor Enhances ANG II Induced Renal Vascular Sensitivity and Reduces Renal Function During Pregnancy

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Preeclamptic women produce agonistic autoantibodies to the Angiotensin II type 1 receptor (AT1-AA) and exhibit increased blood pressure (BP) and vascular sensitivity to angiotensin II (ANG II). Although, together AT1-AAs and ANGII increase the BP, renal artery resistant index, and vasoconstriction of renal afferent arterioles in pregnant rats; the renal hemodynamics in the presence of the AT1-AAs during pregnancy has not been examined. Thus the objective of this study was to examine the changes in the glomerular filtration rate (GFR) and renal blood flow (RBF) during pregnancy in the presence of AT1-AAs and/or ANGII. Methods: Pregnant Sprague Dawley rats were divided into 4 groups: Normal Pregnant (NP, n=6), Pregnant + ANG II (Preg + ANG II, n=6), Pregnant + AT1-AA (Preg + AT1-AA, n=8), and Pregnant + ANG II + AT1-AA (Preg + ANGII + AT1-AA, n= 6). On day 13 of pregnancy, rats were implanted with mini-pumps infusing ANG II (50 ng/kg/min) and/or AT1-AA (1:40 dilution). On day 19 of pregnancy, rats were subjected to terminal renal function surgeries using FITC labeled Inulin. During the surgery, the BP was recorded and a transonic flowmeter probe was placed on the left renal artery to measure RBF. Results: BP was elevated in all pregnant rats administered ANG II and/or the AT1-AA. Although GFR was reduced, it was not significant between Preg + ANG II and Preg + AT1-AA vs. NP rats (1.5 ±0.24, 1.60 ±0.17 vs. 1.90 ±0.16 ml/min). However, the GFR was further decreased in Preg + ANGII + AT1-AA rats (1.20 ±0.08). No difference was observed with the RBF between Preg + ANG II and Preg + AT1-AA vs NP rats (14.4 ±2.96, 14.4 ±1.48 vs. 15.4 ±1.75 ml/min). RBF was decreased in Preg + ANGII + AT1-AA vs NP rats (7.4 ±1.09 vs. 15.4 ±1.75 ml/min). No change in RVR between Preg + ANG II and Preg + AT1-AA vs. NP rats (9.7 ±2.69, 8.3 ±0.58 vs. 6.4 ±0.77). However, the RVR was drastically increased between Preg + ANGII + AT1-AA vs NP rats (18.4 ±2.91 vs. 6.4 ±0.77). Conclusion: Together ANG II and AT1-AA drastically decreases renal function by 37%, RBF by 50%, and caused a 3 fold increase in RVR vs NP rats. These data indicate the importance of AT1-AAs to drastically enhance ANG II induced renal vascular sensitivity and reduce renal function during preeclampsia. Research Supported by T32HL105324 and RO1HD067541

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P091

Vitamin D Supplementation Inhibits Blood Pressure and Uterine Artery Resistance Induced by Autoantibodies to the AT1 Receptor

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Studies in our lab have previously shown that Vitamin D supplementation in the RUPP rat model of preeclampsia lowers blood pressure and reduces autoantibodies to the AT1 receptor (AT1-AA). Therefore, we sought to determine if the effects of Vitamin D supplementation to inhibit endothelial dysfunction and hypertension during pregnancy were mediated by AT1-AA reduction. We hypothesized that Vitamin D supplementation to AT1-AA-induced hypertensive pregnant rats would reduce anti-angiogenic factor soluble FMS-like tyrosine kinase-1 (sflt-1) and uterine artery resistance index (UARI) while improving blood pressure (MAP). Purified rat AT1-AA was infused (1:40) into Sprague-Dawley rats via miniosmotic pump from gestational day (GD) 12 to GD19. On GD14-18 we administered Vitamin D2 or D3 (VD2 or VD3) to AT1-AA rats (50ul/ml) by oral gavage. On GD18 indwelling carotid catheters were inserted and UARI assessed by Doppler sonography and MAP was measured on GD19. Consistent with previous studies, MAP was 119.0 mmHg in AT1-AA infused pregnant rats. MAP was unchanged with VD2 treatment at 121.7 mmHg (n=3), however, reduced to 109.3 mmHg (n=3) in AT1-AA+VD3 rats. Pup and placental weights were 1.79 and 0.46 g (n=3), respectively, in AT1-AA rats and were increased with VD2 treatment to 2.33 and 0.54 g (n=3) and to 2.39 and 0.56 g (n=3) in AT1-AA+VD3 rats. UARI was 0.577 (n=2) in AT1-AA rats but reduced with VD2 treatment to 0.491 (n=3) and VD3 to 0.452 (n=2). Plasma sflt-1, which is increased with AT1-AA infusion, was measured with ELISA and was >1050 pg/ml in AT1-AA rats (n=3) and greatly reduced in both AT1-AA+VD2 at 42.3 pg/ml (n=2) and AT1-AA+VD3 at 241.0 pg/ml (n=3). Our preliminary data demonstrate that Vitamin D supplementation improves uterine artery vascular resistance and sflt-1 and that these are potential mechanisms for improving fetal weight and hypertension that is induced by AT1-AA during pregnancy.

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P092

Proliferation of Endogenous T-regulatory Cells Improves the Pathophysiology Associated with Placental Ischemia of Pregnancy

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Preeclampsia (PE), new onset hypertension during pregnancy, is associated with pro-inflammatory cytokines and decreased regulatory immune mechanisms such as Tregs, IL-10 and IL-4. We believe this decrease in immune regulatory mechanisms leads to an uncontrolled proinflammatory response which contributes to most of the pathophysiology associated with PE. The Reduced Uterine Perfusion Pressure, RUPP, rat model of induced placental ischemia exhibits similar characteristics as women with PE including high blood pressure, elevated pro-inflammatory cytokines and cells and decreased Tregs, IL-4 and IL-10. Therefore, we hypothesized that stimulating Tregs by administration of a superagonist (SA) would increase the Treg profile in the RUPP rats which could reduce pro-inflammatory cytokines and blood pressure. The RUPP procedure was performed at gestation day 14 (GD14); SA was administered intraperitoneally at GD15, GD18 carotid catheters inserted, and GD19 MAP and pup weight, serum and tissues were collected. MAP in NP rats was 91 +/- 3, 119.6 +/- 2 in RUPPs which was improved to 111 +/- 1 mmHg in RUPP+SA. Circulating FoxP3+ Treg cells were 6 +/- 1.7% in NP, 0.77% +/- 0.75 in RUPP rats but increased to 11% +/- 3 in RUPP+SA; IL-6 was 33.65+/- 3.4 in NP, 117.2 +/- 37.8 in RUPP, and 43.66+/- 6.1 pg/mL in RUPP+SA. Placental Pre-pro Endothelin-1 (PPET-1) was increased 44.42+/- 0.269 fold in RUPP compared to NP 1+/- 0.255, but was decreased to 18.78+/- 0.48 in RUPP+SA. These data suggest an important role for up-regulating Treg cells to enhance the immune regulatory interactions and lower the hypertension without causing further reduction in fetal weight in response to placental ischemia during pregnancy.

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P093

The Sflt-1/plgf Ratio Associates with Prolongation of Pregnancy

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Objective: To evaluate whether a single determination of the serum sFlt-1/PlGF ratio associates with pregnancy prolongation in women with suspected or confirmed preeclampsia (PE).
Methods: In this ongoing observational Dutch multicenter study (600 pts to be enrolled) blood was drawn at admission. Values of sFlt-1 and PlGF were measured postpartum using the automated Elecsys system to prevent influence of this information on decision-making of the treating physicians and the defining time point of delivery. Clinical characteristics and pregnancy outcomes were retrieved from medical records. Cutoffs of < 33 to rule out and > 85 to rule in the occurrence of delivery for selected time points were used.
Results: We have included 412 patients, so far complete data of 147 patients (age 18 to 48 yrs. singleton pregnancies, median pregnancy duration 32 weeks (range 20-36 weeks) is available. At time of inclusion 67 pregnancies were complicated with PE, of which 12 with

superimposed PE, 8 with HELLP, 20 with gestational hypertension (GH) and 60 without pregnancy induced hypertension (PIH). The median (range) ratio in patients who delivered within 7 days was 146 (9-1803) compared to 14 (1-469) in those who delivered after > 7 days (p< 0.001). Delivery within 7 days occurred in 59% patients with a ratio > 85 compared to 7% with a ratio <33 (p=0.002) and 39% with a ratio 33-85 (p=0.048).
Conclusion: In this high risk group a low ratio is inversely correlated with prolongation of pregnancy. 

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P094

Vasopressin Infusion in Mice During Pregnancy Results in Immune Alterations Consistent with Human Preeclampsia

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The immediate and long-term maternal and fetal health complications of preeclampsia (PreE) have been linked to an aberrant T helper immune response and pro-inflammatory cytokine profile. AVP secretion is both stimulated by and stimulates pro-inflammatory cytokine secretion and activation of lymphocytes. Our AVP infusion mouse model of PreE closely replicates human PreE. The objective of this study was to investigate if AVP infusion induces the maternal-fetal immune alterations in PreE.
Immune responses were evaluated in wild-type C57BL/6 female mice chronically infused with AVP (24 ng/hr or saline s.c.) throughout pregnancy. By flow cytometry, we observed an increase in the frequency of IL-17 (1.2% vs. 2.6%, p<0.05), TNFα (0.9% vs. 1.6%, p<0.0001), and IFNγ (1.8% vs. 3.4%, p<0.05) expressing T cells in AVP dams. An increase in the frequency of IL-12 producing cells (1.0% vs. 2.4%, p<0.05) and elevated expression of co-stimulatory molecules on dendritic cells (DCs) from AVP dams suggested enhanced DC function. The maternal plasma revealed a significant reduction in IL-17 (7.1e+6 vs. 3.4e+5 ng/g, p<0.05) and IL-6 (3.4e+5 vs. 0.0 ng/g, p<0.01) production in AVP dams by ELISA as seen in human PreE. Pro-inflammatory TNFα was also significantly decreased in the maternal kidney of AVP dams (2.3e+9 vs. 1.3e+9 ng/g, p<0.05). IL-4 production was significantly reduced in the maternal kidney of AVP dams (5.7e+7 vs. 3.4+7 ng/g, p<0.05) suggesting a blunted Th2 response as seen in human PreE. AVP infusion did not result in alterations of cytokine production in the maternal liver, or fetal liver and kidney. Interestingly, IL-17 production was increased in the amniotic fluid (3.0e+6 vs. 1.3e+7 ng/g, p<0.05) and lower in the placenta (2.5e+7 vs. 1.3e+7 ng/g, p<0.0001) of AVP dams. IL-4 production was decreased in both the amniotic fluid (4.5e+5 vs. 2.0e+5 ng/g, p<0.05) and placenta (3.1e+6 vs. 2.0e+6 ng/g, p<0.05) of AVP dams again suggesting a lower Th2 response.
These data support our hypothesis that AVP induces maternal-fetal immune alterations consistent with human PreE. Ongoing

experiments are aimed at identifying the lymphocytes and cytokines involved as well as local vs. systemic immune dysfunction induced by AVP in pregnancy.

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P095

Effects of Obesity on Placental Ischemia-induced Hypertension

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Although it is known that obesity is a risk factor for preeclampsia (PE), the mechanisms are not clear. Placental ischemia stimulates the release of the antiangiogenic factor sFlt-1 into the maternal circulation eliciting vascular dysfunction and hypertension. Therefore, we tested the hypothesis that placental ischemia (reduced uterine perfusion pressure, RUPP)-induced hypertension and sFlt-1 levels are exaggerated in obese rats. MC4R-deficient obese rats (MC4R+/-) and wild-type Wistar Hannover controls (MC4R+/+) were maintained on NIH31 standard chow; mated at 17 weeks old; RUPP surgeries performed at gestational day (GD) 14; and mean arterial blood pressure (MAP) and pregnancy weights assessed at GD 19. This resulted in 4 groups: normal pregnant (NP) MC4R+/+ (N=10), RUPP MC4R+/+ (N=12), NP MC4R+/- (N=12) and RUPP MC4R+/- (N=11). Body weight was greater in NP MC4R+/- than NP MC4R+/+ (371±10 vs. 340±6, P<0.05), which was reduced by RUPP in both rat strains but MC4R+/- were still heavier (327±13 vs. 297±8, P<0.05). Total body fat mass by EchoMRI was greater in NP MC4R+/- than MC4R+/+ (72±6 vs. 45±4, P<0.05) whereas fat mass was not altered in RUPP MC4R+/+ (38±4g) but was reduced in RUPP MC4R+/- (56±5) (P<0.05). Fetal weights were similar between NP MC4R+/+ (1.93±0.04) and MC4R+/- (1.95±0.02), which was reduced to a greater extent in RUPP MC4R+/- than MC4R+/+ (1.62±0.03 vs. 1.74±0.03, P<0.05). MAP was slightly elevated in NP MC4R+/- over MC4R+/+ (107±2 vs. 101±1). RUPP significantly increased MAP in MC4R+/+ (117±2, P<0.05) but not MC4R+/- (113±3). Plasma leptin levels were greater in NP MC4R+/- over MC4R+/- (6.7±1.0 vs. 3.6±0.4, P<0.05) whereas RUPP had no effect on these levels in MC4R+/+ (3.8±0.7) or MC4R+/- (6.5±1.1). Plasma and placental levels of sFlt-1 were similar in all groups. Clinically, not all PE women have elevated sFlt-1 levels. Contrary to our hypothesis, MAP was not exaggerated by placental ischemia in obese rats having MC4R deficiency. In fact, MAP was not significantly increased following placental ischemia in MC4R-deficient rats indicating that intact MC4R signaling, which has been shown to promote the development of hypertension in several animal models by sympathetic mechanisms, mediates the development of PE independent of sFlt-1.

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P096

Vascular Protein Oxidation and Redox Proteomics in Human Hypertension

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Oxidative stress has been implicated in the pathophysiology of hypertension (HTN) through

redox-sensitive processes that cause vascular damage. Despite recent advances in the field of vascular redox signalling in HTN, it still remains unclear exactly how ROS cause vascular injury. We hypothesise that regulation of redox-sensitive protein tyrosine phosphatases (PTP) through post-translational oxidative modification, is impaired in HTN where ROS levels are increased. Vascular smooth muscle cells (VSMC) from small arteries of normotensive (NT) and hypertensive (HT) individuals were stimulated with AngII (10-7 M) and ET-1 (10-7 M). Irreversible oxidation of proteins and PTPs was assessed by oxyblot and immunoblotting, respectively. Differential gel electrophoresis (DiGE) and CyDye thiol labelling were employed for screening of reversibly oxidised proteome. Irreversible protein oxidation was not affected by AngII or ET-1 in VSMCs from NT and HT subjects. PTP hyperoxidation tended to increase in VSMCs from NT upon stimulation with AngII (FC=2.12 at 60min) and ET-1 (FC=1.60 at 60min), whereas a similar trend was observed only after AngII treatment (FC=1.38 at 60min) in HTN ($p>0.05$). Proteomic data, filtered for FC>2, detected 2051 spots with 1899 (92.5%) being equally oxidised between NT and HT. In addition, oxidation of 57 (2.9%) spots was increased, while 95 (4.6%) were decreased in HT. Candidate proteins exhibiting consistent changes across three experimental replicates included β-actin (FC=-2.86), annexin A1 (-2.23), galectin-1 (-1.67), FK506 binding protein (-2.35) and polymerase I and transcript release factor (PTRF, -1.92). Stimulation with AngII altered the redox status in 2-3% of proteins, both in HT and NT. However, vimentin was the only target changing consistently across the replicates (FC=2.48). Our findings indicate that pro-hypertensive agents may not impact significantly on irreversible protein and PTP oxidation in health and disease, but may have effects on reversible oxidation. Our proteomic data, in agreement with our previous rat studies, support decreased reversible thiol oxidation in HTN. Moreover, these novel findings identify differentially oxidised proteins which may contribute to oxidative vascular injury in HTN.

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P097

Molecular Mechanisms of VEGFR Inhibition-induced Endothelial Cell Damage

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VEGF/VEGFR inhibitors, used as anti-angiogenic drugs to treat cancer, induce severe hypertension. Molecular mechanisms whereby VEGF inhibitors cause hypertension are unclear, but nitric oxide (NO) and oxidative stress may be involved. We questioned whether reactive oxygen species (ROS) and Ang II, important regulators of vascular function in hypertension, also play a role in VEGF inhibitor-induced vascular dysfunction. Human microvascular endothelial cells (HMECs) were stimulated with vatalanib (VAT-VEGFR inhibitor) and gefitinib (GEF-EGFR inhibitor) in the absence/presence of Ang II. Activation of eNOS and MAPKs were assessed by immunoblotting. Antioxidant enzyme mRNA was analysed by qPCR. Microparticle levels were measured by flow cytometry. Endothelial microparticles,

biomarkers of endothelial damage, tend to increase in subjects treated with VEGFR inhibitors. Phosphorylation of eNOS activation site (Ser1177) (28.3% ± 7.1) was decreased by VAT, while no changes were observed after exposure of HMECs to GEF ($p<0.05$). VAT decreased mRNA expression of Nox4 (0.5 ± 0.2) and H2O2-regulating antioxidants enzymes such as catalase (0.4 ± 0.1) and glutathione peroxidase (0.4 ± 0.1), while increased mRNA levels of Nox5 (3.35±1.1) ($p<0.05$ vs. veh). Ang II stimulation increased eNOS (171.2% ± 17.4) and ERK1/2 (177.5% ± 38.5) activation ($p<0.05$); all effects were blocked only by GEF. Inhibition of VEGFR also blocked Ang II effects on SOD1 (1.33 ± 0.1), HO-1 (1.6 ± 0.3) and NQO1 (1.6 ± 0.3) mRNA levels ($p<0.05$). In addition, Ang II increased Nox4 mRNA expression through VEGFR-dependent mechanisms. VEGFR1/2 and AT2R, but not AT1R, were expressed in HMEC. Ang II effects on eNOS phosphorylation were inhibited by PD123319 (AT2R antagonist) but not by losartan (AT1R antagonist). In conclusion, our data identify novel mechanisms whereby AngII, possibly through AT2R-dependent VEGFR transactivation, regulates eNOS activation, MAPK signalling and H2O2-related antioxidant enzymes. In addition to changes in NO availability, VEGFR inhibition may interfere with the redox status of endothelial cells, leading to vascular dysfunction and hypertension.

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P098

G-protein Coupled Estrogen Receptor Stimulates Capillary Formation by Human Umbilical Vein Endothelial Cells via ALK1-SMAD 1/5/8 Pathway Activation

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Estradiol (E2) induces vascular repair by promoting endothelial growth and capillary formation. Based on our previous findings that the capillary stimulating effects of E2 are mimicked by its non-permeable analog, BSA-tagged E2, we hypothesize that the stimulatory effects are potentially mediated via the newly discovered membrane bound G-protein coupled estrogen receptor (GPER). To investigate this, we assessed capillary formation by endothelial cells in response to E2, and GPER agonist (G1)and antagonist (G15) in a 2-D matrigel based assay. Capillary formation was assessed microscopically by quantifying junction/sprout formation and capillary length. E2 (10nM) increased capillary formation and this effect was mimicked by G1 (10nM; stimulated from 100% to 517±46% and 210±41%, respectively; $p<.05$ vs control). The effects of E2 and G1 were significantly abrogated by G15 (100nM; $p<.05$), suggesting a role for GPER in mediating the capillary stimulatory effects of E2. Because G-protein coupled mechanisms, Akt, ALK1 and SMAD1/5/8 are involved in capillary formation, we investigated their role in GPER induced capillary formation. ECs expressed GPER, ALK1 and SMAD1/5/8. Treatment with G1 (10-100nM) up-regulated the expression of ALK1 from 100% to 168±21% ($p<.05$ vs control) and phosphorylated SMAD1/5/8 from 100% to 208±36% ($p<.05$ vs control). Similar to capillary formation the stimulatory effects of G1 on SMAD1/5/8 were blocked significantly ($p<.05$) by G15 (100nM), Pertussis Toxin (0,1ng/ml; G protein pathway inhibitor), LY294002 (5µM; Akt/PI3K inhibitor) and ALK1Fc (100ng/ml; specific ALK-1 antagonist). Silencing of GPER

and SMAD1 by siRNA (50nM) abrogated the stimulatory effects of E2 and G1 on capillary formation, and SMAD1/5/8 and Akt phosphorylation. Moreover, treatment with G1(100nM) upregulated ID-1 expression from 100% to 179±26% (p<.05 vs control), a downstream target of ALK1/pSMAD1/5/8. We conclude that E2 via GPER promotes EC-mediated capillary formation by a mechanism that involves non-genomic activation of ALK1→ pSMAD1/5/8 ↔ pAkt → ID-1. GPER agonists in general may promote healing of injured vascular beds by promoting EC activity leading to more rapid endothelial recovery and capillary formation following injury.

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P099

Angiotensin Type I Receptor (AT1R) and (Pro)renin Receptor (PRR) Functional Heterodimer Mediates ERK Phosphorylation

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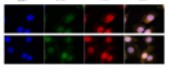
Receptor dimerization was shown to enhance the receptor efficacy, trafficking and signal transduction. Angiotensin II type 1 receptor (AT1R) and (Pro)renin receptor (PRR) are expressed in the kidney. Studies demonstrated that these two receptors have some similarities in their signaling mechanisms and effects. In the present study we hypothesized that the AT1R and PRR form functional heterodimer to enhance cellular ERK phosphorylation. Using Immunoprecipitation technique, confocal immunofluorescence staining (IF), and fluorescence resonance energy transfer (FRET), we evaluated the presence of AT1R and PRR heterodimer in rat renal mesangial (RMC) and PC12W cells transfected with AT1R. These two receptors coimmunoprecipitated at 80kD band. IF demonstrated AT1R and PRR co-localization as shown in figure below and FRET demonstrated the physical distance between them to be less than 10nm (<100 Å), providing strong evidence for these receptors heterodimerization. To evaluate the function of the AT1R/PRR dimer, PC12W cells were treated with scramble siRNA or PRR siRNA . There were no significant differences in phosphorylation of ERK between control and scramble siRNA. PRR siRNA treatment significantly reduced the ERK phosphorylation by 35%, P<0.05. There was 60% (P<0.01) increase in ERK phosphorylation in PC12W cells transfected with AT1R. In these cells, PRR siRNA treatment attenuated the increase in ERK phosphorylation by 33% (P< 0.05). We conclude that AT1R-PRR form a heterodimer that is functionally active to enhance ERK phosphorylation. 

S. Quadri: None. **C. Li:** None. **S. Culver:** None. **H.M. Siragy:** None.

P100

(Pro)renin Receptor (PRR) Mediates Renal Inflammation in Renovascular Hypertension

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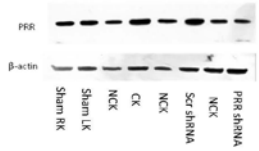
We hypothesized that PRR plays a role in renal inflammation in 2-kidney, 1-clip (2K1C)

hypertension rat model. Male Sprague-Dawley rats were fed normal sodium diet. BP was obtained before and 28 days after left renal artery clipping. Renal expressions of PRR, TNF-α and COX-2 were assessed in sham and 2K1C rats with or without left renal interstitial administration of scramble shRNA or PRR shRNA. At baseline there were no significant differences in BP between different animal groups. Compared to sham, mean arterial blood pressure significantly increased in 2K1C (2K1C 131.8 ± 3.09 mmHg, vs. sham 108 ± 1.9 mmHg, P<0.0.05) at day 28 and was not influenced by scramble shRNA or PRR shRNA treatment. Compared to sham and contra lateral (non-clipped) kidney, there were increases in mRNA and protein expressions of PRR (90% and 45%, P<0.01), TNF-α (72% and 50%, P<0.05), COX-2 (72% and 39%, P<0.05) in the clipped kidney. These expressions were not influenced by scramble shRNA treatment. Compared to 2K1C (no treatment) and scramble shRNA, PRR shRNA treatment in the clipped kidney caused significant reductions in mRNA and protein expressions of PRR (60% and 54%, P<0.01, shown in figure below), TNF-α (54% and 51%, P<0.05), COX-2 (51% and 53%, P<0.05). We conclude that PRR mediates renal inflammation in renovascular hypertension independent of blood pressure reduction.



S. Quadri: None. **C. Li:** None. **S. Culver:** None. **H.M. Siragy:** None.

P101

The Role of Primary Cilia in Vascular Endothelial Cells

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Primary cilia are mechanosensory organelles that are projected into the lumen of blood vessels. It has been demonstrated that vascular endothelia require primary cilia to sense and transmit external mechanical stimuli into internal biochemical reactions. One of these reactions includes the biosynthesis and release of nitric oxide, which is one of the most potent endogenous vasodilators. This idea has *only* been investigated in cultured endothelial cells *in vitro*. Based on this finding, however, a very bold hypothesis is formed to test that abnormal cilia function results in vascular hypertension. Our laboratory has recently generated and obtained several conditional mouse models to specifically study the function and structure of primary cilia in vascular endothelia. These models include **1)** mice without cilia function (*Pkd1* or *Pkd2*); **2)** mice without cilia structure (*Tg737* or *Kif3a*). Our data indicate that mice with abnormal cilia function (*Pkd1*) or structure (*Tg737*) show significantly higher systolic (150±19 for *Pdgfbcre:Pkd1*$^{flox/flox}$ and 147±10 for *Tie2Cre:Tg737*$^{flox/flox}$ vs. 128±9 for wild-type) and diastolic (120±21 for *Pdgfbcre:Pkd1*$^{flox/flox}$ and 120±11 for *Tie2Cre:Tg737*$^{flox/flox}$ vs. 102±7 for wild-type) blood pressure than the corresponding wild-type mice. Because there is a positive and continuous correlation between blood pressure and cardiovascular diseases, satellite hypotheses are developed to look at the pathophysiological roles of endothelial cilia in cardiac functions and focal vascular diseases *in vivo*. Our data clearly point towards deteriorating phenotypes in the cardiac muscle, including cardiac fibrosis due to an increased cardiac workload. As a result, a heart-to-body weight ratio was significantly increased by 17 weeks old (0.008 *PdgfbCre;Pkd1*$^{f/f}$ vs. 0.006

Pkd1$^{f/f}$).The present study will very likely provide new insights for hypertension and offer advanced scientific understanding of vascular endothelial cilia in other cardiovascular diseases.

H. Saternos: None. **Z. Hossain Saad:** None. **W. AbouAlaiwi:** None.

P102

Type I Diabetes Mellitus is More Sensitive to Ang II Due to Increased AT1 Receptor Expression in Afferent Arteriole

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The prevalence of hypertension is about twice in diabetic subjects than that in non-diabetics. In the presence of hypertension, the progress of diabetic nephropathy has been shown to be more severe and rapid. But the mechanisms for the development of hypertension in diabetes have not been elucidated. We hypothesized that angiotensin II receptor type 1 (AT1 receptor) expression level in renal afferent arteriole is elevated, which enhances the responses to angiotensin II (Ang II) stimulation and contributes to the development of hypertension in diabetes.
First, we measured mean arterial pressure (MAP) with telemetry in diabetic and non-diabetic mice with Ang II infusion for 8 weeks. MAP was 26.7 mmHg higher in diabetic mice (157.4±11.3 mmHg) than in non-diabetic mice (130.7±6.4 mmHg). Next, we induced 2-kidney-1-clip Goldblatt hypertension in diabetic mice and non-diabetic mice and measured MAP with telemetry for 8 weeks. MAP was 17.6 mmHg higher in diabetic mice (151.1±9.5 mmHg) than in non-diabetic mice (133.5±4.2 mmHg), indicating blood pressure of diabetic mice is also more responsive to both exogenous and endogenous Ang II than no-diabetic mice.
To investigate the mechanisms of the enhanced sensitivity to Ang II, we dissected afferent arterioles from non-diabetic mice and diabetic mice and measured the expression of AT1 receptor mRNA by real-time PCR. AT1 receptor mRNA level was 6 times higher in diabetic mice that in non-diabetic control. AT1 receptor protein abundance in renal cortex was also 5 times higher in diabetic mice than controls.
In conclusion, the blood pressure of the diabetic mice are more sensitive to Ang II than non-diabetic controls. Increased AT1 receptor expression in the afferent arterioles may contribute to the development of hypertension in diabetes.

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P103

Cellular Stretch Induces Higher Intracellular Calcium Increases in Dahl Salt-Sensitive Than Salt-Resistant Rat Thick Ascending Limbs: Role of Transient Receptor Potential Vanilloid 4

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The thick ascending limb (TAL) reabsorbs 30 % of the filtered NaCl playing a pivotal role in the regulation of salt homeostasis. Abnormal reabsorption of NaCl in this segment causes salt-sensitive hypertension. We showed that flow-induced cellular stretch stimulates superoxide production in epithelial cells from TALs. Dahl salt-sensitive rat (DSS) TALs produce more superoxide than those from salt-resistant

rats (DSR). Mechanical stimulation exerted by cellular stretch increases intracellular calcium (Cai) in several types of cells. We have shown that luminal flow-induced increases in Cai is mediated by Transient Receptor Potential Vanilloid (TRPV4). Therefore, we hypothesized that cellular stretch increases Cai in TALs from DSS rats more than those from DSR rats due to elevated TRPV4 activity. We measured Cai using the calcium-sensitive dye Fura-2 in isolated, perfused DSR and DSS TALs. Cellular stretch increased Cai by 243 ± 51 nM in DSS TALs (n=9; $p<0.0008$ vs. no stretch conditions) and by 124 ± 27 nM in DSR TALs (n=10; $p<0.0005$ vs no stretch conditions). Cellular stretch-induced Cai increases were significantly higher in the DSS group ($p<0.05$ vs DSR). When these animals were fed a high-salt diet (4% NaCl) similar responses were obtained. Cellular stretch increased Cai by 236 ± 58 nM in DSS TALs (n=7; $p<0.004$ vs no stretch conditions) and by 92 ± 15 nM in DSR TALs (n=6; $p<0.0008$ vs no stretch conditions). To study whether DSS and DSR TALs express different amounts of TRPV4 we performed Western blots. We found that TRPV4 protein expression was similar in TALs lysates from both strains fed either a control standard or a high-salt diet. We then tested the effects of transfecting DSS TALs with TRPV4-small hairpin (sh) RNA on stretch-induced Cai increases. Under these circumstances, the difference in stretch-induced Cai increases between DSS and DSR TALs was not significant (75 ± 15 nM in DSS vs 56 ± 28 nM vs DSR, n=4 for each group) . We conclude that DSS TALs are more sensitive to increases in cellular stretch than DSR TALs possibly due to enhanced TRPV4 activity.

P. Cabral: None. **A. Gonzalez-Vicente:** None. **N. Hong:** None. **J. Garvin:** None.

Renin Angiotensin System (RAS) Modulation in Hypertension Program by Maternal Intrauterine Malnutrition

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Intrauterine malnutrition (IM) during the early stages of development can alter the function of organs and tissues and can predict a lifetime of increased risk for adverse health outcomes, such as diabetes and hypertension. The kidney plays a key role in the development of hypertension programmed by IM, with the participation of the RAS. Our objectives were to study ACE activity and angiotensin peptides levels in tissues. Pregnants Wistar rats were separated into two groups: control group (C), fed ad libitum, and malnourished group (D) submitted to food restriction (diet 50% of the amount of feed consumed by the group C). After birth the offspring were kept as experimental groups C and D, respectively. At 4 months of age, the animals were sacrificed, heart and kidney tissues were collected to quantify angiotensin peptides and ACE activity. The offspring born with low birth weight. Kidney ACE activity was higher in group D compared to group C (299 ±86.7 vs. 253.4 ±84.82 mU/mg, $p<0.05$), differing from Heart (D versus C: 0.15 ± 0.08 vs. 0.24 ±0.09 mU/mg). Group D presented high blood pressure values compared to group C (140.6 ±2.8 vs. 124,3±2.6 mmHg). Kidney and heart Ang II levels were increased in group D being significant when compared to group C (238.26 ±25.1 vs. 161.85 ±45.6 pmol/g and 397.89±74.9 vs. 223.33±48.7

pmol/g, p<0.05, respectively). The same was observed for Ang I. The vasodilator peptide Ang1-7 levels in group D from kidney and heart were lower in comparison with group C, thus emphasizing an enabling environment for hypertension (220.74 ± 48.74 vs. 288.09 ± 47 pmol/g and 152.1±41.2 pmol/g vs. 228.93±41.2 pmol/g, p<0.05, respectively). Our results indicate that perturbed maternal nutritional status alters tissue RAS resulting in higher blood pressure in the offspring, demonstrated by increased renal ACE activity and Ang II levels, with reduced Ang 1-7. The increase of Ang I and II in the heart, despite low ACE activity in this tissue suggests the activation of RAS alternative pathways. This study describes for the first time that low levels of Ang 1-7 contributed to the early development of hypertension.

R.S. Banti: None. **R. Yokota:** None. **D.S. Aragão:** None. **A. Souza:** None. **A. Pedroso:** None. **F.A. Ronchi:** None. **E.B. Ribeiro:** None. **D.E. Casarini:** None. **M.C. Andrade:** None.

P105

ACE2 Activator, Diminazene Aceturate, Reduces Adiposity, but Preserves Lean Mass in Young and Old Rats

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Obesity is severely debilitating, and increases the risk for cardiovascular events. Recent evidence suggests increased Ang-(1-7)/Mas activity in obese animal models leads to significant reductions in body weight. We hypothesize that activation of ACE2, via Diminazene Aceturate (DIZE), will significantly reduce body weight of both young and aged rats fed 60% high fat diet.

Male Fisher 344 x Brown Norway rats, ages 4 (n=12) and 23 months (n=17) were fed 60% high fat diet for one week, whereupon, animals were further divided, and given either 15mg/kg/day DIZE s.c. (Young DIZE, n=6; Old DIZE, n=9) or vehicle (Young Control, n=6; Old Control, n=8). Body weight and food intake were measured throughout the experiment. Time Domain-Nuclear Magnetic Resonance (TD-NMR) was used to assess body composition 1 week and 3 weeks after the start of DIZE treatment.

DIZE treatment resulted in a significant reduction of food intake and change in body weight in both young and old animals. TD-NMR results determined the weight-loss was primarily a result of decreased body fat percentage, with a preservation of lean mass as indicated by a reduced fat/lean mass ratio. Tissue weights at the time of sacrifice confirm the significant loss of white adipose tissue (WAT), with no significant difference in tibialis anterior (TA) muscle weights. Importantly, when assessing heart weights, we observed a significant reduction in aged rats treated with DIZE when compared to age matched controls, suggesting a prevention of cardiac hypertrophy associated with obesity. Our data suggests DIZE may be a useful tool in the treatment of obesity along with associated co-morbidities.

	Cumulative KCAL intake from start of DIZE	Δ Body Weight from start of DIZE to Sacrifice (g)	% Body Fat Mass (1wk :: 3wks)	% Body Lean Mass (1wk :: 3wks)	Fat/Lean Ratio (1wk :: 3wks)	WAT mass (g)	TA mass (g)	Heart Weight/Tibia Length (g/cm)
Young Control	675.9±23.4	+25.2±3.5	25.3±0.4 :: 25.1±0.5	58.2±0.4 :: 55.8±0.2#	0.44±0.01 :: 0.46±0.01	20.3±0.7	0.73±0.03	0.26±0.01
Young DIZE	565.6±16.7*	-30.2±4.3*	24.4±0.5 :: 22.1±0.4*#	58.3±0.4 :: 55.8±0.2#	0.42±0.01 :: 0.40±0.01*	11.6±1.2*	0.68±0.02	0.25±0.01
Old Control	874.4±29.8	+43.0±4.8	30.8±0.8 :: 31.0±0.4	51.1±0.7 :: 51.2±0.8	0.60±0.01 :: 0.61±0.01	42.2±1.7	0.71±0.03	0.33±0.01
Old DIZE	533.6±25.4*	-84.4±5.4*	29.4±0.3 :: 26.4±0.3*#	53.7±0.6* :: 57.0±0.5*#	0.55±0.01 :: 0.46±0.01*#	23.4±1.1*	0.67±0.02	0.28±0.01*

All data analyzed using two-way ANOVA. P<.05 *significantly different from age matched Control; #significantly different from 1 week timepoint

E. Bruce: None. **Y. Sakarya:** None. **N. Kirichenko:** None. **H.Z. Toklu:** None. **D. Morgan:** None. **N. Tumer:** None. **C.S. Carter:** None. **P.J. Scarpace:** None.

P106

The Role of Inflammasomes and Angiotensin II Type 1 Receptor in the Pressor Responses of Aged Mice to Angiotensin II

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Introduction: The prevalence of hypertension increases with age. Chronic low-grade inflammation commonly occurs with ageing, and inflammasomes are important initiators of inflammatory responses. We tested whether aged mice have enhanced pressor responses to angiotensin II (Ang II) and whether this is associated with exaggerated pro-inflammatory and vascular contractile responses. We also tested whether MCC950, a NLRP3 inflammasome inhibitor, reduces blood pressure (BP) in Ang II-treated aged mice. Methods: Young (8-12 weeks) and aged (24-30 months) male C57Bl6 mice were left untreated, or either treated with vehicle or a slow-pressor dose of Ang II (0.28 mg/kg/day) for 28 days. Another group of aged mice were treated with either Ang II + saline or Ang II + MCC950 (10 mg/kg/day) for 10 days. We measured systolic BP, mRNA expression of inflammatory markers and components of the renin-angiotensin system, and vascular contractile responses. Results: In young mice, Ang II caused a gradual increase in BP (final BP: 141.6 ± 8.3 mmHg), whereas BP increased by ~20 mmHg from baseline in aged mice, and continued to increase for 28 days (final BP: 155 ± 12.4 mmHg) (n=8-9, $P<0.05$). Ageing alone increased renal expression of AT1a receptors, NLRP3, Caspase-1, IL-1β, IL-33, CCR2, CCL7 and CCL8 by at least 1.5-fold (n=7-8, all $P<0.05$). Maximum contractile responses of mesenteric artery were 1.8-fold greater to Ang II and 1.2-fold greater to phenylephrine in aged vs young mice (n=4, both $P<0.05$). BP was not different between Ang II + saline-treated aged mice (BP: 138.8 ± 6.8 mmHg) and Ang II + MCC950-treated aged mice (BP: 144.7 ± 9.6 mmHg) (n=6-7, $P>0.05$). Conclusions: Aged mice have enhanced pressor responses to Ang II and this is associated with exacerbated pro-inflammatory and vascular contractile responses. The NLRP3 inflammasome does not appear to contribute to Ang II-induced hypertension in aged mice.

Q.N. Dinh: None. **G.R. Drummond:** None. **B.K. Kemp-Harper:** None. **H. Diep:** None. **A.A.B. Robertson:** None. **M.A. Cooper:** None. **C.G. Sobey:** None. **S. Chrissobolis:** None.

P107

Metabolic Syndrome and Human AT1R Expression in Transgenic Mice: Implications of Haplotype-dependent Transcriptional Regulation

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Angiotensin II (Ang II) contributes to the pathophysiologies of cardiovascular and renal systems. Angiotensin receptor type 1 (AT1R) mediates these effects, and genetic variations that increase AT1R can increase these pathological outcomes. Physiological variables like age or pathologies like the metabolic syndrome alter the transcriptional milieu of cells and can provide for feedback activation of

genes. In this regard, we have identified two haplotype blocks of single nucleotide polymorphisms (SNPs) in the hAT1R gene: haplotype II (Hap II: -810A, -713G, -214C, -153G) and I (Hap I: -810T, -713T, -214A, -153A). In clinical studies, Hap I is linked to human hypertension. We have generated transgenic mice (TG) with haplotypes-II and I of the hAT1R gene to study its regulation during metabolic syndrome (MetS). At baseline, ChIP assay shows increased RNA-Pol II binding (~1.6 fold higher) to the chromatin extracts from renal tissues of adult (4-6 months) male Hap I-TG mice with increased hAT1R expression (~6 fold higher). This was accompanied by higher baseline blood pressure in Hap I-TG mice (Hap I- 126±3 vs. Hap II- 115±4, $p<0.05$). To induce MetS, these mice were fed Western diet (45% Kcal from fat and carbohydrate each) for 12 wks. Change in body weight is higher ($p<0.05$) in Hap I (161.5 gm.) vs. Hap II (10.41.2 gm.) mice. MetS phenotype is characterized by increase in blood pressure that is significantly greater in Hap I mice (1362 vs. 1203 in Hap II). Transcription factors, p38/MAPK and STAT3, were induced by MetS to similar extent in both groups. However, MetS-induced up regulation of the hAT1R gene is significantly higher in vascular tissues of Hap I mice (≈6 fold), when compared to Hap II. Complementary experiments show increased inflammatory and redox markers in vascular tissues of Hap I mice, when compared to Hap II, during MetS; including, IL1 (2.2 fold), IL6 (1.8 fold), NOX1 (3.5 fold), VEGF (1.5 fold), and ICAM1 (14 fold). Thus, haplotype-dependent transcriptional regulation of the hAT1R gene causes increased hAT1R expression and blood pressure, in Hap I TG mice. Importantly, MetS exacerbates this differential gene-expression regulation, further increasing hAT1R and promoting a prooxidant/inflammatory milieu in mice with Hap I.

A. Rana: None. **S. Jain:** None. **D. Eren:** None. **N. Sirianni:** None. **M. Kaw:** None. **A. Kumar:** None.

P108

Caveolin-1 is Required for Vascular Remodeling Induced by Angiotensin Ii

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We have recently reported that caveolin-1 (Cav1) enriched membrane microdomains in vascular smooth muscle cells (VSMC) mediates a metalloprotease ADAM17-dependent EGF receptor (EGFR) transactivation, which is linked to vascular remodeling induced by AngII. We have tested our hypothesis that Cav1, a major structural protein of caveolae, plays a critical role in AngII-mediated vascular remodeling via regulation of ADAM17 and EGFR. 8 week old male Cav1-/- and control Cav+/+ wild-type mice (WT) were infused with AngII (1 μg/kg/min) for 2 weeks to induce vascular remodeling and hypertension. Upon AngII infusion, histological assessments demonstrated medial hypertrophy and perivascular fibrosis of coronary and renal arteries in WT mice compared to sham-operated mice. The AngII-infused WT mice also showed a phenotype of cardiac hypertrophy with increased HW/BW ratio (mg/g: 8.0±0.6 vs 5.7±0.7 $p<0.01$) compared with sham-operated WT control. In contrast, vascular remodeling but not cardiac hypertrophy were attenuated in Cav1-/- mice with AngII infusion; HW/BW ratio (8.6±0.5 vs 6.4±0.2 $p<0.05$) compared to sham-operated mice. However, AngII induced similar levels of hypertension in both WT and Cav1-/- mice as assessed by telemetry (MAP mmHg: 142±9 vs 154±20). AngII infusion in WT mice

enhanced ADAM17 and phospho-Tyr EGFR staining in heart and kidney vasculature, whereas AngII-infused Cav1-/- mice showed diminished ADAM17 and phospho-Tyr EGFR staining within the vasculature. In addition, IHC analyses revealed reduced vascular ER stress in heart and kidney samples of AngII-infused Cav1-/- mice compared to WT mice. Expression of Cav1 was predominantly observed within the endothelium and was enhanced upon AngII infusion in WT mice. These data suggest that Cav1, and presumably endothelial caveolae microdomains, plays a critical role in vascular remodeling via vascular induction of ADAM17 and activation of EGFR independent of blood pressure or cardiac hypertrophy regulation.

T. Kawai: None. **T. Obama:** None. **T. Takayanagi:** None. **S.J. Forrester:** None. **K.J. Elliot:** None. **K. Crawford:** None. **S. Eguchi:** None. **V. Rizzo:** None.

P109

Angiotensin-(1-9) Reverses Cardiac Dysfunction in a Model of Angiotensin II-Induced Hypertensive Heart Disease by Acting as a Positive Inotrope

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Angiotensin II (AngII) is involved in the pathophysiology of cardiovascular diseases (CVD) such as hypertension and heart failure. The counter-regulatory axis of the renin angiotensin system is centred on ACE 2 generating angiotensin-(1-7) [Ang-(1-7)] opposing the pathological actions of AngII in the heart. We recently showed that angiotensin-(1-9) [Ang-(1-9)] is part of this axis potentially acting *via* the angiotensin type 2 receptor to inhibit AngII-induced cardiomyocyte hypertrophy *in vitro* and cardiac remodelling in the SHRSP rat. Here, we assessed whether Ang-(1-9) can reverse chronic AngII-induced cardiac pathology.

C57BL/6J mice were infused with H_2O (control) or 48µg/kg/hr AngII for 2 weeks to induce cardiac contractile dysfunction as measured by a reduction in fractional shortening (FS) [control 54.8±3.0%; AngII 35.3±1.9%; $p<0.05$]. Minipumps were replaced and mice received either H_2O, AngII or AngII with Ang-(1-9) (48µg/kg/hr) for a further 2 weeks. Mice receiving Ang-(1-9) in addition to AngII showed a recovery in FS [control 50.5±2.2%; AngII 33.6±1.9%; AngII+Ang-(1-9) 44.0±3.5%; $p<0.05$]. However, Ang-(1-9) did not affect AngII-induced cardiac hypertrophy [heart weight/tibia length (mg/mm): control 10.6±0.4; AngII 11.6±0.4; AngII+Ang-(1-9) 13.32±0.9], cardiomyocyte size [control 23.2±0.9µm; AngII 26.1±1.0µm; AngII+Ang-(1-9) 28.3±1.2µm] or myocardial fractions of collagen I [control 2.3±0.4%; AngII 6.5±0.9%; AngII+Ang-(1-9) 5.0±0.5%] and collagen III [control 2.0±0.3%; AngII 4.1±0.7%; AngII+Ang-(1-9) 3.0±1.3%]. To determine if Ang-(1-9) directly alters cardiac contractility, isolated rat hearts were Langendorff perfused at a constant heart rate (320 bpm) and intra-ventricular pressure was measured. Perfusion with 1µm Ang-(1-9) for 10min induced a significant and sustained increase in developed pressure [max. response: 105.8% normalised to control; $p<0.05$]. In contrast, perfusion with 1µm AngII only led to a small transient increase in developed pressure whereas Ang-(1-7) had no effect.

These results demonstrate for the first time that Ang-(1-9) reverses chronic AngII-induced

cardiac dysfunction and acts directly as a positive inotrope suggesting therapeutic potential in various CVDs.

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P110

Mrgd Expression in Cardiovascular Related Areas

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Recently a new peptide was discovered as part of the Renin Angiotensin System, the heptapeptide alamandine. This new component is a product of the catalytic hydrolysis of Angiotensin A or can also be formed from decarboxilation of Angiotensin-(1-7). Interestingly, alamandine effects were independent of Angiotensin-(1-7) receptor Mas and the Mas-related G protein receptor member D (MrgD) was described as an alamandine receptor. Previous studies have reported that MrgD is expressed predominantly in small diameter IB4(+) neurons in the dorsal root ganglion (DRG). However, functional experiments performed in our lab showed that alamandine has antihypertensive and cardioprotective effects, indicating that MrgD might be also expressed in another tissues. Objective: Identify the anatomical localization of MrgD receptors in blood vessels, heart and in cardiovascular- related centers of the mouse brain. Materials and Methods: Mice were euthanized and then perfused. Brain, Aorta and heart were removed and postfixed. Serial sections were made in a cryostat, hydrated with PBS and permeabilized with 0.2% Tween 20, blocked with 5% of BSA, followed by a rabbit anti-MrgD and an Alexa 488-labeled anti-rabbit antibodies. Immunostaining specificity was characterized by performing experiments using MrgD receptor knockout mice as control. Results: Differently than described before, MrgD expression is not restricted to neurons at DRG. Immunofluorescence for MrgD receptor was detected throughout the trigeminal tract, Cerebellum, Cortex, Insular Cortex, Hypothalamus and in some nuclei of the brainstem. In addition, corroborating previous functional studies from our laboratory showing that Alamandine has an endothelium-dependent vasorelaxant effect we also observed positive fluorescence in aorta endothelial and smooth muscle cells Moreover, MrgD-positive immunofluorescence was also detected in cardiomyocytes, in the membrane and particularly in the nuclear and perinuclear area expanding the evidence that alamandine-induced effects are mediated by MrgD receptor. These observations are in keeping with previous functional studies and unmasked new targets for exploring the biological relevance of alamandine/MrgD.

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P111

Cross-Inhibition of the Vasorelaxing Effect of Ang-(1-9) and Ang-(1-7) in Aortic Rings

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Angiotensin-(1-9) is a nonapeptide formed by the hydrolysis of angiotensin I by ACE2 that seems to counter-regulate the classical RAS axis. The effect produced by Ang-(1-9), has an important role in the cardiovascular system promoting effects similar to those of Ang-(1-7). As vasodilation, anti-hypertrophic action in cardiomyocytes and antihypertensive effect. Because it has been reported that the Ang-(1-9) effects are independent of the Ang-(1-7) receptor Mas, in this study we investigated a possible synergistic additive effect of Ang-(1-9) and Ang-(1-7) in aortic rings.
Materials and methods: The endothelium-dependent vasodilatory response to Ang-(1-7) and Ang-(1-9) (10-10 - 10-6 moles/l) alone or in combination was tested in aortic rings taken from Sprague-Dawley rats, pre-contracted with phenylephrine (0.1 umoles/L).
Results: In aortic rings from SD rats, Ang-(1-7) and Ang-(1-9) induces vascular relaxation (Emax= 13.8±4.5% and 15±2.5%, n=5, respectively). Interestingly, pre-incubation with Ang-(1-9) abolished the vasodilatation induced by Ang- (1-7) (Emax= -2.7 ±1.4%, n=5). Likewise, pre-incubation with Ang-(1-7) abolished the vasorelaxation induced by Ang- (1-9). Indeed in aortic rings pre-incubated with Ang-(1-7),addition of Ang-(1-9) induced contraction instead of relaxation of the aortic rings (Emax= -10±1.7%, n=5). To test the effect of acute combination of the peptides in aortic rings, Ang-(1-7) was prepared in a 10-7M solution of Ang-(1-9) or vice-versa. As observed with pre-incubetion, the vasodilator effect induced by Ang (1-7) or Ang-(1-9) was abolished (Emax= -2.9±2.6 %, n=6 and 4±1.3%, n=6, respectively). These results suggest that these two peptides counter-regulate the vascular effects of each other. Whether these phenomenon in due to receptor internalization or to a signaling-related mechanism, remain to be clarified.

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P112

Mechanism of the Blood Pressure Response to a Novel Vascular Disrupting Combretastatin A1-Diphosphate (OXi4503)

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Background: Hypertension has been observed in cancer clinical trials of vascular disrupting agents (VDAs) but the mechanism is unknown; hence, prevention and management are difficult. Clearly, an improved understanding of the pathophysiology of blood pressure (BP) changes with VDAs would improve the standard of care for this novel class of anti-cancer agents. Accordingly, we hypothesized that VDAs may influence BP via activation of the angiotensin-renin system.
Methods: Escalating doses of combretastatin A1-diphosphate (OXi4503) were given intravenously over 10 minutes on days 1,8,15 and 22 in 28-day cycles in a Phase IA trial of acute myeloid leukemia (AML) patients (NCT01085656). BP and plasma levels of angiotensin II (angII) were measured prior to and 0.5, 1, 2, 3, 4, 5, 6 and 24 hrs after infusion. If BP was ≥120/80 at baseline, amlodipine was administrated 60 minutes before OXi4503.
Results: A total of 16 patients were treated:

median age 63.5 years (range, 24-79); 12 were men. Two subjects received 2.5 mg/m^2, two-3.75 mg/m^2, nine-5 mg/m^2 and three-6.25 mg/m^2. Most (9/16) had a history of HTN. SBP rose in all patients, reaching 140-159 mmHg in 5/16 (31.2%) and ≥160 mmHg in 3/16 (18.7%). In 11 patients pretreated with amlodipine, 5/16 (45.4%) developed SBP ≥140 mmHg. Prior HTN history and increasing age were predictors of the SBP response. In those with a HTN history, peak SBP was 151.717.2 (mean±SD) versus those without a HTN history (126.711.4, P = 0.003). At one hr after OXi4503 infusion, SBP correlated with the level of angII (Spearman: 0.53, CI95% (0.04-0.81) P= 0.03). Although there was no significant association between OXi4503 dose and percent maximal increase in SBP or level of angII, compare with baseline, there was a significant correlation between the dose and level of angll 0.5 hours after infusion (Spearman: 0.54, CI95% (0.07-0.82), P=0.02). In all patients, SBP returned to baseline by 24 hrs. ***Conclusion:*** The VDA OXi4503 increased BP and this may be mediated, in part, by angII signaling. Our findings suggest, for the first time, that selective angII receptor blockade is worth exploration for patients receiving VDAs.

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P113

Angiotensin Converting Enzyme 2 Modulates Bradykinin B1 Receptor Function During DOCA-Salt Hypertension

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DOCA-salt hypertension is associated with reduced angiotensin converting enzyme 2 (ACE2) and increased bradykinin B1 receptor (B1R) expression in the brain. ACE2 hydrolyzes des-Arg(9)-bradykinin, the endogenous B1R agonist, into inactive metabolites. Therefore, we hypothesized that ACE2 overexpression or deletion modulates B1R function in the brain during neurogenic hypertension. To test this hypothesis, we used mice overexpressing ACE2 in neurons (SA), ACE2 knockout and B1R knockout mice. Blood pressure (BP) was monitored using telemetry probes in conscious animals. While baseline BP was not different between strains, DOCA-salt treatment (1 mg/g body weight DOCA, 1% NaCl for 3 weeks) resulted in a significantly lower BP in B1R knockout mice (121 ±2 mmHg, n=5) compared to wildtype (WT) mice (138 ±3 mmHg, n=8). DOCA-salt hypertension resulted in 27% decrease (74 ±6 vs. 54 ±2 Fluorescence Units (FU)/min/µg of protein, $p<0.05$) in ACE2 activity in the hypothalamus, but not in B1R knockout mice with DOCA (69 ±6 vs. 65 ±3 FU/min/µg of protein). In DOCA-treated WT mice, B1R mRNA (Real time PCR) and protein expression (Western blot) in the hypothalamus were increased by 3 and 2 fold (n=6, $p<0.01$), respectively. This increased B1R expression was blunted in SA mice with DOCA (3.2 ±0.4 vs. 0.9 ±0.1 fold, $p<0.001$) but not in ACE2 knockout mice with DOCA (3.2 ±0.4 vs. 3.1 ±0.9 fold). Moreover, DOCA-salt treatment resulted in an increased expression of pro-inflammatory ADAM17 in the brain by 2.2 fold (n=6, $p<0.01$ vs. WT). ACE2 overexpression or B1R knockdown blunted this ADAM17 expression (1.4 ±0.1 and 1.1 ±0.6 fold, respectively, n=3, $p<0.01$ vs. WT+DOCA), while it was remain increased in ACE2 knockout mice (2.6 ±0.7 fold, n=3, $p<0.01$) compared to control mice. Together, our data provide novel evidence to

support a role for ACE2 in the modulation of central B1R function in the development of DOCA-salt hypertension.

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P114

Mas Receptor Deficiency Increases Diastolic Blood Pressure and Reduces Cardiac Function in Obese Female Mice

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Objective: We recently demonstrated that protection of female mice from obesity-induced hypertension was associated with increased plasma concentrations of angiotensin-(1-7) (Ang-(1-7)). Ang-(1-7) is a ligand for Mas receptors (MasR), where the peptide has been reported to promote endothelial release of nitric oxide and reduce blood pressure. In this study, we hypothesized that MasR deficiency will abolish protection of female high fat (HF)-fed mice from the development of obesity-induced hypertension.
Methods and Results: Female (8 weeks, C57BL/6) wild type (MasR+/+) and whole body MasR deficient mice (MasR-/-) were fed a HF diet (60% Kcal as fat) for 16 weeks. MasR deficiency had no effect on the development of obesity or glucose intolerance in HF-fed female mice. At week 16 of HF feeding, blood pressure was quantified by radiotelemetry. Deficiency of MasR resulted in a significant decrease in pulse pressures of HF-fed females (24hr average: MasR+/+, 37.7 ± 1.5 mmHg; MasR-/-, 33.1 ± 1.0 mmHg; $P<0.05$). Diastolic blood pressures (DBP) of HF-fed female MasR-/- mice were modestly elevated during the night cycle compared to controls (DBP night: MasR+/+, 91.5 ± 2.0 mmHg; MasR-/-, 96.6 ± 1.3 mmHg; $P=0.06$). However, systolic blood pressure, mean arterial pressure and heart rate were not significantly different between genotypes. Assessment of left ventricular function by ultrasound demonstrated significant reductions in stroke volume (MasR+/+, 44.3 ± 1.6 µl; MasR-/-, 36.3 ± 2.1 µl), ejection fraction (MasR+/+, 57.1 ± 1.2; MasR-/-, 50.1 ± 2.7%) and fractional shortening (MasR+/+, 29.7 ± 0.8; MasR-/-, 25.3 ± 1.7%) in HF-fed MasR-/- females compared to controls. Conclusion: These results demonstrate that MasR deficiency promotes elevated diastolic blood pressures and reduced cardiac function in obese female mice, suggesting that the Ang-(1-7)/MasR axis protects females from obesity-hypertension. Moreover, these results suggest that MasR agonists may be effective therapies for obesity-associated cardiovascular conditions.

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P115

Effects of Exercise Training on Renal Damage and Renin-angiotensin System in Dahl Salt-sensitive Rats

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Exercise training (Ex) has anti-hypertensive and renal protective effects. Renin-angiotensin system (RAS) are involved in the regulation of blood pressure and renal damage. In this study, we investigated the effects of the Ex on renal RAS in Dahl salt-sensitive rats. Six-week-old, male Dahl salt-sensitive rats were divided into four groups: 1) normal salt diet (NS); 2) NS + Ex; 3) high salt diet (HS); 4) HS+ Ex. NS or HS groups were fed diet containing 0.6% or 8% NaCl, and treadmill running was performed in Ex groups for 8 weeks (5 days/week; 60 min/day at 16-20 m/min, 0% grade). Systolic blood pressure (SBP) was monitored by tail-cuff method. Urine samples were collected on ice for 24 hours with metabolic cage. After 8 weeks, protein expression of RAS components in renal cortex (CO) and outer medulla (OM) were investigated by Western blotting. HS significantly elevated SBP (209±6 vs. 114±3 mmHg), and Ex did not change SBP (205±8 mmHg). HS significantly decreased creatinine clearance (0.96±0.04 vs. 2.57±0.07 ml/min/g kidney weight), but Ex significantly mitigated creatinine clearance (1.35±0.18 ml/min/g kidney weight). HS significantly increased urinary protein excretion (433±37 vs. 16±2 mg/day), but Ex significantly suppressed urinary protein excretion (327±22 mg/day). HS induced glomerular sclerosis, but Ex suppressed it. HS increased angiotensinogen expression (138 % and 328 %) and decreased renin expression (47% and 24%) in the CO and OM. HS increased angiotensin II type 1 (AT1) receptor expression in the OM (149%) and Mas receptor expression in the CO (198%), but decreased angiotensin II type 2 (AT2) receptor expression in the CO and OM (53% and 36%) and Mas receptor expression in the OM (20%). Ex improved HS-increased angiotensinogen and AT1 receptor expressions only in the OM. Ex improved HS-decreased renin expression in the CO and OM, and HS-decreased AT2 and Mas receptor expressions only in the OM. These results indicated that Ex improves HS-induced renal damage independently of SBP with specific changes of RAS components in the OM. Ex may have beneficial effects in HS-induced renal damage.

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P116

Effects of Febuxostat on Blood Pressure and Renal Functions in Dahl Salt-Sensitive Rats

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Several clinical and basic studies have reported that febuxostat (Fx), xanthine oxidase (XO) inhibitor, has anti-hypertensive and renal protective effects. However, these effects of Fx are controversial, and the mechanisms have not been clarified. In this study, we investigated the effects of Fx on blood pressure and renal functions in Dahl salt-sensitive rats. Eight-week-old, male Dahl salt-sensitive rats were divided into three groups: 1) normal salt diet (NS) group; 2) high salt diet (HS) group; 3) HS + Fx group. The NS or HS groups were fed diet containing 0.6% or 8% NaCl, and the Fx group treated with Fx (3 mg/kg/day in drinking water). After 8 weeks, the systolic blood pressure (SBP), renal functions, and renal histology were

examined. HS significantly increased the SBP, urinary protein excretion, and plasma creatinine and uric acid ($p<0.01$), and exacerbated glomerular sclerosis. HS also significantly increased urinary H_2O_2 and XO activity in the renal cortex (3.27±0.37 vs. 0.78±0.16 mU/mg protein) and outer medulla (1.84±0.15 vs. 0.76±0.04 mU/mg protein) ($p<0.01$). The Fx treatment significantly ameliorated the HS-increased SBP (174±6 vs. 209±4 mmHg), urinary protein excretion (152±21 vs. 325±40 mg/day), plasma creatinine (0.29±0.01 vs. 0.41±0.03 mg/dl), uric acid (0.53±0.06 vs. 1.67±0.21 mg/dl) ($p<0.01$) and glomerular sclerosis. The Fx treatment significantly suppressed the HS-increased urinary H_2O_2 (339.6±27.8 vs. 483.8±47.4 nM/day, $p<0.01$), and almost completely inhibited XO activity in the renal cortex and outer medulla. In additional study, we investigated the effects of Fx on blood pressure and renal functions in spontaneously hypertensive rats. The Fx treatment (3 mg/kg/day in drinking water) for 8 weeks did not significantly change the SBP (189±6 vs. 205±4 mmHg), plasma creatinine (0.19±0.01 vs. 0.18±0.01 mg/dl), or urinary albumin excretion (214±20 vs. 206±33 μg/day). These results indicate that anti-hypertensive and renal protective effects of Fx in Dahl salt-sensitive rats fed high-salt diet, suggesting that Fx has anti-hypertensive and renal protective effects in salt-sensitive hypertension.

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P117

Lysine Specific Demethylase-1 Deficiency Accelerates the Development of Renal Damage and Hypertension During Long Term Exposure to Sodium

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Long term exposure to salt is a demonstrated risk factor for hypertension (HT), as well as cardiovascular and renal (CVR) outcomes. We recently proposed a novel contributor to the etiology of salt-mediated HT: the Lysine Specific Demethylase-1 (LSD1). We showed that LSD1 deficiency (in mice) and LSD1 gene variants (in humans) associate - in response to short term (one week) of sodium loading - with dysregulated renal sodium handling, volume expansion and HT, and RAAS dysfunction. However, the timeline and severity of these changes during long term exposure to sodium, and the protective effects of sodium restriction in LSD1 deficient states have yet to be determined. This study aimed (1) to evaluate the timing of onset for changes in CVR health during long term sodium loading in LSD1 deficient mice and (2) to assess whether a low salt (LS) diet can prevent these effects. LSD1 heterozygous (HET) and WT mice were randomized to high salt (HS) or LS and followed longitudinally for 6 months. BP, plasma aldosterone (Aldo) and albumin/creatinine ratios (A/C) were assessed monthly.
The SBP (mm Hg) increased progressively during the study, and reached significance on the 5th and 6th month for HS-HET and HS-WT (141±4 and 134±3, respectively, both $p<0.05$ vs.

baseline) but not for LS-HET and LS-WT (128±6 and 120±6, respectively). The SBP effects were driven by a significant interaction between genotype and age ($p<0.05$). Similar results were obtained for DBP ($p<0.05$), suggesting a volume mediated effect. HS plasma Aldo was appropriately suppressed.
The A/C (µg/mg) was progressively increased in both HS groups, one month prior to the BP change. Namely, A/C reached significance on the 4th and 5th month for HS-HET and HS-WT (46±4 and 48±9, respectively, both $p<0.05$ vs. baseline). The LS diet prevented these changes in both genotypes.
Our novel study shows that long term exposure to HS induces kidney damage followed by BP increase, and that these changes are initiated earlier in the LSD1 HET, suggesting LSD1 as a critical component of mechanisms involved in CVR health. Moreover, long term sodium restriction prevented the development of both target organ damage and HT in this model, suggesting that this dietary intervention may be particularly efficient in human carriers of LSD1 gene variants.

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P118

Differential Effects of Renal α- and β-Adrenoreceptors in the Development of Sodium Chloride Cotransporter (NCC) Dysfunction and Salt-sensitive Hypertension

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Aim: Determine the contribution of α vs. β-adrenoeceptors in NCC dysfunction & the development of salt-sensitive hypertension (HTN).
Methods: Sprague-Dawley rats receiving a sc saline, NE (600ng/min), NE with propranolol (Pro; 9.9mg/kg/day) or NE with prazosin (Praz; 2.5mg/kg/day, orally) infusion were fed a 0.4% (NS) or 8% NaCl (HS) diet for 14 days (N=6). On day 14, MAP & NCC activity (peak natriuresis to iv hydrochlorothiazide infusion; HCTZ; 2mg/kg) was assessed. Immunoblotting for NCC, STE-20 Alanine/Proline kinase (SPAK), & oxidative stress response-1 (OxSR1) was performed on kidney cortex tissue.
Results: NE infusion results in HTN that is exacerbated by HS (MAP [mmHg] NE+NS 151±3; NE+HS 171±4; $p<0.05$). Excess NE prevents HS-evoked suppression of NCC function and NCC & SPAK expression. NE:Pro co-infusion significantly decreased MAP (MAP NE:Pro+NS 136±4; NE:Pro+HS 136±4), regardless of Na+ intake, when compared to rats receiving the NE infusion, but failed to restore HS-evoked suppression of NCC activity (similar to results observed when AT1Rs are chronically antagonized during NE infusion; data not shown), or NCC, SPAK, or OxSR1 expression despite reduced baseline SPAK & OxSR1 compared to NE alone. NE:Praz co-administration abolished the salt-sensitive component of NE-induced HTN (MAP NE:Praz+NS 159±5; NE:Praz+HS 156±5) and restored HS-evoked suppression of NCC function and expression (Peak ΔUNaV [µeq/µl] NE:Praz+NS 10.7±1.2; NE:Praz+HS 6.14±1.2; $p<0.05$). NE+Praz co-administration resulted in HS-evoked suppression of SPAK, but not OxSR1 expression, in addition to decreased baseline

expression of SPAK/OxSR1.
Discussion: Our data suggests that α, but not β, adrenoreceptors are predominantly responsible for NE-mediated NCC dysregulation and the development of salt-sensitive HTN. During a HS intake, α-adrenoreceptors mediate NE-evoked salt-sensitivity via a SPAK-NCC dependent pathway. Chronic antagonism of β-adrenoreceptors decreased MAP independent of direct effects on NCC possibly via actions on the RAS. Our data shows a synergistic relationship between α-/β-adrenoreceptors to upregulate SPAK/OxSR1 during chronic adrenergic stimulation with SPAK as the primary driver of NCC dysfunction & salt-sensitivity.

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P119

Human Podoplanin+/vegfr-3+/lyve-1+ Monocytes are Lymphatic Endothelial Precursors

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Lymphatic vessels are involved in the development of various inflammatory disorders, and lymphatic vessel regeneration has been increasingly investigated to develop therapies for lymphatic diseases. Here we report that Podoplanin+/VEGFR-3+/LYVE-1+ is a valid marker for human lymphatic endothelial precursors and the triple-positive cells can be used in lymphatic regeneration. During 5-day culture on an ultra-low attachment surface dish, human peripheral blood mononuclear cells (PBMCs) underwent exponential growth, aggregating into a sphere-like structure and expressing several lymphatic endothelial cell (LEC) markers and lymphangiogenic transcription factors. When dissociated from the aggregate and cultured on a gelatin-coated dish, the cells were attached to the surface. The attached cells were triple positive for LEC markers e.g. Podoplanin, LYVE-1, VEGFR-3. Furthermore, seeded in Matrigel with LECs, the 5-day aggregate-derived cells were incorporated into lymphatic endothelial network. The 5-day aggregates were largely positive for CD14+, a monocyte marker. The CD14+ population was sorted into Podoplanin-positive and negative group for further characterization. Notably, CD14+/Podoplanin+ cells showed increased expression of lymphangiogenic molecules (e.g. VEGFR-3, LYVE-1) both at the genetic and protein levels. Also, CD14+/Podoplanin+ cells secreted higher levels of lymphangiogenic cytokines (VEGF, HGF, PDGF-BB). ELISA results showed that CD14+/Podoplanin+ cells produced more lymphangiogenic cytokines than CD14+/Podoplanin- cells. Local injection of monocyte aggregates significantly increased lymphatic neovascularization and facilitated healing of the skin wound model of nude mice, with CD14+/Podoplanin+ group showing the most dramatic result. Our data suggests that Podoplanin-positive monocytes can be transdifferentiated into lymphatic endothelial precursor cells, and cells with triple positivity

for Podoplanin, VEGFR-3, and LYVE-1 can be a promising cell source for therapy against human lymphatic vessel diseases.

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P120

Improving the Angiogenic Abilities of Mobilized Peripheral Blood Stem Cells Achieved by Priming with Activated Platelet Supernatant for Regenerative Cell Therapy

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Platelets play a critical role in hemostasis and also have ability to promote angiogenesis and tissue repair by secreting of numerous cytokine and making angiogenic condition. We investigated whether autologous 'activated platelet supernatant (APS)' has effect on enhancing pro-angiogenic potential of peripheral blood stem cells (PBSC) for stem cell-based therapy for ischemic diseases. Granulocyte-colony stimulating factor (G-CSF) mobilized peripheral blood stem cells (mobPBSC) were isolated from healthy volunteers, while APS was collected from platelet rich plasma by thrombin activation. mobPBSCs were primed with APS (APS primed mobPBSCs) for 6 hours, and APS primed mobPBSCs characterized their angiogenic ability. For the safety analysis, we estimated the thrombogenicity of platelets in whole blood mixed with APS primed mobPBSCs by expression of glycoprotein IIb and IIIa on platelets. APS had a higher level of various cytokines, such as IL8, IL17, PDGF and VEGF than naïve platelet supernatants. And APS primed mobPBSCs had more expression of angiogenic factors, surface markers (i.e. CD34, CD31, and CXCR4) and integrins (integrin α5, β1 and β2) than Veh primed and Pre primed mobPBSC. Also APS primed mobPBSCs were polarized toward CD14++/CD16+ pro-angiogenic monocytes. And result in adhesion to endothelial cells and fibronectin which represents cell to cell and cell to extracellular matrix adhesion, respectively. The culture supernatant of APS-primed mobPBSCs contained high levels of IL8, IL10, IL17 and TNFα, and augmented proliferation and capillary network formation of HUVEC. In-vivo transplantation of APS-primed mobPBSC into athymic mice ischemic hindlimbs and Matrigel plugs elicited vessel differentiation and tissue repair. In thrombogenicity test, platelet activity increased after mixing whole blood with mobPBSC regardless of the priming agent. However, this was reduced by pretreatment of aspirin, which is an antiplatelet agent prescribed to patients with ischemic diseases. Our data demonstrate that mobPBSCs primed with APS improve angiogenic potential, and that can be adjunctive strategy to enhance the efficiency of stem cell therapy for ischemic diseases.

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P121

Vascular Protective Effects of Telomerase Activity Are Independent of Nuclear Function

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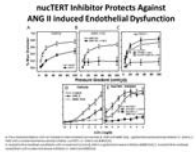
Rationale: The contributions of nuclear telomerase activity to cardiovascular protection we have previously described are not defined. We used a novel inhibitor of nuclear telomerase activity (nucTERT I) to test the hypothesis that nuclear telomerase activity is not critical to the protective effects of telomerase.
Methods and Results: To confirm the effect of nucTERT I cultured cells were treated for 24 h and subjected to standard cell fractionation procedures. Telomerase activity (TRAP assay) confirmed decreased nuclear telomerase activity after treatment. nucTERT I reduced nuclear telomerase activity (44% +/- 7) compared to untreated control.
To evaluate the effect of nucTERT I on vascular function, human microvessels were dissected from discarded surgical tissue from patients with no history of coronary artery disease and used for videomicroscopy. Vessels were challenged with ANG II and their ability to dilate in response to flow and acetylcholine was evaluated. Vessels pre-treated with nucTERT I (24 h) were significantly less sensitive to ANG II-induced endothelial dysfunction when stimulated with either acetylcholine or increased flow. The telomerase activity inhibitor, BIBR1532, abrogated these effects (figure).
Conclusions: Our data suggest that nuclear telomerase activity is not necessary for the protective effects of telomerase on ANG II induced endothelial dysfunction. Treatment with BIBR1532 confirms that vascular stress resistance is conferred by catalytically active telomerase. These data suggest that modulation of telomerase may be a useful strategy in the treatment of vascular disease.



J.D. Ebben: H. Other; Modest; Provisional patent on nucTERTi. **J. Hockenberry:** None. **M. You:** None. **A.M. Beyer:** H. Other; Modest; Provisional patent on nucTERTi.

P122

Mitochondrial Transcription Factor 2B Regulates Endothelial Cell Morphology

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Mitochondrial dysfunction, such as observed in endothelial cells, has been implicated in various cardiovascular diseases including, hypertension and atherosclerosis. Mitochondrial transcription factor 2B (TFB2M) is an essential component to maintain proper transcriptional and functional control of mitochondrial DNA. As well, elongation of endothelial cells is a characteristic of atheroprotective regions within the vasculature, and the relationship between the mitochondria and EC shape is currently unknown. The aim of our study is to investigate the hypothesis that TFB2M has a novel role in enhancing endothelial function. Human umbilical vein endothelial cells (HUVECs) were harvested 72 hours after adenoviral transduction with TFB2M (100 moi). HUVECs transduced with TFB2M showed an elongated

cell morphology when compared to GFP control. To further investigate the effect of TFB2M on regulating mitochondrial function and cell shape, immunoblotting was carried out for markers involved in mitochondrial function/dynamics and markers indicative of cytoskeleton reorganization. TFB2M transduction resulted in increased expression of mitochondrial biogenesis marker VDAC (2.6 fold increase), mitochondrial fusion protein MFN2 (2.1 fold increase), and phosphorylated myosin phosphatase targeting protein MYPT1 at Thr850 (2.2 fold increase, $p < 0.05$ for all proteins). Additionally, fluorescence microscopy showed enhanced mitochondrial fluorescence in TFB2M transduced cells using mitotracker red staining (3.5 fold increase, $p < 0.001$). These data indicate that TFB2M has a previously undiscovered function contributing to altered EC function and shape, potentially through a novel mitochondrial retrograde signaling mechanism. Further research will focus on distinguishing the exact mechanisms culminating in a protective EC phenotype and the beneficial role of endothelial TFB2M-mediated enhanced mitochondrial function in the treatment of EC dysfunction associated with various cardiovascular diseases.

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P123

Retinol-Binding Protein 7 (RBP7) Regulates Endothelial Function via an Adiponectin-Dependent Mechanism in Angiotensin II-Infused Mice

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The transcription factor peroxisome proliferator-activated receptor gamma (PPARγ) regulates vascular function and protects against endothelial dysfunction. We reported that mice expressing dominant-negative PPARγ in endothelium exhibit an enhanced pressor response to angiotensin II (Ang II). Given that RBP7 is a PPARγ target expressed in endothelium, we hypothesized that loss of RBP7 would augment endothelial dysfunction caused by Ang II. Using osmotic minipumps, RBP7 knockout (KO) mice and wild type (WT) littermates were infused with subpressor Ang II (120 ng/kg/min, 2 weeks) or saline. Systolic blood pressure (by tail cuff) was not different between KO and WT mice and was not altered by infusion of saline or Ang II. Acetylcholine (ACh, 30 μM) mediated relaxation of carotid arteries was not different between groups during saline infusion, but was selectively impaired in KO mice during Ang II (44±5% in KO vs 71±3% in WT, $p<0.05$). Aortic superoxide, assessed by hydroethidine staining, was higher after Ang II in KO compared with WT mice. Pre-incubation of carotid arteries with a superoxide scavenger Tempol (1 mM, 30 min) restored ACh-induced relaxation in Ang II-infused KO mice (74±6% in Tempol-KO vs 46±8% in KO). To identify molecular mechanisms, RNA Sequencing was performed using carotid arteries from WT and KO mice fed high fat diet (HFD) or normal chow for 8 weeks. Adiponectin (Adipo), a known PPARγ target gene, was increased ~6-fold in HFD-fed WT mice, a response that was markedly blunted in KO mice. Immunohistochemistry revealed that Adipo was expressed specifically in endothelial cells (co-immunostaining with endothelial specific CD31). Rosiglitazone, a PPARγ ligand, increased

carotid artery Adipo protein in WT, but not KO mice. To test the importance of Adipo, carotid arteries from Ang II-infused WT and KO mice were incubated with vehicle or full-length Adipo (5 μg/mL) for 12 hrs. Adipo incubation ameliorated Ang II-induced endothelial dysfunction in KO mice (ACh, 30 μM: 86±3% Adipo-KO vs 58±8% KO) while having no effect in WT mice (ACh, 30 μM: 83±1% Adipo-WT vs 87±3% WT). In conclusion, RBP7 protects the endothelium from oxidative stress-induced dysfunction caused by Ang II. Our data suggest an important role for Adipo in mediating this protective response.

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P124

Human Small Artery MicroRNA Expression Profiles: Changes in Type 2 Diabetes and Associations with Endothelial Function

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Background: Studies of experimental models and human blood samples support an important role of microRNAs (miRNAs) in the development of vascular dysfunction in hypertension and diabetes mellitus (DM). Information on miRNA expression in clinically directly relevant tissues such as human small arteries and its relationship with impaired vascular endothelial function is currently lacking.
Methods and Results: 38 subjects (18 type 2 DM, 20 controls) underwent gluteal adipose pad biopsy to obtain small arteries for miRNA expression profiling by small RNA deep sequencing. In vivo conduit artery endothelial function was measured by brachial artery reactivity. In vitro microvascular endothelium dependent vasodilation was measured by videomicroscopy. Correlations between miRNA expression and measurements of endothelial function were calculated using generalized linear models. Several miRNAs correlated with measurements of vascular structure and function. Endothelium dependent vasodilation was impaired in type 2 DM subjects compared to controls based on both the vasodilatory response to peak dose acetylcholine (44±25 vs. 69±18 %, P=0.04) and by analyses of the entire acetylcholine dose-response curve. Several miRNAs were differentially expressed in small arteries from type 2 DM subjects, two of which were verified by real-time PCR. Cross-referencing the top 30 miRNAs (P<0.015) with prior studies of plasma miRNA expression in DM subjects identified 7 miRNAs differentially expressed in both human small arteries and plasma, all of which have some reported role in vascular regulation.
Conclusions: Multiple miRNAs are differentially expressed in human small arteries in DM patients and correlated with in vivo or in vitro measurements of endothelial function, suggesting an important role of microvascular miRNAs in the development of endothelial dysfunction in humans.

D.M. Jensen: None. **M.E. Widlansky:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Merck Sharp & Dohme Corp.. **C. Chu:** None. **P. Liu:** None. **Y. Liu:** None. **A. Kriegel:** None. **J. Wang:** None. **M. Malik:** None. **R. Ying:** None. **A. Couillard:** None. **M. Liang:** None.

P125

Visceral Adipose Tissue-derived Serine Protease Inhibitor Prevents the Development of Hypertension Through the Inhibition of Vascular Remodeling via Anti-Oxidative Mechanisms in Spontaneously Hypertensive Rats

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Visceral adipose tissue-derived serine protease inhibitor (vaspin) is an adipokine originally identified in visceral adipose tissue of obese type 2 diabetic Otsuka Long-Evans Tokushima Fatty rats. We have been focusing on the direct vascular effects of vaspin and found that vaspin inhibits: 1) tumor necrosis factor-α-induced inflammatory responses in vascular smooth muscle cells (SMCs) via the anti-oxidative mechanisms, 2) platelet derived growth factor-BB-induced migration of SMCs, 3) apoptosis of vascular endothelial cells (ECs) mediated by methylglyoxal, a metabolite of glucose. Since vascular remodeling via inflammation and migration of vascular SMCs as well as apoptosis of ECs is an important process for the development of hypertension, it is suggested that vaspin has preventive roles on the pathogenesis of hypertension. However, it is not revealed whether vaspin affects the development of hypertension in *in vivo* hypertensive model. The aim of the present study was to explore it. Five-week-old male spontaneously hypertensive rats (SHR) were received an intraperitoneal injection of vaspin (1 μg/kg) or saline once daily for four weeks. Age-matched male Wistar-Kyoto (WKY) rats were used as a control. Blood pressure (BP) was measured using a tail-cuff method weekly. Isolated superior mesenteric arteries were used for the examination of vascular structural changes by a hematoxylin and eosin staining. Reactive oxygen species (ROS) generation was examined by an immunohistochemical staining to 4-hydroxy-2-nonenal (4-HNE), an end-product of lipid peroxidation by ROS. Vaspin significantly inhibited age-dependent elevation of BP in SHR (from 165.5 ± 3.9 mmHg to 153.1 ± 2.5 mmHg, $n = 3$, $P < 0.05$). Vaspin significantly inhibited vascular wall hypertrophy in SHR mesenteric artery (from 1.35±0.05 to 0.98±0.08-fold relative to WKY, $n = 3$, $P < 0.05$). Moreover, vaspin inhibited an increase of 4-HNE-positive area to vessel area ratio in SHR mesenteric artery (from 13.1±4.4 % to 4.6±0.4 %, $n = 3$). The present results demonstrate that vaspin inhibits the increase of BP through inhibiting vascular remodeling via anti-oxidative mechanisms in SHR.

S. Kameshima: None. **Y. Sakamoto:** None. **M. Okada:** None. **H. Yamawaki:** None.

P126

Angiotensin II-induced Arterial Hypertension, Vascular Dysfunction and Inflammation is Promoted by Platelet-localized Coagulation Factor XI

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Multicellular interactions of platelets, leukocytes and the vessel wall play pivotal roles in activating coagulation and precipitating arterial and venous thrombosis. High levels of angiotensin II (ATII) cause arterial hypertension by a complex inflammatory pathway requiring inflammatory leukocyte recruitment and reactive oxygen species production within the vessel wall coupled to vascular dysfunction. Here we delineate a novel non-thrombotic, pro-inflammatory coagulation pathway that substantially regulates vascular tone and endothelial function. We demonstrate that ATII (1 mg/kg/d s.c.) induces an upregulation of tissue factor, thrombin-dependent endothelial cell VCAM-1 expression and integrin α4- and platelet-dependent leukocyte adhesion to arterial conductance vessels. The resulting vascular inflammation and dysfunction unexpectedly involves the activation of FXI that was independent of FXII (maximal relaxation (ACh) [%] of C57BL/6 +ATII vs. FXI-/- +ATII vs. FXII-/-+ATII mice: 41.09±4.246 vs. 67.83±7.117 vs. 37.70±4.612). We discovered that the platelet FXI receptor glycoprotein Ibα supports the upregulation of thrombin feedback activation in ATII-treated mice. Importantly, pharmacologic inhibition by an antisense oligonucleotide of FXI synthesis (FXI Aso) is sufficient to prevent thrombin propagation on platelets (endogenous thrombin potential (ETP) [nMxmin] of C57BL/6 +ATII vs. C57BL/6 +FXI Aso +ATII mice: 703.0±67.90 vs. 438.2±86.54), to reduce vessel wall leukocyte infiltration, and to diminish ATII-induced endothelial dysfunction (maximal relaxation (ACh) [%] of C57BL/6 +ATII vs. C57BL/6 +FXI Aso +ATII mice: 30.4±3.061 vs. 63.08±3.141) and arterial hypertension (Systolic blood pressure [mmHg] of C57BL/6 +ATII vs. C57BL/6 +FXI Aso +ATII mice: 152.8±4.82 vs. 140.0±1.472).
Our results provide novel insight into coagulation-inflammation circuits promoting vascular dysfunction and point to a broader utility of specific FXI-targeted anticoagulants beyond indications as antithrombotic agents in cardiovascular diseases.

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P127

Angiotensin II Priming Enhances Prostaglandin E2 Vasoconstrictor Effects

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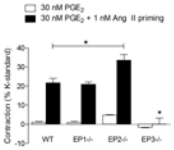
Prostaglandins are key modulators of blood pressure and arterial tone. Prostaglandin E_2 (PGE_2), is a prostanoid that has vasodepressor effects; however, under certain circumstances PGE_2 can induce vasopressor responses. Recent reports demonstrated that sub-threshold concentrations of vasoconstrictors augment PGE_2-mediated constriction in rat femoral

arteries. However, whether angiotensin II (Ang II) could affect PGE_2-mediated contraction is not known. Using a wire myograph, we demonstrated that PGE_2 had no significant effect on mouse femoral arterial rings at doses up to 1 µM. However, priming of arterial rings with 1 nM Ang II potentiated PGE_2-evoked constriction in a concentration dependent manner (Area Under the Curve, $AUC_{untreated}$ 1.784 ± 0.353, $AUC_{Ang\ II}$ 23.27± 9.820, $P<0.05$). We tested femoral arteries from EP1, EP2, and EP3 receptor knockout mice. Only the EP3-/- arteries were unable to respond to PGE_2 after Ang II priming (figure below). Pretreatment of arterial rings with 1 µM losartan, an angiotensin receptor antagonist, blocked PGE_2-induced constrictor effects primed with Ang II (% of KCl, Ang II 21.72 ± 5.296, Ang II + losartan 3.025 ± 1.046, n=3). We have determined that re-addition of extracellular Ca^{2+} to a Ca^{2+}-free artery restores PGE_2-induced contractions (n=5) and that the Rho-kinase inhibitor Y-27632 blocks contraction (n=3). Taken together these data are consistent with angiotensin AT1 and prostaglandin EP3 receptors mediating a synergistic Rho-kinase-dependent contractile response. We are continuing to investigate the relationship between Ang II and PGE_2 to determine the physiological relevance this may have in modulating blood pressure.



M.P. Kraemer: None. **F. Lamb:** None. **R.M. Breyer:** None.

P128

RhoBTB1, a Novel PPARγ Target Gene Regulates Vascular Function

Masashi Mukohda, Stella-Rita C Ibeawuchi, Chunyan Hu, Frederick W Quelle, Curt D Sigmund, Univ of Iowa Carver Coll of Med, Iowa City, IA

Peroxisome proliferator-activated receptor gamma (PPARγ) is a ligand activated transcription factor regulating metabolic and vascular function. We previously reported that mice (S-DN) expressing a dominant-negative PPARγ mutation in smooth muscle cells (SMC) are hypertensive, exhibit impaired vascular relaxation and enhanced contraction, and display reduced expression of a novel PPARγ target gene, RhoBTB1. We hypothesized that RhoBTB1 may play a role in the PPARγ-mediated regulation of vascular function that is disrupted in S-DN mice. To test this, we generated transgenic mice (R+) with tamoxifen-inducible, Cre-dependent expression of RhoBTB1 in SMC. These mice were crossed with S-DN to produce mice (S-DN/R+) in which tamoxifen-treatment (75 mg/kg, ip, for 5 days) increased RhoBTB1 RNA expression in aorta from the reduced level seen in S-DN mice, and restored it to the level of non-transgenic mice. Thoracic aorta from S-DN showed impaired acetylcholine (ACh)-induced endothelial-dependent relaxation, which was reversed by replacement of RhoBTB1 in SMC (43.3±4.4 vs 74.2±1.1 %, $p<0.01$, n=6). A similar improvement was observed in basilar artery (19.9±6.7 vs 48.1±12.3 %, $p<0.05$, n=6). Aorta from S-DN mice also displayed severely decreased NO donor (sodium nitroprusside, SNP)-induced endothelial-independent relaxation with a right-shifted SNP dose-response, which was also reversed in aorta from tamoxifen-treated S-DN/R+ mice ($p<0.01$, n=6). To confirm that these effects were specifically due to replacement of RhoBTB1, we assessed vascular function in tamoxifen-treated S-DN

mice. Notably, tamoxifen itself did not affect relaxation in response to ACh or SNP, or contraction in response to KCl, endothelin-1 (ET-1) or Prostaglandin F2α in aorta or basilar artery from S-DN (n=4). Interestingly, contraction induced by ET-1, but not KCl, was enhanced in S-DN aorta, and was not improved by restoring RhoBTB1 expression (n=6). This suggests that RhoBTB1 may function specifically by regulating vasodilation pathways. We conclude that RhoBTB1 plays an important role in facilitating vasodilatation in aorta and basilar artery, and loss of RhoBTB1 function explains the vascular dysfunction observed in response to interference with PPARγ in smooth muscle.

M. Mukohda: None. **S.C. Ibeawuchi:** None. **C. Hu:** None. **F.W. Quelle:** A. Employment; Significant; University of Iowa. **C.D. Sigmund:** A. Employment; Significant; University of Iowa. B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH Grants.

P129

20-HETE Antagonist, 20-SOLA, Restores Coronary Collateral Growth in the Metabolic Syndrome

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We have previously shown that transient and repetitive ischemia-induced (RI) coronary collateral growth (CCG) was severely impaired in a metabolic syndrome rat model (JCR rat). Levels of 20-hydroxyeicosatetraeonic acid (20-HETE), a cytochrome (CYP)-derived arachidonic acid metabolite are greatly elevated in hypertensive animal models and loosely associated with obesity in humans, but its levels in metabolic syndrome, especially in cardiovascular tissues, as well as its possible involvement in the regulation of collateral growth are unknown. In rats, CYP4A1 is the major enzyme responsible for the production of 20-HETE. In this study, we demonstrated that cardiac CYP4A1 expression (RT-PCR, Western blot and immunohistochemistry) and 20-HETE levels were markedly (10-fold) elevated in JCR vs. Sprague-Dawly (SD) rats in response to RI. Importantly, administration of an antagonist of 20-HETE, 20-SOLA, completely restored CCG in JCR rats (collateral flow was 86±1% of that in the normal zone (JCR+SOLA) vs. 21±2% (JCR) vs. 84±5% (SD), $p<0.05$). We conclude that 20-HETE is an important modulator of CCG in the metabolic syndrome where its myocardial tissue levels are highly elevated.

A.A. Soler: None. **B. Hutcheson:** None. **M.A. Devila-Molina:** None. **I. Hunter:** None. **V.G. Garcia:** None. **M.L. Schwartzman:** None. **P. Rocic:** None.

P130

The Influence of Maternal Obesity on Perivascular Adipose Tissue Function in Offspring

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Objective: Maternal obesity pre-programs offspring to develop obesity and associated cardiovascular disease although the underlying mechanism is currently unknown. This study investigated the effect of maternal obesity on

perivascular adipose tissue (PVAT) regulation of resistance artery tone.
Design and method: 8 week old female SD rats were fed a 10% fat diet (controls) or 45% fat obesogenic diet (HFD) for 12 weeks before mating, then continued on their respective diets during pregnancy and lactation. Male offspring were provided with 10% fat diet until sacrifice at 12 weeks old. PVAT-intact or -denuded mesenteric arteries (250-300µm internal diameter) from offspring were mounted on a wire myograph. Cumulative concentration-response curves were constructed to the thromboxane A2 receptor agonist U46619 (10nM-3µM) ± 10µM A769662, an activator of AMP-activated kinase (AMPK) or 100µM L-NMMA, a nitric oxide synthase (NOS) inhibitor.
Results: Body weight, systolic and diastolic blood pressure were significantly increased in HFD dams compared to age-matched controls (391±11 vs 348±12 g; 154±1 vs 145±1 mmHg; 116±1 vs 103±103 mmHg respectively) but no differences were observed between offspring. However, fat pads and insulin levels were increased in both HFD dams versus controls (13.4±1.6 vs 6.5±0.8 g; 1.18±0.09 vs 0.76±0.14 nmol/L respectively) and their offspring (7.2±0.3 vs 6.0±0.2 g; 0.56±0.15 vs 0.17±0.05 nmol/L respectively) versus controls. PVAT exerted an anti-contractile effect in artery segments from offspring of control dams ($p<0.001$), an effect which was lost in offspring of HFD dams. AMPK activation decreased contractility of both PVAT-denuded and -intact arteries from control offspring ($p<0.01$; $p<0.0001$ respectively, n=8); this effect was abolished in PVAT-intact vessels of offspring of HFD dams. Inhibition of NOS increased contractility of both PVAT-denuded and -intact arteries from control offspring ($p<0.0001$ and $p<0.0001$ respectively, n=8), revealing a contractile effect of PVAT in control offspring ($p<0.01$, n=8) but not in arteries from offspring of HFD dams.
Conclusions: In summary, the diminished anti-contractile effects of PVAT in 12 week old offspring of HFD dams may be due to reduced AMPK and nitric oxide activity.

K.E. Zaborska: None. **C. Austin:** None. **M. Wareing:** None. **G. Edwards:** None.

P131

Mef2C-MYOCD and Leiomodin1 Suppression by miRNA-214 Promotes Smooth Muscle Cell Phenotype Switching in Pulmonary Arterial Hypertension

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Background: In pulmonary arterial hypertension (PAH), smooth muscle cell (SMC) phenotype switching from a terminally differentiated contractile to synthetic state is gaining traction as our understanding of disease progression improves. While maintenance of SMC contractile phenotype is reportedly orchestrated by a MEF2C- Myocardin (MYOCD) interplay, little is known regarding molecular control at this nexus. Moreover, the burgeoning interest in microRNAs (miRs) provides a basis for exploring their modulation of MEF2C-MYOCD signaling, and, in turn, a pro-proliferative, synthetic SMC phenotype. We hypothesized that suppression of SMC contractile phenotype in pulmonary hypertension is mediated by miR-214 via repression of the MEF2C-MYOCD-leiomodin1 signaling axis.
Methods and Results: In SMCs isolated from a PAH patient cohort and commercially obtained

hPASMCs exposed to hypoxia, miR-214 expression was upregulated approx. ~1.5 fold compared to controls ($p<0.05$). These increases in miR-214 were paralleled by downregulation of MEF2C, MYOCD and SMC-specific contractile proteins, leiomodin1 and smoothelin. Of these, leiomodin1 was directly targeted by the miR. MicroRNA-214 overexpression mimicked the PAH profile, downregulating MEF2C (1±0.054 vs 0.696±0.026, $p<0.05$) and leiomodin1 (1± 0.051 vs 0.281±0.095, for scrambled control vs miR-214 mimic, $p<0.05$). Hypoxia significantly reduced expression of SMC-specific contractile proteins, leiomodin1 and calponin1 (5 of 10 percent), and smoothelin (approx. 3 of 10 percent), and miR-214 antagomiR abrogated hypoxia-induced suppression of the contractile phenotype. We also found that hypoxia-induced hPASMC proliferation was significantly attenuated by the anti-miR (approx. 2-fold less compared to hypoxia control). Further, anti-miR-214 also restored PAH-PASMCs to a contractile (approx.5 of 10 percent reversal of MEF2C and leiomodin1 expression), and less proliferative phenotype seen during vascular homeostasis.
Conclusions: Our findings illustrate a key role for miR-214 in modulation of MEF2C-MYOCD-leiomodin1 signaling and suggest that an antagonist of miR-214 could mitigate SMC phenotype changes and proliferation in vascular hyperproliferative disorders including PAH.

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P132

MEF2C-MYOCD and Leiomodin1 Suppression by miRNA-214 Promotes Smooth Muscle Cell Phenotype Switching in Pulmonary Arterial Hypertension

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Vascular hyperproliferative disorders are characterized by excessive smooth muscle cell (SMC) proliferation leading to vessel remodeling and occlusion. In pulmonary arterial hypertension (PAH), SMC phenotype switching from a differentiated contractile to a synthetic state contributes to disease progression. While SMC contractile phenotype is reportedly maintained by a MEF2C-Myocardin (MYOCD) transcription factor interplay, its molecular control is poorly understood. MicroRNAs (miRs) have emerged as modulators of many cellular processes and some evidence associates them to MEF2C-MYOCD signaling. It is, therefore, plausible that miRs can regulate the synthetic SMC phenotype. We hypothesized that suppression of SMC contractile phenotype in PAH is mediated by miR-214 via repression of signaling by MEF2C-MYOCD and downstream contractile proteins leiomodin1 and smoothelin. Using qRT-PCR, the levels of miR-214 expression were shown to be upregulated in pulmonary artery SMC (PASMCs) from PAH- vs. control human subjects as well as in commercially obtained human PASMC (hPASMCs) exposed to hypoxia (~1.5 fold, $p<0.05$). These increases in miR-214 were paralleled by downregulation of MEF2C, MYOCD, and SMC-specific contractile proteins, leiomodin1 and smoothelin. MicroRNA-214 overexpression mimicked the PAH profile, downregulating MEF2C (1.00±0.054 vs. 0.696±0.026, $p<0.05$) and leiomodin1 (1.00± 0.051 vs. 0.281±0.095, $p<0.05$) protein levels for

control vs miR-214 mimic, respectively. Hypoxia significantly reduced expression of SMC-specific contractile proteins leiomodin1, calponin1 (~50%) and smoothelin (~30%), and miR-214 antagomiR abrogated this response. We also investigated whether miR-214 participates in the induction of hPASMC proliferation, and found that hypoxia-induced hPASMC proliferation was significantly attenuated by the anti-miR (~2-fold). Further, anti-miR-214 restored PAH-PASMCs to a contractile (~50% reversal of MEF2C and leiomodin1 expression) and less proliferative phenotype. Our data illustrate a key role for miR-214 in modulation of MEF2C-MYOCD-leiomodin1 signaling and suggest that an antagonist of miR-214 could mitigate SMC phenotype changes in vascular hyperproliferative disorders including PAH.

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P133

Mitochondrial- Dependent Apoptosis in Arterial Remodeling: Link to Hypertension

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Hyperhomocysteinemia (HHcy) has been observed to promote hypertension through endothelial dysfunction and vascular remodeling, but the mechanisms are unclear. Previously, we showed that elevated homocysteine levels disturbed mitochondrial dynamics facilitating excessive mitochondrial fission with consequent endothelial cell loss and collagen deposition in the mesenteric artery. In the present study, we hypothesize that HHcy-induced excessive mitochondrial fission promotes mitochondrial apoptosis through Bax activation that up- regulates downstream apoptotic proteases (Caspase-9, Caspase-3), causing endothelial cell loss and arterial remodeling that predispose to hypertension. To test this hypothesis, we used 12 week old C57BL/6J mice (WT) as a control; Cystathionine-β-synthase deficient mice (CBS+/-) with genetic HHcy; C3H/HeJ (C3H) mice, that are resistant to oxidative stress and CBS+/-/C3H mice. Blood pressure and vascular reactivity measurements, western blotting (Caspase-9 and Caspase-3), q-PCR (Bax, Bcl-2), immunohistochemistry (cleaved Caspase-3) and TUNEL assay were used in this study. Blood pressure values were up-regulated in CBS+/- mice (diastolic: 118.4 ± 9.8 mmHg; systolic: 153.9 ± 12.7 mmHg; mean: 130 ± 10 mmHg) compared to WT mice (diastolic: 101 ± 15 mmHg; systolic: 139 ± 10 mmHg; mean: 113 ± 14 mmHg). Interestingly, blood pressure values were decreased in C3H mice (diastolic: 74.8 ± 6.5 mmHg; systolic: 118.6 ± 9 mmHg; mean: 89 ± 7 mmHg) compared to control (diastolic: 96 ± 7 mmHg; systolic: 143.4 ± 2.4 mmHg; mean: 111.4 ± 5.5 mmHg). q-PCR showed 11 fold up-regulation of Bax mRNA expression in the mesenteric artery of CBS+/- mice compared to control. Western Blotting validated two- fold increase of Caspase-9 and Caspase-3 protein expressions in the mesenteric artery of CBS+/- mice compared to WT mice. TUNEL assay further indicated the presence of DNA fragments in the mesenteric artery of CBS+/- mice. In conclusion, our data suggested that HHcy-induced mitochondrial fission promotes Bax activation followed by mitochondrial apoptosis with activation of downstream proteases (Caspase-9, Caspase-3), leading to endothelial cell loss and arterial remodeling that contributes to hypertension.

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P134

Identification of Tissue Inhibitor of Metalloproteinases (TIMP)-4 as a Novel PPARγ Target in Smooth Muscle Cell

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Patients with PPARγ mutations develop severe early onset hypertension, type 2 diabetes and lipodystrophy. Our recent findings demonstrated that transgenic mice expressing a dominant negative (DN) PPARγ mutant in vascular smooth muscle (S-P467L) exhibited exacerbated DOCA-salt induced hypertension and vascular remodeling in conduit and resistance arteries. Here, we hypothesized that predisposition to vascular injury during hypertension in S-P467L mice is through altered expression of PPARγ target genes in smooth muscle cells (SMC). Gene expression profiling in aorta and mesenteric arteries revealed a significant loss of Tissue Inhibitor of Metalloproteinases (TIMP)-4 in S-P467L compared to non-transgenic (NT) littermates. Interference with PPARγ activity either by treatment with PPARγ inhibitor, GW9662 or expressing P467L PPARγ markedly suppressed TIMP-4 in primary SMC, suggesting that loss of TIMP-4 in S-P467L arteries is a direct result of PPARγ inhibition. Downregulation of TIMP-4 in SMC by GW9662 was correlated with a significant increase in total MMP activity (Fluorescent signal subtracted from negative control; vehicle: 1±0.5, GW9662: 2.2±0.7, $p<0.05$), consistent with TIMP-4 function as an endogenous inhibitor of MMPs. Overexpressing TIMP-4 in SMC significantly blunted cell migration compared to those with empty plasmid (change in open area after 8 hr; Empty: 1±0.05, TIMP-4: 0.8±0.051, $p<0.05$), whereas decreased TIMP-4 expression caused by mutant PPARγ resulted in increased migration (change in open area after 10 hr; GFP: 1±0.25, P467L: 1.63±0.26, $p<0.05$). The significance of TIMP-4 was also underscored during hypertension since the compensatory increase in TIMP-4 during DOCA-salt in NT mesenteric arteries was lost in S-P467L. We identified two highly conserved potential PPAR response elements (PPREs) close to TIMP-4 promoter region using a sequence-based model. Chromatin immunoprecipitation assay showed strong binding of PPARγ at one of the suggested PPREs in SMC (% input±sem; GFP: 37±0.6, P467L: 66±0.7, $p<0.05$ vs. IgG), suggesting that TIMP-4 is a direct target of PPARγ. Our findings highlight one of protective mechanisms of PPARγ during hypertension and provide a novel link between PPARγ and TIMP-4.

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P135

Deletion of the Mas Receptor Aggravates Vascular Dysfunction and the Development of Atherosclerosis Through a NO-dependent Mechanism in Apolipoprotein E-deficient Mice

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Recently, we have shown that chronic Ang-(1-7) treatment acting through the Mas receptor improves vascular dysfunction in atherosclerotic apolipoprotein E-deficient (apoE-KO) mice by increasing NO bioavailability. To test whether deletion of the Mas receptor aggravates atherosclerosis and to examine the underlying mechanism, we generated apoE/Mas-KO mice.
12 weeks old ApoE-KO and apoE/Mas-KO mice fed on a lipid-rich Western diet were treated via osmotic minipumps with either saline or Ang-(1-7) (82 µg/kg/h) for 6 weeks. Aortae were stained with red oil and quantified for atherosclerosis. To examine whether Ang-(1-7) modulates the development of atherosclerosis through a NO dependent mechanism, 8 weeks old apoE-KO mice treated with L-NAME (20mg/kg/d) were infused either with Ang-(1-7) or saline for 6 weeks. Tissue nitrite, a marker for NO generation was measured by HPLC. Endothelial dependent vasodilation and atherosclerosis was significantly impaired in apoE/Mas-KO mice compared to apoE-KO (relative lesion area of the aortic arch: 38.7±3.0 vs. 25.4±2.0%; P<0.01; specific lesion area 11.7±0.9 vs. 8.1±1.0mm2 P<0.01). Moreover, nitrotyrosin and urinary 8-Isoprostane levels, both markers for oxidative stress were significantly increased in apoE/Mas-KO compared to apoE-KO mice. In contrast, chronic Ang-(1-7) treatment attenuated atherosclerotic lesion in apoE-KO (11.1±2.6% vs. 25.4±2.0, P<0.01 and 3.1±0.8mm2 vs. 8.1±1.0, P<0.01) but not in apoE/Mas-KO mice (38.7±3.0 vs. 38.0±14.2% and 11.7±0.9 vs. 11.4±5.0mm2). Additionally, aortic nitrite content was increased in Ang-(1-7) treated apoE-KO compared to untreated apoE-KO mice (180±31 vs. 311±47µM/g, P<0.05). L-NAME treatment increased blood pressure (BP) and reduced aortic nitrite content significantly compared to sham-treated apoE-KO mice. However, Ang-(1-7) treatment did not affect BP (127±3 vs. 128±3mmHg), aortic nitrite (861±16 vs. 1004±174 µM/g) content and the development of atherosclerosis in L-NAME treated apoE-KO mice.
In conclusion, our findings indicate that Ang-(1-7) improves atherosclerosis via Mas receptor activation. Moreover, these effects seems to mediated through a NO-dependent mechanism as Ang-(1-7) failed to affect atherosclerosis in L-NAME treated mice.

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P136

What is the Impact of Arterial Stiffness on Brain's Health

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Arterial stiffness is an important risk factor for cognitive decline. However, its specific effects on brain homeostasis are unknown. Hence, the objective of the study is to explore the effects of arterial stiffness on brain's health, especially on oxidative stress, inflammation, cerebrovascular regulation and cognitive functions.
Approach and Results: Arterial stiffness was induced by applying calcium chloride to carotid arteries of C57BL6 male mice. The control group received sodium chloride. Cerebral inflammation was assessed by quantifying

immunoreactivity to activated glia markers ; Iba-1, CD68 and s100β. Oxidative stress was determined with dihydroethidium. Cerebral blood flow (CBF) was monitored by laser-Doppler flowmetry in anesthetized mice equipped with a cranial window and spatial memory was tested using the Morris water maze. Results show that arterial stiffness activates microglia in the hippocampus, and astrocytes in the hippocampus and the frontal cortex. Superoxide anion production was elevated in the hippocampus of these mice. Arterial stiffness attenuated the CBF increase produced by stimulation of the vibrissae or by topical application of the endothelium-dependent vasodilator acetylcholine. The results indicate learning and spatial memory deficit induced by arterial stiffness in comparison to the sodium chloride group. Conclusions: This study shows that arterial stiffness, induced by carotid calcification, leads to cerebral inflammation and increased oxidative stress mainly in the hippocampus. Arterial stiffness also alters CBF regulation and cognitive functions. This suggests that arterial stiffness has an impact on cerebral homeostasis and should be considered as a therapeutical target for the prevention of cerebral dysfunctions in the aging population.

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P137

Accelerated Arterial Stiffness During the Menopausal Transition - Results from Study of Women's Health Across the Nation

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Background: Arterial stiffness is an independent marker of cardiovascular disease (CVD). We hypothesized that arterial stiffness would increase in women undergoing menopausal (MP) transition.
Methods: 349 healthy, peri-MP participants from SWAN-Heart, an ancillary study of SWAN (Study of Women's Health Across the Nation) were evaluated for subclinical atherosclerosis at baseline and again at 2 years. Subjects using hormone replacement (15) or vasodilators (38) were excluded. Radial tonometry waveforms were digitized and a transfer function was utilized to calculate Aortic Augmentation index, adjusted for heart rate of 75bpm (AIx@75). Pulse wave velocity (PWV) was recorded as carotid-to-femoral arterial pulse propagation time. Carotid intima-media thickness (cIMT) was measured by ultrasound. Women were grouped into pre/early-MP (PMP) and late peri/post-MP (LMP). Kruskal-Wallis test was utilized to compare differences among groups.
Results: Mean duration of follow up was 2.4 years with a retention rate of 69% (203/296). Age at baseline was (mean±SD) 51±3, BMI 29±6; 46 subjects transitioned from PMP to LMP, 82 remained at PMP and 75 at LMP. Peripheral blood pressure (BP) readings did not differ among groups at baseline or follow up (mean, 120/77 mmHg at both). Augmentation index (ΔAIx@75) changed from 35±6 to 40±6%, for a difference of 5±8, for women transitioning from PMP to LMP vs 34±8 to 34±6% in women staying in PMP and 36±8 to 38±9% in women staying at LMP, p=.04. The trend for ΔPWV was

similar in PMP to LMP, changing from 6.9 to 8.4 m/s (Δ = 1.5±2.1) compared to PMP, from 7.4 to 8.3 m/s (Δ = 0.9±1.9) and LMP, from 8 to 8.4 m/s (Δ = 0.4±2.5), p=.19. There was no difference in cIMT change between all groups; from 0.67 to 0.7 mm (Δ = 0.03±0.05) in PMP to LMP, from 0.64 to 0.68 mm (Δ = 0.04±0.05) in PMP and from 0.64 to 0.68 mm (Δ = 0.04±0.07) in LMP.

Conclusion: Augmentation index, a physiologic measure of arterial stiffness, increased through the menopausal transition. There was no significant difference in cIMT change, showing that physiological changes occur before detectable morphologic changes in arterial vasculature in this setting. Changes in arterial stiffness occur first and may mediate the increased CVD risk in women undergoing menopause.

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Novel Arterial Stiffness Index Was Associated with Pulmonary Function

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Introduction

Recent studies have shown that the results of vascular function tests, such as pulse wave velocity or cardio-ankle vascular index (CAVI), were associated with pulmonary function in children or hypertensive patients, and increased CAVI might be correlated with progression of chronic obstructive pulmonary disease. However, the association between vascular function and pulmonary function remains unclear, especially in healthy adult people. Arterial velocity index (AVI) is a novel arterial stiffness index that can be measured more easily than previous methods. The aim of this study was to investigate the association between AVI and pulmonary function test results in the healthy adult population.

Methods

We conducted a cross-sectional survey of healthy adults aged 20 years or older at a single large medical center in Hachinohe, Japan between April 2014 and March 2015. We measured AVI using cuff oscillometry. AVI means the characteristics of pulse waves at higher cuff pressure than systolic BP. The outcome measure was forced expiratory volume in one second (FEV1), which was measured with spirometry. We used log-transformed values of AVI and FEV1, as the distributions of these values were skewed. We calculated a correlation coefficient between AVI and FEV1, and performed multiple linear regression analyses to adjust for effects of age, sex, height, and smoking status.

Results

In total, 777 men and 530 women participated in this study. The mean age of total participants was 44.9 years (SD = 5.9), and the percentage of current smokers was 32.0 % (418 out of 1,307). The mean AVI was 15.6 (SD = 4.9), and the mean FEV1 was 3.13 L (SD = 0.65). AVI was negatively correlated with FEV1 ($r = -0.21$, $p < 0.001$). In fully-adjusted models, AVI was independently associated with FEV1 ($\beta = -0.03$, $p = 0.017$, 95 % CI = −0.06 to −0.01).

Conclusions

Our study showed that an increase of arterial stiffness as assessed via AVI was independently

associated with a decrease in FEV1 in the healthy Japanese population. Further study is warranted to confirm these findings in cohort studies.

M. Okamoto: None. **F. Nakamura:** None. **Y. Kobayashi:** None. **T. Musha:** None.

P139

Ft3 Level Correlates with Arterial Stiffness and Systolic Blood Pressure: A Cross-sectional Study

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Background: Previous studies have suggested that thyroid dysfunction was associated with numerous risk factors of cardiovascular diseases, but whether thyroid function status could influence arterial stiffness and blood pressure (BP) has not been investigated sufficiently.
Methods: From January to December 2011, we enrolled 1732 patients who took physical examination in our center consecutively. The exclusion criteria were: previous histories of cardiovascular and cerebrovascular diseases, current usage of any anti-hypertensive drugs, anti-thyroid drugs, or thyroxin. All patients underwent testing for thyroid function status, brachial-ankle pulse wave velocity (baPWV), and ankle brachial index (ABI). We investigated the relationship between thyroid hormone levels, arterial stiffness markers (baPWV and ABI) and BP.
Results: There were 97 patients (5.6%) who had thyroid dysfunction, including low-T3-syndrome (11 patients, 0.64%), subclinical hypothyroidism (51 patients, 2.94%) and clinical hypothyroidism (19 patients, 1.10%), subclinical hyperthyroidism (16 patients, 0.92%). After adjusting for conventional risk factors (age, gender, smoking, diabetes mellitus, dyslipidemia, previous hypertension), free triiodothyronine (FT3) was negatively correlated with baPWV ($r=-0.482$, $P<0.001$) and positively correlated with ABI ($r=0.290$, $P<0.001$), indicating that the lower FT3 level correlates with arterial stiffening. FT3 also had a moderate negative correlation with systolic BP ($r=-0.375$, $P<0.001$). TSH was significantly associated with baPWV ($r=0.327$, $P=0.002$), but it did not have any relationship with ABI and BP. No significant correlation was found between other thyroid hormone levels (TT4, FT4 and TT3), baPWV, ABI and BP.
Conclusions: Lower FT3 and higher TSH were associated with arterial stiffening markers. In addition, FT3 is negatively correlated with systolic BP. These results indicate that thyroid hormone levels play an important role in arterial stiffness and hypertension. Further study is warranted to investigate whether thyroid hormone therapy could benefit people with arterial stiffening and hypertension.

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P140

Alamandine Signaling in Cardiomyocytes in Health and Disease

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Alamandine is a new component of the renin-angiotesin system generated from angiotensin A or angiotensin-(1–7). Its biological actions include vasodilation, and antihypertensive effects. In the heart, the molecular pathways activated by alamandine have not been characterized. Our goal was to investigate the signaling pathways activated by alamandine in ventricular myocytes isolated from both healthy and hypertensive models. Cardiomyocytes isolated from C57BL/6 mice and from rats that overexpress renin (TGRmRen) were treated with 100nmol/L alamandine. Intracellular nitric oxide (NO) and Ca^{2+} levels were recorded in cells loaded with DAF-FM and Fluo4-AM, respectively. Protein phosphorylation was assessed by western-blot. In cardiomyocytes, alamandine induced an increase in phosphorylation of PDK1 (arbitrary units (a.u.): control=0,20±0.06 versus alamandine=0,42±0.08 n=6, p<0.05) and Akt Ser473 (a.u.: control= 0,40 ±0,01 versus alamandine= 0,52±0,10 n=5 p<0.05). Moreover, alamandine induced a significant increase in NO (Fluorescence a.u.: control=4.8±0.8 n=44 versus alamandine=12.19±1.47 n=85 p<0.05), without any effect on Ca^{2+} transient amplitude. Ventricular myocytes treated with alamandine showed decreased GSK3β phosphorylation (a.u.: control=0,62±0.19 versus alamandine=0,50±0.25 n=5 p<0.05) and increased phosphorylation of ERK1/2 (a.u.: control=0,48±0.24 versus alamandine= 0,80±0.29, n=7 p<0.05) when compared to control. Conversely, TGRmRen cardiomyocytes exposed to alamandine showed increased GSK3β phosphorylation and decreased phosphorylation of ERK1/2. The effect of alamandine on Akt phosphorylation was preserved in TGRmRen cardiomyocytes. Furthermore, alamandine treatment prevented both cardiomyocyte hypertrophy (area (μm^2): control=447±23 n=58; angiotensin II= 609±26 n=70; angiotensin II+alamandine= 491±31 n=45 p<0.05), and nuclear translocation of GRK5 induced by 100nmol/L angiotensin II. Our data show that alamandine signaling in cardiomyocytes changes according to different pathophysiological condition, and includes the activation of cardioprotective pathways. These data highlight the therapeutic potential of alamandine in the heart.

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P141

Cardiac TRH Partly Mediates Angiotensin II-induced Fibrotic and Hypertrophy Effects in "in vivo" and "in vitro" Models

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Cardiac TRH (cTRH) is overexpressed in the hypertrophied ventricle (LV) of the SHR. Additionally in vivo siRNA-TRH treatment induced downregulation of LV-TRH preventing cardiac hypertrophy and fibrosis demonstrating that TRH is involved in hypertrophic and fibrotic processes. Moreover, in a normal heart, the increase of LV TRH expression alone could induce structural changes where fibrosis and hypertrophy could be involved, independently of any other system alterations.
Is well-known the cardiac hypertrophy/ fibrotic effects induced by AII, raising the question of whether specific LV cTRH inhibition might attenuates AII induced cardiac hypertrophy and fibrosis in mice.
We challenged C57 mice with AII (osmotic

pumps,14 days; 2 mg/kg) to induce cardiac hypertrophy vs saline. Groups were divided and , simultaneously to pump surgery, injected intracardiac with siRNA-TRH and siRNA-Con as its control. Body weight, water consume and SABP were measured daily.
As expected, AII significantly increased SABP ($p<0.05$) in both groups treated , although cardiac hypertrophy (heart weight/body weight) was only evident in the group with the cardiac TRH system undamaged, suggesting that the cardiac TRH system function as a necessary mediator of the AII-induced hypertrophic effect.
As hypothesized, we found an AII-induced increase of TRH ($p<0.05$) gene expression (real-t PCR) confirmed by immunofluorescence that was not observed in the group AII+siRNA-TRH demonstrating the specific siRNA treatment efficiency.
Furthermore, AII significantly increase ($p<0.05$) BNP (hypertrophic marker), III collagen and TGFB (fibrosis markers) expressions only in the group with AII with the cardiac TRH system intact. On the contrary, the group with AII and the cTRH system inhibited, shows genes expressions similar to the saline control group. We confirmed these results by immunofluorescence.
Similar fibrotic results were observed with NIH3T3 cell culture where we demonstrated that AII induced TRH gene expression ($p<0.05$) and its inhibition impedes AII-induced increase of TGFB and III/I collagens expressions telling us about the role of the cTRH in the AII fibrosis effects.
Our results point out that the cardiac TRH is involved in the AII-induced hypertrophic and fibrotic effects.

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P142

Regulation of Brain Derived Neurotrophic Fractor (BDNF) Expression by Angiotensin II in the Adrenals and the Brain

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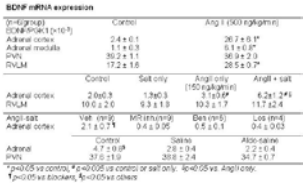
Circulating Ang II activates central angiotensinergic pathways, and an amplifying aldosterone (aldo)-MR-ENaC-AT_1R neuromodulatory pathway which are critical for several forms of hypertension. We hypothesized that in addition Ang II increases BDNF expression in both the CNS and adrenal which plays a balancing role against Ang II excitatory actions. In the 1st exp, Wistar rats were sc infused with Ang II at the high dose of 500 ng/kg/min for 7days. In the 2nd exp, rats were treated with regular salt diet (0.4% NaCl), high salt diet (2% NaCl), sc Ang II at the low dose of 150 ng/kg/min, or sc Ang II with high salt diet for 14 days. In the 3rd exp, MR blockers (eplerenone, spironolactone), ENaC blocker (benzamil), AT_1R blocker (losartan) or vehicles (Veh) were icv infused combined with Ang II-salt. In the 4th exp, rats were sc infused with aldo (1µg/hr) with saline as drinking fluid. mRNA levels of BDNF were measured by real-time qPCR. In the adrenals, Ang II dose-related increased BDNF mRNA, and Ang II-salt further enhanced BDNF mRNA. High dose Ang II increased BDNF mRNA in the RVLM but not in the PVN. Central blockades markedly decreased Ang II-salt induced BDNF expression in the adrenal cortex. In contrast, saline alone or aldo-

saline decreased BDNF mRNA in the adrenal cortex with no effect in the PVN. Central blockades lower Ang II-salt induced BDNF expression in the adrenal, indicating that a central regulatory mechanism plays a role in adrenal BDNF expression. Activation of BDNF by Ang II -modulated by salt-, may provide an important balancing mechanism both centrally and peripherally for Ang II associated hypertension.


BDNF mRNA expression

(n=4/group)	Control	Ang I (500 ng/kg/min)
BDNF/GAPDH (x10⁻³)		
Adrenal cortex	2.4 ± 0.1	26.7 ± 6.1*
Adrenal medulla	1.1 ± 0.3	6.1 ± 0.8*
PVN	39.2 ± 1.1	36.9 ± 2.0
RVLM	17.2 ± 1.6	28.5 ± 0.7*

	Control	Salt only	AngII only (150 ng/kg/min)	AngII + salt
Adrenal cortex	2.0±0.3	1.3±0.3	5.1±0.6*	6.2±1.2*¶
RVLM	10.0 ± 2.0	9.3 ± 1.9	10.3 ± 1.7	11.7 ± 2.4

AngII-salt	Vehicle (n=9)	MR blocker (n=9)	Ben (n=5)	Los (n=4)
Adrenal cortex	2.1 ± 0.7¶	0.4 ± 0.05	0.5 ± 0.1	0.4 ± 0.03

	Control	Saline	Aldo-saline
Adrenal	4.7 ± 0.8¶	2.6 ± 0.4	2.2 ± 0.4
PVN	37.6 ± 1.9	28.8 ± 2.4	34.7 ± 0.7

*p<0.05 vs control, ¶ p<0.05 vs control or salt only; #p<0.05 vs AngII only; ¶p<0.05 vs blockers, §p<0.05 vs others

H. Wang: None. **J. Lu:** None. **M. Ahmad:** None. **F.H. Leenen:** None.

P143

The Angiotensin AT2 Receptor Agonist Compound 21 Is a Low Affinity Thromboxane A2 Receptor Antagonist

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Objective: The purpose of this study was to investigate potential vasorelaxant effects of angiotensin AT2-receptor stimulation by the specific AT2-receptor agonist C21 in pericardial resistance arteries from cardiovascular disease patients.
Methods: Parietal pericardium was obtained during coronary artery bypass-grafting and/or valve replacement in patients. Pericardial arteries from pigs and mesenteric arteries from C57BL-6J and AT2R deficient (AT2R-/y) mice were used for comparison.
Isolated arteries were mounted onto wire myographs, pre-contracted with either K+, phenylephrine, endothelin-1 (ET-1) or the thromboxane agonist U46619, and the relaxing effect of C21 (0.1nM - 10µM) was investigated in the absence or presence of 3nM valsartan (AT1R antagonist), 10µM PD123319 or 10µM M132 (AT2R antagonists).
Results: C21 significantly relaxed ET-1 contracted porcine arteries (Emax -37 ± 6 %; -0.87 vs -0.45 N/m relaxation; N=6; P<0.05, two-way ANOVA) and phenylephrine contracted mouse mesenteric arteries (Emax -52 ± 10 %; -0.26 vs -0.09 N/m relaxation; N=6; P<0.01, two-way ANOVA), and this was inhibited by PD123319 or M132 and absent in arteries from AT2R-/y. C21 significantly induced vasorelaxation in U46619 contracted vessels from all species and vascular beds (e.g. in human pericardial arteries: Emax -78 ± 10 %; -0.67 vs -0.07 N/m relaxation; N=17; P<0.01, two-way ANOVA), and this effect was not blocked by AT2R antagonists and still present in AT2R-/y. C21 further inhibited U46619 induced platelet aggregation. An Arrestin Biosensor Assay revealed that C21 binds to the TXA2 receptor with a Ki of 3.74 µM.
Conclusion: Depended on species, vascular bed and contractile stimulus, C21 relaxes resistance-sized arteries by AT2R stimulation or by TXA2 antagonism. These data together with the low affinity binding of C21 to the TXA2 receptor and its effect on platelet aggregation strongly suggest that C21 is not only a high affinity, selective AT2R agonist, but also a low affinity TXA2 receptor antagonist.

U.M. Steckelings: C. Other Research Support (includes receipt of drugs, supplies, equipment or other in-kind support); Modest; Free drug supply (Compound 21) by Vicore Pharma. **M. Fredgart:** None. **T. Leurgans:** None. **M. Stenelo:** None. **M. Nybo:** None. **L.M. Rasmussen:** None. **J.G.R. De Mey:** None.

P144

Effects of Alamandine in Post-ischemic Function

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Alamandine, a biologically active peptide of the renin-angiotensin system (RAS), was recently described and characterized. Further it has been shown to present effects similar to those elicited by Ang-(1-7). It has been described that Ang-(1-7) decreases the incidence and duration of ischemia-reperfusion arrhythmias and improved the post-ischemic function in isolated perfused rat hearts. In this study we aimed to evaluate the effects of Alamandine in isolated rat hearts subjected to myocardial infarction (MI). Wistar rats weighing between 250-300g were euthanized and their hearts were placed on Langendorff apparatus to evaluate the cardiac parameters. Hearts were submitted to 30min of stabilization, 30min of partial ischemia by occlusion of the left descending coronary artery and 30min of reperfusion. Drugs (alamandine 22pM, d-pro7-ang-(1-7) 220pM) were added to the perfusion setting from the beginning of the experiment until the end. 2,3,5-tryphenyltetrazolium chloride were used to evaluate the extension of infarcted area. In control hearts (CON), there was a decrease on the left ventricular systolic pressure (LVSP) on ischemic period (54,6 ± 6,9mmHg) compared to the baseline period (84,6 ± 11,6mmHg). Alamandine (ALA) attenuated that decrease in the ischemic period (66,9 ± 7,9mmHg) vs (82,3 ± 8,9mmHg). Further, ischemia led to a decrease in the left ventricular developed pressure (dLVP), dP/dt maximum and minimum when compared to baseline values. ALA, once more, kept the ischemic parameters of dLVP and dP/dt max and min (58,9 ± 8mmHg; 1629 ± 202,2mmHg/s; 1101 ± 130mmHg/s, respectively) similar to those of baseline period (68,9 ± 8,92; 1682 ± 248,8; 1179 ± 118,6 mmHg, respectively). Ischemia/reperfusion induced an arrhythmia severity index (ASI) in control hearts (4,9 ± 1,26) higher than in hearts treated with ALA (1,10 ± 0,58). ALA also reduced infarcted area (19,64 ± 2,61%) compared with CON (33,85 ± 4,55%). All those effects were blocked by D-PRO7-Ang-(1-7). In conclusion, our data shown that Alamandine exert cardioprotective effects in post-ischemic function in isolated rat hearts by preventing LVSP, dLVP , dP/dt max and min decrease. Furthermore it reduced the infarcted area and I/R arrhythmias, apparently involving MrgD receptor participation.

J.F.Q. Almeida: None. **R.A.S. Santos:** None.

P145

Immuno-neutralization of Endogenous Ouabain Lowers Blood Pressure in Angiotensin II-dependent Models

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Circulating angiotensin II (Ang II) activates the brain RAAS. The activated brain RAAS increases sympathetic drive and elevates plasma endogenous ouabain (EO). Increased heart rate and vascular constriction as a result of increased sympathetic nerve activity are widely recognized, but the functional significance of the high EO, when Ang II is elevated, is unknown. We employed DigiFab, fab fragments that bind, and selectively immuno-neutralize ouabain, to determine its acute and chronic effects on BP (telemetry) in two rodent models with elevated Ang II: 1. Normal mice infused with Ang II (350 ng/kg/min; sc minipump) and

fed high salt (HS; 4% NaCl) to raise BP; and 2. Normal rats fed low salt (LS; 0.04% NaCl) to activate the peripheral RAAS. CroFab, which immuno-neutralizes crotalus toxin, was used to control for protein and excipients. Neither acute DigiFab nor CroFab (10-40 mg/kg ip) lowered mean daytime BP (MBP) in control mice fed a normal salt (0.4% NaCl) diet. Both Ang II+HS and LS elevated plasma EO (from 0.07±0.09 to 1.30±0.71, *n*=8, and from 0.21±0.05 to 0.70±0.19 nM, *n*=8, respectively, both *P*<0.05; radioimmunoassay). The Ang II+HS increased mouse MBP from 104 ± 1 at baseline, to 123 ± 1 mm Hg (*P*<0.001; *n*=8) at 16 days. Acute DigiFab treatment (10 mg/kg, ip injection at 0 and 4 hrs) reversibly lowered MBP to 112 ± 2 mm Hg (*P*<0.001) within 2-3 hr, but CroFab (10 mg/kg, ip at 0 and 4 hrs) had no effect in the same mice (MBP = 120 ± 2 mm Hg). Chronic DigiFab treatment (sc minipump, 10.7 mg/kg/day x 7 days) in mice with Ang II+HS hypertension (MBP = 133 ± 1 mm Hg; *n*=8) reversibly lowered MBP (125 ± 2 mm Hg, *P*<0.01 vs CroFab, 136 ± 1 mm Hg), When Ang II was elevated by 16 days of low salt in rats, MBP was 107 ± 2 mm Hg. Acute DigiFab treatment (10 mg/kg, ip at 0 and 4 hrs) reversibly lowered MBP to 98 ± 2 mm Hg (*P*<0.001; *n*=6), whereas CroFab (10 mg/kg, ip at 0 and 4 hrs) did not in the same animals (MBP = 106 ± 1 mm Hg). Conclusion: Increased circulating EO augments the elevation of BP in Ang II+HS hypertension, and also helps to sustain BP during salt restriction. EO is a previously unrecognized functional component of the physiological and pathological actions of Ang II.

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P146

Serum Soluble (Pro)Renin Receptor Levels in Patients Undergoing Hemodialysis

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Background: The (pro)renin receptor [(P)RR] plays an important role in regulating the tissue renin-angiotensin system (RAS) through the non-proteolytic activation of prorenin, the precursor of renin. (P)RR is cleaved by furin to generate soluble (P)RR [s(P)RR], which is secreted into the extracellular space, and serum s(P)RR has been reported to reflect the status of tissue RAS. Hemodialysis (HD) patients have poor prognosis due to increased prevalence of cardiovascular diseases resulting from severe atherosclerosis. It is speculated that activation of tissue RAS by (P)RR may be associated with this condition, it remains speculative. In the present study, we investigated the relationships

between serum s(P)RR levels and background factors including indices of atherosclerosis in HD patients. Methods: Serum s(P)RR levels were measured in 258 maintenance HD patients and these values were compared with 25 subjects with normal renal function. Western blot analysis was done using HD waste water samples, and clearance of s(P)RR through the membrane of dialyzer was examined. Furthermore, relationships between serum s(P)RR levels and background factors were assessed in HD patients. Results: Serum s(P)RR levels were significantly higher in HD patients (30.4±6.1 ng/ml) than those in subjects with normal renal function (16.5±4.3, P<0.0001). s(P)RR was detected in HD waste water by Western blot analysis, and the clearance of s(P)RR and creatinine were 56.9±33.5 and 147.6±9.50 ml/min, respectively. Serum (P)RR levels were significantly higher in those with ankle-brachial index (ABI) of < 0.9, an indicator of severe stenosis or obstruction of lower limb arteries, than those of ≧0.9 (32.2±5.9 and 30.1±6.2 ng/ml, respectively; P<0.05). The association between low ABI and high serum s(P)RR levels were observed even after the correction with age, history of smoking, HbA1c, and LDL-C. Conclusions: Serum s(P)RR levels are significantly higher in HD patients when compared with subjects with normal renal function, although s(P)RR are dialyzed to some extent. High serum s(P)RR levels may be associated with atherosclerosis independent of other risk factors, suggesting that serum s(P)RR could be used as a marker for atherosclerotic condition in HD patients.

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Office Blood Pressure: An Inadequate Guide to the Diagnosis and Treatment of Hypertension

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Hypertension is a cardiovascular (CV) disease with high risk for CV morbid events (ME) that benefits from anti-hypertensive therapy. Resting blood pressure (BP) >140/90 mmHg serves as the diagnostic criterion for hypertension, and management has been aimed at BP reduction. Progression of CV disease in the absence of elevated blood pressure identifies individuals who might benefit from CV-protective therapy but are not currently being recognized as in need of treatment.
In 2017 asymptomatic individuals evaluated for early functional and structural CV abnormalities, 1534 not taking anti-hypertensive drugs were available to determine the relationship between office blood pressure and the severity of CV abnormalities, as defined by a 10-test non-invasive disease score (DS) of 0-20. Previous studies have documented the high predictive value of DS for future CVME. The population was 53% male, average age 50±11 years, BP 122/77mmHg, LDL cholesterol 129±38 mg/dL, HDL 52±17mg/dL, triglycerides 109 mg/dL. DS was adjusted by eliminating the score for BP, but 9-test DS was still directly related to BP: 2.3 in those (n=550) <120/80 mmHg (Group I), 3.2 in those 120-129/80-85 mmHg (n=600) (Group II), 4.1 in those 130-139/85-89 mmHg (n=236) (Group III), and 5.7 in those 140+/90+mmHg (n=148) (Group IV). Nonetheless, DS of >6 indicative of high risk was present in 10% of Group I, 20% of Group II and 30% of Group III. BP was largely overlapping in

individuals with no CV disease (DS 0-2), early disease (DS 3-5) and advanced disease (DS 6+). Therefore, reliance on resting BP leaves many at-risk individuals undiagnosed and untreated for early CV disease likely to progress. The hypertensive state exists in the absence of elevated BP and should be recognized and treated to prevent CVME.

J. Cohn: F. Ownership Interest (includes any stock, stock option, partnership, membership or other equity position in an entity regardless of the form of the entity, or any option or right to acquire such position, and any rights in any patent or other intellectu; Significant; Cardiology Prevention, LLC. **S. Duval:** None. **N. Florea:** None. **L. Hoke:** None. **D. Duprez:** None.

P148

Ambulatory Blood Pressure in Hypertensive Patients Treated with Different 2-Drug and 3-Drug Combinations

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An elevated proportion of patients require 2-drug and 3-drug combinations to achieve BP control. There is scarce evidence regarding if there are differences in such BP control among different types of combinations in daily practice. We aimed to assess office and ambulatory BP values achieved, as well as the proportion of controlled patients, depending on the type of drug combination used.
From the Spanish ABPM Registry we selected 17187 patients treated with the 6 most common types of 2-drug combinations and 9724 treated with the 6 most common types of 3-drug combination. We looked for differences in achieved office and ambulatory BP, as well as nocturnal dip, and the proportion of controlled patients among types of combinations, after adjusting for confounders (age, gender, BMI, smoking, diabetes, dyslipidemia, and previous CV disease).
With respect to the combination of renin angiotensin system (RAS) blockers and diuretics (reference), none of the other combinations achieved lower BP values or better BP control. Ambulatory BP control (including 24-hour, daytime and nighttime) was worse with combinations of RAS blockers/beta blockers (OR: 1.06; 95%CI: 1.01-1.11), with combinations of calcium channel blockers (CCB)/beta blockers (1.1; 1.04-1.16) and with combinations of RAS blockers and CCB (1.38; 1.23-1.55). Nondipping was also more frequent in combinations other than RAS blockers/diuretics. In patients receiving 3-drug combinations, and with respect to RAS blockers/CCB/diuretic combinations (reference), ambulatory BP were higher and non dipping more frequent in other types of combinations. Ambulatory BP control was worse in RAS blockers/CCB/alpha blockers, RAS blockers/diuretics/alpha blockers, and CCB/beta blockers/diuretics combinations. No differences in office BP control were observed among types of 2-drug or 3-drug combinations.
We conclude that RAS/diuretic combinations and RAS/diuretic/CCB combinations are associated with better ambulatory BP control and more pronounced dipping in comparison with other types of 2-drug or 3-drug combinations, even with same rates of office BP control. These results can be helpful in deciding the way to combine antihypertensive agents in patients who require combination therapy.

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P149

Distinct Profiles of Brain Medullary Metabolites Detected by ^{1}H-Magnetic Resonance Spectroscopy Correlate with Indices of Autonomic Function and Visceral Fat in Healthy Adults

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Higher forebrain myoinositol (mIns), a marker of glial inflammation/proliferation as detected by proton magnetic resonance spectroscopy (MRS), correlates with systemic inflammation. Elevated circulating markers of inflammation are associated with lower baroreceptor reflex sensitivity (BRS) and heart rate variability (HRV) as well as visceral fat or central obesity. To determine whether transmitters/metabolites in a cardiovascularly (CV) relevant brain region correlate with age-related declines in BRS and HRV and markers of abdominal fat, autonomic profiles were determined by spectral and sequence analysis from continuous blood pressure and HR values in the supine position of healthy subjects 22 - 76 yrs old (12F, 4M); subjects later underwent a single voxel (10 x 7 x 20mm) proton MRS scan of dorsal medulla on a 3T magnet (n = 11; 9F, 2M) and measures of abdominal fat by computerized tomography (CT) (n = 9; 7F,2M). The mean arterial pressure was 88 ± 3 mm Hg, HR 64 ± 3 beats/min and BMI 27 ± 1 kg/m^2. Glutamate (Glu) correlated directly with vagal (HF_{RRI} r = 0.72, p < 0.02) and inversely with sympathetic (LF_{RRI} r = -0.72, p < 0.02) control of HR. Markers of Glu metabolism and neuronal integrity/activity, N-acetyl-aspartate acid (NAA) + N-acetyl aspartyl glutamate (NAAG), did not correlate with age, but did correlate inversely with BRS (Seq ALL: r = -0.69, P < 0.02), HRV (rMSSD: r = -0.76, p < 0.008) and directly with HR (r = 0.68, p < 0.03). Total visceral fat had a negative correlation with BRS (Seq Up: r = -0.70, p < 0.02). mIns and markers of demyelination and reduced axonal integrity such as Glycerophosphocholine (GPC) and total choline containing compounds (GPC+PCh) exhibited striking positive correlations with percent visceral fat (r = 0.97, 0.81 and 0.82, P < 0.02). BMI and GPC correlated with HR (r = 0.55, 0.72, p < 0.04), but neither these nor mIns or choline compounds correlated with autonomic function. Thus, in healthy adults, Glu concentration in dorsal medulla directly correlates with cardiac vagal function, whereas markers of Glu metabolism inversely correlate with BRS and HRV. In contrast, markers of glial inflammation directly associate with increases in visceral adiposity, but not autonomic dysfunction. P30-AG21332, Farley Hudson, Hypertension & Vasc Res Ctr

D.I. Diz: None. **J. Olson:** None. **J. Bourland:** None. **D. Kitzman:** None. **J. Carr:** None. **S.B. Kritchevsky:** None. **L. Groban:** None. **H.A. Shaltout:** None.

P150

Podocyte Derived Urinary Microparticles Are Elevated in Angiotensin II Induced Hypertension

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Background: Early and non-invasive biomarkers of kidney damage are needed to identify hypertensive patients at risk for kidney damage. Urinary micropaticles (UMPs) have gained significant attention as potential novel biomarkers for kidney damage, and have already been identified in pre-albuminuric diabetic glomerular injury. These vesicles are less than 1 micron in size and carry markers of the parent cell. We hypothesized that podocyte derived UMPs are elevated in angiotensin II-induced hypertension (HTN)
Methods: Primary podocytes were isolated and grown in culture. Wild-type mice were treated with AII (400ng/kg/min) via mini-osmotic pumps. Untreated WT mice served as controls. 24 hour urines were collected after 5 days of AII treatment. Enumeration and phenotyping of MPs was done of podocyte culture supernatant and urine. Podocalyxin (Pcal), podoplanin (Ppla) and annexin 5 (AV) were used as surface markers.
Result: Pcal and Ppla positive MPs as well as AV positive and negative MPs were detectable in supernatant from primary podocyte cultures. Compared to untreated controls (n=3), AII treated mice (n=2) had an increase in systolic blood pressure (SBP) by 33 mmHG (p=0.02). Despite similar urinary albumin/creatinine ratios between groups, there was a trend of higher levels of total numbers of Ppla and Pcal positive MPs in hypertensive mice compared to untreated (Figure 1). In addition, Annexin negative but Ppla and Pcal positive MPs were also numerically higher in hypertensive mice.
In conclusion, podocyte derived UMPs are detectable in AII HTN. These findings need to be confirmed in a larger group of animals. UMPs can be potential marker for kidney end-organ damage in HTN.
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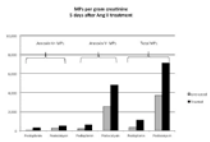

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P152

Acute Effect of Unilateral Unipolar Electrical Carotid Sinus Stimulation in Patients with Treatment-Resistant Arterial Hypertension

Karsten Heusser, Julia Brinkmann, Jan Menne, Jessica Kaufeld, Silvia Linnenweber-Held, Hannover Med Sch, Hannover, Germany; Joachim Beige, Hosp St. Georg, Leipzig, Germany; Mathias Wilhelmi, Hannover Med Sch, Hannover, Germany; André Diedrich, Vanderbilt Univ, Nashville, TN; Hermann Haller, **Jens Jordan**, Jens Tank, Hannover Med Sch, Hannover, Germany

Electrical carotid sinus stimulation has been developed for treatment of resistant arterial hypertension. The first-generation device (Rheos™) relying on bilateral placement of bipolar electrodes acutely reduced muscle sympathetic nerve activity (MSNA) and blood pressure (BP) but is no longer available. The second-generation device (Neo™) utilizes a smaller unilateral unipolar disk electrode to reduce invasiveness while saving battery life. We tested acute effects of the latter on BP and MSNA.
We studied 18 treatment-resistant hypertensive patients (9 women, 53±11 years, 34±5 kg/m²) on stable medication who had been implanted with the second-generation device. We

assessed acute BP (oscillometry), heart rate (HR, ECG), and MSNA (microneurography) responses to electrical stimulation in the supine position.
Without stimulation, BP was 165±31/91±18 mmHg, HR was 75±17 bpm, and MSNA was 48±14 bursts/min. Acute stimulation with intensities producing side effects that were tolerable in the short term elicited variable changes in systolic BP (SBP: -16.9±15.0 mmHg, range: 0.0 to -40.8 mmHg, p=0.002), HR (-3.6±3.6 bpm, p=0.004), and MSNA (-1.9±5.3 bursts/min, p=0.194). Stimulation intensities had to be lowered in 12 patients to avoid side effects at the expense of efficacy (SBP: -6.3±7.0 mmHg, range: 2.8 to -14.5 mmHg, p=0.028; HR: -1.5±2.3 bpm, p=0.078; comparison against responses with side effects). Reductions in diastolic BP and MSNA (total activity) tended to be correlated (r^2=0.202, p=0.093).
In our patient cohort, unilateral unipolar electrical baroreflex stimulation acutely lowered BP. Side effects may limit efficacy. The novel approach should be tested in a controlled comparative study.

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P153

Impact of Antibiotic Treatment on Antihypertensive Drug Adherence

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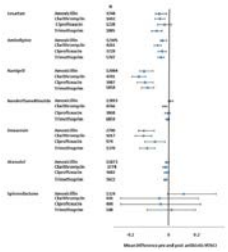
Background
Suboptimal adherence reduces the effectiveness of antihypertensive therapy and is recognised as a significant obstacle in achieving better patient outcomes. The impact of concomitant therapy on adherence levels is unknown. The aim of this study was to identify changes in antihypertensive drug adherence after antibiotic treatment.
Methods
We analysed refill prescriptions (2004-2013) from 361,021 patients who attended 2 hospitals in Glasgow. Antihypertensive drug adherence over the preceding and following 12 months of first antibiotic therapy in each patient were analysed. Drug adherence was measured as drug usage over the study period and calculated from the amount of drug dispensed and the defined daily dose for each antihypertensive drug. Comparisons were made using paired-t tests.
Results
There were 190,699 subjects prescribed amoxicillin, 63,292 ciprofloxacin, 69,756 clarithromycin and 94,832 trimethoprim. The mean difference in adherence for each antihypertensive drug before and after

amoxicillin, clarithromycin, ciprofloxacin and trimethoprim are presented in the Figure. There is a general reduction in adherence following any antibiotic therapy for the antihypertensive drugs studied, with bendrofluomethiazide and atenolol showing the least changes in adherence and doxazosin and ramipril associated with the highest decreases in adherence. No differences between antibiotics was observed for any antihypertensive drug.

Conclusion

A single course of antibiotic therapy can have a sustained adverse impact on antihypertensive drug adherence over the following year. The impact of this on BP control and outcomes need to be further study.



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P154

Ultrasound Imaging for Serial Measurements of Venous Diameters in Rats

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The purpose of our study was to investigate serial ultrasound imaging in rats as a means to quantify the diameters of splanchnic veins in real time and the effect of drugs on venous capacitance. A 21 MHz probe (Vevo 2100 imaging system,Visual Sonics Inc.) was used to collect images containing the portal vein (PV) and the superior mesenteric vein (SMV) in anesthetized male Sprague-Dawley rats maintained at 37°C. Stable landmarks were established and we were able to repeatedly locate specific cross-sections of PV and SMV. When controlled for respiratory and cardiac cycles during measurements, respective diameters of these vessels remained within 0.75±0.15% and 0.2±0.10% of baseline (PV: 2.02±0.15 mm; SMV: 1.67±0.05 mm) when located and measured every 5 minutes over 45 minutes (n=3 rats). PV and SMV remained within 1.0±0.6% and 0.38±0.9% from baseline, respectively, when measured on separate days over 10 weeks in a preliminary study using 2 rats. The consistency of raw vessel measurements allowed these vessels to serve as their own control during subchronic pharmacologic interventions.

In a second study, the vasodilator sodium nitroprusside (2 mg/kg, i.v. bolus) was administered to anesthetized rats (n=3) following collection of baseline vessel measurements. PV and SMV diameters increased 37.23±2.4% and 29.77±8.8% from baseline by 30 minutes post drug administration while mean arterial pressure decreased 10.32±1.7 mmHg. Conversely, the administration of the venoconstrictor sarafotoxin (S6C) (5 ng/kg, i.v. bolus) to other anesthetized rats (n=3) decreased PV and SMV diameters 22.10±2.4% and 9.44±1.6% from baseline within 5 minutes, associated with an increase in mean arterial pressure of 12.85±3.2 mmHg.

Together these results support serial ultrasound imaging as a reliable technique to accurately measure acute and subchronic changes in the diameter of splanchnic veins concurrent with blood pressure changes in intact rats. The ability to follow rat abdominal vein diameters in real

time will assist in determining the role of the venous circulation in blood pressure regulation.

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P155

Inadequate Blood Pressure Control in Hypertensive Patients Referred for Cardiac Stress Test

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Introduction: Hypertension (HTN) is a powerful risk factor for fatal and nonfatal cardiovascular events. Achieving adequate blood pressure (BP) control can reduce morbidity and mortality from cardiovascular diseases.The current study examined the degree of BP control and incidence of myocardial ischemia in hypertensive patients referred for cardiac stress test.
Methods: We retrospectively analyzed 2,039 consecutive patients with the diagnosis of HTN referred to New York Hospital Medical Center of Queens/Weill Cornell Medical College nuclear cardiology laboratory for stress testing from January 2007 through July 2010. Patients were categorized into well-controlled (<140/90 mmHg), poorly-controlled (140-160/90-100 mmHg), and very poorly-controlled (>160/100 mmHg) groups according to their resting BP measured by an IntelliVue MP70 (Royal Philips Electronics, the Netherlands) non-invasive oscillometric BP monitor. The incidence of ischemia was defined as the presence of at least one reversible perfusion defect on stress/rest single photon emission computed tomography scan
Results: Mean age [±SEM] = 68 ± 13 years, 885 (43.4%) were males. Prevalence of well-controlled HTN was 47.2%, poorly-controlled HTN, 29.5% and very poorly-controlled HTN, 23.3%. Evidence of ischemia was seen in 19.8% and 19.3% of the well-controlled and poorly-controlled group respectively. The very poorly-controlled group had the lowest incidence of ischemia (14.3%) ($p < 0.05$) compared to the other two groups.
Conclusions: Symptoms mimicking ischemic heart disease in hypertensive patients may be partly explained by poorly controlled BP. Quality of care might be improved by optimally controlling BP in patient with angina symptoms prior to ordering diagnostic testing associated with radiation exposure and cost

T.M. Mousa: None. **O.A. Akinseye:** None. **T.C. Kerwin:** None.

P156

A Novel Gene-based Tool to Predict the Risk of Essential Hypertension and Initial Validation

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Background
According to the CDC, 70 million American adults suffer from high blood pressure. With hypertension costing the United States $46 billion annually, efforts to screen, prevent, and treat hypertension are warranted.
Objective

To create and evaluate the ability of a novel scoring algorithm derived from genotypic and phenotypic factors to predict the risk of essential hypertension.
Subjects:
For this multi-center, observational, retrospective study, 462 subjects were chosen from 14 clinical research sites across the U.S from the period of September 30, 2014 to December 17, 2014. The first study group of 126 subjects had a diagnosis of essential hypertension (ICD-9 codes 401, 401.1, and 401.9); and the first study set of 131 controls had no diagnosis of essential hypertension. The second study consisted of 95 subjects with a diagnosis of essential hypertension and 110 controls were used.
Methods:
Subjects were genotyped using TaqMan® SNP genotyping assays (Life Technologies, Carlsbad, CA). A scoring algorithm was developed using a logistic regression model with age as a phenotypic factor and 6 single nucleotide polymorphisms (COMT rs4680; PTGS1 rs1330344; SHMT1 rs1979277; LEPR rs1137101; and VKORC1 rs8050894). A second study was conducted to independently apply the algorithm and validate results.
Results:
For the first study group, the receiver operating characteristic (ROC) for the algorithm was statistically significant (AUC 0.917, p=0.000). From a scoring range of 0 - 17, 8.5 was determined to be the best cut-off score indicating a high risk of essential hypertension with a sensitivity of 83.17% (84 of 101); specificity 91.45% (107 of 117); PPV 89.36% (84 of 94); and NPV 86.29% (107 of 124). For the second study group, the ROC was also statistically significant (AUC 0.969, p=0.000). As in the first study group, a score of 8.5 was the best cut-off score for essential hypertension risk with a sensitivity of 91.58% (87 of 95); specificity 95.45% (105 of 110); PPV 94.57% (87 of 92); and NPV 92.92% (105 of 113).
Conclusion:
This scoring algorithm can reliably predict the risk of essential hypertension in two separate study groups. Implementing such a tool in clinical practice may guide treatment decisions for patients at risk of essential hypertension.

T. Onojighofia: None. **N. Anand:** None. **B. Meshkin:** A. Employment; Modest; I am the CEO of Proove Biosciences. F. Ownership Interest (includes any stock, stock option, partnership, membership or other equity position in an entity regardless of the form of the entity, or any option or right to acquire such position, and any rights in any patent or other intellectu; Modest; I own stocks in Proove Biosciences. **S. Silverman:** None. **D. Holman:** None. **J. Hubbard:** None. **M. Hafez:** None. **S. Kantorovich:** None.

P157

Tractography of White Matter Connections Predicts for Vascular Cognitive Impairment in Hypertensive Patients

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Vascular cognitive impairment (VCI) results by several vascular risk factors and, particularly, hypertension (HTN). The identification of early changes associated with later development of dementia is demanding. Great part of research has primarily focused on brain changes occuring in grey matter. However, more recent data highlighted that HTN may determine cognitive

decline, even before manifest neurodegeneration. Diffusion tensor imaging (DTI) on magnetic resonance, opened the possibility to predict white matter connections that correlate with specific cognitive functions. In this study, we used DTI and cognitive assessment (CA), in order to identify a regional pattern of fractional anisotropy (FA) changes that could predict for VCI in hypertensive patients (HT).
We have examined 15 HT (moderate to severe, with antihypertensive medications) vs 15 normotensive (NT), subjecting them to DTI and CA. HT had significant higher SBP (138±4 vs 118±3 in NT) and DBP (87±2 vs 75±2 in NT) ($p<0.001$), displayed a significant LV hypertrophic remodeling (LVM/BSA 112±5 vs 83±3 for NT) ($p<0.0001$), with a significant moderate increase in albuminuria (15.7±2.6 mg/24h vs 8.8±1.6 for NT) ($p<0.03$). When subjected to CA, HT had significantly worsen performance on both MoCA (22.66±0.97 vs 26.21±0.57 NT) and Stroop Test (34.50±3.87 vs 17.75±2.57 NT) ($p<0.01$). Conversely, tests regarding Verbal Fluency and Instrumental Activities of Daily Living revealed normal performance of HT, thus indicating a selective impairment of memory. Brain imaging showed that, while none of the patients had abnormal signal intensity on T1/T2-weighted MRI,
DTI indices FA were significantly reduced in HT as vs NT. In particular, HT had lower FA in projection fibers related to impairment for non-verbal materials (Anterior Thalamic Radiation: 0.358±0.012 vs 0.330±0.006, $p<0.05$), association fibers involved in executive functioning and emotional regulation (Superior Longitudinal Fasciculus: 0.388±0.013 vs 0.356±0.007, $p<0.05$), limbic system fibers involved in attention tasks (cingulate gyrus: 0.364±0.009 vs 0.328±0.010, $p<0.01$).
Our data highlight a novel paradigm of combined DTI/CA of HT patients, capable to identify, with great sensitivity, predictive signs of HTN-induced VCI.

L. Carnevale: None. **G. Selvetella:** None. **D. Cugino:** None. **G. Grillea:** None. **G. Lembo:** None. **D. Carnevale:** None.

P158

Increased Circulating Cathepsin K in Patients with Chronic Heart Failure

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Background: Cysteinyl cathepsin K (CatK) is one of the most potent mammalian collagenases involved in cardiovascular disease. We investigated the clinical predictive value of serum CatK levels in patients with chronic heart failure (CHF).
Methods and Results: We examined 134 patients with CHF, measuring their serum CatK, troponin I, high-sensitive C-reactive protein, and pre-operative N-terminal pro-brain natriuretic peptide levels. The patients were divided into two groups: the 44 patients who showed a left ventricular (LV) ejection fraction (LVEF) < 40% (the “lowLVEF” group) and the 90 patients showing LVEF values ≥ 40% (the “highLVEF” group). The lowLVEF patients had significantly higher serum CatK levels compared to the highLVEF patients (58.4 ± 12.2 vs. 44.7 ± 16.4, $P < 0.001$). Overall, a linear regression analysis showed that CatK levels correlated negatively with LVEF ($r = -0.4$, $P < 0.001$) and positively with LV end-diastolic dimensions ($r = 0.2$, $P < 0.01$), LV end-systolic dimensions ($r = 0.3$, $P < 0.001$), and left atrial diameters ($r = 0.2$, $P < 0.01$). A multiple logistic regression analysis

showed that CatK levels were independent predictors of CHF (odds ratio, 0.90; 95% confidence interval, 0.84-0.95; P = 0.001). Conclusions: These data indicate that elevated levels of CatK are closely associated with the presence of CHF and that the measurement of circulating CatK provides a noninvasive method of documenting and monitoring the extent of cardiac remodeling and dysfunction in patients with CHF.

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P159

Effect of Pharmacological Kinin Receptor Activation on Brain Damage and Mortality in Experimental Cerebral Ischemia in Non-diabetic and Diabetic Mice

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Brain ischemia is a major complication of arterial diseases and has a poorer prognosis in diabetic patients. As activation of the kallikrein-kinin system has been shown to enhance cardiac and renal tolerance to ischemia we tested effect of kinin receptor activation by pharmacological agonists, selective B1R or B2R, in a mouse model of transient middle cerebral artery occlusion [C57bl6 male, 10 week-old, anaesthesia, occlusion 60 min (MCAO)]. Treatment with the B1R agonist NG29 (B1Rago) or the B2R agonist NG291 (B2Rago) was started at reperfusion using osmotic micropumps. Neurological deficit (ND) was evaluated at 1 and 2 days using a panel of 8 established tests combined in a 0-30 deficit score. Brain infarction was quantified at day 2 using TTC and hematoxylin-eosin staining. In some mice diabetes was induced by streptozotocin 8 weeks before MCAO.

MCAO induced bradychardia, mild hypotension (mean -11.3 mmHg), ND (19.7 ± 2.4) and resulted in partial brain infarction (18 ± 1.4 %), all $p<0.05$ compared to sham, n=10/group. B2Rago (720 nmol/kg.day-1) increased ND to 27 ± 1.8 at day 1 and mortality to 60% at day 2 (both $p<0.05$, n=10) while decreasing brain infarct size by 66% ($p<0.01$). B2Rago reduced BP by 16 mmHg and increased plasma creatinine (73 ± 25 µmol/l, $p<0.05$). Although B1R mRNA level increased by 1.3 fold in the ischemic brain B1Rago had no effect on ND, mortality or brain infarction.

In diabetic mice MCAO increased ND (28 ± 1), mortality (25%) and infarct size (40 ± 3 %) more than in non-diabetic mice (n=8, $p<0.05$). B2Rago increased mortality to 80% ($p<0.05$, n=9). B1Rago, tested at two different dosages (720 or 240 nmol/kg.day-1, n= 8/group) reduced ND (22 ± 2 at day 2 for the low dosage, $p< 0.05$) and did not increase mortality or alter renal function. B1Rago reduced infarct size by 66 and 71 %, at the two dosages, respectively ($p<0.01$).

Thus, B2R activation reduces brain infarction but paradoxically increases mortality by mechanisms that may involve brain oedema and renal insufficiency. B1R activation has no effect in non-diabetic mice but in diabetic animals it reduces infarct size and improves ND without adverse effect on renal function and survival. Longer follow-up studies are in progress for further evaluating interest and limitation of B1R activation in MCAO.

D. Desposito: None. **C. Taveau:** None. **G. Zadigue:** None. **C. Adam:** None. **N. Bouby:** None. **F. Alhenc-Gelas:** None. **R. Roussel:** None.

P160

Role of Uncoupling Protein 2 in Stroke Susceptibility of Stroke-prone Spontaneously Hypertensive Rat

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Mitochondrial dysfunction causes severe cellular derangements potentially underlying tissue injury and consequent diseases. Evidence of a direct involvement of mitochondrial dysfunction in hypertensive target organ damage is still poor.
The gene encoding Uncoupling Protein 2 (UCP2), a inner mitochondrial membrane protein, maps inside stroke QTL/STR1 in stroke prone spontaneously hypertensive rat (SHRSP). We explored the role of UCP2 in stroke pathogenesis of SHRSP. Male SHRSP, stroke resistant SHR (SHRSR) and reciprocal STR1/congenic rats were fed with stroke permissive Japanese style diet (JD). A group of SHRSP received JD plus fenofibrate (150 mg/kg/die). Rats were sacrificed at stroke occurrence. Additional SHRSR and SHRSP rats were sacrificed at 1, 3, 6, 12 months of age upon regular diet. SBP, BW, proteinuria, stroke signs were monitored. Brains were used for molecular analysis (UCP2 gene and protein expression, Nf-kB protein expression, oxidative stress quantification) and for histological analyses.
As a result, brain UCP2 expression was reduced to 20% by JD only in SHRSP (showing 100% stroke occurrence by 7 weeks of JD). Fenofibrate protected SHRSP from stroke and upregulated brain UCP2 (+ 100%). Congenic rats carrying STR1/QTL showed increased (+100%) brain UCP2 expression, as compared to SHRSP, when resistant to stroke, and, viceversa, decreased (-50%) brain UCP2 levels, as compared to SHRSR, when susceptible to stroke. Brain UCP2 expression progressively decreased with aging only in SHRSP, down to 15% level at one year of age (when SHRSP showed spontaneous stroke). Both brain Nf-kB expression and oxidative stress levels increased when UCP2 expression was downregulated, and viceversa. Histological analysis showed both ischemic and haemorrhagic lesions at stroke occurrence.
Our results highlight a role of UCP2 in stroke predisposition associated to hypertension in an animal model of complex human disease.

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P161

Angiotensin Receptor Blockade Decreases Glutamate-induced Brain Inflammation

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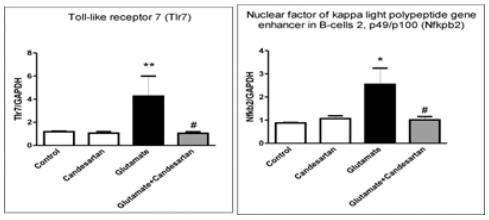
Chronic Kidney Disease (CKD) is very frequently associated with brain inflammation and cell injury leading to cognitive loss. At present a combined treatment of related kidney and brain injury has never been proposed. We focused on glutamate-induced excitotoxicity and neuronal inflammation, and on the effects of candesartan, a renoprotective Angiotensin Receptor Blocker (ARB) ameliorating hypertension and diabetes-induced kidney

damage, and with accompanying neuroprotective efficacy. Primary cerebellar granule cells (CGC) were exposed to 100 µM glutamate and pre-treated for one hour with candesartan at neuroprotective concentrations (10 µM). Gene expression was quantified by qPCR. Candesartan significantly reduced glutamate-induced inflammation. Multiple group comparisons were performed by one-way ANOVA followed by Newman-Keuls post-test. Exposure to glutamate significantly reduced neuronal viability while up-regulating the expression of multiple genes on pro-inflammatory pathways, including Toll-like receptor 7 (Tlr7) and Nuclear factor of kappa light polypeptide gene enhancer in B-cells 2, p49/p100 (Nfkpb2). * p<0.01, ** p< 0.05, vs control; # p< 0.01 vs glutamate. In all cases, pretreatment with candesartan completely prevented glutamate-induced neurotoxicity. Our results indicate that Angiotensin II receptor blockade with candesartan is strongly and directly neuroprotective, significantly ameliorating neuronal injury as a result of glutamate excitotoxicity. These results support the use of candesartan and other ARBs for the concomitant treatment of CKD and associated neuronal injury.



J. Saavedra: None. **A.G. Elkahloun:** None. **R. Hafko:** None.

P162

Nicotine via Cd36, Cox2 and Oxidative Stress Promotes Podocyte Injury: Link Between E-cigarettes and Renal Disease Progression?

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Cigarette smoking (CS) accounts for 175,000 annual CV deaths in US. CKD is a major CV risk factor. Epidemiologically link between CS proteinuria and progression of diabetic and of hypertensive nephropathy is documented. We showed that Nicotine (NIC) in concentrations achieved in CS and E- smokers a)Increased proteinuria (100%) renal Nox4 & Nitrotyrosine in diabetic db/db mice (AJP '10) and b) Promoted in human macrophages O_2^- production and foam cell formation associated with upregulation of B scavenger receptor CD36 and oxLDL uptake (AJP'13). Podocytes (POD) are vulnerable to diabetes and to hypertension; POD injury results in detachment and glomerulosclerosis. We demonstrated in human POD NIC receptors α2, 3,4 and β3 **Methods:** We treated human POD with NIC, 100nmol/L, a concentration attained in serum of CS and E-Cigarettes smokers, and determined O_2^- production with lucigening; some POD were pre-incubated with Hexametonium: blocker of NIC receptors; DPI: inhibitor of NADPH; Catalase: inhibitor of H2O2 and AICAR: activator of AMPK, a suppressor of NADPH. By Western Blot (WB) we determined in Control and NIC exposed POD, CD36, COX2 known to induce POD injury (JASN '08) and Synaptopodin (Synpo) a stabilizer of POD actin skeleton. In POD incubated with 50ug/ml oxLDL we measured POD apoptosis (APOPT) by flow cytometry. Stat. ANOVA- Bonferonni's- Scheffe's. **Results:** NIC increased POD O_2^- production 225% (200 to 450 CPM/ug); Synpo expression was reduced 50% (1to 0.5) (p< 0.05). DPI and AICAR prevented Synpo reduction and increase in O_2^- (p<0.05). NIC upregulated expression of COX2 300% (0.5-

1.5) and CD36 40 % (1-1.4) ($p<0.05$). DPI, Catalase, and Hexametonium inhibited CD36 and COX2 upregulation. The COX inhibitor NS-398 prevented O_2^-production and CD36 upregulation .POD incubation with oxLDL plus NIC significantly increased POD oxLDL uptake and APOPT 10% over baseline (2%),($p<0.05$) both inhibited by CD36-SiRNA. **Conclusion:** NIC *activated* in human POD a reciprocal *interplay* between NADPH oxidases and COX2 that increased O2- production, reduced Synpo and upregulated the B scavenger receptor CD36 that, via increased uptake of oxidized LDL, promoted podocyte *apoptosis.* We surmise that these novel findings implicate NIC as a risk factor for CVD and CKD progression.

L. Raij: None. **R. Tian:** None. **M. Zhou:** None.

P163

Endothelin-1 induces Epithelial-Mesenchymal Transition (EMT) in Human Renal Tubular Cells via Activation of RhoA /ROCK Kinase and Inhibition of YAP Pathways

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We tested the hypothesis that tubulo-interstitial fibrosis (TIF), the final outcome of most kidney diseases, involves activation of epithelial mesenchymal transition (EMT) through angiotensin II- and endothelin-1 (ET-1)-mechanisms.
In a transgenic model of fulminant angiotensin II-dependent hypertension with early prominent renal damage, the TG(mRen2)27 rat (TGRen2), we found that the development of renal damage implied a decrease of the epithelial marker E-cadherin and an increase of the mesenchymal markers alpha SMA and vimentin, which indicated the occurrence of EMT. As treatment with the angiotensin II type 1 receptor antagonist irbesartan, or with the mixed ET-1 receptor antagonist bosentan, prevented these changes an involvement of both angiotensin II and ET-1 in EMT is suggested.
To confirm this contention we exposed HK-2 human kidney tubular cells to ET-1. This showed that ET-1 blunted the expression of E-cadherin, increased that of alpha SMA and vimentin, enhanced the synthesis of collagen, and also the activity of metalloproteinases (MMP). These changes implicated activation of Rho-kinase signaling pathway and de-phosphorylation of Yes-associated protein (YAP).
Hence, ex vivo and in vitro experiments demonstrated that EMT is a fundamental process in the TIF that accompanies the development of angiotensin II-dependent hypertension. Moreover, they suggested that EMT involves ET-1 acting via ETA and ETB receptors through activation of Rho-kinase and de-activation of YAP pathways.

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P164

White-coat Hypertension is a Factor Relating Postural Blood Pressure Dysregulation and Cardiovascular Risks

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Backgrounds Postural blood pressure (BP) dysregulation particularly orthostatic hypotension is a phenomenon that is frequently observed in elderly persons and has been reported to be a prognostic factor for incidence of cardiovascular diseases and mortality. White-coat and masked hypertension are another indices of blood pressure dysregulation and were also known to be a risk factor for cardiovascular outcomes. However, relationship between these common phenomena is yet unclear. Here we conducted a cross-sectional study to clarify the relationships.
Methods Study subjects were 818 general persons. Homed BP was calculated from ambulatory monitored BPs as a mean of BPs measured during 1 hour after wake up. Orthostatic BP change was measured at 1 and 3 minutes after standing up and a maximum BP difference was used in the analysis.
Results Home-to-clinic SBP differences were linearly and inversely associated with orthostatic SBP change ($r=-0.321$, $p<0.001$). Therefore, white-coat hypertensive subjects (10.3%, -9.3±13.3 mmHg) showed larger orthostatic BP decline than normotensive subjects (30.9%, -3.0±11.1 mmHg) and masked hypertensive subjects (24.7%, -0.4±12.1 mmHg, $p<0.001$). Home-to-clinic SBP differences ($\beta=-0.150$, $p<0.001$), but not orthostatic SBP change ($\beta=-0.046$, $p=0.169$), was significantly associated with carotid hypertrophy independently of basic covariates including clinic SBP.
Conclusion Home-to-clinic SBP differences may be partially involved in the prognostic significance of orthostatic hypotension.

Y. Tabara: None. **M. Igase:** None. **T. Miki:** None. **K. Kohara:** None.

Incident and Associated Factors of Restenosis After Percutaneous Transluminal Renal Angioplasty in Renovascular Hypertensive Patients

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Background: Percutaneous transluminal renal angioplasty (PTRA) is one of the standard treatments for renal artery stenosis (RAS). Restenosis after PTRA may influence disease prognosis, but little is unknown about the frequency or its associated factors.
Methods: This study included 174 renovascular hypertensive patients (mean age= 59.5 years; 33.9% women) who underwent PTRA and were followed more than 12 months after PTRA. Data collection including blood pressure (BP), intensity of antihypertensive medication, and duplex ultrasonography (DUS) was performed before and 3, 6, and 12 months after PTRA. Cure of hypertension was defines as a BP below 140/90mmHg without antihypertensive medication. Diagnosis of restenosis was based on DUS, by applying a renal aortic ratio >3.5 in conjunction with a renal artery peak systolic velocity > 250cm/s.
Results: At 12 months after PTRA, BP (156±25/82±15 to 133±16/75±13mmHg) as well as antihypertensive medications (2.5±1.2 to 2.1±1.3 types) decreased significantly ($p<0.01$, respectively), and the incident of restenosis was 34 (19.5%). Compared with patients without

restenosis, baseline clinical characteristics in those with restenosis showed significantly younger (46.1±23.6 vs 62.8±16.3 years), higher prevalence of female (50.0 vs 30.0%), fibromuscular dysplasia (FMD) diagnosed by angiography (58.8 vs 23.6%), and balloon PTRA without stenting (44.1 vs 18.6%), and lower numbers of antiplatelet and/or anticoagulant agents administrated after PTRA (1.3±0.6 vs 1.7±0.6 types of drug) ($p<0.05$, respectively). Compared with atherosclerotic RAS patients (n=121), the rates of cured (25.9 vs 9.0%) and the cumulative incident rates of restenosis at 3 (20.8 vs 0 %), 6 (35.9 vs 6.6%), and 12 (37.7 vs 11.6%) months after PTRA were significantly higher in FMD ($p<0.01$, respectively). FMD without restenosis showed a significantly greater decrease in systolic BP than that with (159±21 to 131±17 vs 154±28 to 141±25 mmHg, $p<0.05$), whereas no significant difference was found in atherosclerotic RAS.
Conclusions: Incident of restenosis after PTRA was higher in FMD than in atherosclerotic, and its incident pattern seemed to be different by causes. Especially for FMD, assessment of restenosis is important for treatment success.

Y. Iwashima: None. **T. Fukuda:** None. **F. Yoshihara:** None. **H. Kusunoki:** None. **M. Kishida:** None. **S. Hayashi:** None. **S. Nakamura:** None. **K. Kamide:** None. **T. Horio:** None. **Y. Kawano:** None.

P166

The Effects of V2 Receptor Antagonist Treatment on the Renal Medullary Circulation and Urinary Sodium Excretion in Rat

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Objective; V2 receptor (V2R) antagonist increases aquaresis, and was reported to have renoprotective and natriuretic effect, although the mechanism is not fully clarified. Renal medullary hemodynamics contributes sodium retention and renal injury. Therefore, the present study was designed to evaluate the effect of V2R antagonist on renal medullary blood flow.
Methods; Catheter was inserted in femoral artery and vein of anesthetized SD rats to monitor blood pressure (BP), heart rate (HR) and to infuse drugs, respectively. Renal medullary blood flow (MBF) and renal medullary oxygen pressure (pO2) were measured with laser-Doppler flowmetry or oxygen microelectrode, respectively. V2R antagonist, OPC-31260 (OPC, 0.25mg/kg bw/h) or furosemide (Furo, 0.5mg/kg bw/h) was intravenously administrated for 90min. Urine was collected in 30 min interval and urinary sodium (UNaV), hydrogen peroxide (UH2O2V) and [nitrate + nitrite] (UNOxV) excretion were measured.
Results; OPC and Furo treatment did not change BP and HR. Urine volume was significantly increased by OPC (1.1+0.2 to 6.1+0.5 g/30 min) and Furo (1.4+0.6 to 4.7+0.3 g/30 min) treatment but was not different between groups. MBF was significantly decreased in Furo (12+4% decrease from baseline), while OPC did not changed MBF (1+3% increase from baseline). pO2 was significantly increased by both OPC and Furo treatment (20+6 and 27+10% increase from baseline, respectively). UNaV was significantly increased in OPC (0.10+0.02 to 0.44+0.05 mEq/30 min) and Furo (0.14+0.08 to 0.69+0.06 mEq/30 min) treatment, the increase of UNaV was significantly higher in Furo than OPC group. UH2O2V was significantly increased by Furo treatment (16+4 to 28+6 nmol/30 min), while

did not change in OPC treatment (10+2 to 19+4 nmol/30 min). UNOx was significantly increased in OPC treatment (211+30 to 376+45 nmol/30 min), while did not change in Furo treatment (142+27 to 237+75 nmol/30 min). Conclusion; OPC treatment increased NO production. Increased NO could contribute to decrease of sodium reabsorption, result in increase of renal medullary pO2. This scheme could be one on the mechanisms of renal protective effect by V2R antagonist treatment.

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P167

Conditional Deletion of the Na+/H+ Exchanger 3 in the Proximal Tubule of the Kidney Promotes Pressure Natriuresis in Mice

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The development and progression of most, if not all, forms of hypertension appear to converge on a final common pathway or mechanism, i.e., increased renal salt retention due to significantly impaired pressure natriuresis responses. Yet the factors responsible for resetting the pressure natriuresis response in hypertension remain to be determined. The present study tested the hypothesis that the upregulation of the sodium and hydrogen exchanger 3 (NHE3) impairs while selective knockout of this transporter in the proximal tubule promotes pressure natriuresis and lowers blood pressure in mice. Proximal tubule-specific NHE3 knockout mice (PT-NHE3-KO) were generated by the Cre/Lox approach, and confirmed by complementary PCR, Western blot, and immunofluorescent imaging of NHE3 expression in the proximal tubule of the kidney, respectively. No abnormal histological phenotypes were observed in small intestines and the kidney of PT-NHE3-KO mice. Compared with wild-type mice (WT, n=12), PT-NHE3-KO mice (n=10) had significantly lower basal systolic blood pressure (WT: 116 ± 3 mmHg versus PT-NHE3-KO: 103 ± 3 mmHg, $p<0.01$). Mean intra-arterial pressure was also significantly lower in anesthetized PT-NHE3-KO mice (WT: 91 ± 3 mmHg versus PT-NHE3-KO: 77 ± 5 mmHg, $p<0.01$) (n=8 each). The lower basal blood pressure in PT-NHE3-KO mice was associated with higher basal urine flow (WT: 0.81 ± 0.09 ml/day versus PT-NHE3-KO: 1.12 ± 0.06 ml/day, $p<0.05$), urinary sodium (WT: 120.1 ± 17.5 µmol/day versus PT-NHE3-KO: 197.1 ± 24.5 µmol/day, $p<0.01$), potassium (WT: 171.7 ± 26.0 µmol/day versus PT-NHE3-KO: 310.3 ± 24.3 µmol/day, $p<0.01$), and chloride excretion (WT: 125.7 ± 22.5 µmol/day versus PT-NHE3-KO: 222 ± 29.2 µmol/day, $p<0.05$) (n=8-12 for each group). In response to ~25 mmHg increase in renal perfusion pressure in both strains, urinary sodium excretion was increased by 4-fold in WT mice (n=12, $p<0.01$),

whereas it increased by 7-fold in PT-NHE3-KO mice (n=10, p<0.01). We concluded that NHE3 in the proximal tubule of the kidney plays an important role in the regulation of proximal tubular sodium reabsorption and blood pressure homeostasis, and that selective deletion of NHE3 in the proximal tubule promotes the pressure natriurestic response and lowers blood pressure in mice.

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P168

ChIP-seq Analysis of Genomic Binding Sites for the Transcription Factor Fosl2 in Kidney Collecting Duct Cells

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Kidney collecting duct principal cells play a key role in blood pressure regulation and water balance in part through the action of vasopressin. We integrated data from prior proteomics and transcriptomics studies of cultured mouse mpkCCD cells using Bayes' Rule to rank transcription factors (TFs) likely to be involved in vasopressin-mediated transcriptional regulation. The top ranked TF was the AP-1 component Fos-like 2 (Fosl2) and the third ranked TF was Sox4. We carried out ChIP-seq analysis (HiSeq 2000 sequencer) to identify genomic sites of Fosl2 binding using an antibody successfully employed for ChIP-seq in the Mouse ENCODE Project. mpkCCD cells treated with the vasopressin analog dDAVP or vehicle were separately analyzed. The success of the immunoprecipitation of Fosl2 was confirmed by western blotting and LC-MS/MS of the immuno-precipitated material (ChIP-MS). ChIP-MS identified two different Fosl2 peptides, but there were no peptides corresponding to other AP-1 proteins. ChIP-MS also identified Bub1b, a nuclear protein kinase known to phosphorylate histones, as a Fosl2-interacting protein. Fosl2-binding sites successfully mapped to genes by ChIP-seq included a Fosl2 binding site, centered at 87 bp upstream from the transcription start site [TSS] of Sox4, that was strongly downregulated in response to dDAVP, n=2; confirmed by ChIP-PCR. TF binding site prediction (Genomatix) identifies putative Sox4 binding sites in vasopressin-regulated genes beta-ENaC (-822 bp from TSS) and aquaporin-2 (-515 bp from TSS). These data provide an initial step in identification of the vasopressin-regulated transcriptional network in renal collecting duct cells.

H. Jung: None. **V. Raghuram:** None. **C. Chou:** None. **M. Knepper:** None.

P169

Antenatal Betamethasone Attenuates the Angiotensin-(1-7)/Nitric Oxide Axis in Adult Male but not Female Renal Proximal Tubule Cells

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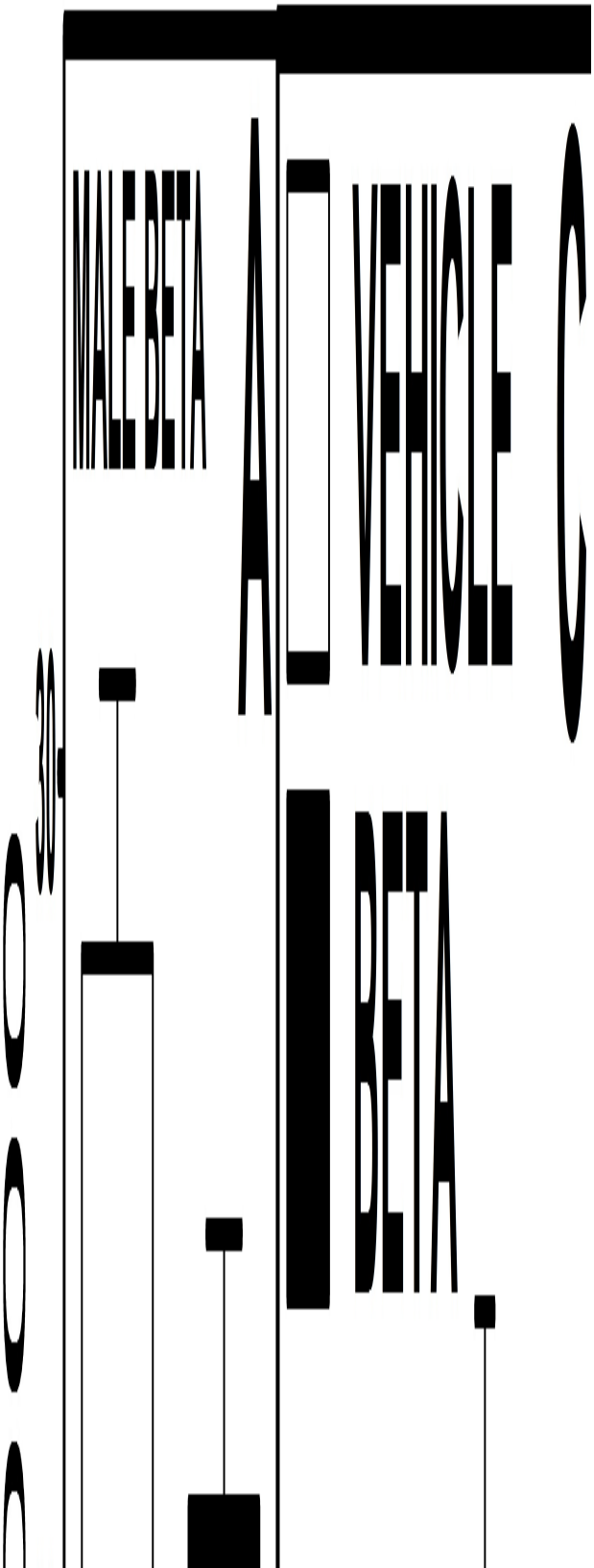
Our studies have revealed a sex-specific effect of fetal programming on sodium (Na+) excretion in adult sheep whereby the males exhibit reduced Na+ excretion and an attenuated natriuretic response to Ang-(1-7) as compared to the females. We hypothesize that the renal proximal tubules are a key target for the early programming effects of glucocorticoids exposure to regulate Na+ handling in the adult males. Therefore, we

isolated and cultured cortical proximal tubule cells (RPTC) from adult male and female sheep antenatally exposed to betamethasone (Beta) or vehicle. Na+ uptake and nitric oxide (NO) were assessed with Sodium Green and DAF fluorescence prior to and following a low dose of Ang-(1-7) (1x10-11 M) in isolated RPTC from sheep at ~1.5 years of age. Data are expressed as % of basal uptake or area under the curve (AUC) for Na+ or % of control for NO. Male Beta RPTC exhibit greater Na+ uptake than male vehicle cells (427±32%, n=13, vs. 315±28%, n=14, $p<0.05$; however, Beta had no effect on Na+ uptake in the female cells (242±18%, n=9, vs. 250±15%, n=10, $p>0.05$). Ang-(1-7) inhibited Na+ uptake in RPTC from vehicle male (255±40%) and from both vehicle (191±14%) and Beta (209±11%) females (Figure 1B), but failed to attenuate Na+ uptake in Beta male cells (Figure 1A). Beta exposure also abolished NO stimulation by Ang-(1-7) in male but not female RPTC (Figure 1C). We conclude that an Ang-(1-7)-NO-dependent pathway contributes to the sex-dependent consequences of programming on Na+ regulation in the proximal tubules of the kidney. Moreover, the RPTC retain both the sex and Beta-induced phenotype of the adult and may reflect an

appropriate cell model of fetal programming.



Y. Su: None. **V.M. Pulgar:** None. **J. Bi:** None. **M. Chappell:** None. **J.C. Rose:** None.

P170

Portfolio Analysis on Sex and Gender Differences Research in Hypertension at the National Heart, Lung and Blood Institute

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Objectives: While epidemiological studies show that blood pressure levels and the prevalence of hypertension are subject to sex-differences, the mechanisms responsible for this sexual dimorphism are poorly understood. To gain a better understanding of funding trends and topics in the field of sex/gender differences in hypertension research, we performed a portfolio analysis of National Heart, Lung and Blood Institute (NHLBI) awards related to “sex differences” active between FY1991-2014. **Analysis:** A list of NHLBI awards (including subprojects) active in FY1991-2014 was obtained through an NIH RePORTER search using the following terms: “sex differences”, “gender differences”, “X chromosome”, “Y chromosome”, “sex hormones”, “preeclampsia”, “pregnancy hypertension” and “menopause”. The abstracts and specific aims of all the awards were read by staff in order to eliminate “false positives” (grants unrelated to sex/gender differences). Applications were further analyzed and categorized according to specific disease focus (e.g. hypertension, cardiovascular disease, preeclampsia etc.). **Results:** The number of NHLBI research awards related to sex and gender differences in hypertension being funded has progressively increased from FY 1991 (40 awards) to 2014 (140 awards), for a cumulative total of 2231 awards. Similarly, the overall dollar investment

has also progressively increased from $6.1 million (FY 1991) to $76.3 million (FY2014), for a cumulative $837 million. According to the disease focus, in FY 2014, only 15% of active awards directly relate to sex/gender differences in hypertension, with 55% of awards relating to cardiovascular disease, 19% to pregnancy-related hypertension and 11% to other diseases (e.g. pulmonary hypertension).
Conclusions: NHLBI is funding an increasing number of awards related to sex/gender differences in hypertension. However, the majority of these awards are only broadly related to hypertension per se, more being related to other cardiovascular diseases and pregnancy-related conditions. Our findings warrant more detailed analyses to identify potential knowledge gaps in need of support to further this important research field.

C. Maric-Bilkan: A. Employment; Modest; Government Employee. **Z. Galis:** A. Employment; Modest; Government Employee.

P171

Mast Cell Inhibition Attenuates Cardiac Remodeling and Diastolic Dysfunction in Ovariectomized, Middle-aged Fischer344×Brown Norway Rats

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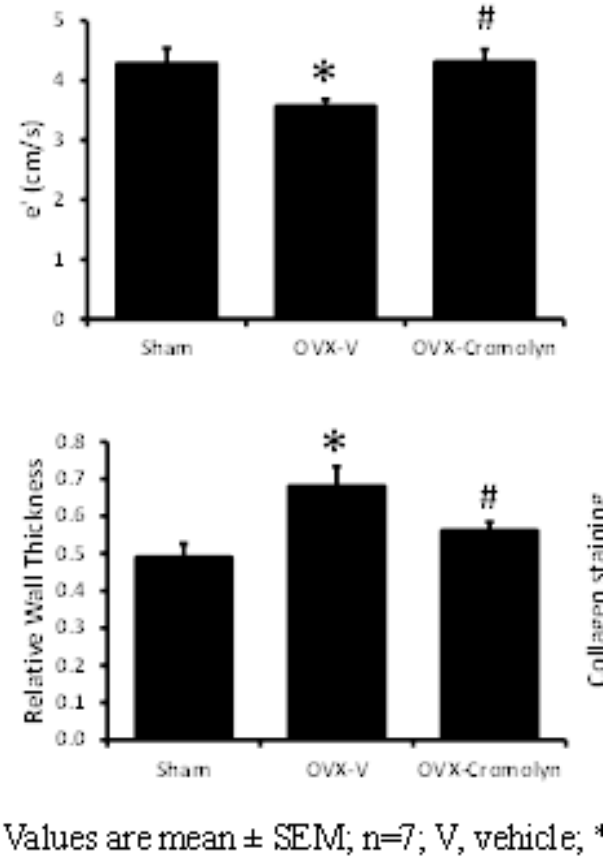
The incidence of left ventricular hypertrophy (LVH) and diastolic dysfunction (LVDD) increases in postmenopausal women, but the mechanisms are not yet clear. This study determined the cardioprotective effects of chronic mast cell inhibition by the mast cell stabilizer, cromolyn sodium, in middle-aged (18-month-old), female Fischer344×Brown Norway (F344BN) rats after estrogen (E2) loss by ovariectomy (OVX). Eight weeks after OVX, systolic blood pressure increased in OVX vs. sham-operated rats (141±6 vs. 108±4 mmHg, $P<0.05$), and cromolyn treatment (30 mg/kg/day x 4 weeks, s.c. via osmotic minipump, n=7/group), initiated one month after OVX, attenuated this effect (115±4 mmHg). Myocardial relaxation (e') was reduced, LV filling pressures (E/e') were increased, and LV mass, wall thicknesses, and percent interstitial fibrosis were increased in OVX vs. sham rats. All of these cardiac adverse effects of E2 loss were mitigated by cromolyn treatment (Figure). Cardiac mast cell number was increased after OVX, irrespective of cromolyn. While no differences in plasma angiotensin (Ang) II levels were observed between OVX and sham rats (33.7±4.6 vs. 32.0±4.5 pg/mL), plasma levels of Ang II were reduced in cromolyn-treated OVX rats (21.3±3.0 pg/mL) ($P<0.05$ vs. sham and OVX-vehicle). Ang II content was significantly increased in hearts of OVX vs. sham rats, and cromolyn attenuated this effect. Moreover, cromolyn prevented the increase in cardiac Ang II type 1 receptor (AT1aR) mRNA expression in OVX rats. Our findings demonstrate that mast cell inhibition with cromolyn attenuates adverse LV remodeling and LVDD in OVX-F344BN rats possibly through

a chymase/Ang II-mediated mechanism.



Values are mean ± SEM; n=7; V, vehicle; *

H. Wang: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; PO1-HL-051952 from NHLBI of the NIH. B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; R01-AG033727-06. **J. da Silva:** None. **A. Alencar:** None. **M.S. Lin:** None. **X. Sun:** None. **S. Ahmad:** None. **C.M. Ferrario:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; R01 AG033727-06. B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NHLBI/NIH PO1 - HL-051952. **L. Groban:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; NHLBI/NIH PO1-HL--051952. B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIA/NIH R01 AG033727.

P172

Sex Differences in Obesity Associated Diastolic Dysfunction in Western Diet Fed Mice

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Premenopausal women are protected against cardiovascular disease (CVD); however, this protection is lost in the setting of obesity, insulin resistance and type 2 diabetes. The mechanisms responsible for abrogation of the sex-related CVD protection are not clearly understood. We have recently developed a translational model in which female mice fed a diet high in fat and refined carbohydrates (western diet - WD) develop cardiac stiffness and diastolic dysfunction earlier than males consuming a WD. We hypothesized that these earlier adverse effects in females are mediated via increased mineralocorticoid (MR) activation/oxidative stress mediated activation (phosphorylation) of the serine kinase, S6K1 which promotes cardiac growth and fibrosis. Accordingly, four week old male and female C57BL6/J mice were fed a WD (containing high fat [46%], sucrose [17.5%], and high fructose corn syrup [17.5%]) or control diet (CD) for 8 and 16 weeks. Two-dimensional echocardiograms were used to evaluate diastolic function. Immunohistochemistry and

western blotting were used to evaluate MR receptor expression and S6K1 phosphorylation. Diastolic dysfunction, indicated by prolonged isovolumic relaxation time (IVRT) and abnormal myocardial performance (increased myocardial performance index [MPI]) was present at 8 weeks in WD-fed female, but not male mice. Although male mice fed a WD exhibited diastolic dysfunction at 16 weeks, diastolic function as assessed by IVRT was more pronounced in female mice. The magnitude of cardiac fibrosis and oxidative stress was greater in females consuming a WD. Moreover, levels of plasma aldosterone, expression of MR (WD female 1.60 fold, WD male 1.26 fold), phosphorylation of S6K1 (WD females 2.92 fold, WD males 1.97 fold) and levels of mRNA for monocyte chemoattractant protein 1 (MCP-1, WD females 1.86 fold, WD males 1.16 fold) were higher in WD-fed female mice compared to WD-fed male mice. These results suggest enhanced cardiac MR mediated S6K1 activation and increased immune and inflammatory responses contribute to enhanced fibrosis and abrogation of cardiac protection in female mice fed a WD high in fat and fructose.

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Serum Soluble and Placental (Pro)Renin Receptor in Normal and Preeclamptic Pregnancy

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Objective: Preeclampsia (preE), a syndrome of hypertension, proteinuria and edema, has many elusive triggers. The renin-angiotensin system (RAS) has been implicated in preE pathogenesis. Recently, we have demonstrated that (pro)renin levels are increased in preE patients and that levels of (pro)renin and (pro)renin receptor ((P)RR) are elevated in a rat model of preE. It has also been demonstrated that high circulating levels of soluble (P)RR at delivery is associated with preE. We evaluated the placental expression of (P)RR in preE patients as well as in a nonhuman primate. We also evaluated the serum levels of soluble (P)RR in preE patients and in a rat model of preE. **Study Design:** (1) Blood and placental samples were collected from 15 normal pregnant (NP) and 15 preE consenting patients in an IRB approved prospective study. (2) We used NP rats (n=10) and pregnant rats receiving weekly injections of desoxycorticosterone acetate and whose drinking water was replaced with 0.9% saline (preE, n=10). (3) The placental samples from

owl monkey (both early and term, NP, n= 2) were collected. The (P)RR expression was measured both by western blotting (WB) and Immunohistochemistry (IHC) with anti-ATP6IP2 (detect (P)RR). The extracellular-signal-regulated kinase ½ (ERK ½) expression was evaluated by WB. The levels of serum soluble (P)RR were measured by a commercially available ELISA kit. **Results:** The placental expression of (P)RR was higher ($p<0.05$) in preE compared to NP patients. The ERK1/2 expression was higher ($p<0.05$) in preE placenta compared to NP. The soluble (P)RR levels were higher in preE (preE patients: 29.2 ± 4.5; PDS rats: 16.9 ± 1.9 ng/mL) compared to NP (NP human: 19.3 ± 4.2; NP rats: 10.4 ± 3.7 ng/mL). The early placenta of owl monkey expressed higher (P)RR compared to term. **Conclusions:** These data suggest that increased expression of (P)RR and ERK1/2 in preE placenta is related to the occurrence of preE. These data also reconfirmed that high level of circulatory soluble (P)RR is associated with preE. The higher expression of (P)RR in early pregnancy compared to term placenta in owl monkey suggests that the (P)RR is important for normal placental development. The expression of (P)RR in nonhuman primates reveals the approach of future studies on owl monkey preE model.

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P174

Comparison of Groups With and Without Diabetes Mellitus and Preeclampsia in Pregnancy: A Retrospective Case-control Comparison

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Objective: Preeclampsia (PreE) and diabetes mellitus (DM) in pregnancy share many risk factors and consequences. Thus, the interactions between these two disease-processes need to be further examined. We compared normal pregnancies to those complicated with preE, gestational diabetes (GDM), and/or pre-existing DM to assess the effect of DM on placental development and outcomes when this condition is complicated by preE. **Methods**: Chart reviews were performed in an IRB approved retrospective case-control design with pregnancies resulting in live born singletons. Total 178 subjects with preE with and without DM or GDM and live born singletons were selected from deliveries in 2008 through 2011 at Scott & White Memorial Hospital, Temple, Texas. These were compared to 443 without preE and with and without DM or GDM. Statistical analysis was performed using ANOVA and Duncan's post-hoc test. **Results:** Patients who developed preE had higher systolic and diastolic pressures compared to groups without preE ($p < 0.05$). Patients with DM or GDM were older ($p < 0.05$). There was no difference among groups for gravidity ($p = 0.21$) with the average gravidity of 2.7 (1.8 SD) for 621 subjects having a range of 1 to 14 pregnancies. Patients with preE delivered earlier in pregnancy than those without preE regardless of diabetes status. However, those

with preE and DM delivered earlier at 35.0 ± 0.4 weeks than the other two preE groups ($p < 0.05$), suggesting a more severe condition. Patients with DM who developed preE delivered smaller ($p < 0.05$) babies (correcting for gestational age at delivery) than those with DM without preE (1.00 ± 0.03 versus 1.16 ± 0.04, respectively). Development of GDM did not result in smaller babies for those pregnancies with preE (1.07 versus 1.09). **Conclusions**: The development of preE in those with pre-existing DM led to growth restriction and more severe disease as evidenced by lower birth weights corrected for gestational age and earlier gestational ages at delivery. These differences were not seen in GDM pregnancies. This observation supports the concept that elevated glucose levels during first trimester placental development may alter the placenta and lead to restriction later in pregnancy when a second stimulus triggers preE.

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P175

Impairment of Autonomic Function Precedes Blood Pressure Elevation in Rat Model of Pre-eclampsia

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Hypertensive disorders of pregnancy including pre-eclampsia affect about 5-7% of all pregnant women and can cause severe acute morbidity, long-term disability and death among mothers and babies. Impairment of heart rate variability predicts risk of cardiovascular disease and all cause mortality. In this study we investigated the hypothesis that impairment of the autonomic function at early time point during pregnancy precedes and may lead to the elevation of blood pressure.

To test this hypothesis, blood pressure and heart rate were recorded under 2% isoflurane in 7 day pregnant rats generated via mating of the transgenic female rat containing the human angiotensinogen gene with the male transgenic containing human renin (hREN), the hAGT×hREN rat (TgA, n=8) which develop increased blood pressure, proteinuria and increased sensitivity to angiotensin II in the last half of gestation. The reverse mating of male containing the human angiotensinogen gene with the female transgenic containing human renin (TgR, n=8) which do not develop preeclampsia, and pregnant SD (n=8) rats at day 7 of gestation were used as controls.

By the 7th day of pregnancy, TgA rats had significantly impaired heart rate variability measured as root of mean successive differences (rMSSD) compared to SD and TgR (2.39 ±0.3 ms vs. 3.4 ± 0.2 in SD or 3.4 ±0.1 in TgR) and impaired baroreflex sensitivity measured as HF alpha (1.2±0.3 ms/mmHg vs. 2.5±0.4, SD or 1.7±0.26, TgR)

There was no difference in systolic arterial pressure or heart rate among the three groups at this time point but diastolic pressure was higher in TgA and TgR (91± 3.5 vs. 85±2.4 or vs. 75 ±2 mmHg in SD rats)

Although the predictive value of impaired baroreflex and heart rate variability in preeclampsia development needs further

investigation, our findings suggest that these changes at early pregnancy precede and may contribute to the significant rise in pressure in the last half of gestation in this rat model of pre-eclampsia which may have significant clinical implications.

H.A. Shaltout: None. **L.M. Yamaleyeva:** None. **M. Bader:** None. **R. Dechend:** None. **B. Brosnihan:** None.

P176

Predicting Preeclampsia Using Copeptin in Women at Low Risk of Disease

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Preeclampsia annually kills 76,000 mothers and 500,000 babies worldwide often due to delay in diagnosis secondary to the lack ofsimple, early gestation tests. Elevated circulating copeptin (CPP), the pro-segment of vasopressin, is associated with preeclampsia (PreE). We have demonstrated that CPP is robustly predictive of PreE as early as the 6th week of gestation in all mothers. Development of PreE is increased 3 fold by a history of PreE. Currently, no test robustly predicts PreE in women without a history of PreE. To evaluate if CPP is predictive in this low risk setting when a predictor is most needed, a nested case-control study was performed to evaluate the predictive characteristics of CPP of women with and without a history of PreE. Maternal plasma CPP concentrations throughout gestation were measured by ELISA. Univariate comparisons were performed. Receiver operating characteristic (ROC curves were constructed to determine sensitivity, specificity, positive and negative predictive values for particular cutoffs. Multivariable logistic regression was performed to control for confounding to examine if CPP was significantly predictive of PreE. Apart from a difference in prior history of PreE, no significant demographic or clinical differences were observed between groups. In all trimesters, CPP predicted PreE similarly or better in women with no history of PreE as evidenced by an elevated ROC Area Under the Curve in comparison to values of women with a history of Pre (1st trimester: 0.96 vs. 0.85; 2nd trimester: 0.95 vs. 0.94 ; 3rd trimester: 0.82 vs. 0.67; $p<0.05$). Despite controlling for significant covariates such as maternal age, BMI, diabetes, chronic hypertension, and twin gestation, logistic modeling demonstrate that trimester specific CPP cutoffs throughout gestation are significantly associated with the development of PreE in women with no history of PreE (all models $p< 0.001$). Our data clearly support copeptin as an early predictor of preeclampsia in a low risk cohort. The ability to predict PreE in a low risk cohort with CPP is clinically significant as women in whom the diagnosis of preeclampsia is delayed or missed may now receive the appropriate interventions.

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P177

The Myotrophoblast of the Rat Placenta Ex Vivo Study of Nitric Oxide Synthase Inhibition

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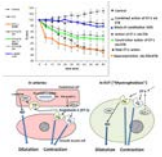
In pregnancy spiral arteries, are invaded by endovascular trophoblasts (EVT) and remodeled. Previously, NOS, and of smooth muscle proteins expression in EVT, and endothelin-1 (ET1) ex-vivo contraction of the remodeled artery were demonstrated, mediated by ET1 receptors A and B (ETA, ETB)
Placentas on gestational day 21, were dissected, spiral artery rings devoid of smooth muscle were fixed to a silicon-coated 8-well chamber slide in oxygenated solution.
Rings cut surface area (CSA) was observed under laser scanning confocal microscope.
Following baseline, L-NAME and 10- 5 M, ET-1 10-7 M were added to some chambers. In other wells, also with ETA antagonist at 10-6 M (BQ-123).
CSA was measured using ImageJ software.
L-NAME alone, reduced CSA by 2.5% p=0.002.
Addition of ET-1 to L-NAME, reduced CSA area immediately, compared with a plateau at 60min by ET1 p=0.001 .L-NAME, followed by ET-1 and ETA antagonist, the isolated constrictive effect of ET-1 via ETB, 7.2%, was earlier and stronger n than via ETA, 6.1% p< 0.001 (figure).
L-NAME + ET-1 causes contraction of the arterial ring via ETA and ETB, without the dilatory effect of NO.
ET-1 alone shows an earlier, immediate CSA reduction, compared to that of ET-1 without L-NAME, achieved at 40-60 minutes. This is in accordance with the instantaneous NO effect through ETB, compared with the gradual ET-1 induced CSA reduction .To isolate the contracting effect via ETB, we added L-NAME + ETA+ ET-1. The ETB contraction is earlier and stronger than that via ETA.
Thus, EVT of the rat remodeled spiral artery react to ET-1 like vascular smooth muscle of non-modified arteries: contraction via receptors A and B and relaxation via receptor B through NOS.



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P178

Depletion of Gamma-Delta T Cells Protects Against Toll-Like Receptor-Induced Preeclampsia in Mice

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Preeclampsia (PE), a hypertensive disorder of pregnancy, is associated with vascular endothelial dysfunction and excessive immunity and inflammation. However, it is unclear which innate immune cells propagate the pro-inflammatory state. Gamma-delta T (gdT) cells can secrete tolerogenic anti-inflammatory cytokines or cytotoxic pro-inflammatory cytokines depending on their activation status. gdT cells from women with PE produce significantly more IFNg and perforin and are less susceptible to apoptosis than gdT cells from normal pregnant women. We hypothesized that Toll-like receptor (TLR) activation in gdT cells induces inflammation and causes PE-like features in mice and that gdT cell KO mice

would be resistant to developing TLR-induced PE-like features. Activation of splenocytes isolated from day 14 normal pregnant mice with the TLR3 agonist poly I:C or the TLR7 agonist R837 for 24 hours significantly increased gdT cells as well as IFNg and TNFa production. We have reported that poly I:C or R837 treatment of normal pregnant mice elicits a pregnancy-dependent PE-like syndrome by inducing a pro-inflammatory immune response. Pregnant poly I:C-treated and R837-treated mice had significantly increased splenic levels of gdT cells and plasma levels of IFNg and TNFa compared to pregnant vehicle-treated mice. Pregnant gdT cell KO mice treated with poly I:C or R837 did not develop hypertension or endothelial dysfunction. These data demonstrate that gdT cells mediate the TLR-induced PE-like features in mice and depletion of gdT cells may reduce the severity of PE in women.

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P179

miR-214 Reduces Termination Efficiencies of Alternative Transcriptional Termination Sites

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Alternative polyadenylation signals are genomic sites where transcription can be terminated, resulting in the production of transcripts often with shorter 3' sequences. While it is clear that alternative transcriptional termination impacts the action of microRNAs, it is not known whether the reverse is true. We identified miR-214, a microRNA upregulated in the kidneys of Dahl salt-sensitive rats, as a potent regulator of the use of alternative transcriptional termination sites. miR-214 targeted human and rat cleavage and polyadenylation specific factor 4 (CPSF4), a component of the CPSF complex that plays a key role in transcriptional termination. miR-214 reduced the termination efficiency of the classic alternative termination site in human cyclin D1 gene from 66% to 47% ($P<0.05$). Of genes in HeLa cells that showed large abundance shifts between transcript isoforms with shorter and longer 3' sequences in response to miR-214, a significantly greater portion (69%, 35 of 51 pairs) shifted to longer isoforms ($P<0.01$). Interestingly, an alternative polyadenylation site was predicted and confirmed in the genomic region upstream of the sequence encoding miR-214 itself. The use of the alternative polyadenylation site correlated with decreased miR-214 abundance in rat tissues in vivo. The termination efficiency of the alternative termination site was reduced from 60% to 32% ($P<0.001$) by miR-214. The termination efficiency was not reduced by miR-214 if CPSF4 had been knocked down by siRNA. These data indicate that miR-214 can relieve alternative polyadenylation-dependent transcriptional termination signals including one that influences its own expression.

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P180

Dopamine D2 Receptors Regulate Wnt Signaling and Apoptosis in Human Renal Proximal Tubule Cells

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Previous work from our laboratory indicates that the dopamine D2 receptor (D2R) in the kidney has a direct role in regulating renal inflammation and injury and blood pressure. Some common single nucleotide polymorphisms (D2R SNPs; rs 6276, 6277, and 1800497) in the human *DRD2* gene are associated with decreased D2R expression and function. Immortalized renal proximal tubule cells (RPTCs) from subjects carrying D2R SNPs (RPTC-D2R SNPs) express less D2Rs than RPTCs carrying no D2R SNPs (RPTC-D2R WT) (62±4 vs 100±6%; P<0.04) and a pro-inflammatory and pro-fibrotic phenotype with markers of epithelial mesenchymal transition. RPTC-D2R SNPs showed increased apoptosis compared with RPTC-D2R WT (11± 0.8 vs 2.3±0.4% TUNEL positive cells, P<0.01, n=5/group). We hypothesized that the D2R regulates renal cell survival through effects on Wnt signaling. We found that Wnt3 expression was increased in RPTC-D2R SNPs compared with RPTC-D2R WT (mRNA: 2.6±0.35 vs 1±0.11 fold; P<0.05; protein: 133±4 vs 100±5%; P<0.05). RPTC-D2R SNPs showed activated Wnt3/β-catenin signaling pathway demonstrated by decreased β-catenin phosphorylation (64±4 vs 100±8%; P<0.05) and increased expression of downstream pro-apoptotic factors Bax (136±4.6 vs 100±5%, P<0.05) and FasL (128±5.6 vs 100±6.5%, P<0.05). Silencing D2R in RPTC-D2R WT (siRNA; 0.30±0.02 vs 1±0.07 fold, P<0.05) increased Wnt 3 expression, decreased β-catenin phosphorylation, and increased expression of Bax and FasL. By contrast, treatment of RPTC-D2R WT with a D2R agonist (quinpirole,1μM, 24h) or transfection of RPTC-D2R SNPs with a *DRD2* which restored D2R expression decreased Wnt3 expression, increased β-catenin phosphorylation, and decreased Bax and FasL expression. Moreover, Wnt3 silencing (siRNA) in RPTC-D2R SNPs increased β-catenin phosphorylation (132±5 vs100±9%, P<0.05), decreased Bax and FasL expression, and reduced the number of apoptotic cells (6±1.0 vs12± 0.9 % TUNEL positive cells, P<0.01). Our results indicate that D_2R function is important in the regulation of the Wnt pathway and that the alterations in D_2R function result in modifications in the pathway potentially leading to fibrosis, cell death, and hypertension. These results may have clinical relevance for subjects bearing D2R SNPs.

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P181

Circulating Second-messenger Glycerophosphocholines and Cardiovascular Risk Factors in a Population-based Sample of Adolescents

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Circulating second-messenger glycerophosphocholines (smGPCs), including lysophosphatidylcholines and platelet-activating factors, are low-abundance plasma phospholipids that modulate atherosclerosis and inflammation and, in turn, the risk for cardiovascular disease (CVD). Although CVD is a slow-progressing disease culminating in middle-to-late adulthood, its initial stages may be seen already in adolescence. Here, we investigated whether circulating smGPCs are associated with classical CVD risk factors - excess body fat, elevated BP, insulin resistance and low-grade inflammation - during adolescence. We studied a population-based sample of 1029 adolescents (52% females, 12-18 years), as part of the Saguenay Youth Study. We used targeted serum lipidomics (LC-ESI-MS) to identify and quantify circulating smGPCs within the 440-640 Da range. In all participants, we also measured: (i) visceral fat with MRI and total body fat with bioimpedance; (ii) blood pressure (BP) beat-by-beat for five minutes under standard clinical conditions; and (iii and iv) fasting serum insulin (as an index of insulin resistance) and CRP (as an index of low-grade inflammation). We identified a total of 81 smGPCs that varied by the length and saturation of their fatty acyl residues and the type of linkage these residues are attached to the glycerol backbone. Over 30 of them were associated with multiple CVD risk factors ($p<6x10^{-4}$). Most of these associations were inverse and involved 'medium' mass smGPCs. Positive associations were also seen and these involved 'low' or 'high' mass smGPCs. Most strongly *inversely* associated smGPCs were: (i) PC(20:6/0:0) and PC(O-18:6/2:0), which were associated with total body fat ($p<3x10^{-14}$) and CRP ($p<8x10^{-36}$); and (ii) PC(16:0/2:0), which was associated with visceral fat ($p=2x10^{-18}$) and BP ($p<1x10^{-5}$). The most strongly *positively* associated smGPC was PC(14:1/0:0), which was associated with visceral fat ($p=8x10^{-8}$) and fasting insulin ($p=2x10^{-24}$). Thus, specific circulating smGPCs are strongly associated with multiple CVD risk factors in adolescence; some of these associations may be 'protective' whereas others 'adverse'. Circulating smGPCs may serve as novel biomarkers of early risk for CVD.

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P182

Advanced Atherosclerosis is Associated with Systemic and End-organ Inflammation, Vascular Oxidative Stress and Endothelial Dysfunction but not Hypertension in Mice

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Introduction: Evidence suggests that hypertension involves underlying inflammation, however whether atherosclerosis - a chronic inflammatory condition - can cause hypertension is unknown. We tested whether blood pressure (BP) is higher in high-fat fed ApoE-/- vs chow-fed wild-type (WT) mice, and whether advanced atherosclerosis is associated with systemic and end-organ inflammation, oxidative stress and endothelial dysfunction. Methods: Male ApoE-/- and WT mice were placed on high fat and chow diets, respectively, from 5-56 weeks. To clarify the effects of ageing alone, aged WT mice were compared to young

chow-fed WT mice (8-12 week old). We measured systolic BP, plasma cytokine levels, mRNA expression of inflammatory markers, vascular superoxide and endothelial function. Results: There was no difference in BP of aged ApoE-/- (104.2 ± 2.9 mmHg) and age-matched WT mice (113.2 ± 1.3 mmHg) (n=13-18, P>0.05). However, plasma IL-6, TNF-α and IFN-γ were elevated in ApoE-/- by more than 2-fold vs age-matched WT (n=9-10, all P<0.05), as was brain expression of IL-1β, IL-6, TNF-α, IFN-γ, TGFβ1, CCR2, CCL2, CCL7, CCL8, CCL12 and IL-10 (n=9-10, all P<0.05), and aortic expression of IL-6, CCR2, CCL8 and CCL12 (n=6-8, all P<0.05). Ageing, but not atherosclerosis, increased renal expression of IL-1β, IL-6, TNF-α, CCR2, CCL2, CCL7, CCL8, CCL12 and Foxp3, and aortic expression of CCL2, IL-10 and Foxp3 by at least 2-fold (n=6-10, all P<0.05). In ApoE-/- aorta, Nox2-dependent superoxide production was 4-fold greater than in WT (n=5-6, P<0.05), and endothelium-dependent vasorelaxation to carbachol was markedly reduced by more than half (n=5-7, P<0.05). Ageing alone had no effect on BP, systemic inflammation or endothelial function.
Conclusions: Despite the systemic and end-organ inflammation, oxidative stress and endothelial dysfunction, advanced atherosclerosis does not result in elevated BP.

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P183

Myeloid-Derived Suppressor Cells Prevent Cyclosporine A-Induced Hypertension, Endothelial Dysfunction, and Renal Injury in Mice

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Cyclosporine A (CsA) is an immunosuppressive drug used to treat focal segmental glomerulosclerosis, reduce autoimmune diseases, and prevent allograft rejection; however a limitation of CsA is that it can induce vascular and renal injury as well as hypertension. Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of immature granulocytes, macrophages, and dendritic cells that suppress pro-inflammatory immune responses by secreting anti-inflammatory cytokines, inhibiting innate and adaptive immune cells, and inducing regulatory T cells (Tregs). We hypothesized that adoptive transfer of MDSCs would prevent CsA-induced vascular and renal injury and hypertension. Daily treatment of male C57BL6/J mice for 1 week with CsA (50 mg/kg/day, i.p. injection) significantly increased systolic blood pressure (Day 7 SBP in mmHg: Con=97±2 vs. CsA=145±3, p<0.05 vs. Con), decreased aortic endothelium-dependent relaxation responses, increased aortic fibronectin levels, increased renal glomerular mesangial expansion, increased renal fibronectin levels, and decreased splenic Treg levels. Adoptive transfer of 1 million MDSCs by i.p. injection on days 1, 4, and 7 partially prevented the CsA-induced rise in systolic blood pressure (CsA+1M MDSCs=120±3 mmHg) and the detrimental vascular and renal effects. However, adoptive transfer of 2 million MDSCs fully prevented the CsA-induced hypertension (CsA+2M MDSCs=105±1 mmHg) and vascular and renal effects. The anti-hypertensive and vascular and renal protective effects of 2 million MDSCs were independent of Treg induction as splenic Treg levels remained

significantly decreased. These data suggest that MDSCs can prevent the hypertension and toxicity caused by CsA independent of Tregs and may be a therapeutic target for CsA-treated patients.

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P184

Effects of Vagus Nerve Stimulation on Hemodynamics and Inflammation in a Rheumatoid Arthritis Model in Rats

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The electrical stimulation of neural pathways has been considered a reliable alternative for treating some pathophysiological conditions, for instance, rheumatoid arthritis. Recently, the concept of electroceuticals has emerged and suggests that proper anatomic mapping associated with identification of electrical stimulation patterns for each neural pathway would tailor this tool. The effects of electrical stimulation of the vagus nerve (VNS) on hemodynamics and inflammatory responses were assessed in a rheumatoid arthritis experimental model. Male Wistar rats (280 g body mass) were anesthetized (ketamine: 57 mg/kg; xylazine: 10 mg/kg) and instrumented with a polyethylene catheter into the femoral artery combined with a stainless steel bipolar electrode around the left vagus nerve. Animals were kept anesthetized and had the pulsatile arterial pressure recorded at baseline during 10 minutes, followed by a single session (2-minutes long) of VNS under three different conditions: A) 5 Hz, 0.1 ms, 1 V; B) 10 Hz, 0.1 ms, 1 V; and, C) 20 Hz; 0.1 ms; 3 V. Immediately after VNS ceased, rats were injected with zymosan (100 µg/50 µL) into the femorotibial joint to elicit rheumatoid arthritis. Rats were allowed to rest over the next six hours and afterwards were killed and had the synovial fluid collected for neutrophil count. Control rats were subjected to similar procedures but without VNS. The VNS condition A and B elicited a transitory bradycardia (A: 23±3, B: 23±2 Δbpm; as compared to baseline). No changes in blood pressure were seen following VNS at conditions A and B. VNS under C condition elicited pronounced transient bradycardia (101±13 Δbpm; as compared to baseline) and produced a transient hypotensive response (26±6 ΔmmHg, as compared to baseline). VNS at conditions A and B had anti-inflammatory effect showed by neutrophil count (A: -44±8, B: -38±7; percentage change as compared to control rats). However, VNS at C condition had no anti-inflammatory effect. Therefore, VNS was an effective anti-inflammatory tool that did not elicit remarkable hemodynamic alterations.

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P185

Cardiometabolic and Inflammatory Biomarkers in the Dahl Salt-sensitive Rat

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The Dahl salt-sensitive (Dahl/SS) rat is a genetic model of salt-sensitive hypertension that

develops left ventricle (LV) hypertrophy after five to six weeks and cardiac failure with LV dilation and contractile dysfunction after 10 to 12 weeks of high-salt intake. The aim of the present study was to evalute cardiometabolic and inflammatory biomarkers in the Dahl/SS rat fed a normal-salt diet (NS-0.3% NaCl) and a high-salt diet (HS-8% NaCl).
For that purpose, eight week-old Dahl/SS rats were randomized into two groups, one group received a HS and the other a NS diet for 10 weeks. In the last week of treatment, 3 out of 10 animals from the HS diet group died. All animals from the NS diet group survived (n=8). The high-salt diet induced heart and kidney hypertrophy, as shown by higher heart/body weight (3.86±0.05 vs 2.96±0.05 mg/g, $p<0.05$) and kidney/body weight ratios (5.23±0.29 vs 3.41±0.06 mg/g, $p<0.05$), as compared to the NS diet group counterparts. Rats in the HS diet group presented proteinuria (273.1±26.8 vs 34.5±2.6 mg/day, $p<0.05$), hypercholesterolemia (4.45±0.23 vs 3.07±0.22 mmol/l, $p<0.05$), reduced levels of free fatty acids (0.23±0.02 vs 0.35±0.02 mmol/l, $p<0.05$) and normal levels of triglycerides in plasma (1.87±0.29 vs 1.28±0.15 mmol/l, $p>0.05$), as compared to animals on a NS diet. Insulin plasma levels in the HS group were lower than in the NS group (1.82±0.13 vs 4.03±0.40 ng/ml, $p<0.05$), though the glucose plasma levels were unchanged (162±5 vs 150±7 mg/dl, $p>0.05$). The plasma levels of monocyte chemoattractant protein (MCP)-1 were higher in the HS than in LS diet group (105.0±10.4 vs 67.6±2.8 pg/ml, $p<0.05$). A multiplex inflammatory cytokine assay was used to profile expression of 23 inflammatory mediators. The plasma levels of nine inflammatory mediators, including IL-1β, IL-4 and VEGF, were significantly increased in rats in the HS group.
In conclusion, HS diet feeding in the Dahl/SS rat deteriorate cardiometabolic and inflammatory biomarkers.

B. Igreja: A. Employment; Modest; BIAL - Portela & Cª S.A. **N. Pires:** A. Employment; Modest; BIAL - Portela & Cª S.A. **L.C. Wright:** A. Employment; Modest; BIAL - Portela & Cª S.A. **P. Soares-da-Silva:** A. Employment; Modest; BIAL - Portela & Cª S.A..

P186

Aging Induces Endothelial Dysfunction and Leukocyte Trafficking via Downregulation of Calpastin

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The vascular endothelium of the microcirculation plays a crucial role in tissue homeostasis and inflammation by regulating organ perfusion and leukocyte recruitment. Aging is associated with chronic endothelial dysfunction, vascular inflammation, hypertension and atherosclerosis. Calpains are a family of calcium-dependent proteases that have been recently implicated in the inflammation of aging organs. We tested the hypothesis that aging increases calpain activation with endothelial dysfunction by deregulating the endogenous calpain inhibitor calpastatin. Studies in human lung microvascular endothelial cells (HMVEC-L) revealed that 12 passage aging HMVEC-L experience suppressed mRNA and protein levels of calpastatin, thereby increasing the activity of the endothelial expressed μ-calpain isoform ($p<0.01$ vs young endothelial cells), as assessed by western blot analyses of cleaved u-calpain. Additional in vitro and in vivo studies in aging endothelial cells and F1-F344xBN rats, respectively, demonstrated that the calpain-

dependent endothelial dysfunction depresses endothelial nitric oxide bioavailability and increases expression of the proinflammatory endothelial cell adhesion molecules ICAM1 and VCAM1 ($p<0.01$ vs young endothelium). Calpain activation and actions in the aging vascular endothelium were prevented by either pharmacological inhibition of calpain or genetic calpastatin overexpression. These data demonstrate that downregulation of calpastatin is causative of endothelial dysfunction and inflammation in the aging microcirculation.

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P187

The Salt Sensitivity of Human GRK4 Transgenic Mice Is Associated With Renal Oxidative Stress

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Variants of hGRK4 gamma are associated with human essential hypertension. hGRK4gamma^{142V} transgenic mice are hypertensive without oxidative stress on normal salt intake. hGRK4 gamma^{486V} transgenic mice have salt-sensitive hypertension. Because renal oxidative stress is increased in some rodent models of salt-sensitive hypertension, we quantified the renal expression of reactive oxygen species related proteins in *hGRK4* gamma^{486V} transgenic mice and non-transgenic (NT) littermates, on normal (NS, 0.8%*)* and high (HS, 4%) NaCl diets*.* Systolic blood pressure (measured under anesthesia) was similar in *hGRK4* gamma^{486V} (89.83±2.7, mm Hg, n=9) and NT (94.7±2.5) mice on NS diet and elevated in *hGRK4* gamma^{486V} (114±6.1) but not in NT mice (94.1±2.8) on HS diet. The renal expressions of NOX1, 2, and 4 were similar in both strains on NS diet but NOX2 was decreased by HS in NT (28±7, % of NT on NS diet, n=5-6/group). On NS diet, CuZnSOD and ECSOD were similar in the two mouse strains while MnSOD (66±3%) was lower in *GRK4* gamma^{486V} than NT mice. However, on HS diet, CuZnSOD (87±7%), MnSOD (70±3%), and ECSOD (55±7%) were decreased in *GRK4* gamma^{486V} mice but not altered in NT mice. HO-2, not HO-1, was slightly greater in *GRK4*gamma^{486V} than NT mice on NS diet (117±7%) but this difference was abolished by HS diet. Urinary 8-isoprostane was lower in *GRK4*gamma^{486V} than NT mice (57±2.5 vs 70±0.1, ng/mg of Cr) on NS diet but increased to a greater extent in *GRK4*gamma^{486V} than NT mice (197±18 vs128±6) on HS diet. Renal SOD activity and superoxide production were similar in both mouse strains on NS diet. HS diet decreased SOD activity (81.6±2.7%) and increased superoxide (138.9±6.6%) production in *GRK4* gamma^{486V}mice but not in NT mice. The renal tubular immunofluorescence of ECSOD was reduced to a greater extent in hGRK4 gamma^{486V} than NT by HS diet. HS diet also decreased the renal expression of NOS3, not NOS 1 and 2, in *GRK4* gamma^{486V} mice (49±2%) but not in NT mice. These changes were not observed in the mice with hGRK4 gamma^{wildtype} and hGRK4 gamma^{142V} transgenic mice, suggesting that the salt-sensitive hypertension of *GRK4* gamma^{486V}mice is related to renal oxidative stress.

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P188

Mitochondrial Redox Phenotype Contributes to Angiotensin Induced Hypertension

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Oxidant stress contributes to the initiation and progression of hypertension by contributing to endothelial dysfunction and/or causing perturbations in nitric oxide homeostasis. Differences in mitochondrial function may contribute to this process and provide insight into why age of onset and clinical outcomes differ amongst individuals form distinct ethnic groups. We have previously demonstrated that variations in normal mitochondrial function and oxidant production exist in endothelial cells from individuals of Caucasian and African American ethnicity and that this variation contributes to endothelial dysfunction. To model these distinct mitochondrial redox phenotypes we used the C57Bl/6J (6J) and C57Bl/6NJ (NJ) that also display unique mitochondrial redox phenotypes due to the differential expression nicotinamide nucleotide transhydrogenase. Interestingly, we found that the 6J animals had a significantly higher systolic blood pressure compared to the NJ animals (116 $\pm$ 0.96 vs 102 $\pm$ 2.39 mmHg) that was exacerbated by angiotensin II induced hypertension (159 $\pm$ 8.25 vs 136 $\pm$ 4.33 mmHg). Vascular oxidative stress was assessed in these animals by evaluating mitochondrial oxygen utilization and oxidant production in primary aortic endothelial cells (MAEC) from these animals. Bioenergetic analysis indicates that compared to NJ, 6J MAEC utilized significantly less oxygen for ATP production, possess a lower maximal respiratory capacity (17.31 $\pm$ 1.84 vs 41.87 $\pm$ 2.95 pmol O_2/min/ug protein), and have reduced electron leak. 6J MAEC also produce more superoxide compared to NJ in response to angiotensin II stimulation (0.08 $\pm$ 0.004 vs 0.11 $\pm$ 0.006 nmol/mg protein). Additionally, isolated renal mitochondria and vessel myography indicated differences in respiratory coupling ratios and endothelial dependent vasodilation amongst the groups. Taken together, these data indicate that differences in “normal” mitochondrial function amongst ethnic groups could contribute to a unique mitochondrial redox phenotype that influences individual susceptibility by contributing to endothelial dysfunction, providing important insights into the mechanisms that contribute to the development of hypertension.

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P189

Angiotensin II - Endocannabinoid Interactions in the Nucleus of the Solitary Tract are Important for Regulation of Baroreflex Control of Heart Rate

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Hypertension resulting from elevated brain angiotensin (Ang) II is associated with impaired functioning of neural reflexes regulating sympathetic and parasympathetic outflow. Restoration of normal baroreflex sensitivity (BRS) for control of heart rate (HR) is achieved in a rat model of Ang II - dependent hypertension [*(mRen2)27 transgenic rats*] by local injection of the cannabinoid CB_1 receptor antagonist rimonabant (SR141716A), a CB_1 receptor antagonist, into the solitary tract nucleus (NTS) of anesthetized rats or by chronic oral rimonabant treatment, which has central and peripheral sites of action. Together with elevated brain dorsal medullary tissue concentrations of 2-arachidonylglycerol present

in the (mRen2)27 rats, these findings are consistent with an activated endocannabinoid system contributing to the impaired BRS in these animals. To further explore acute interactions between Ang II - mediated suppression of BRS and the endocannabinoids, Ang II was injected into NTS of anesthetized Sprague-Dawley rats 10 minutes following NTS injection of rimonabant or aCSF (120 nL, bilaterally). In the presence of aCSF, Ang II reduced BRS by ~50% (In msec/mm Hg: 1.14 ± 0.14 before versus 0.53 ± 0.16; n = 7, $p < 0.008$); this effect was abolished in the presence of rimonabant (In msec/mm Hg: 0.92 ± 0.16 before versus 0.86 ± 0.21; n = 4, p = 0.8). There was no difference in mean arterial pressure or heart rate before or after Ang II treatment in either aCSF or rimonabant groups. Thus, the data support the interpretation that Ang II - mediated attenuation of BRS for control of HR involves release of endocannabinoids. Others report that the pressor actions following acute local injections of Ang II into the hypothalamic paraventricular nucleus are prevented by blockade of CB_1 receptors. We conclude that functional interactions between these two systems occur at multiple brain sites relevant to blood pressure control mechanisms and together the findings support a role for elevated brain endocannabinoids as contributors to the altered reflexes characteristic of Ang II - dependent hypertension. Support: HL-51952, DA-024863 and DA-03690, the Hypertension & Vascular Research Center, Farley-Hudson Foundation

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P191

Increases in Cerebrospinal Fluid NaCl Concentration Excites Neurons of the Organum Vasculosum of the Lamina Terminalis to Elevate Sympathetic Outflow and Blood Pressure

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Accumulating evidence suggests salt-sensitive hypertension is mediated partly by an increase in cerebrospinal fluid (CSF) NaCl concentration and elevation in sympathetic nerve activity (SNA). Increased NaCl concentration or osmolality is sensed by specialized neurons in the organum vasculosum of the lamina terminalis (OVLT). The present study investigated the contribution of these neurons to the SNA and arterial blood pressure (ABP) responses during acute increases in CSF NaCl concentrations. Male Sprague-Dawley rats were anesthetized with Inactin (120mg/kg, IV) and prepared for SNA and ABP recordings. Lateral ventricle infusion of 1M NaCl (5uL over 10 min) increased CSF [Na+] by 5±1 mM and elevated mean ABP (9±1 mmHg), lumbar SNA (125±3%) and adrenal SNA (121±5%) but decreased renal SNA (-9±1%, n=8) and did not alter splanchnic SNA (102±3%). Inhibition of the OVLT with injection of the GABAA agonist muscimol (2.5mM per 20nL, n=5) significantly attenuated the NaCl-induced increase in ABP (2±1 mmHg), lumbar SNA (102±1%), adrenal SNA (103±2%) and decrease in renal SNA (-3±1%). In vivo single-unit recordings demonstrate that lateral ventricular infusion of 1M NaCl (5uL per 10 min) significantly increased the firing rate in 75% (3/4) of OVLT neurons from 1.2±0.4Hz to 5.1±1.2Hz ($P<0.05$). Furthermore, direct injection of NaCl (100nL over 20s, n=3) into the OVLT produced dose-dependent increases in mean ABP (0.15M: 0±1mmHg; 0.5M: 2±1mmHg; 1.0M: 4±1mmHg; 2.0M: 7±1mmHg) and lumbar

SNA (0.15M: 101±2%; 0.5M: 107±1%; 1.0M: 112±3%; 2.0M: 118±4%). Altogether, these findings suggest that increases in CSF NaCl concentrations excite OVLT neurons to elevate lumbar and adrenal SNA and ABP.

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P192

Both Mineralocorticoid Receptor and Angiotensin II type 1 Receptors in the Subfornical Organ Mediate Angiotensin II Induced Reactive Oxygen Species (ROS) in Brain Angiotensinergic Pathways

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Activation of angiotensinergic pathways and central aldosterone (aldo)-MR-ENaC-endogenous ouabain (EO)-AT_1R pathway play a critical role in Ang II associated hypertension. The SFO contains both MR and AT_1R and can relay the signals of circulating Ang II to downstream nuclei such as the PVN, SON and RVLM. We evaluated the effect of knockdown of MR and AT_1R specific in the SFO on reactive oxygen species (ROS) production in downstream nuclei. Wistar rats were intra SFO infused with AAV-MR- or $AT_{1a}R$-siRNA and after 7 days received a sc infusion of Ang II at 500 ng/min/kg for 2 weeks. MR and AT_1R expression were measured by real-time qPCR and western blotting. ROS was assessed by DHE staining. Ang II increased AT_1R mRNA expression in the SFO. Both MR- and AT_1R-siRNA in the SFO prevented this increase. Ang II decreased MR mRNA but increased protein expression in the SFO. Both MR- and AT_1R-siRNA further decreased MR mRNA expression. Ang II significantly increased ROS in the SFO, magno- and parvocellular parts of the PVN, SON and RVLM. Both MR- and AT_1R-siRNA in the SFO prevented ROS increases in the PVN and RVLM. In contrast, MR- but not AT_1R-siRNA in the SFO prevented the Ang II-induced ROS in the SON. Both MR- and AT_1R-siRNA in the SFO prevented most of the Ang II-induced hypertension. These results suggest that aldo-MR signaling in the SFO is needed for the activation of Ang II-AT_1R signaling from the SFO to the PVN and RVLM. Considering that only MR-siRNA in the SFO prevents circulating Ang II induced ROS in the SON, activation of aldo-MR signaling from the SFO to the SON may via EO enhance AT_1R dependent activation of pre-sympathetic neurons in the PVN, and thereby to Ang II-hypertension.

ROS and AT_1R and MR mRNA expression

		Ang II (500 ng/kg/min)		
(n=6-8/group)	SCM	SCM	AT_1R-siRNA	MR-siRNA
ROS (relative DHE staining vs control)				
mPVN	1.00±0.05	1.26±0.07*	1.06±0.09	0.96±0.05
pPVN	1.00±0.03	1.16±0.05*	0.81±0.09	0.83±0.03
SON	1.00±0.09	1.42±0.09†‡	1.47±0.13†	1.12±0.11
RVLM	1.00±0.03	1.16±0.10*	0.86±0.03	0.94±0.03
mRNA/PGK1 (×10⁻³)			SFO	
AT_1R	8.0±0.4	16.2±3.7*	9.6±1.9	11.0±1.9
MR	4.1±0.2	3.2±0.2#	2.2±0.3#	2.7±0.2#
MR protein (/β-actin vs. control)	1.00±0.10	1.59±0.13#		

*p<0.05 vs others, †p<0.05 vs SCM or MR-siRNA, #p<0.05 vs SCM

H. Wang: None. **R. White:** None. **B.S. Huang:** None. **A. Chen:** None. **M. Ahmad:** None. **F.H. Leenen:** None.

P193

Upregulation of Brain Renin-angiotensin System and Inflammation Mediates Leptin Sensitization of Angiotensin Ii-induced Hypertension

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Leptin, an adipocyte-derived hormone, contributes to the increase in blood pressure (BP) associated with obesity. Obesity has also been shown to promote the renin-angiotensin system (RAS) activity and inflammation in the hypothalamus that increase BP and sympathetic activity. Our previous studies using an Induction-Delay-Expression experimental design demonstrated that a central leptin pretreatment resulted in an enhanced hypertensive response to subsequent treatment with angiotensin (ANG) II. The present study tested whether this central leptin sensitization of ANG II-induced hypertension is mediated by an increase in RAS acitivity and inflammation in the brain. Male rats prepared for telemetry BP recording and were pretreated during Induction with central leptin (20 ng/kg/min, ICV) alone or with either ANG II type 1 receptor (AT1R) antagonist irbesartan (125 µg/d) or the TNF-α synthesis inhibitor pentoxifylline (10 µg/h) for one week. After one week Delay, rats were treated during Expression with subcutaneous ANG II (120 ng/kg/min) for 2 weeks. The animals pretreated with central leptin responded with enhanced hypertension to ANG II (Δ39.3±3.8 mmHg vs. Δ21.2±5.2 mmHg). Central infusion of pentoxifylline or irbesartan during Induction blocked the sensitization produced by central leptin. RT-PCR analysis of tissue from the lamina terminalis indicated that leptin up-regulated mRNA expression of several components of the RAS and proinflammatory cytokines including AT1R, angiotensin converting enzyme, TNF-α and interleukin 1β. The results indicate that leptin-induced sensitization of ANG II-elicited hypertension is mediated by upregulation of central RAS and proinflammatory cytokines.

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P194

A Novel Signalosome in the Alpha1a-Adrenergic Receptor and its Genetic Variant Signaling Pathway

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Objectives: Human $\alpha_1$adrenergic receptors (AR), members of G protein-coupled receptor superfamily (GPCR) regulate blood pressure via smooth muscle cell (SMC) proliferation and vasoconstriction. We showed that α_{1a}AR-G247R (247R) genetic variant, identified in a hypertensive patient, constitutively couples to β-arrestin1/MMP/EGFR transactivation pathway leading to hyperproliferation in fibroblasts, cardiomyoblasts and SMCs. A scaffolding protein spinophilin (SPL) serves as a docking site for many regulatory proteins including RGS2 (negative **R**egulators of **G**-protein **S**ignaling). SPL also binds 3rd intracellular loop of some GPCRs.
Hypothesis: differential interaction of 247R or α_{1a}AR-WT (WT) with SPL/RGS2/β-arrestin signalosome mediates unique signaling of 247R and hyperproliferation in cardiovascular cells.
Methods: Receptor-protein interactions were determined by co-immunoprecipitation from HEK293 cells expressing HA-α_1ARs and full length Myc-SPL, its fragments or Flag-RGS2. Protein levels were analyzed by Western. SPL knockdown or RGS2 overexpression was performed using SPL-specific or scrambled siRNA or Flag-RGS2.
Results: Our results reveal that in SMCs or

cardiomyoblasts 247R but not WT upregulates endogenous SPL. SPL exhibits stronger (~2-fold) interaction with WT compared to 247R or α_{1b}, recruits RGS2 and inhibits receptor signaling. In contrast, weak SPL-247R interaction diminishes RGS2 inhibitory effect permitting hyperproliferation of 247R cells. SPL knockdown inhibits 247R-induced proliferation by allowing RGS2 directly bind to 247R as observed with RGS2 overexpression. Overexpression of SPL with RGS2 restores hyperproliferation suggesting that in 247R cells SPL binds RGS2 preventing RGS2-247R interaction. **Conclusions:** We present SPL/RGS2/β-arrestin as a novel signalosome responsible for α_{1a}AR-247R genetic variant triggered hyperproliferation in different cardiovascular cells. We reveal that SPL regulates α_{1a}AR signaling by differentially binding WT or 247R receptors and recruiting RGS2 protein to the receptor. These novel findings unravel critical roles of SPL and RGS2 in α_1AR signaling, as well as identify SPL as a potential novel target for treatment of α_1AR-mediated cardiovascular disorders.

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P195

Transforming Growth Factor beta 1-Mediated Repression of Guanylyl Cyclase/Natriuretic Peptide Receptor-A Gene Expression and Function Involving TGF-beta 1 and Delta EF1 in Vascular Smooth Muscle Cells

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The binding of atrial and brain natriuretic peptides (ANP and BNP) to guanylyl cyclase-A/natriuretic peptide receptor-A (GC-A/NPR-A) produces second messenger cGMP, which lowers blood pressure and prevents cardiovascular events. The objective of the present study was to examine the repressive effect of transforming growth factor (TGF-β1) in the regulation of Npr1 (coding for GC-A/NPRA) gene expression and function. The rat thoracic aortic vascular smooth muscle cells (RTASMC) were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and treated with TGF-β1. The luciferase assay results showed that TGF-β1 significantly repressed Npr1 promoter activity in a dose- and time-dependent manner by 82% and 85% (2.5 ng/ml, 25.51 ± 2.2 and 24 h, 7.0 ± 0.6 vs. untreated control 70.06 ± 4.6, $p < 0.001$, respectively). Treatment with TGF-β1 decreased NPRA mRNA and protein levels by 62% (treated, 0.42 ± 0.05 vs. control, 0.9 ± 0.02, $p < 0.01$) and 55% (treated, 9603 ± 860 vs. untreated, 22211 ± 1449, $p < 0.01$), respectively. TGF-β1 attenuated ANP-dependent intracellular accumulation of cGMP by 59% (TGF-β1 + ANP-treated, 8.66 ± 0.9 pmol/1x 106 cells vs. ANP-treated cells 23.51 ± 2.2; $p < 0.001$). Chromatin immunoprecipitation and electrophoretic mobility shift assay showed that TGF-β1 enhanced the recruitment of transcription factor delta EF1 (δEF1) to form a transcriptional repressor complex with their binding sites in Npr1 promoter. Western blot analysis showed significant increase in δEF1 protein expression by 2.4-fold (treated, 907.9 ± 36.5. vs. untreated, 378.5 ± 10.3; $p < 0.001$) and phosphorylation of mothers against decapentaplegic homolog 2/3 (SMAD2/3) proteins by 2.3-fold (treated, 620.9 ± 10.6 vs. untreated, 269 ± 9.7; $p < 0.01$) in TGF-β1-treated cells. Collectively, the present results demonstrate that TGF-β1 represses Npr1 gene transcription and expression by interactive actions of δEF1 and phosphorylated SMADs. Together, the present results suggest that TGF-

β1 and δEF1 inhibit Npr1 gene expression and function, which may be critical in regulating the blood pressure and cardiovascular homeostasis.

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P196

Chemerin Activates Procontractile Pathways in Isolated Arteries

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In the vasculature, the adipokine chemerin is present in perivascular adipose tissue and causes arterial contraction through activation of the heptahelical receptor ChemR23. We presently test the hypothesis that ChemR23 is linked to procontractile signaling pathways in isolated arteries. The isolated, endothelium-denuded thoracic aorta of both male and female rats was used for measurement of isometric contraction and biochemical analyses. The peptide analog chemerin-9 (1 nm -3 μM) caused concentration-dependent contraction in both isolated aorta from male and female, with a maximum of ~80% of a phenylephrine (10 μM) contraction. The L type voltage dependent calcium channel inhibitors verapamil (1 μM) and nifedipine (1 μM), as well as the protein kinase C (PKC) inhibitor chelerythrine chloride (10 μM), abolished chemerin-9 induced contraction relative to vehicle-incubated tissues (male and female). Next most effective in inhibiting chemerin-9-induced contraction were the Rho Kinase inhibitors Y27632 (1 μM) and fasudil (1 μM), both effective in male and female aorta. The p38 MAPK inhibitor SB203850 (10 μM) and phosphoinositide-3-kinase inhibitor LY294002 (10 μM) were significantly less effective in reducing chemerin-9-induced contraction in both sexes, reducing maximum contraction in male and female by less than 50%. Finally, the phospholipase C inhibitor U73122 (5 μM) and Erk MAPK inhibitor PD098059 (1 μM) did not shift or reduce chemerin-9-induced contraction compared to control. Western analyses of chemerin-9-contracted tissues validated activation of phosphoinositide-3-kinase and lack of activation of the Erk MAPKs. These data are the first to associate ChemR23 with L type channel and Rho Kinase activation, and support the hypothesis that ChemR23 taps into some but not all of the well-recognized contractile pathways. This is important knowledge for understanding the mechanisms by which chemerin could support obesity-associated hypertension

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P197

Extracellular Ubiquitin Blocks CXCL12-Induced Proliferation of Cardiac Fibroblasts

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CXCR4 receptors mediate in part hypertension-induced cardiac fibrosis. This suggests that endogenous CXCR4-receptor agonists stimulate

cardiac fibroblast (CF) proliferation; however, this possibility is unexplored. Although CXCL12 (aka SDF-1α) is the best described endogenous CXCR4 agonist, ubiquitin$_{1-76}$ (U-76) exists in the extracellular compartment (10 to 100 nM) and is reported to be a CXCR4 agonist. Therefore, we investigated the ability of both CXCL12 and U-76 to stimulate growth of rat CFs. Low concentrations of CXCL12 (10 nM x 4 days) increased CF proliferation (from 38,973 ± 384 to 44,429 ± 774 cells/well, n=6, p<0.0001), and this effect was augmented by sitagliptin, a dipeptidyl peptidase 4 (DPP4) inhibitor (% increase by CXCL12: without sitagliptin, 14 ± 2 (n=6); with 1 μM of sitagliptin, 38 ± 9, n=6, p<0.0001). This finding is consistent with the known ability of DPP4 to metabolize CXCL12, i.e., sitagliptin inhibits the metabolism of CXCL12 and enhances CXCL12-induced effects. Not only did U-76 at low physiological concentrations (10 nM) not stimulate CF proliferation, U-76 surprisingly nearly abolished CF proliferation induced by CXCL12 + sitagliptin (% increase by CXCL12 + sitagliptin: without U-76, 58 ± 5, n=12; with U-76, 10 ± 7, n=12, p<0.0001). Ubiquitin$_{1-74}$ (U-74), a metabolite of U-76, inhibited the pro-growth effects of CXCL12 + sitagliptin 10-fold more potently than did U-76. CFs expressed insulin degrading enzyme (IDE) mRNA (qRT-PCR), protein (western blot), and activity (IDE activity assay) and inhibition of IDE with the newly discovered potent IDE inhibitor 6bK (1 μM) prevented U-76 from blocking the growth effects of CXCL12 + sitagliptin. Analysis by mass spectrometry (selected ion monitoring) demonstrated that CFs converted U-76 to U-74 and this conversion was attenuated by 6bK. Conclusions: CFs express IDE and therefore convert U-76 to U-74; U-74 blocks CXCR4 receptors thus protecting against CXCL12 + DPP4 inhibitor induced CF proliferation. Implications: In patients with low IDE activity (due to genes or drugs) who are treated with DPP4 inhibitors, U-74 (CXCR4 antagonist) levels would be low and CXCL12 (CXCR4 agonist) levels would be high, thus possibly creating a “perfect storm” for CF over-proliferation and cardiac fibrosis.

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P198

Differential Regulation of Endothelial Nitric Oxide Synthase Phosphorylation by Protease-Activated Receptors in Adult Human Endothelial Cells

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Protease-activated receptors (PARs) have been shown to regulate endothelial nitric oxide synthase (eNOS) through the activation of specific sites on the enzyme. It has been established that phosphorylation of eNOS-Ser-1177 leads to the production of the potent vasodilator nitric oxide (NO), and is associated with PAR-2 activation; while phosphorylation of eNOS-Thr-495 decreases NO production, and is coupled to PAR-1 activation. In this study, we demonstrate a differential regulation of the eNOS/NO pathway by the PARs using primary adult human coronary artery endothelial cells (HCAEC). Thrombin and the PAR-1 activating peptide, TFLLR, which are known to phosphorylate eNOS-Thr-495 in bovine and human umbilical vein endothelial cells, phosphorylated eNOS-Ser-1177 in HCAECs, and increased NO production. The PAR-1 responses were blocked using SCH-79797, a PAR-1 inhibitor, and L-NAME was used to inhibit NO production. A PAR-2 specific ligand, SLIGRL, which has been shown to phosphorylate eNOS-

Ser-1177 in bovine and human umbilical vein endothelial cells, primarily regulated eNOS-Thr-495 phosphorylation and suppressed NO production in the HCAECs. PAR-3, known for its non-signaling potential, was activated by TFRGAP, a PAR-3 mimicking peptide, and only induced phosphorylation of eNOS-Thr-495 with no effect on NO production. In addition, we confirmed that PAR-mediated eNOS-Ser-1177 phosphorylation was calcium-dependent using the calcium chelator, BAPTA, and eNOS-Thr-495 phosphorylation was mediated via Rho kinase using the ROCK inhibitor, Y-27632. These data suggest a vascular bed specific differential coupling of PARs to the signaling pathways that regulate eNOS and NO production that may be responsible for the modulation of endothelial function associated with cardiovascular disease.

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P199

Uric Acid Promotes Vascular Stiffness, Immune Inflammatory Response and Proteinuria in Western Diet Fed Mice

Annayya Aroor, Guanghong Jia, Vincent G DeMarco, Javad Habibi, Zhe Sun, Mona Garro, Luis Martinez-Lemus, Ravi Nistala, Adam Whaley-Connell, Gerald Meininger, James Sowers, Univ of Missouri, Columbia, MO

Increased consumption of a diet high in fructose and fat (western diet, WD) is associated with an increase in cardiovascular disease (CVD) and kidney injury. In this regard, excess hepatic production of uric acid generated from excess fructose consumption is emerging as a risk factor for vascular stiffness, which underpins CVD and kidney injury. We hypothesized that a WD would increase uric acid levels and cardiovascular and renal xanthine oxidase (XO) activity and associated increased vascular stiffness and proteinuria. Furthermore, we proposed that inhibition of XO activity would prevent arterial stiffening and reduce proteinuria in a clinically relevant model of WD-induced CVD and renal injury. Four week-old C57BL6/J male mice were fed a WD containing high fat (46%), sucrose (17.5%), and high fructose corn syrup (17.5%) with or without allopurinol (125mg/L), a potent XO inhibitor for 16 weeks. XO inhibition significantly attenuated WD-induced increases in plasma and urine uric acid levels and aortic XO activity (WD, 0.225 + 0.031 mU/mL WD + allopurinol, 0.097+ 0.026mU/mL, $P<0.05$), as well as proteinuria (WD, 20.92 + 2.66 mg/ mg creatinine, WD + allopurinol, 13.48 + 1.56 mg/mg creatinine, $P<0.05$). XO inhibition had no effect on increases in body weight, fat mass, and HOMA-IR promoted by the WD. Blood pressure was not different between any of the groups. Stiffness of aortic endothelial cells, extracellular matrix and vascular smooth muscle cells, as determined by atomic force microscopy, was significantly increased in WD mice and this was prevented by XO inhibition. WD induced a significant macrophage pro-inflammatory response in aorta that was significantly suppressed by XO inhibition. Collectively, these findings support the notion that increased XO activity in the vasculature and kidney and increased hepatic production of uric acid secondary to consumption of a WD promotes vascular stiffness, vascular inflammation and a maladaptive immune response that lead to vascular stiffness and kidney injury.

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P200

Endothelial Estrogen Receptor Alpha Does Not Protect Female Mice Against Western Diet Induced Vascular Stiffness

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BACKGROUND: Women with obesity, insulin resistance and type 2 diabetes mellitus (T2D) lose the cardiovascular disease protection normally afforded by female sex hormones, but the underlying mechanism(s) remain unknown. Increases in vascular stiffness occur with aging, but conditions of insulin resistance such as obesity and T2D are characterized by accelerated development of this phenomenon. Under physiological conditions, vascular estrogen signaling via estrogen receptor alpha (ERα) increases endothelial bioavailable nitric oxide which decreases stiffness. Nevertheless, in conditions of insulin resistance, the effects of ERα signaling may be deleterious. METHODS: We used a novel rodent model lacking ERα in the endothelial cells (ECERαKO). The genomic region encompassing exon 3 of the ERα gene was flanked by loxP sites. ECERαKO mice were generated by crossing ERα doubled floxed mice with Cad-Cre+ mice (VE-Cadherin promoter driving expression of Cre-recombinase). Female ECERαKO mice and littermates were fed a high fructose/high sucrose (Western diet - WD) for 8 weeks. The WD diet consisted of 60% fat and 20% sucrose. At the end of the intervention period, mice underwent in vivo and ex vivo assessment of vascular stiffness. RESULTS: The absence of EC ERα did not impact whole body insulin sensitivity (examined by HOMA-IR). Females lacking the endothelial specific ERα had less vascular stiffness when assessed in vivo via aortic pulse wave velocity than the littermates fed with a WD (3.43 ± 0.184 m/s vs. 4.080 ± 0.172 m/s, $p<0.05$). Similarly, ex vivo evaluation of aortic endothelial cell stiffness using atomic force microscopy (AFM) revealed increased stiffness in the females with intact EC ERα when compared with ECERαKO females (1.91 ± 0.60 kPa vs. 13.09 ± 2.61 kPa) ($p<0.05$). Resistant vessel (femoral artery) also revealed less stiffness (decreased modulus of elasticity) in ECERαKO mice fed a WD.
CONCLUSION: Endothelial ERα does not protect females from vascular stiffness induced by a WD. Indeed, the present data suggest a predisposition toward protection of rodent lacking ERα in conditions of insulin resistance.

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P201

Divergent Angiotensin Receptor Signaling in a Mouse Model of Post-Traumatic Stress Disorder (PTSD)

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Independent of their beneficial effects on hypertension and cardiovascular related disease, angiotensin receptor type 1 (AT1R) blockers can improve stress-related symptoms. AT1R receptor-mediated actions can be counteracted directly or indirectly by the angiotensin receptor type 2 receptor (AT2R). Our recent studies in a mouse model of PTSD have shown that AT1R blockade increases the extinction (learned inhibition) of a traumatic fear memory and that AT1R mRNA expression is reduced in fear related brain regions of animals treated with the AT1R antagonist losartan. These data imply that downstream AT1 signaling events maybe important in consolidation of fear memory extinction. Therefore we investigated the acute effects of AT2R inhibition and AT2R stimulation on fear memory and baseline anxiety in mice. We performed classical Pavlovian fear conditioning pairing auditory cues with foot shocks and examined fear extinction (% freezing to conditioned stimulus) in the presence of the AT2R antagonist PD 123319 and agonist Compound 21. Twenty-four hours following fear conditioning, PD 123319 (15 mg/kg IP), Compound 21 (10 mg/kg IP) or vehicle was administered prior to fear memory extinction. The PD treated group exhibited significantly less percent freezing (68%; 68 of 100) compared to vehicle control (47%; 47 of 100) during fear expression ($F_{10, 300} = 1.9$; $p<0.05$, n=15) while no effect was observed during extinction retention, an index of long-term fear memory. On the other hand, Compound 21 had no effect on fear expression, extinction or basal levels of anxiety. Moreover, following fear conditioning, qPCR data revealed that mRNA expression of AT2R and angiotensin converting enzyme 2 (ACE2) in the central amygdala were elevated (5 fold and 3 fold respectively, $P<0.05$, n=6) compared to home cage control, however the AT1R and angiotensin converting enzyme (ACE) gene expression pathways were unaltered. PD 123319 and Compound 21 had no effect on basal levels of anxiety as determined by open field testing. These data indicate that AT1R and AT2R may have divergent effects on short and long-term fear memory formation. Further studies are required to understand the differential regulation of angiotensin receptor signaling in PTSD.

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P202

Angiotensin Receptor-binding Protein ATRAP as a Possible Candidate for Therapeutic Strategy of Functionally Selective Modulation of Angiotensin Receptor Signaling

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Drug discovery targeting GPCRs is no longer limited to seeking agonists or antagonists to stimulate or block cellular responses associated with a particular receptor. GPCRs are now known to support a diversity of pharmacological profiles, a concept broadly referred to as functional selectivity. Therefore, if possible, functionally selective modulation of receptor signaling may be a safer, better tolerated, and more efficacious therapeutic strategy. Recent research progress is reported in the field of functional selectivity such as receptor biased ligands, and receptor binding molecules for the therapy of cardiovascular and renal pathophysiology.

The renin-angiotensin system including plays a critical role in the regulation of cardiovascular and renal function in physiological homeostasis via activity of its effector AT1R. While exaggerated activation of AT1R promotes organ damages by BP elevation and insulin resistance via enhancement of oxidative stress, inflammation and fibrotic response, genetic total inactivation of the renin-angiotensin system components, such as angiotensinogen, renin and AT1R, reportedly results in significant hypotension and in renal morphological alteration even under baseline condition from birth, indicating that baseline AT1R signaling activity is indispensable for the maintenance of cardiovascular and renal physiology.

In the course of an investigational search for a sophisticated means to regulate AT1R signaling at local tissue sites, we have focused our analysis on the AT1R-associated protein (ATRAP; *Agtrap* gene), which is a molecule that directly binds to the carboxyl-terminal domain of AT1R. In contrast to the classical components of the renin-angiotensin system (i.e. angiotensinogen, renin and AT1R), alteration of ATRAP expression exerts no evident effects on baseline BP and renal morphology *in vivo* such as in ATRAP-transgenic mice and ATRAP-deficient mice. However, accumulating results in these mice indicate that ATRAP exerts inhibitory effects on the exaggerated activation of tissue AT1R signaling in response to pathological stimuli, in order to protect cardiovascular and renal tissues under pathological stimuli, in spite of no influence of ATRAP on physiological AT1R signaling.

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P203

ICV Angiotensin-(1-7) Decreased TNF-α and Increased IL-10 in the Hypothalamus of (mRen2)27 Transgenic Hypertensive Rats

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Hypertensive rats subjected to chronic intracerebroventricular (ICV) infusion of angiotensin-(1-7) [Ang-(1-7)] presented attenuation of arterial hypertension, improvement the baroreflex sensitivity and restoration of cardiac autonomic tonus. In the

present study we evaluated whether chronic increase in Ang-(1-7) in the brain modulates inflammatory mediators in the hypothalamus of the transgenic hypertensive rats that present overexpression of renin [(mRen2)27; TGR]. Sprague Dawley (SD) and TGR were subjected to 14 days of ICV infusion of Ang-(1-7) (200 ng/h) or 0.9% sterile saline (0.5 µl/h) through osmotic mini-pumps. The animals were euthanized by decapitation and the hypothalamus was quickly removed and frozen on dry ice. Cytokine levels were evaluated through Elisa assay and enzymes of RAS were measured by fluorimetric assays. As expected, levels of pro-inflammatory cytokines (IL-1α, IL-6 and TNF-α) were increased in TGR (48 ± 3.5 pg/mg, 61 ± 2.7 pg/mg, 76 ± 4.3 pg/mg, respectively) as compared to SD rats (23 ± 1.8 pg/mg, 38 ± 3.4 pg/mg, 39 ± 3.5 pg/mg, respectively), while IL-10 was not altered. Interestingly, ICV infusion of Ang-(1-7) reduced levels of TNF-α (48 ± 3.4 pg/mg vs 76 ± 4.3 pg/mg in untreated TGR) and increased the levels of IL-10 (32 ± 2.6 pg/mg vs 19 ± 1.2 pg/mg in untreated TGR), without affecting IL-1α or IL-6 levels. No difference was found in ACE activity in plasma, on the other hand, the increased ACE activity in the hypothalamus of TGR (207 ± 25.5 nmoles His-Leu/ min/ mg of protein vs 173 ± 13.0 nmoles His-Leu/ min/ mg of protein, in SD rats) was significantly reduced (134 ± 7.9 nmoles His-Leu/ min/ mg of protein) by ICV infusion of Ang-(1-7). These data show that long term increase in Ang-(1-7) levels in the brain modulates inflammatory mediators in the hypothalamus, suggesting a possible additional mechanism for Ang-(1-7) antihypertensive action in the central nervous system.

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Food Restriction Increases the Cardiovascular Response Evoked by Angiotensin-[1-10] and Angiotensin-[1-7] in Female Fischer Rats

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Anorexia is associated with cardiovascular dysfunction including hypotension, bradycardia and cardiac arrhythmias. In a rat model of food restriction (FR)-induced hypotension and bradycardia, we previously demonstrated that the activity of endothelial α1 adrenergic receptors and brain type 1 angiotensin receptors (AT1R) were increased. The aim of this study was to determine the role of the vasodilator, angiotensin (Ang)-[1-7] and the precursor Ang-[I-10] in the periphery and brain. Methods: Female Fischer rats (220g, 4 mo) were maintained for 14 days on a FR diet, that was 40% of the control group (C) food intake. Cannulas were implanted in the lateral ventricle (LV) on day 7. On day 14, the animals were catheterized with a polyethylene tube in the femoral artery for recording of mean arterial pressure (MAP) and heart rate (HR). Results: After 14 days, body weight (g) [C: 202±2, n=13 vs. FR: 176±2, n=14; $p<0.05$], MAP (mm Hg) [C: 105±1, n=35 vs. FR: 102±2, n=36; $p<0.05$] and HR (bpm) [C: 386±5, n=35 vs. FR: 353±8, n=36, $p<0.001$) were reduced in the FR compared to control rats. Injection of Ang-[1-7] caused a greater increase in MAP (Δ mm Hg) in the FR compared to C group when delivered either into the LV (25pmol) (C: Δ -2±1, n=8 vs. FR: Δ 7±1, n=10; $p<0.05$) or the femoral vein (1910pmol) [C: Δ 1±1, n=9 vs. FR: Δ 3±1, n=6; $p<0.05$]. Moreover, there was a greater increase in HR (Δ

bpm) in the FR compared to the C group after LV injection (C: Δ 9±3, n=8 vs FR: Δ 29±9, n=10; p<0.05). Injection of Ang-[I-10] caused a greater increase in MAP (Δ mm Hg) in the FR compared to C group when delivered into the LV (25pmol) (C: 5±1, n=7 vs. FR: 13±2, n=7; p<0.005) or i.v (1910pmol) (C: Δ 1±1, n=7 vs. FR: Δ 15±2, n=8; p<0.05); however, there was no effect on HR. This Ang-[I-10]-induced increase in MAP was attenuated by administering captopril into the LV, in both the FR and C groups, suggesting this increase in MAP was mediated by angiotensin converting enzyme. Conclusion: These results suggest that increased Ang-[1-7] and Ang-[I-10] activity both in the periphery and brain play a role in the increased sympathetic activity observed in anorexia.

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P205

Angiotensin II Metabolic Pathways In Isoproterenol-Induced Heart Failure: Myocytes vs Non-myocytes

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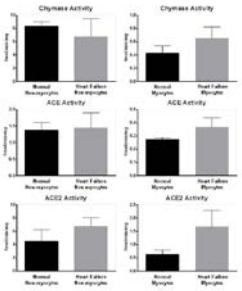
Although myocytes represent three-fours of the ventricular volume in mammalian hearts 90% of the remaining myocardial cells are interstitial cardiac fibroblasts. To shed light into the paracrine mechanisms that contribute to the expression and function of the cardiac angiotensins, we investigated the comparative activity of chymase, ACE, and ACE2 in freshly isolated myocytes and non-cardiac myocytes (NCM) from the left ventricle of rats in which heart failure (HF) was induced by two injections of isoproterenol (Iso) (170 mg/kg body weight, s.c.) spaced 24 h apart. Consistently, chymase enzymatic activity was approximately 10-fold higher in NCM (8.37 ± 0.66 in normal and 6.76 ± 2.7 fmol/min/mg in Iso-induced HF, P < 0.05) compared to myocytes obtained from normal (0.43 ± 0.11 fmol/min/mg) or Iso-induced HF rats (0.65 ± 0.17 fmol/min/mg). Compared to chymase, ACE activity was several folds lower in both NCM (1.37 ± 0.21 in normal and 1.45 ± 0.49 fmol/min/mg in Iso-induced HF) and myocytes (0.28 ± 0.01 fmol/min/mg in normal and 0.37 ± 0.07 fmol/min/mg in Iso-induced HF). As illustrated in the Figure, both chymase and ACE2 activity tended to be higher in myocytes from HF rats. These data suggest that Iso-induced HF causes selective changes in the activity of chymase and ACE2 in myocytes but not in NCM. The clinical significance of these novel findings suggest that chymase rather than ACE inhibitors will have a greater benefit in the management of adverse cardiac remodeling.



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P206

Structural Analysis of Glycosyl Chain at 14th Amino Acid Of Human Angiotensinogen in Plasma

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The Renin angiotensin system (RAS) is a major regulator of body fluid balance and control of blood pressure. The protease enzyme renin secreted from kidneys cleaves specifically angiotensinogen (Aogen) circulating in the blood to produce angiotensin I (Ang I). Human Aogen is a heterogeneous glycoprotein constitutively secreted by the liver. In addition, human Aogen contains four putative asparagine-linked glycosylation sites (Asn 14, 137, 271, 295) and contains four cysteines (Cys 18, 138, 232, 308), with Cys18 and Cys138 linked by a disulfide bridge. The glycosyl chains and cysteines position are very important for binding of the renin.
Big angiotensin-25 (Bang-25) is a consisting of 25 amino acids with glycosyl chain (14th amino asid) and added cysteine (18th amino acid), which we recently isolated from human urine.
To compare glycosyl chain of Bang-25 and Aogen, we analyzed of structure glycosyl chain at position 14th amino acid of human Aogen in plasma.
To determine of glycosyl chain at position 14th amino acid, we performed lysyl endopeptidase digestion and reduction on human plasma Aogen. Then, Aogen after digest was purified by reverse-phase high-performance liquid chromatograpy (RP-HPLC), and glycosyl chain structure analyzed by the two-/three-dimensional HPLC mapping method.
We show that plasma Aogen has three types of glycosyl chain at position 14th amino acid. One glycosyl chain structure is identical to Bang-25 in urine. N-linked glycosylation on 14th amino acid of Aogen plays an important role about renin reaction. In addition, Bang-25 is rapidly cleaved by chymase to Ang II, but is resistant to cleave by renin. These results suggest that the structure of the glycosyl chain at position 14th amino acid of the human Aogen may be involved in the substrate specificity for renin or chymase.

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P207

Role of (Pro)Renin Receptor in the Pathogenesis of Colon Cancer

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(Pro)renin receptor ((P)RR) is a component of the Wnt receptor complex (Science, 2010). We have recently demonstrated that (P)RR plays an important role in the tumorigenesis of pancreatic ductal adenocarcinoma via the activation of Wnt/β-catenin signaling pathway (Shibayama et al. Sci Rep. 2015). Since the patients with colon cancer often show aberrantly activated Wnt/β-catenin-dependent signaling pathway by the mutations of its components, we investigated the possible role of (P)RR and Wnt/β-catenin signaling pathway in carcinogenesis of colon cancer. Real-time PCR was used for measuring mRNA levels of (P)RR. Protein levels of (P)RR was determined by Western blotting and immunohistochemistry. Activated β-catenin levels were determined by Western blotting. Cell proliferative ability was evaluated by counting the cell number in cultured colon cancer cell lines, HCT116 and DLD-1 cells. As compared to normal colon tissues (n=6), mRNA and protein levels of (P)RR were increased by 2.6- and 2.2-fold, respectively, in colon cancer tissues (n=9), which were associated with increased activated β-catenin levels (by 2.8-fold, $P<0.05$). However, plasma soluble (P)RR levels were not changed in patients with colon cancer (n=9). (P)RR and activated β-catenin levels were also increased in HCT116 (by 2.2- and 2.7-fold, n=5, respectively) and DLD-1 cells (by 1.9- and 2.8-fold, n=5, respectively). In these cells, inhibiting (P)RR with an siRNA attenuated the activity of β-catenin and reduced the proliferative abilities ($n=5$, $P<0.05$, respectively). These data suggest that (P)RR contributes to the tumorigenesis of colon cancer through the activation of Wnt/β-catenin signaling pathway.

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P208

Effect of Rosiglitazone on Renal Neprilysin Activity and Protein Expression in Db/db Diabetic Mice

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Alteration in renin-angiotensin system has been implicated in the pathophysiology of diabetic kidney disease. The deleterious actions of angiotensin II (Ang II) could be antagonized by the formation of Ang (1-7) partly generated by the actions of angiotensin converting enzyme 2

(ACE2) and neprilysin (NEP). NEP is a member of the zinc-containing metallopeptidase family, and has a main role in the degradation of several peptides, including natriuretic peptides, bradykinin, amyloid beta, and Ang I. Our previous studies demonstrated increased shedding of renal ACE2 in db/db mouse model of type 2 diabetes. We also showed that treatment with the PPAR_ agonist, rosiglitazone normalized hyperglycemia, decreased urinary ACE2 and attenuated albuminuria in db/db mice. The aim of the study was to test the hypothesis that hyperglycemia down-regulates renal NEP in db/db diabetic mice and that treatment with rosiglitazone will normalize renal NEP expression and activity. Seven-week-old db/db male mice were subjected to rosiglitazone treatment (20 mg/kg/day) for 10 weeks. Treatment with rosiglitazone significantly lowered blood glucose levels in db/db mice ($p<0.001$). Western blot analysis and immunohistochemistry demonstrated a significant decrease in renal and urinary NEP protein expression in 17wk db/db mice compared to lean control mice ($p<0.0001$). Treatment of db/db mice with rosiglitazone attenuated albuminuria, increased renal NEP protein expression and activity. In conclusion, the renoprotective effects of rosiglitazone could be mediated by up-regulation of renal NEP expression and activity in db/db mice. Alteration in the balance between Ang II and Ang (1-7) forming enzymes could contribute to the development of albuminuria in db/db diabetic mice.

L. Alawi: None. **S. Emberesh:** None. **K. Elased:** None.

P209

RAS-Equilibrium Analysis: Simplified Biochemical Characterization of the Renin-angiotensin-system and Implications for Diagnosis and Treatment of Hypertension

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The concentrations of angiotensins are maintained by equal rates of formation and degradation. The resulting steady-state angiotensin levels are affected by local molecular factors including tissue, endothelium or blood cell associated angiotensin receptors and processing enzymes as well as plasma soluble RAS components. The high concentration of the pro-hormone angiotensinogen in human plasma in combination with reported ranges for plasma renin activity result in a long-lasting and stable Ang I formation rate without significantly reducing angiotensinogen levels within several hours. This phenomenon can be utilized for the generation of an ex vivo situation that is characterized by significantly higher but stable angiotensin peptide levels. These ex vivo equilibrium angiotensin levels provide an integrated picture about plasma angiotensinase activities and therefore represent a powerful diagnostic tool for analyzing the systemic RAS in clinical samples. Moreover, these ex vivo levels remain stable over hours of incubation at 37°C and show a very high correlation (>90%) with angiotensin levels obtained by a state-of-the art sample collection procedure using complex inhibitor cocktails for stabilizing angiotensin peptides during blood collection.
The quantification of equilibrium angiotensin levels does not require any special sample collection procedures and can be applied to frozen serum and heparin plasma samples. It turned out that ex vivo angiotensin levels represent a measure for the consumption of

angiotensin metabolites by the organism, therefore providing a powerful and versatile tool for assessing in vivo angiotensin signaling on the patient specific level enabling new diagnosis based rationales for anti-hypertensive therapies. The application of ex vivo RAS-Fingerprinting in clinical studies could substantially enhance our understanding of the regulation and physiology of the human RAS and could further lead to the development of powerful personalized approaches in the future treatment of hypertension.

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P210

Association of Perceived Stress and Aldosterone and Adiponectin Levels in a Rural Cohort of African Americans

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African Americans (AA) have the highest prevalent rates of ESRD than any other group in this country. AA males have the highest risk with more than a two-fold greater risk of kidney injury compared to their female counterparts. The underlying pathophysiology that predisposes AA males to renal injury remains to be definitively determined. However, recent findings from our laboratory have shown that stress may exacerbate the renal injury associated with hypertension. Using an animal model of nitric oxide deficiency hypertension we showed that renal injury associated with this model of hypertension was increased following four weeks of intermittent stress (Pointer et al. 2012). The current study sought to determine whether we could find a similar association of stress and renal injury in a population prone to renal injury. To achieve this we recruited African Americans (11 males; 36 females) attending a health fair. We administered a perceived stress scale; measured resting blood pressure; and collected a blood sample for cortisol, adiponectin, and aldosterone measurement. Aldosterone (ALDO) is a hormone released from the adrenal gland in response to stress. ALDO levels have been linked to increased cardiovascular injury such as acute myocardial infarction and kidney disease. Adiponectin is an adipokine and appears to have protective actions in metabolic disease. In this sampling we found that non-normotensive (blood pressure above 120/80 mmHg) AA females had similar PSS scores as the males (24 ± 2 vs. 21 ± 2) and similar plasma cortisol levels (9.4 ± 0.5 vs. 9.8 ± 1µg/dL, respectively). However, in males cortisol was positively associated with ALDO ($p=0.032$) and negatively associated with adiponectin ($p<0.03$). There was no significant association of cortisol with ALDO and adiponectin in females. These findings suggest that AA males may be predisposed to renal injury as a consequence of stress leading to 1) increased injury promoting (ALDO) and 2) decreased disease protective (adiponectin) agents. Further studies are needed to confirm these findings in a larger sampling.

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P211

Normo-aldosteronemic Aldosterone-producing Adenoma: Immunochemical Characterization and Diagnostic Implications

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Background. Primary Aldosteronism (PA), the most common form of endocrine hypertension, is usually identified by a high aldosterone-renin ratio (ARR) alongside elevated plasma aldosterone concentration (PAC) values. Normo-aldosteronemic PA is also being diagnosed when only the high ARR was driven by low renin because PAC was normal. However, whether this entity truly exists remains contentious since most such cases did not undergo surgical confirmation and even when adrenalectomy was performed no demonstration of an aldosterone-producing adenoma (APA) could be obtained at immunohistochemistry.
Case Description. In 2003 a young lady presented with severe hypertension, low plasma renin activity (PRA), but consistently normal PAC values. She undertook a magnetic resonance that revealed a small adenoma on the right side, but adrenal vein sampling (AVS) showed left lateralization of aldosterone secretion. Therefore, she underwent left laparoscopic adrenal surgery, which determined complete normalization of blood pressure and PRA long term follow up (12 years). In 2014 the development of novel monoclonal antibodies for the human aldosterone synthase CYP11B2 and 11β-hydroxylase CYP11B1, allowed us to immunochemically characterize the resected adrenal gland. Double immunostaining for CYP11B2 and CYP11B1 showed a small CYP11B2-positive adenoma, thus unequivocally proving the presence of an APA.
Conclusions. This case provides compelling evidence for the existence of normo-aldosteronemic APA and suggests that many cases that we dismiss as “low renin-essential hypertension” might instead have an undetected surgically curable APA.

G. Rossi: None. **F. Gioco:** None. **A. Fassina:** None. **C.E. Gomez-Sanchez:** None.

P212

A Meta Analysis of Somatic KCNJ5 Mutations in 1636 Primary Aldosteronism Patients

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Background. We meta-analysed the available studies reporting on KCNJ5 mutations in Aldosterone Producing Adenoma (APA) to determine the clinical characteristics of APA patients with a mutation of the KCNJ5 gene.
Methods. We applied the PICO strategy using predefined terms (Population: primary aldosteronism patients with aldosterone producing adenoma; Intervention: adrenalectomy, sequencing for KCNJ5 mutations; Control: APA without KCNJ5 mutations; Outcome: clinical and pathological correlates of KCNJ5 mutations) to extract relevant studies from the PubMed, Scopus, Web of Science e Cochrane databases until January 2015. To allow for independent replication of the results, we elected to use the commercially available software.
Results and Conclusions. By this PICO strategy we could identify 13 studies involving a total of 1636 patients (age 49 years ± 4; 55% females). The overall prevalence of KCNJ5 mutations was 43%; it was lower frequency ($p<0.003$) in European, USA and Australian studies (35%) than in Japanese and Chinese studies (63%),

and correlated (r=0.70, p=0.008) with the mean daily urinary sodium excretion. The meta-analysis comparison of wild-type and KCNJ5 mutated patients demonstrated that the latter were younger (45±3 vs 52±5 years), had higher plasma aldosterone levels (42±8 vs 33±8 ng/dl), bigger tumors (16.1±6.4 vs 14.9±7.4 mm) and were more females (67% vs 44%) ($p<0.05$ for all). At variance, no significant effect of KCNJ5 mutations on systolic and diastolic blood pressure, and on serum potassium could be found.
Therefore, the meta-analysis of a large dataset comprising all studies available thus far showed that features associated with the presence of KCNJ5 mutations in PA due to APA entail young age, female gender, bigger tumor size and more prominent hyperaldosteronism.

G. Rossi: None. **L. Lenzini:** None. **G. Rossitto:** None. **G. Maiolino:** None. **C. Letizia:** None. **J. Funder:** None.

P213

Western Diet May Modulate Kidney Injury and Albuminuria Differentially in Female Mice Deficient in Mr in the Endothelium versus the Smooth Muscle

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Activation of the mineralocorticoid receptor (MR) has been implicated in kidney injury and precipitation of proteinuria. In this regard, diet induced obesity (DIO), a condition of MR activation is characterized by increase in kidney injury and proteinuria. DIO and other conditions of MR activation also manifest vascular dysfunction that may play a role in kidney injury and proteinuria. Vascular dysfunction may be endothelial or smooth muscle mediated. Moreover, MR signaling in the endothelium versus smooth muscle may be important in vascular function. Data from the Jaffe lab and our preliminary data show that deficiency of smooth muscle and endothelial MR plays a protective role from vascular dysfunction such as increased pulse wave velocity and stiffness. However, the role of endothelial specific versus smooth muscle specific MR in kidney injury and proteinuria is not known. Hence, we hypothesized that deficiency of endothelial and smooth muscle specific MRs (ECMRKO and SMMRKO) will protect the mice from Western diet-fed (high fat/high sucrose, WD) kidney injury and proteinuria. We fed female ECMRKO/SMMRKO and their littermate controls WD for 16wks and collected urine and performed imaging, molecular and morphological analyses. We observed significantly less proteinuria in the ECMRKO mice fed WD when compared to their littermates (2.4mg/mg vs. 3.5mg/mg creatinine) ($p<0.05$), however there was no change in the SMMRKO mice fed a WD when compared to their littermates. Furthermore, we observed significantly less impairment in aortic/renal pulse wave velocity and stiffness in both the ECMRKO/SMMRKO models. Western blots showed that there was a tendency to suppression of MR protein in the ECMRKO on WD. This suppression of MR expression was contemporaneously observed with decreased phosphorylation of ribosomal protein S6 along with reduction in membrane localization suggesting endothelial MR may regulate S6 activation. In summary, our study suggests endothelial specific MR may mediate kidney injury in conditions of MR activation and a lesser role for smooth muscle specific MR.

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P214

Meta-analysis of the Effect of Mineralocorticoid Receptor Antagonists on Proteinuria and Progression of Chronic Kidney Disease

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Background/Objective
ACE-inhibitors (ACE-I) and angiotensin receptor blockers (ARB) are standard of care for patients with chronic kidney disease (CKD) and persistent proteinuria. Mineralocorticoid receptor (MR) activation has numerous "off target" effects on the kidney and vasculature and a number of studies have evaluated the use of MR antagonists (MRAs) in CKD. We conducted a meta-analysis of randomised controlled trials of MRA in addition to ACE-I and/or ARB in CKD to evaluate the potential reno-protective effects and risks of hyperkalaemia.
Methods
MEDLINE (1966-2014) and EMBASE (1947-2014) were searched using a pre-specified strategy, and unpublished data were obtained from original study authors where possible (8 authors, 10 studies). Studies including dialysis or transplant patients and studies of less than 4 weeks duration were excluded from analysis. Results were pooled using random effects meta-analysis.
Results
Eighteen trials (1438 patients) were included. Addition of MRA reduced blood pressure (-5.9, [95% CI 9.6, -2.2mmHg] for systolic; -2.3, [95% CI -3.6, -1.0mmHg] for diastolic) and weighted mean protein/albumin excretion (- 40.6%) at the cost of a small reduction in glomerular filtration rate (GFR) (-3.4, [95% CI -5.7, -1.1 ml/min/1.73m^2]). There was a 3-fold higher relative risk of hyperkalaemia above predefined trial limits (RR 3.21, [95% CI 1.19, 8.71]) equating to number needed to harm over one year of 23 [95% CI 7-267]. Average potassium increase was 0.21mmol/L [95% CI 0.08-0.33]. No studies reported life threatening hyperkalaemia events or hospitalisations as a result of hyperkalaemia. Baseline creatinine or diabetes status had no effect on hyperkalaemia risk in our analysis (p=0.15 for creatinine; p=0.38 for diabetes status).
Conclusion
Addition of MRA is a promising therapeutic strategy for reducing blood pressure and proteinuria in patients with CKD, with a quantifiable risk of hyperkalaemia. This reduction in proteinuria could translate into reduced risk of progressive renal disease and cardiovascular events but appropriately designed larger studies with long term follow up are required.

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P215

Angiotensin II-Mediated Cardiac Remodeling in cChAT Mice

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It has been demonstrated that the cardiac non-neuronal cholinergic system (NNCS) plays a role in regulating cardiac homeostasis under physiological conditions. To examine a possible role played by NNCS upon cardiac remodeling, we submitted mice with genetic deletion of cardiomyocyte-specific choline acetyltransferase (cChAT) to chronic subcutaneous infusion of angiotensin II (Ang II). Male mice aged six months were assigned into four experimental groups: wild-type (WT) + Saline; WT + Ang II; cChAT + Saline and cChAT + Ang II. Ang II did not change the heart weight in WT mice (10.4±0.3 vs. 8.5±0.2 mg/mm in WT+Saline) but determined an increase in cChAT mice (12.8±1 vs. 8.8±0.6 mg/mm in cChAT+Saline). In WT mice, Ang II decreased left ventricular (LV) fractional shortening (23±2 vs. 35±2 % in WT+Saline) and LV ejection fraction (54±4 vs. 73±2 % in WT+Saline). However, cChAT+Ang II mice exhibited greater decrease in both LV fractional shortening (12±1.8 vs. 23±2 % in WT+Ang II) and ejection fraction (31±4 vs. 54±4 % in WT+Ang II). cChAT mice displayed cardiomyocyte hypertrophy (2002±116 vs. 1377±48 µm2 in WT+Saline) even when receiving saline. Ang II increased the cardiomyocyte surface area in both WT and cChAT mice; however, the cChAT mice exhibited greater myocyte hypertrophy (3035±181 vs. 2603±122 µm2 in WT+Ang II). Hematoxylin and eosin staining revealed that Ang II promoted greater disruption of myocardial structure in cChAT mice. Additionally, Trichrome C staining revealed that WT+Ang II mice presented increased collagen deposition (2.86±0.2 vs. 0.45±0.07 % in WT+Saline); nevertheless, the fibrotic response in cChAT+Ang II mice was greater than that observed in WT animals (6.16±1.1 vs. 2.86±0.2 % in WT+Ang II). Therefore, mice with deficiency for ChAT displayed exacerbated ventricular dysfunction induced by chronic Ang II administration, providing support for a role of the NNCS in the progression of cardiac remodeling.

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P216

Thoracic Epidural Administration of Resiniferatoxin Improves Cardiac and Autonomic Dysfunction in Post-MI Rats

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Our recent study demonstrated that chronic ablation of the cardiac sympathetic afferent reflex (CSAR) at the time of myocardial infarction (MI) by destroying TRPV1-expressing CSAR afferent nerve endings by epicardial delivery of the afferent neurotoxin, resiniferatoxin (RTX) significantly improved cardiac and autonomic dysfunction post MI. In this study, we provide an alternative route of epidural peri-ganglion administration of RTX for CSAR ablation thus destroying TRPV1-expressing CSAR afferent neuronal soma at the level of the T1-T4 DRGs in post-MI rats. This strategy completely abolished the CSAR for up to 6 months, significantly longer than epicardial application (~3-4 months). We compared the cardioprotective effects of epidural application of RTX with [Sar^{9},$Met(O_2)^{11}$]-substance P (SSP)-saporin (SAP) to determine if SP-containing CSAR afferents ablation mimics the cardioprotective effects of RTX in post-MI rats.

Echocardiographic data demonstrated that both epidural RTX and SSP-SAP treatments significantly slowed LV chamber dilation in MI rats. Epidural application of RTX significantly decreased cardiac sympathetic tone (%max: 44±5 (n=6) vs. 11±1 (n=9), vehicle vs. RTX, P<0.01) and improved baroreflex sensitivity at 12-weeks post-MI whereas SSP-SAP had less effect on autonomic dysfunction. Compared to vehicle-treated MI rats (n=16), epidural RTX (n=13) reduced left ventricular end diastolic pressure (LVEDP: 22±1 vs. 7±1 mmHg, P<0.01) whereas epidural SSP-SAP only partially reduced LVEDP (15±2 mmHg, n=10, p<0.05) in MI rats. All groups had similar infarct size. Cardiac hypertrophy and lung edema in MI rats were reduced by epidural RTX whereas they were only partially reduced by SSP-SAP. Pressure-volume analysis data showed that epidural RTX significantly improved cardiac diastolic dysfunction in MI rats to a greater extent than SSP-SAP, neither of which improved cardiac systolic dysfunction. These data suggest that 1) epidural peri-ganglion RTX ablation of the CSAR can be used to reduce cardiac remodeling and autonomic dysfunction post-MI; 2) SP-containing thoracic afferents partially but not completely mediate the cardioprotective effects of thoracic afferent ablation post MI.

H. Wang: None. **I. Zucker:** None.

P217

Western Diet Promotes Cardiac Diastolic Dysfunction by Increasing Cardiomyocytes Calcium Sensing Receptor via Activation of Parathyroid Hormone, Parathyroid Related Hormone and Their Receptor- 1 in Female Mice

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Background- Data from our Lab and others indicate that consumption of a western diet (WD), high in fat and refined carbohydrates promotes cardiac hypertrophy and diastolic dysfunction. Recently, a role for calcium sensing receptor (CaSR) and the fibroblast growth factor 23 (FGF 23)/ Klotho axis has been increasingly recognized in the pathogenesis of cardiac hypertrophy and diastolic dysfunction. However, the role of these factors in WD induced cardiac dysfunction has not been elucidated. Therefore, the purpose of this study was to determine mechanisms and signaling pathways underlying WD-induced cardiac diastolic dysfunction in female C57-Bl6/J mice. Methods and Results- Four week-old female C57-BL6/J mice were fed a control diet (CD) or WD containing fat (46%) and fructose (17.5%) for 16 weeks. Then, left ventricular (LV) and serum were harvested and processed for immunohistochemistry and serum analysis. Four µm paraffin embedded sections of the LV were incubated with primary antibodies (CaSR), parathyroid hormone (PTH), parathyroid related hormone (PTHrP), parathyroid hormone receptor-1 (PHTR-1), parathyroid hormone receptor-2 (PHTR-2), (FGF23), klotho and appropriate secondary antibodies. Images were captured with a bi-photon confocal microscope and signal intensities were quantified as gray scale intensities. Analysis of immunofluorescence images revealed that consumption of a WD resulted in significantly higher expression level of CaSR, PTH, PTHrP, PTHR-1 and FGF23. Also, consumption of WD significantly increased the serum PTH level. Furthermore, analysis of WGA stained images showed significant hypertrophy of LV cardiomyocytes. Interestingly, WD did not

increase the expression of PTHR-2 and Klotho in LV but levels of this protein in the coronary arteries.
Conclusion- These findings support the preliminary notion that there is a role of PTH, PTHR-1 and PTHrP signaling in dietary promotion of cardiomyocytes hypertrophy and diastolic dysfunction. These effects could be mediated via modulation of cardiac calcium metabolism.

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P218

Role of Metabolic Biomarkers in Early Detection of Diabetic Cardiomyopathy in West Virginian Population

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Background: Diabetes Mellitus is a significant risk factor for heart failure. With the increasing global prevalence of diabetes, diabetic cardiomyopathy (DCM) represents a significant public health issue. Elevated levels of Insulin-like growth factor-binding protein 7 (IGFBP7) is an early-stage biomarker in DCM but studies are lacking on the association between IGFBP7 and DCM. Most patients with DCM remain asymptomatic until late-stage disease, therefore it becomes of utmost importance to identify biomarkers to detect early stage of DCM. The aim of this study is to determine if IGFBP7 and other biomarkers can detect the onset of preclinical DCM relative to echocardiographic irregularities exhibiting diastolic dysfunction. **Methods:** Eighty patients were grouped equally into four categories based on predetermined clinical diabetic and cardiac parameters: *Normal, Diabetes (DM), Diastolic Heart Failure (DHF)*, and *DM+DHF,* the last group being the preclinical DCM group. **Results:** Echocardiography images indicated severe diastolic dysfunction in patients with DHF + DM as compared to DHF patients alone. In the *DM* and *DM+DHF* groups, IL-6, TNF-alpha, isoprostane, and leptin were elevated compared to the control, as were clinical markers HDL, glucose and hemoglobin A1C. More importantly fibrotic markers IGFBP7 and TGF-β followed the same trend. The *Normal* group showed higher levels of beneficial biomarkers adiponectin and bilirubin, which were reduced in the *DM* and *DM+DHF* groups. **Conclusion:** This novel study demonstrates that West Virginia patients with early onset DCM were more likely to have elevated levels of detrimental biomarkers, including IGFBP7 and TGF- β, indicating potential diastolic dysfunction. The present study provides new and exciting evidence supporting the potential clinical applications of using IGFBP7 and other biomarkers in diagnosing early stage DCM in the West Virginia population.

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P219

Differences in Phosphoproteins in Rodent versus Human Hearts: Implications for Translational Studies of Hypertensive Heart Disease

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Background: Rodent models are commonly used to study hypertensive heart disease. Several recent studies have probed the level of correlation between specific signaling pathways and proteins in human and rodents. Current evidence is overwhelming that protein phosphorylations play a key role in cardiac remodeling.
Methods: Left ventricular tissue samples were obtained from human systolic failing (n=5) and control (n=5) hearts and 3 rat models of hypertensive heart failure (aortic banding, Dahl salt-sensitive, and spontaneously hypertensive rats (SHR)) and corresponding controls. Total proteins were extracted and and phosphoenrichment performed. Phosphoproteins were separated by 2D-DIGE with Cydye staining. Gel images were registered and rectified for composite analysis and statistical comparisons using pixel intensity. Phosphoproteins were identified by MALDI-TOF/TOF Mass Spectrometry.
Results: The patterns of overall protein abundance from normal and failing hearts were not statistically different. However, when the composite of human hearts were compared with composite patterns of phosphoproteins in normal and failing rodent hearts, there were profound differences in the phosphoprotein patterns in 26% of pixels in registered images ($P < 0.05$). Targeted pair wise analyses showed differences ($P < 0.05$) between human and rodent hearts for troponin T, myosin light chain, peroxiredoxin, and haptoglobin phosphorylations.
Conclusions: Together, the present results indicate significant differences in cardiac phosphoproteins in human versus rodent heart and the importance of confirming findings from rodent studies in humans for translational studies of kinases, phosphatases, and phosphoproteins. This may specifically relate to studies of phosphorylation of myosin light chain and troponin.

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P220

Congestive Heart Failure in the Rat Induces Subtle Renal Damage via Neurogenic Pathways

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Background: Cardiomyopathy in experimental renal insufficiency is putatively influenced by neurogenic pathways of renal origin. We wondered if cardiac neurogenic effects in congestive heart failure could likewise harm the kidney. We hypothesized that increased renal sympathetic nerve activity (RSNA) in rats with congestive heart failure after myocardial infarction (CHF) induces renal structural damage.
Methods: 21 day after induction of CHF renal morphology was evaluated by immunohistology (interstitial and glomerular mononuclear cell infiltration (ED1), cell proliferation (PCNA),

collagen I,III,IV,V,VI, laminin und fibronectin). RSNA was assessed by volume challenge (VE) to decrease RSNA. CHF and control rats were investigated with and without renal denervation (DNX). Blood pressure (BP), heart rate (HR) and RSNA were recorded. Nodose ganglion neurons (NGN) with vagal cardiac afferents were cultured for 1 day. Whole cell recordings were obtained and current-voltage relationships established. Cells were characterized by osmomechanical stress with a mannitol solution.
Results: In CHF rats with intact renal nerves (nonDNX) formation of collagen I occurred, that was reduced after DNX (12.2+0.7 %area vs. 9.1+1.1 %area*, n=6, * p<0.05). VE-induced RSNA decreases were impaired in CHF vs controls suggesting increased RSNA (-α 34+8% vs. -α 54+6% *, n=6, * p<0.05). NGN from CHF exhibited altered conductance in response to mechanical stress as compared to controls (change in holding current at -80 mV: control_normoosmotic: -144±30 pA; control_hypoos.: -282±34 pA vs CHF_normosmotic: - 230±55 pA; CHF_hypos.: -540±100* pA; *p<0.05 CHF vs. control).
Conclusion: CHF induced subtle renal structural damage due to increased renal sympathetic tone which was likely due to altered NGN mechanosensitivity. Afferent nerve units from cardiovascular organs obviously form a complex sympathomodulatory network.

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P221

Physicians are More Prone to Causing White Coat Hypertension than Nurses or Cardiovascular Technicians: An Observational Study

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Introduction: Accurate assessments of blood pressure (BP) are critical for the effective diagnosis and treatment of hypertension. A substantial portion of patients labelled hypertensive have been shown to instead have White Coat Hypertension (WCH), where their BP is elevated exclusively when assessed in a clinic. In this study, we assessed whether the type of healthcare provider measuring individuals' blood pressure impacted the incidence of WCH.
Methodology: Following collection of baseline demographics, 106 participants had their BP

measured by a physician, a nurse and a cardiovascular technician. The order of measurements was randomized. All healthcare providers used the same BP cuff for measurements and were instructed to measure BP following a standardized method. Following BP readings taken by healthcare providers, a 24-hour Ambulatory Blood Pressure Monitor (ABPM) was applied to all participants. The average of the daytime readings of the ABPM served as the control for this study.
Results: Patients whose BP were greater than 140/90 mm Hg when measured by a healthcare provider, but whose control readings by ABPM were less than 135/85 mm Hg were classified as having WCH. Physicians caused 33% of participants (35 of 106) to have WCH. Nurses caused 23.5% of participants (25 of 106) to have WCH. Cardiovascular technicians caused 5.6% of participants (6 of 106) to have WCH. ($p<0.0001$).
Similar trends were observed based on analysis examining the percentage of accurate readings and the average of readings compared to the control ABPM, with technicians having the most accurate readings, nurses having moderately accurate readings, and physicians having the least accurate readings.
Conclusions: The results of this study suggest that the incidence of WCH and BP measurement inaccuracy occur more frequently when BP is assessed by certain types of healthcare providers, potentially because patients may feel more anxious or stressed around these individuals. It may therefore be advisable for BP to be assessed by cardiovascular technicians, instead of nurses or physicians, to reduce the risk of White Coat Hypertension and inaccurate BP readings.

A.K. Pandey: None.

Venous Dilation Contributes to 5-HT-induced Hypotension

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Serotonin (5-hydroxytryptamine, 5-HT) infusion in a normal conscious rat decreases mean arterial pressure (MAP), in part by reduction in total peripheral resistance. Microsphere experiments have shown 5-HT increases blood flow within the splanchnic vascular bed, with the greatest being in the intestine and spleen. Interestingly, 5-HT does not cause a direct relaxation of resistant (small or large) mesenteric arteries. The present study addresses the possibility of the venous circulation contributing to the 5-HT induced fall in blood pressure. Our working hypothesis is venous dilation, specifically dilation of veins measurable within the splanchnic vascular bed, contributes to 5-HT-induced hypotension. Using an ultrasound imaging system (Vevo 2100 imaging system; 21 MHz probe,Visual Sonics Inc.), telemetry-implanted, anesthetized male Sprague Dawley rats underwent cross-sectional imaging which was controlled for respiration and cardiac cycles. The following vessels were imaged: abdominal aorta (AA); portal vein (PV); abdominal inferior vena cava (IVC); and superior mesenteric vein (SMV). Following the collection of baseline MAP and vessel diameter measurements, Alzet osmotic mini-pumps containing vehicle (saline; n=9) or 5-HT (25 ug/kg/min; n=9) were implanted for 1 week. After, 24 hours of infusion, 5-HT increased the vein diameter (SMV 17.48±2%; PV 17.67±2%; IVC 46.87±8%) and maintained the AA diameter (AA 0.93±1%) from baseline while reducing MAP (vehicle 101.93±3; 5-HT 84.68±2 mm Hg;

p<0.05).One-week post removal of all osmotic mini-pumps, there was no difference in the MAP or diameter of all noted vessels between the two treatment groups. To correlate with in vivo findings, the PV and IVC, when isolated in a tissue bath for measurement of isometric force and contracted with endothelin 1, relaxed in a concentration dependent fashion to 5-HT and 5-carboxamidotryptamine (5-HT 1/7 receptor agonist;1 nM-10 uM). Collectively, these findings highlight the contribution of splanchnic venous dilation in 5-HT-induced hypotension and propose a possible mechanism for 5-HT reduction in blood pressure.

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P223

How Does Patient Engagement and Gamification Correlate With Hypertension Control? Results From a Large-Scale Nationwide Network of Ambulatory Blood Pressure Kiosks

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Background: Ambulatory blood pressure (ABP) is known to provide prognostic information about cardiovascular disease better than office BP. Not much is known about the correlation of ABP control and patient engagement with gamification.
Objective: To examine the relationship between ambulatory blood pressure and patient engagement with a nationwide ABP kiosk network.
Methods: De-identified historic data from a nation wide ABP kiosk network (higi SH, llc www.higi.com) was analyzed from September 2012 to May 2015. At time of this abstract submission 9,926 ABP kiosks were deployed within the network. Only patients with initial BP readings in the hypertensive range and those who opted-in to share data for research purposes were included in the study. Level of engagement was defined as the total number of achievements, or badges, earned on the gamification platform. BP changes were defined as the difference between patients first and last reading on the kiosk network. Patient demographics, level of engagement with gamification platform and their ABP trends were analyzed and correlation measured.
Results: A total of 153,092 patients qualified the inclusion criteria for the study. Mean age was 52 years with 56% (85,361) male and 44% (67,731) female. Almost half the patients were obese (49%, 74,587). The patients on the gamification platform earned a total of 898,130 achievements. There was a statistically significant difference in drop in systolic and diastolic blood pressure with number of achievements earned by patients (systolic BP: p-value < 0.0001, diastolic BP: p-value = 0.0033). Patients achieving greater than 20 achievements showed an average drop of systolic BP of 16.2 mmHg (p<0.01) and a drop of diastolic BP of 10.6 mmHg (p<0.01). Of the patients earning greater than 20 achievements 84.8% moved from hypertensive classification to normotensive classification.
Conclusion: The results showed a statistically significant relationship between level of achievements earned on the gamification platform and lowering of blood pressure.

K. Siddiqui: A. Employment; Significant; Co-Founder and CTO, higi SH, llc. **R. Goglia:** A.

Employment; Significant; Product Manager, higi SH, llc.

P224

Zamicastat Prevents the Deterioration of Cardiometabolic and Inflammatory Biomarkers in a Genetic Model of Salt-sensitive Hypertension

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The Dahl salt-sensitive (Dahl/SS) rat, a genetic model of salt-sensitive hypertension and heart failure, develops left ventricle (LV) hypertrophy after five to six weeks and cardiac failure with LV dilation and contractile dysfunction after 10 to 12 weeks of high-salt (HS) intake. The aim of the present study was to evaluate the effect of zamicastat, a selective peripheral dopamine β-hydroxylase inhibitor, on cardiometabolic and inflammatory biomarkers in Dahl/SS during chronic HS intake.
Eight week-old Dahl/SS rats were randomized into three groups; two groups received for 10 weeks a HS (8% NaCl) diet, one of which received 30 mg/kg/day zamicastat (HS+ZAMI); a third cohort received normal-salt diet (NS-0.3% NaCl) and served as controls. In the last week of treatment, 3 out of 10 animals from the HS group and 1 out of 10 from HS+ZAMI group were found dead. All animals from the NS group survived (n=8). Rats in the HS and HS+ZAMI groups showed heart and kidney hypertrophy as indicated by higher heart/body weight ratios (3.86±0.05 and 3.63±0.09 vs 2.96±0.05 mg/g) and kidney/body weight ratios (5.23±0.29 and 4.80±0.24 vs 3.41±0.06 mg/g), as compared to the NS group. A multiplex inflammatory cytokine assay was used to profile expression of 23 inflammatory mediators. Plasma levels of IL-1β, IL-4, IL-5, IL-7 and GM-CSF were significantly increased in the HS, but not in the HS-ZAMI group. Plasma levels of monocyte chemoattractant protein (MCP)-1 were higher in the HS and HS+ZAMI than in NS group (105.0±10.4 and 85.2±5.9 vs 67.6±2.8 pg/ml). The HS group, but not HS+ZAMI, showed hypercholesterolemia (4.45±0.23 and 3.72±0.37 vs 3.07±0.22 mmol/l). Both HS and HS+ZAMI cohorts had reduced levels of free fatty acids (0.23±0.02 and 0.23±0.02 vs 0.35±0.02 mmol/l) and normal levels of triglycerides in plasma (1.87±0.29 and 1.69±0.18 vs 1.28±0.15 mmol/l). Insulin plasma levels in HS and HS+ZAMI groups were lower than in NS group (1.45±0.26 and 1.90±0.22 vs 4.03±0.40 ng/ml), but the glucose levels were similar in all three groups (162±5 and 168±7 vs 150±7 mg/dl).
In conclusion, chronic HS intake deteriorates several cardiometabolic and inflammatory biomarkers in Dahl/SS rats, which can be prevented by dopamine β-hydroxylase inhibition with zamicastat.

B. Igreja: A. Employment; Modest; BIAL - Portela & Cª S.A. **N. Pires:** A. Employment; Modest; BIAL - Portela & Cª S.A. **L.C. Wright:** A. Employment; Modest; BIAL - Portela & Cª S.A. **P. Soares-da-Silva:** A. Employment; Modest; BIAL - Portela & Cª S.A..

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Time of Food Intake is an Important Determinant of Blood Pressure Circadian Rhythm

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Blood pressure (BP) exhibits a 24-hour rhythm. Loss of BP oscillation is associated with significantly higher risk of target organ injuries. However, the mechanisms underlying the BP circadian rhythm remain incompletely understood. While light is a well-established prominent external cue that entrains intrinsic clock and circadian rhythm, recent studies indicate food intake is also an important external cue that can potently entrain intrinsic clocks especially those in peripheral tissues. However, whether BP circadian rhythm is affected by the time of food intake is unknown. If yes, via what mechanisms: are peripheral clocks and vascular functions involved? To address these specific questions, we used 12-14 weeks old male Per2::LUCIFERASE knock-in mice and investigated the effects of a two-week long, 10 hours light phase time restricted feeding (TRF, food only available from ZT2 to ZT 12) on BP circadian rhythm, clock gene oscillations and vascular contractile function. In the TRF group, food intake was initially decreased but gradually recovered to the ad libitum level by day 5. TRF did not alter body weight but significantly decreased non-fasting blood glucose. The BP was monitored continuously using radiotelemetry from 2 days prior to until 14 days after the TRF. Interestingly, the normal 24 hour BP oscillating rhythm transformed to a 12 hour oscillating rhythm by day 3 after TRF. Using LumiCycle, we investigated the clock gene Per2 expression in various isolated tissues. We found that the phase of Per2 luciferase oscillations was significantly shifted in liver and suprachiasmatic nucleus (SCN) containing brain slices, but was not significantly changed in aorta or mesenteric arteries. The oscillation amplitude of Per1 at mRNA level was suppressed in mesenteric arteries. Interestingly, the isometric contractile responses to high potassium depolarization, alpha1 agonist phenylephrine, and 5-HT were significantly suppressed in the abdominal aorta isolated from TRF group compared to those from ad libitum feed group. In summary, our results demonstrate that the time of food intake is an important determinant of blood pressure circadian rhythm. Moreover, time of food intake affects vascular clock gene oscillations and function.

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P226

Biochemical Properties of the N-terminally Palmitoylated Adrenomedullin

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Purpose: Adrenomedullin (AM) is a potent vasodilator peptide having pleiotropic effects including cardiovascular protection and angiogenesis. Because of these beneficial effects, AM appears to be a promising therapeutic tool for human diseases such as myocardial infarction or peripheral artery disease, while intravenous injection of AM stimulates sympathetic nerve activity due to the short-acting potent vasodilation resulting in increased heart rate and renin secretion. To lessen those acute unfavorable actions, we conjugated human adrenomedullin N-terminally with palmitic acid, and examined biological effects of palmitoylated AM in the present study.
Methods: Synthesized human AM peptide was conjugated with palmitic acid, and then

palmitoylation AM was purified by HPLC. Biological effects in vitro stimulating intracellular cAMP, a major second messenger of AM, were examined using cultured human embryonic (HEK)-239 cells stably expressing a specific AM receptor. Blood pressure-lowering effects in vivo were tested by intravenous injections of palmitoylated AM or native AM peptides into anesthetized rats. Plasma disappearance curve of peptides were evaluated by the two compartment model.
Results: Palmitoylated AM stimulated intracellular accumulation of cAMP in cultured HEK-293 cells, as did native human AM peptide, in a dose-dependent manner.
pEC50 of palmitoylatted AM was lower than natibe AM (8.49±0.12 vs. 9.17±0.12, mean±SEM, $P<0.05$), but no difference was noted in the maximum response of cAMP (579.9±24.5 vs. 667.2±24.5 pmol/well) . The first and second phases of plasma half-lives of native AM were 0.276 sec and 780 sec, while those of palmitoylated AM were 1.15 min and 599 min, respectively. Both half-lives of the palmitoylated peptide were significantly prolonged, as compared with the native peptide ($P<0.05$).
Conclusions: N-terminally palmitoylated AM stimulated cAMP production in vitro, showing smaller acute hypotensive action and a prolonged plasma half-life in comparison of native AM peptide in vivo. The present results suggest a possibility for palmitoylated AM as a therapeutic tool with lessened unfavorable effect of acute hypotensin of native AM.

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Safety and Efficacy of Losartan/hydrochlorothiazide Combination in Elderly Patients With Morning Hypertension

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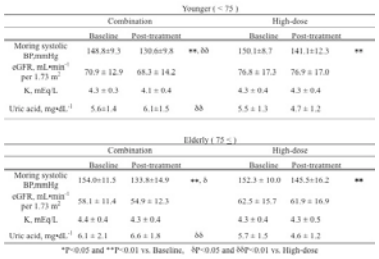
Background: Cardiovascular events occur most frequently in the morning. Elevation of blood pressure (BP) in the early morning (morning hypertension) is characteristic feature of hypertension in the elderly, and is attributed to cardiovascular events. However, treatment of morning hypertension has not been established, especially in the elderly patients. A combination of an angiotensin II receptor blocker (ARB) and hydrochlorothiazide (HCTZ) is a desirable choice of treatment for uncontrollable hypertension by antihypertensive monotherapy.
Purpose: The aim of this study was to compare the safety and effectiveness between an ARB/HCTZ combination therapy and high-dose ARB therapy in the elderly (75 years or more) and younger (less than 75 years) patients.
Methods: This study enrolled 201 (66 elderly and 135 younger) on-treatment patients having morning hypertension evaluated by home BP self-measurement. Patients were randomly assigned to receive 50mg-losartan/12.5mg-HCTZ combination (Combination) or 100mg-losartan (High-dose) therapy.
Results: During the 3-month treatment, the incidence of adverse events of Combination and High-dose therapies was similar in the elderly and younger patients. In the elderly patients, Combination therapy induced greater morning systolic BP reduction than High-dose therapy, whereas the two therapies showed the similar effects on estimated glomerular filtration rate

(eGFR), serum K, and uric acid, as well as in younger patients (Table).
Conclusions: ARB/HCTZ combination therapy was safe and more effective for controlling morning hypertension than high-dose ARB in the elderly patients, as seen in younger patients.



Younger (< 75)

	Combination			High-dose		
	Baseline	Post-treatment		Baseline	Post-treatment	
Morning systolic BP,mmHg	148.8±9.3	130.6±9.8	**, bb	150.1±8.7	141.1±12.3	**
eGFR, mL•min^{-1} per 1.73 m^2	70.9 ± 12.9	68.3 ± 14.2		76.8 ± 17.3	76.9 ± 17.0	
K, mEq/L	4.3 ± 0.3	4.1 ± 0.4		4.3 ± 0.4	4.3 ± 0.4	
Uric acid, mg•dL^{-1}	5.6±1.4	6.1±1.5	bb	5.5 ± 1.3	4.7 ± 1.2	

Elderly (75 =)

	Combination			High-dose		
	Baseline	Post-treatment		Baseline	Post-treatment	
Morning systolic BP,mmHg	154.0±11.5	133.8±14.9	**, b	152.3 ± 10.0	145.5±16.2	**
eGFR, mL•min^{-1} per 1.73 m^2	58.1 ± 11.4	54.9 ± 12.3		62.5 ± 15.7	61.9 ± 16.9	
K, mEq/L	4.4 ± 0.4	4.3 ± 0.4		4.3 ± 0.4	4.3 ± 0.5	
Uric acid, mg•dL^{-1}	6.1 ± 2.1	6.6 ± 1.8	bb	5.7 ± 1.5	4.6 ± 1.2	

*P<0.05 and **P<0.01 vs. Baseline, bP<0.05 and bbP<0.01 vs. High-dose

H. Uchiwa: None. **H. Kai:** None. **T. Ueda:** None. **T. Anegawa:** None. **Y. Aoki:** None. **Y. Iwmoto:** None. **K. Fukuda:** None. **Y. Fukumoto:** None.

P228

Endothelial Dysfunction Markers and Coagulation Factors Are Elevated in Individuals With Hypertensive Emergency

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Introduction: Hypertensive crisis (HC) is characterized as sudden and symptomatic elevation of diastolic blood pressure (BP) > 120mmHg. HC can be classified in emergency (HE), condition that presents target organ damage (TOD) and hypertensive urgency (HU), situation without TOD. Evidences have shown that the coagulation markers and endothelial dysfunction (ED) play an important role in the pathogenesis of chronic elevation of BP. However, scarce studies have demonstrated the involvement of these markers in the complications of acute elevation of BP. Objectives: To characterize biochemical profile of HU and HE, and to evaluate the participation of C-reactive protein (CRP), intercellular adhesion molecule (ICAM-1) and coagulation factors (fibrinogen and PAI-1) in subjects with HC. Methods: It was a cross-sectional study with 74 normotensive (NT), 74 controlled hypertensive (CH), 50 with HU and 78 individuals with HE. It was used MULTIPLEX technique for evaluating the clotting factors. Analysis of variance was used for comparative study among the groups, with significant p-value<0.05. Results: The diastolic BP and heart rate were higher in the HC group (120mmHg and 85bpm, respectively) compared to the CH group (75mmHg and 68 bpm, respectively). Individuals with HE were older. Glycaemia was significantly higher in the HE group (113mg/dL) in comparison to NT and CH (91mg/dL and 98mg/dL, respectively; p<0.05). It was also higher in the HU group (109mg/dL) compared with NT. Potassium was significantly lower in HE group (4,2mEq/l) compared to NT, CH and HU groups (4.5, 4.4 and 4.4mEq/L, respectively). CRP, fibrinogen, and PAI-1 were significantly higher in patients with HE (0.75, 0.04 and 2.46, respectively) in comparison to NT (0.19, 0.01 and 2.1, respectively) and CH (0.14, 0.01 and 2.25, respectively) groups, except ICAM-1. Logistic regression showed that CRP and fibrinogen were markers for HC development with odds ratios of 2.6 (1.24 to 5.50) and 8.72 (4.07 to 18.68), respectively. Conclusions: Individuals with HC present biochemical changes. Markers of ED and coagulation factors are higher in EH group compared to controls. This suggests the role of ED markers and coagulation factors in the pathogenesis of acute hypertensive event.

J.F. Vilela-Martin: None. **D.O. Andrade:** None. **L.N. Cosenso-Martin:** None. **M.F. Godoy:** None. **M.L. Gregório:** None. **D.R.S. Souza:** None. **J.C. Yugar-Toledo:** None.

P229

Is a Multidisciplinary Approach to Hypertension Better Than Traditional Care in Female Hypertensive Patients?

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Background: Rush Heart Center for Women (RHCW) opened in October 2003 to provide a multidisciplinary approach (MDA) for female patients (pts). RHCW provided personalized care plans to address women's heart health, with an emphasis on female-specific risk factors and symptoms. MDA including cardiologists, dietitians and nurse practitioners to treat female pts was compared to a similar cohort using standard practice in terms of HTN treatment.
Methods: A retrospective study identified pts with HTN treated at RHCW using a MDA and compared to a cohort of pts treated by cardiologists and received a standard level of care consistent with guidelines and recommendations (standard practice). Pts seen between 2008 and 2014 with at least 2 visits between 9 and 15 months apart were studied. Demographic moderating variables were race, age, and insurance. Moderating clinical variables were baseline systolic BP, body mass index, diabetes, smoking status, history of coronary disease, stroke, and prior treatment of HTN. Bivariate and multivariate analyses were conducted to determine the effect of treatment type, with pts' follow-up BP.
Results:
A total of 1486 pts were evaluated. Pts seen treated by MDA was younger by <3 years. Table shows descriptive statistics and bivariate analysis. Multivariate analysis revealed that pts treated with MDA had a significantly lower BP in their follow-up systolic BP, 3.8 mmHg less, compared to pts treated with standard practice. ($p<0.002$).
Conclusion:
The multidisciplinary approach had better BP control in female hypertensive pts. Prospective studies comparing MDA to standard practice may help to assess improved quality of life, compliance and outcomes.
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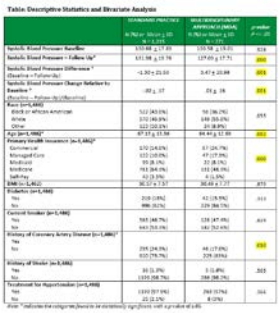

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P230

Glucagon-like Peptide-1-induced Increases in Afferent Renal Nerve Activity and Renal Sodium Excretion: Role of Trpv1

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Glucagon-like peptide 1 (GLP-1), an incretin hormone that has clinically been used to treat type 2 diabetic patients, may cause diuresis and natriuresis. However, the mechanism of GLP-1 effects on the kidney is largely unknown. We test the hypothesis that GLP-1 increases afferent renal nerve activity (ARNA) and renal sodium excretion by activation of the transient receptor potential vanilloid 1 (TRPV1) channels expressed in afferent renal nerves innervating the kidney. Exendin-4 (Ex4, 3*10-7M), a GLP-1 receptor agonist perfused into the left renal pelvis, increased ipsilateral ARNA in wild type (WT) mice when compared to TRPV1-null mutant (TRPV1−/−) mice (ARNA % integrated activity, WT:160±22 vs TRPV1-/-: 109±5, $p<0.05$). Ex4-induced increases in ARNA in WT mice were abolished by capsazepine, a selective antagonist of TRPV1, or by RP67580, a selective antagonist of the neurokinin 1 (NK1) receptor, but not by calcitonin-gene related peptide (CGRP)8-37, a selective antagonist of the CGRP receptor pre-perfused into the renal pelvis. Ex4 increased substance P (SP) and CGRP release from the renal pelvis isolated from WT but not TRPV1-/- mice. Ex4-induced increases in SP and CGRP in WT mice were prevented by Ex9-39, a GLP-1 receptor antagonist, 2,5-dideoxyadenosine, an adenylate cyclase inhibitor (ACI), and brefeldin A (BFA), an EPAC inhibitor, or attenuated by bisindolylmaleimide I (BIM) , a PKC inhibitor, and H89, a PKA inhibitor. Wortmannin (Wort), a PI3K inhibitor, had no effect on Ex4-induced SP or CGRP release. Acute Ex4 treatment (3ug/kg, i.p.) increased renal sodium excretion in both strains with greater degree of increases in WT compared to TRPV1-/- mice (increased % rate, WT: 160±36 vs. TRPV1-/-: 82±7, $p<0.05$). Thus, our data show that Ex4 increases ARNA, renal SP and CGRP release, and urinary sodium excretion in WT mice, and these functions of Ex4 are impaired in TRPV1-/- mice. These data indicate that Ex4-induced enhancement of ARNA and natriuresis attributes to, at least in part, activation of TRPV1-positive afferent nerves possibly via stimulating the GLP-1R/cAMP-PKA/PKC-TRPV1/SP pathway.

B. Zhong: None. **D. Wang:** None.

P231

High Fat Diet-induced Er Stress and Inflammation in the Kidney: Role of Trpv1-positive Afferent Renal Nerves

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Chronic high fat diet (HFD) intake may lead to enhancement in endoplasmic reticulum (ER) stress, reactive oxygen species (ROS) production, and inflammation in the kidney, but the mechanisms of HD effects are largely unknown. This study tests the hypothesis that HFD impairs afferent renal nerves expressing the transient receptor potential vanilloid 1 (TRPV1) channels, and that stimulating/preserving TRPV1-positive afferent renal nerves prevents against HFD-induced increases in ER stress, ROS production, and inflammation in the kidney. HFD decreased levels of TRPV1 in the kidney, levels of urinary calcitonin-gene related peptide (CGRP) and substance P (SP), and responses of afferent renal nerve activity (ARNA) to capsaicin, a TRPV1 agonist, perfused into the renal pelvis.

HFD increased ER stress (XBP-1, CREB2, GRP78, p-JNK), ROS production (urinary 8-isoprostane), and inflammation (TNF-α, IL-1β) in the kidney. N-oleoyldopamine (OLDA, a TRPV1 agonist, 1 ng/kg, daily) or vehicle was given intrathecally (i.t.) via indwelled catheters to segments (T8-L3) supplying the kidneys of rats fed a HFD or normal fat diet (Con) for 8 weeks. OLDA prevented HFD-induced decreases in the levels of renal TRPV1, urinary CGRP and SP, and ARNA responses to capsaicin, as well as HFD-induced increases in renal ER stress, ROS production, and inflammation. OLDA-induced protection during HFD was abolished by degeneration of TRPV1-positive afferent renal nerves by topical application of resiniferatoxin (RTX, 2 μg/ml x 2 times), a potent TRPV1 agonist, on renal nerves (TNF-α, Con: 0.17±0.02, HFD: 0.41±0.03, HFD+OLDA: 0.21±0.02, HFD+OLDA+RTX: 0.46±0.04, $p<0.05$; 8-isoprostane, Con: 8.8±1.2, HFD: 17±2, HFD+OLDA: 11±1, HFD+OLDA+RTX: 22±3 ng/day, $p<0.05$). Thus, our data show that HFD impairs TRPV1-positive afferent renal nerves, and chronic stimulation of TRPV1 via intrathecal injection of OLDA to T8-L3 segments preserves TRPV1-positive afferent renal nerves to against HFD-induced ER stress, ROS production, and inflammation in the kidney given that impairment of this population of afferent renal nerves abolishes OLDA effects. These data indicate that TRPV1-positive afferent renal nerves play a counteractive role against HFD-induced renal injury.

S. Yu: None. **D. Wang:** None.

P232

Dipeptidyl Peptidase-4 (DPP-4) Inhibitor, Linagliptin, Prevents Aortic Stiffening and Vascular and Cardiac Diastolic Dysfunction Caused by Western Diet Feeding in Female Mice

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Aortic stiffness, endothelial dysfunction and diastolic dysfunction (DD) are cardiovascular (CV) abnormalities seen in obesity associated with consumption of high fat/fructose western diet (WD). Moreover, CV dysfunction is increasingly prevalent in obese women. Herein, we examined whether the DPP-4 inhibitor, linagliptin (LINA), improves these outcomes in WD fed female C57BL/6 mice. Four week old mice were fed control diet (CD) or WD with or without LINA for 16 weeks, after which pulse wave velocity (aortic stiffness) (PWV), echocardiography (diastolic function), atomic force microscopy (endothelial stiffness) and wire myography (aortic vascular reactivity) were performed. Compared to CD mice, WD mice exhibited 21% and 353% higher PWV and endothelial stiffness, respectively. WD induced DD, indicated by impaired septal wall motion (<E'/A' ratio), left atrial filling pressure (>E/Vp ratio), prolonged isovolumic relaxation time (IVRT) and impaired myocardial performance index (>MPI). These vascular and cardiac abnormalities were prevented by LINA. LINA also prevented WD-induced impairments in acetylcholine-, sodium nitroprusside-, and insulin-mediated aortic vascular relaxation. These results show that LINA exerts CV protection in a translational model of obesity.

V.G. DeMarco: B. Research Grant (includes principal investigator, collaborator, or

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P233

Sodium Glucose Transporter Type-2 (SGLT-2) Inhibitor, Empagliflozin, Improves Cardiovascular Outcomes in Female Diabetic db/db Mice Independent of Blood Pressure Reduction

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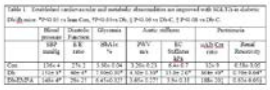
Dysglycemia, proteinuria, vascular stiffness, diastolic dysfunction (DD) and hypertension are abnormalities seen more frequently in the obese, diabetic population. SGLT-2 inhibitors (SGLT-2i), which increase urinary glucose/sodium excretion to lower HbA1c and blood pressure (BP), are emerging as unique diabetes therapies. We examined whether the SGLT-2i, empagliflozin (EMPA), ameliorates hypertension, dysglycemia, proteinuria, aortic stiffness and DD in obese/diabetic female db/db mice. Eleven week old mice were fed a diet with or without EMPA (10mg/kg/day) for 5 weeks. In vivo blood pressure (telemetry), diastolic function (echo), aortic stiffness (pulse wave velocity, PWV), proteinuria, renal resistivity (echo) and HbA1c were evaluated. Db/db had elevated BP that was not affected by SGLT2i. HbA1c, proteinuria and the renal resistivity index (RI) were elevated (P<0.001) in control (Db) mice vs treated mice (Db-EMPA) and lean control mice. Db exhibited DD that was ameliorated by SGLT2i as indicated by reduced LV filling pressure (<E/E') and improved septal wall motion (E'/A' ratio, not shown). Elevated PWV and aortic endothelial cell stiffness in Db-C were abrogated with SGLT2i. These results show that SGLT2i confers BP-independent cardiovascular and renal protective effects in obese diabetic female mice.



J. Sowers: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; National Institutes of Health, Veterans Affairs Merit Award. **A. Aroor:** None. **G. Jia:** None. **R. Nistala:** None. **M. Garro:** None. **G. Meininger:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; National Institutes of Health. **L. Martinez-Lemus:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; National Institutes of Health. **E. Mayoux:** A. Employment; Modest; Boehringer Ingelheim Pharma. **A. Whaley-Connell:** None. **V.G. DeMarco:** B. Research

Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Boehringer Ingelheim.

P234

High Sodium Intake is Associated with Increased Hemoglobin A1C in Young Overweight/Obese African Americans

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Introduction: Whether high sodium intake, assessed by 24-hour urinary sodium excretion (24hrUNaEx), is associated with altered glycemic control, evaluated by hemoglobin A1C (HbA1C), in the African American population remains unknown. We aimed to evaluate the relationship between 24hrUNaEx and HbA1C in young overweight/obese African Americans. Methods: A total of 106 apparently healthy overweight/obese drug-naïve African Americans were recruited. Subjects were asked to discard the first morning urine specimen and collect all remaining urine specimens for next 24-hr including a urine specimen of the following morning. HbA1C was measured from venous blood by ion-exchange chromatography. Results: The means (± SE) of age and body mass index (BMI) of subjects (67%, 71/106 females) were 24.30±0.82 years and 35.51±0.70 kg/m2, respectively. Average 24hrUNaEx was 172.17±7.09 mEq/L/d, which corresponded to average sodium intake of 3.96±1.63 g/d. Pearson's correlation analysis revealed a positive correlation between log transformed 24hrUNaEx and HbA1C after adjusting for age, sex, BMI, and systolic blood pressure (r=0.20, p=0.04). In a subgroup analysis involving subjects with HbA1C ≥5.7% (N=40), the correlation between 24hrUNaEx and HbA1C was stronger even with adjustment for the above variables (r=0.35, p=0.04). Conclusions: High sodium intake is associated with increased HbA1C independent of traditional risk factors in our study population. This relationship was stronger in subjects with HbA1C ≥5.7%, which by definition represents prediabetes (HbA1C ≥5.7 & <6.5%) and diabetes (HbA1C ≥6.5%). Although not fully understood, the possible mechanism by which high sodium intake could contribute to glycemic dysregulation involves cellular dysfunction and apoptosis of pancreatic beta cell induced by high sodium intakes via inflammation and oxidative stress.

S.J. Parikh: None. **J. Bhagatwala:** None. **A. Raed:** None. **Y. Huang:** None. **I. Kotak:** None. **G.A. Harshfield:** None. **Y. Dong:** None. **H. Zhu:** None.

P235

Employing the DASH Diet to Treat Non-dippers: A Two-month Intervention Study

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Introduction: Hypertensive patients with abnormal circadian blood pressure patterns, including a lack of nocturnal blood pressure dipping or rises in blood pressure from daytime to night-time, are at an increased risk for numerous cardiovascular events including strokes, heart failure and renal failure. Currently, limited therapeutic strategies exist to treat non-dippers. In this study, we examine the role of obesity on circadian blood pressure patterns and the impact of lifestyle intervention on nocturnal dip.

Methodology: 24-hour ambulatory blood pressure monitoring was performed before and after a 2-month intervention employing the DASH diet and lifestyle program with a targeted 5% weight loss in 80 volunteers. 20 control patients had a healthy nocturnal dip, 30 patients had a non-dipping blood pressure pattern, and 30 patients had a rise in blood pressure nocturnally from daytime.
Results: At baseline, there was a linear correlation between individuals' BMI and nocturnal blood pressure aberrancies ($r= 0.60$, $p<0.0001$). The control group had the lowest average BMI of 28.1 kg/m2. Non-dippers had a slightly higher average BMI of 30.3 kg/m2, and those with a rise in blood pressure nocturnally had the greatest average BMI of 35.3 kg/m2. After the two-month lifestyle intervention, individuals who achieved weight loss had significantly greater average reductions in nocturnal blood pressure (24.3 mm Hg), compared to daytime (12.1 mm Hg), resulting in the restoration of a more normal nocturnal dip and circadian blood pressure pattern. Non-dippers who achieved a 5% reduction in weight during the intervention had an average 8.31% nocturnal dip by the end of the study. Individuals who lost less than 5% of their weight or who gained weight continued to have a non-dipping blood pressure pattern by the end of the study.
Conclusions: The results of this study would suggest that perhaps reducing weight by adhering to the DASH diet and lifestyle intervention could be examined as therapeutic avenues for non-dippers in the future. The long-term effects of a restoration of normal circadian blood pressure pattern warrants further investigation.

A.K. Pandey: None.

Alginic Acid May Not Play a Major Role in the Mechanism of Alleviating Hypertension by Dietary Saccharina Japonica in 2-kidney, 1-clip Renovascular Hypertensive Rats

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Objective: Saccharina japonica (SJ), one of brown algae, is cultivated or grows wild in East Asia. The extract ‘dashi’ is used for soup stock in Japan. The intake of SJ was reported to decrease blood pressure (BP) in 2-kidney, 1-clip renovascular hypertensive (2K1C) rats in our previous study, as well as in spontaneously hypertensive rats (SHR) in other studies. Some investigators suggested that a major mechanism of reducing BP by dietary SJ in SHR includes alginic acid (AA). However, in our preliminary study it was not confirmed in 2K1C model, because we observed that dashi containing AA as much as 5% of what the original SJ contained alleviated the hypertension, too. To detect how AA in SJ contributes to the mechanism, we observed BP in 2K1C rats fed a diet containing SJ or extracts from SJ.
Methods: Male Sprague-Dawley rats (6 wks) were treated with sham operation (SHAM) or clipping the left renal artery (2K1C). After surgery, the rats started receiving a control diet (C) or a diet containing 5% (w/w) SJ (S), dashi extracted from the same amount of SJ in a correct method (D), extract from SJ equal in amount by boiling for a long time, in which AA was much eluted (B) or the SJ leftover after dashi was extracted (L) for 6 weeks. The systolic BP (SBP) was measured by a tail-cuff method

every week. At the end, mean arterial BP (MAP) was measured in each rat under anesthesia. After euthanasia, the aortas were collected for extracting mRNA and protein. Endothelial nitric oxide synthase (eNOS) mRNA expression (eNOS-M) was evaluated in aortas by reverse transcriptase-polymerase chain reaction. eNOS and phosphorylated eNOS protein expression (eNOS-Ps), was determined in aortas by western blot analysis.
Results: Six weeks after the surgery, SBP was significantly higher in 2K1C-C than in SHAM-C (182 ± 6 vs 126 ± 5 mmHg, P < 0.001). In 2K1C-S, -D, -B and -L (145 ± 4, 148 ± 4, 133 ± 3 and 146 ± 4 mmHg), SBP was significantly lower than that in 2K1C-C (P < 0.001, each). At the end of the protocol, MAP showed similar results to SBP. No significant differences were found in eNOS-M between each groups. eNOS-Ps were enhanced in 2K1C compared to SHAM (P < 0.05), but showed no significant differences by diet.
Conclusion: The role of AA and eNOS may be limited in the mechanism of alleviating hypertension by dietary SJ in 2K1C.

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P237

Dysregulation of Hypothalamic mTORC1, Sympathetic Nerve Activity and Vascular Reactivity in the Hypertensive Obese Prone Rats

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Neurogenic mechanisms play a major role in obesity-induced increase in sympathetic nerve activity (SNA) and arterial pressure, but the molecular pathways involved remain ill defined. Mechanistic target of rapamycin complex 1 (mTORC1) signaling in the hypothalamus has emerged as a critical molecular regulator of SNA, vascular function and arterial pressure. To analyze the status of hypothalamic mTORC1 signaling in obesity we compared the phosphorylated levels of ribosomal protein S6, a downstream effector of mTORC1, in the brain between obesity prone (OP) and obesity resistant (OR) rats. Body weight was elevated (P<0.05) in OP rats (763±22 g) relative to OR rats (575±19 g). OP rats also had higher fat mass. Interestingly, OP rats exhibited increased phospho-S6 in the mediobasal hypothalamus including the arcuate nucleus, but not in other nuclei such as the paraventricular nucleus, the supraoptic nucleus or the subfornical organ. Next, we assessed the hemodynamic and sympathetic parameters in OP and OR rats. Radiotelemetry systolic arterial pressure was greater in OP rats (133±1 mmHg) compared to OR rats (119±2 mmHg) at 8 weeks of age and remained elevated at 42 weeks of age. Ganglionic blockade with hexamethonium produced a dose-dependent decrease in arterial pressure in both the OP and OR rats, but the response was more pronounced (P<0.05) in OP rats. Direct SNA recording revealed elevated (P<0.05) renal and splanchnic SNA in OP rats (86±3 and 55±6 spikes/sec, respectively) compared to OR rats (48±2 and 22±4 spikes/sec). Using ultrasound Doppler, we found that OP rats have altered regional blood flows. Sodium nitroprusside-induced dilation

was attenuated and phenylephrine-evoked constriction was potentiated in the hindquarters vasculature of OP rats relative to OR rats. However, there were no differences in the renal, mesenteric or abdominal aorta vascular beds. Acetylcholine (ACh)-mediated relaxation was impaired in isolated coronary arteries from OP rats (relaxation to 10 µM ACh: 41±8% in OP rats vs 67±10% in OR rats, $P<0.05$). These studies raise the possibility that overactivation of hypothalamic mTORC1 signaling contributes to the altered hemodynamic, sympathetic and vascular functions associated
with the obesity prone phenotype.

C.L. Oltman: None. **K. Muta:** None. **B.L. Dake:** None. **D.A. Morgan:** None. **K. Rahmouni:** None.

P238

High Fat Diet-induced Obesity Disrupts Vascular Homeostasis in Both Kidney and Retina: Role of Epoxyeicosatrienoic Acids

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Obesity-induced vascular inflammation is considered an early and common pathological change for the development of microvascular complications including retinopathy and nephropathy. We have previously demonstrated that obesity down-regulated renal cytochrome P450 epoxygenase and decreased epoxyeicosatrienoic acids (EETs) levels in kidney as well as triggered retinal expression of the pro-inflammatory thioredoxin interacting protein (TXNIP) that coincided with vascular inflammation. The aim of this work is to examine the impact of obesity-induced reduction in EETs levels on TXNIP-inflammasome activation. Because EETs are quickly hydrolyzed by the soluble epoxide hydrolase (sEH) enzyme to inactive metabolites, we use sEH gene deleted mice (Ephx2 -/-) as a model with high EETs levels. WT and Ephx2 -/- were fed normal (14 % fat) or high fat diet (HFD, 60 % fat) for three months. HFD treatment down-regulated cyp2c44, the main epoxygenase for EETs production, and decreased EETs levels in kidney of obese mice and these changes were associated with podocyte loss and increased podocalyxin excretion as markers of glomerular injury. Although HFD treatment decreased EETs levels in Ephx2 -/- mice, it remained significantly higher than obese WT mice. HFD impaired endothelial function and induced TXNIP-inflammasome activation evident by increases in NOD-like receptor-3 (NLRP3) and interleukin-1β (IL-1β) in both retina and kidney. In parallel, HFD also increased leukostasis, retinal vascular permeability and development of acellular capillary, hall mark of ischemia in WT but not in TXNIP-/-. Restoring EETs levels in Ephx2 -/- mice improved endothelial function and was associated with decreased TXNIP-mediated inflammasome activation in the kidney of obese mice. Our data suggest that diet-induced obesity causes homeostatic imbalance by down-regulation of the anti-inflammatory epoxygenase/EETs levels and increasing the inflammatory TXNIP expression resulting in vascular injury in both retina and kidney.

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P239

Could Uric Acid Levels Predict Success In Chronic Weight Loss?

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[Background] Obese individuals have higher prevalence of hyperuricemia (hyperUA) and with weight loss (WL) improved hyperUA. We previously reported that serum uric acid levels (UA) associated plasma norepinephrine (NE) could predict future weight gain and BP elevation, and that WL program combining diet + exercise was most effective on UA, WL and BP reductions, although diet had stronger effects on UA and BP reduction compared to exercise..
[Objective] In this study, we examined whether lower UA levels would predict future success and maintenance of chronic WL compared to higher UA levels in a 2 yr WL regimen with diet and exercise.
[Methods] 154 overweight/obese men (BMI>25 kg/m2) consisting of 89 normotensive and 65 untreated mild hypertensives were enrolled in the WL program with diet + exercise. The subjects had BP, BMI, Fat-mass, UA, plasma NE, insulin, glucose, HOMA-IR measured at entry, 6, 12, and 24 months. WL was defined as ≥10% WL.
[Results] 95 subjects succeeded significant WL at 24 month including 34 subjects in WL Maintenance group (WL was observed at both 6 and 24 m), and 61 subjects with Slow WL (WL was observed at only 24 m). At entry, serum UA and plasma NE in WL group (WL maintenance + Slow WL) were slightly lower compared to those in non-WL groups (WL<10% at 24 m). Especially the slow WL group had significantly lower UA at entry compared to the non-WL group and WL maintenance group. Plasma UA and NE levels in the WL group were significantly lower than the non-WL group. Percent (%) reductions of UA and NE were greater in WL group than non-WL group. In all subjects, baseline UA and % changes of UA correlated with % changes in BMI over 24 m (R=0.205, P<0.05; R=0.230, P<0.05, respectively). Baseline UA correlated with % changes in UA, changes in Fat-mass, and changes in BMI (R=0.210, P<0.05; R=0.197, P<0.05; R=0.185, P<0.05, respectively). Plasma NE at entry correlated with changes in UA and % changes in UA in all subjects (R=0.263, P<0.05; R=0.412, P<0.01). BP reductions at 24 m correlated with WL and baseline UA in all subjects (R=0.345, P<0.05; R=0.247, P<0.05) respectively).
[Conclusions] Serum UA and plasma NE levels may predict success in chronic WL with WL regimens, and may predict BP reduction associated with WL.

K. Masuo: None.

P240

Gender Differences in the Association of Adiponectin and BMI in a Rural Cohort of African Americans

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Gender differences in the association of adiponectin with visceral and subcutaneous fat have been reported among African Americans (AAs). Specifically, adiponectin is negatively associated with visceral and subcutaneous fat in women but positively associated in males. The previous findings were obtained, however, under a controlled environment using expensive scanning equipment. In this study we wanted to determine if similar gender differences could be observed when sampling from a rural cohort of African Americans under less controlled conditions. African Americans (11 males; 36 females) attending a health fair at a local school were recruited and the following

were measured or collected: resting blood pressure, weight, self reported height, blood sample, and spot urine. Both males and females tended to be hypertensive (SBP: 148 ± 6 in males vs 129 ± 3 mmHg in females; p=0.002; DBP: 79 ± 5 and 70 ± 2mmHg, p=0.047) and obese (35.6 ± 2 vs. 33.4 ± 1 in males and females, respectively; p=0.52). Although there was no difference in BMI between males and females, total adiponectin levels were significantly higher in males as compared to females (6.9 ± 1 vs. 3.9 ± 0.5 μg/ml; p=0.026). Interestingly, BMI was positively associated with adiponectin in males (p=0.004) but there was no significant association between BMI and adiponectin in females (p=NS). These findings are similar to what has been previously reported when sampling under controlled conditions and suggest that sampling from the field could be a viable means to further investigate the gender differences in adiponectin and adiposity in African Americans, particularly among rural residents.

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P241

Uric Acid-Induced Adipocyte Dysfunction is Attenuated by HO-1 Upregulation: Potential Role of Antioxidant Therapy to Target Obesity

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Increased uric acid levels have been implicated in the pathogenesis of metabolic syndrome. To examine the mechanisms by which this occurs we hypothesized that an increase in Heme Oxygenase-1, a potent anti-oxidant gene will decrease uric acid levels and adipocyte dysfunction via suppression of ROS and xanthine oxidase levels. We examined the effect of uric acid, on adipogenesis in human mesenchymal stem cells (MSCs) in the presence and absence of cobalt protoporphyrin (CoPP), HO-1 inducer and tin mesoporphyrin (SnMP), HO activity inhibitor. Uric acid increased adipogenesis by increasing NADPH oxidase expression and elevation in the adipogenesis markers C/EBPα, PPARγ, Mest while decreasing small lipid droplets and the expression of Wnt10b. Importantly, we treated MSCs with fructose, a fuel source that increase uric acid levels. Our results showed that fructose increased XO expression as compared to the control and concomitant treatment with CoPP significantly decreased XO expression and also decreased uric acid levels. These beneficial effects of CoPP were reversed by SnMP; supporting a role for HO activity in mediating these effects. These novel findings demonstrate that increased levels of HO-1 appear crucial in modulating the phenotype of adipocytes exposed to uric acid and in down regulating XO and NADPH oxidase levels. Furthermore, this study offers new insight into potential therapies by which targeting the production and/or downstream signaling of uric acid can curtail adipogenesis.

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P242

Role of Human Renal Proximal Tubule Sodium Bicarbonate Cotransporter NBCe2 (SLC4A5) in Salt Sensitivity of Blood Pressure

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The sodium bicarbonate cotransporter NBCe2 (encoded by SLC4A5) partially regulates renal tubular sodium bicarbonate transport. Hypothesis: since SLC4A5 single nucleotide polymorphisms (SNPs, rs10177833 and rs7571842) are associated with salt sensitivity of blood pressure, the gene product, NBCe2, would be involved with the etiology of human salt sensitivity. NBCe2 was localized in freshly fixed renal tissue and in primary and immortalized RPT cell (RPTC) cultures from tissue or isolated from urine. Basal expression of NBCe2 mRNA and protein was not different between RPTCs carrying WT or HV SLC4A5 before or after dopaminergic or angiotensin (II and III) stimulation. However, total transcellular sodium transport, NHE3 protein expression, and Cl-/HCO3- exchanger activity were higher in SLC4A5 HV than WT RPTCs (WT: 8.6±1.2 mM NaCl n=6, 5207.1±386.4 RFU n=36, 0.265±0.006 pH unit/min, n=33 respectively; VS HV: 14.75±0.7 mM NaCl n=4, 6946.2±500.4 RFU n=48, 0.314±0.018 pH unit/min n=35 respectively, $p<0.01$). Aberrant sodium transport was even more evident after increasing intracellular sodium, which resulted in increased NBCe2 mRNA, NBCe2 protein and bicarbonate transport in HV RPTCs compared to WT (WT 146% ± 24% , 109% ± 4.7%, 89% ± 4.5%, respectively, VS HV 214% ± 23%, 128% ± 5.7%, 141% ± 4.8%, respectively N=8-12, $p<0.05$). RPTCs carrying HV variants showed increased binding of HNF4A to SLC4A5 DNA, which was blocked by two HNF4A antagonists. Assays in RPTCs isolated from urine showed increased bicarbonate-dependent pH recovery in RPTCs from salt-sensitive subjects who are HV for SLC4A5. NBCe2 under high sodium is hyper-responsive in RPTCs carrying SLC4A5 HV through an aberrant HNF4A-mediated mechanism.

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P243

A Maternal High Salt Diet During Pregnancy and Lactation Affects Offspring Cardiac Function

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The maternal environment during pregnancy and lactation has important effects on the offspring's cardiovascular system. For example, changes in offspring blood pressure and vascular structure have been reported to occur in response to a maternal high salt intake during pregnancy and lactation. However, it remains unclear whether a maternal high salt intake may affect cardiac function in offspring. We previously demonstrated that high salt diet causes aggravation of hypertension in young spontaneously hypertensive rats (SHR). Therefore, in the present study we investigated the influence of exposure to a maternal high salt diet during gestation and lactation on offspring cardiac function in SHR.
SHR dams were fed either a high salt diet (4% NaCl) or a control (0.3% NaCl) diet. After weaning, the offspring were fed the control diet for 8 weeks. Systolic blood pressure and heart rate of the offspring were measured without heating using a photoplethysmographic tail-cuff system at 11 weeks of age. Indices of both left ventricular (LV) systolic and diastolic function (LV developed pressure [LVDP], and maximum rate of LV decline [-dP/dt]) and coronary flow were determined in 12 week old offspring using a Langendorff isolated perfused working heart system.
Offspring of the high-salt diet-fed dams (High salt) had lower blood pressure, heart rate, and indices of both LV systolic and diastolic function, compared with offspring of the control diet-fed dams (control) (Fig.). There were no significant differences in coronary flow or heart weight between the two groups. These results suggest that a maternal high salt intake is a predisposing factor for disturbance of cardiac function in offspring.

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P244

Role of the Na-H Exchanger Regulatory Factor 1 (NHERF1) in Hypertension of Aging Animals

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Aging animals develop hypertension when challenged with high salt diet due, in part, to desensitization of dopamine receptors (DR) in renal proximal tubules (RPT). We have demonstrated that NHERF1 associates with DR1 and Na-K ATPase (NKA) and is important for regulation of NKA in RPT. Preliminary data showed loss of NHERF1 expression in 22m old F344 rats. We hypothesized that loss of NHERF1 results in increased blood pressure (BP) and lack of natriuretic response to dopamine (DA) in aging animals. To address this hypothesis, Fischer Brown Norway (FBN) rats (1m, 4m, 12m, and 24m old) were fed diet containing 1% or 8% NaCl for one week and, BP was measured in

anesthetized animals using an indwelling left femoral artery catheter. 8% NaCl did not increase BP in 1m or 4 month old rats. By contrast, 8% NaCl diet increased BP in 12m (84.3±3.5 vs 90.8±2.36) and 24m (73.5±7.58 vs 104±1.6) old animals. To determine if lack of NHERF1 is responsible for the increase in BP, we measured BP in 12 m old WT and NHERF1 KO mice. By contrast to WT mice, 8% NaCl diet did not increase BP in NHERF1 KO mice (84±4.9 vs 96.5±3.56 (WT) and 78.2±3.89 vs 81.8±9.2 (NHERF1 KO mice)). To confirm that NHERF1 is required for DA-mediated inhibition of NKA, NKA activity in primary proximal tubule cells (PTC) from young and old mice in culture was measured in the presence or absence of DA. DA decreased NKA activity in PTC from young animals (67.2±3.8 vs 32.7±5.3) but not in PTC from old animals. Transfection of NHERF1 restored NKA regulation by DA in PTC from old rats (58.4±4.2 vs 64.4±4.3 (in untransfected cells) 54.2±3.8 vs 31.1±3.4 (in NHERF1 transfected cells)). We conclude that NHERF1 regulates DA-mediated proximal tubule sodium handling; however, other factors modulate BP response to dietary sodium intake in young and old animals. The contribution of NHERF1 and dopamine signaling to sodium homeostasis requires further study.

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P245

High-salt Diet Induces Catabolic Urea Formation and Muscle Wasting to Enable Renal Salt Concentration

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Background: We showed previously that a high salt diet (HSD) increases cortisol levels in man. We hypothesized HSD induces catabolic urea generation to facilitate renal water conservation during dietary salt excretion.
Methods: 16 male mice were pair-fed either a low-salt diet (<0.1% NaCl plus tap water) or a HSD (4% NaCl plus 0.9% saline water) for two weeks. We investigated urinary concentration, body weight, MRI lean body mass, tissue urea levels, and enzymatic urea and creatine generation in liver, skeletal muscle, skin and kidney.
Results: HSD increased renal Na clearance, decreased urea clearance and resulted in urinary Na concentration. HSD reduced body weight and lean body mass, indicating catabolic muscle wasting. This catabolic state was paralleled by urea accumulation and increased arginase activity in liver and in skeletal muscle, while renal urea excretion was reduced. Expression of L-arginine:glycine amidinotransferase (AGAT), which generates the creatine precursor guanidino-acetate and is representative for anabolic liver/muscle

metabolism, was selectively reduced in the livers of HSD mice.
Conclusion: HSD induces urea production and body urea accumulation to allow for water-economizing urinary Na concentration. The liver regulates Na homeostasis and induces catabolism by favoring urea over creatine production. Catabolic skeletal muscle serves as a glutamine reservoir for urea generation in HSD mice, resulting in sarcopenia.
Perspectives: Cachexia is associated with increased cardiovascular mortality and heart failure in humans. Our findings link this condition to catabolic urea osmolyte generation for maintaining Na balance.
$$MISSING OR BAD IMAGE SPECIFICATION {78679E93-E94A-4466-AD80-48BBA1CCD048}$$

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P246

Salt-sensitivity of Angiogenesis Inhibition-induced Blood Pressure (BP) Rise: Role of Interstitial Sodium Accumulation?

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Objective
Angiogenesis inhibition with the VEGF-inhibitor sunitinib, an established anti-cancer therapy, induces hypertension and proteinuria. Exposed to osmotic stress, the mononuclear-phagocyte-system (MPS) cells produces VEGF-C and exert homeostatic regulatory activity by promoting lymphatic Na+ drainage; interference with this process resulted in salt-sensitive hypertension. Therefore, we hypothesized that sunitinib, via blockade of the VEGF pathway, leads to Na+ accumulation in the skin and salt-sensitive hypertension.
Design and Methods
In male WKY rats, mean arterial pressure (MAP) was monitored telemetrically during oral treatment with sunitinib (7 mg/kg.day, n=7-8) or vehicle (n=7-8) after a normal salt diet (NSD: 0.5-1.0% NaCl and tap water) or a high salt diet (HSD: 8% NaCl and saline water) for 2 weeks. After 8 days of sunitinib or vehicle administration, 24-h urine was collected. After sacrificing, blood was collected for biochemical measurements, skin for Na+ concentration ([Na+]) using dry-ashing, and ears for staining of MPS cells (CD68).
Results
MAP during NSD was 101±0.9 mmHg. HSD increased MAP by 27±3 mmHg ($P<0.05$ vs. NSD). Sunitinib increased MAP by 15±1 mmHg during NSD ($P<0.05$ vs. NSD alone) and by 23±4 mmHg during HSD ($P<0.05$ vs. HSD alone). Although body weight, plasma [Na+] and plasma [cystatin-C] did not change in response to HSD and/or sunitinib, skin [Na+] increased from 90±1 (NSD) to 100±4 (HSD), and 108±4 mmol/L

(HSD+sunitinib), respectively (P<0.01 for linear trend). Skin [Na+] correlated with MAP (r=0.57, P<0.01). Compared to NSD, proteinuria increased during HSD, rising further (P<0.05) with sunitinib. CD68 positive area increased during HSD from 0.29% to 0.43% (P<0.05), and increased even further with sunitinib (0.63%, P<0.05).

Conclusions

Angiogenesis inhibition-induced hypertension is salt-sensitive. The parallel increases in BP and skin [Na+], in the face of unaltered plasma [Na+] and bodyweight, support the existence of a Na+-buffering compartment in the skin that may contribute to the salt-dependent volume and BP homeostasis during VEGF inhibition.

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P247

Tumor Necrosis Factor-alpha Receptors (Type 1 & Type 2) are Differentially Expressed in Renal Tissues During Chronic Dietary Intake of High Salt and Angiotensin II Treatment

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Tumor necrosis factor-alpha (TNF-α) exerts natriuresis that is mediated by its' receptor type 1 (TNFR1) while its' receptor type 2 (TNFR2) is involved in mediating inflammatory renal injury. To determine the differential roles of these receptors in angiotensin II (AngII) induced salt-sensitive hypertension and associated renal injury, protein expressions of TNFR1 and TNFR2 were examined in mice (n=6-7 in each group) chronically treated with or without AngII (25 ng/min; implanted minipump) for 4 weeks which were fed either normal (NS; 0.4% NaCl) or high salt (HS; 4% NaCl) diets. Systemic blood pressure (SBP) in these mice was measured by tail-cuff plethysmography and 24 hour urine collections were made using metabolic cages at the start and at the end of treatment period when the kidneys were harvested after sacrificing the mice with euthanasia. Immuno-histochemical analysis of TNFR1 and TNFR2 proteins in renal slices was performed by measuring the staining area as well as the intensity of receptors' immunoreactivities using NIS Elements Software (Nikon), which allowed the semi-quantitation of positive staining and the intensity of these proteins. The results were expressed in percent area of positive staining and the relative intensity. HS intake alone did not alter mean SBP (HS; 77±1 vs NS; 76±3 vs mmHg) but it caused an exaggeration of AngII induced increases in mean SBP (AngII+HS; 104±2 vs AngII+NS; 95±2 mmHg). The area of TNFR1 staining was higher in HS (6.0± 0.9 vs 3.2 ±0.7%; P<0.05) than NS group but it was not significantly different between AngII+HS (5.0± 0.7%) and AngII+NS groups (6.3±0.7%). Similar qualitative differences were also observed in relative intensity in protein expressions. TNFR2 immunoreactivity was minimal in NS and HS groups but it was high in AngII+NS group and even greater in AngII + HS group. These data suggest that the increases in TNFR1 activity due to HS alone facilitate salt excretion that results no change in SBP in response to HS intake. However, such HS induced increases in TNFR1 activity was compromised in elevated AngII condition causing more salt retention and thus, exaggerated hypertensive response. On the other hand, HS induced increases in TNFR2 activity in elevated AngII condition facilitates enhanced renal injury response.

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P248

Changes in Dipping Pattern of Blood Pressure During the Progression of Renal Injury in Dahl Salt-sensitive Hypertensive Rats

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A growing body of clinical evidence has indicated that non-dipper pattern of circadian rhythm of blood pressure (BP) is a great risk of cardiovascular disease, which is accompanied by impaired renal function and proteinuria. Here, we aimed to investigate the circadian rhythm of BP during the progression of renal injury in Dahl salt-sensitive (DSS) hypertensive rats. DSS rats were treated with a high salt diet (HS; 8% NaCl) for 10 weeks (n=10). Before starting a HS diet, the difference in mean arterial pressure (MAP) between dark and light period was 6.46±0.61 mmHg in normal salt (0.3% NaCl)-fed DSS rats. Treatment with a HS diet for 5 days did not change renal function, but blood pressure was increased. Furthermore, the difference in MAP between dark and light period was significantly increased (11.05±0.87 mmHg, $P<0.05$), suggesting extreme dipping type of circadian BP. However, further HS diet feeding for 10 weeks induced the development of hypertension, renal tissue injury and proteinuria, which were associated with non-dipper pattern of BP. Namely, the MAP was similar between dark and light period (180.47±6.26 vs.177.92±6.33 mmHg). After switching to normal salt diet for 4 weeks, MAP was significantly decreased and circadian rhythm of BP was returned to normal dipper type (157.27±3.96 vs. 149.93±4.11 for MAP in dark and light period, respectively). In conclusion, the present study has demonstrated that treatment with HS diet initially showed extreme dipping pattern of BP in DSS rats, whereas it changed to non-dipping pattern of BP during the progression of renal tissue injury and proteinuria. These data support the hypothesis that non-dipping pattern of BP is associated with the progression of renal injury during the development of salt-dependent hypertension, which may contribute to the cardiovascular events.

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P249

Central Nervous System Control of Plasma Aldosterone and Endogenous Ouabain in Angiotensin II-Salt Hypertension

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High salt intake markedly enhances hypertension induced by Ang II. We evaluated peripheral mechanisms which may amplify central mechanisms activated by Ang II-salt. In the 1st exp, Wistar rats were sc infused with Ang II at low dose of 150 ng/kg/min together with 2% high salt diet for 14 days. In the 2nd exp, MR blockers (eplerenone, spironolactone), ENaC blocker (benzamil), AT_1R blocker (losartan) or vehicles (Veh) were icv infused combined with Ang II-salt. BP was recorded by telemetry. Plasma corticosterone (Cor), aldosterone (aldo) and endogenous ouabain (EO) were measured by RIA. Gene expression was assessed by real-time qPCR. Ang II alone caused a small increase in MAP (112±1 vs. 99±1 mmHg), but BP was markedly increased by Ang II-salt (152±4 mmHg, P<0.05 vs. others). BP increases to Ang II-salt were largely inhibited by central infusion of MR blockers, benzamil or losartan. Only Ang II together with salt increased plasma aldo, Cor and EO. In the adrenal cortex, both Ang II alone and Ang II-salt increased CYP11B2 expression but neither affected CYP11B1, Hsd3b1 or AT_1R mRNA expression. Central blockades significantly (p<0.05) lowered plasma aldo and EO in rats on Ang II-salt. Central blockades had no effect on Hsd3b1, CYP11B1 and AT_1R mRNA but markedly decreased Ang II-salt induced CYP11B2 expression in the adrenal cortex. Together, these results suggest that in Ang II-salt hypertension, Ang II-AT_1R signaling and MR-ENaC pathway in the brain increase plasma aldo and EO, which may amplify BP responses to central mechanisms and contribute to severe hypertension by Ang II-salt vs Ang II alone.

Plasma aldo, Cor and EO and mRNA expression in the adrenal cortex

(n=6-8/group)	Control	Salt only	Ang II only	Ang II + salt
Plasma aldo (pg/ml)	149±39	90±23	155±40	439±113§
Cor (pg/ml)	98±23	115±32	75±27	198±43§
EO (pM)	475±120	513±179	485±68	866±193§
CYP11B1/GE1	5.8±0.4	7.9±0.5	6.7±0.4	6.6±0.6
CYP11B2/GE1(×10^{-3})	3.4±0.5	0.6±0.3¶	9.3±2.4*	7.8±3.5*
Ang II+salt	Veh (n=10)	MR inh. (n=9)	Ben (n=8)	Los (n=6)
Plasma Aldo (pg/ml)	97 ± 21¶	41 ± 9	69± 31	44 ± 13
Cor (pg/ml)	108±35	72±24	113±57	53±17
EO (pM)	401 ±65¶	291 ± 33	300±11	228 ± 41
CYP11B1/GE1	6.0±0.4	6.2±0.3	5.6±0.8	5.6±0.7
CYP11B2/GE1(×10^{-3})	6.3 ±1.1¶	0.2±0.06	1.0± 0.2	0.1 ± 0.05

§ p≤0.05 vs others, * p<0.05 vs control or salt only, ¶ p<0.05 vs Losartan, ‖ p<0.05 vs others

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P250

Central Mechanisms Mediating Angiotensin II-Salt Hypertension in Rats

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Circulating Ang II causes persistent activation of brain angiotensinergic pathways through a neuromodulatory aldosterone (aldo)-mineralocorticoid receptors (MR)-epithelial Na^+ channel (ENaC)-ouabain pathway. The response of BP to circulating Ang II is enhanced by high salt intake. To evaluate the central mechanisms that mediate Ang II-salt induced hypertension, Wistar rats were treated with regular salt diet (0.4% NaCl), high salt diet (2% NaCl), sc Ang II (150 ng/kg/min), or sc Ang II with high salt diet for 14 days. In the 2nd exp, MR blockers (eplerenone, spironolactone), ENaC blocker (benzamil), AT_1R blocker (losartan) or vehicles (Veh) were icv infused combined with Ang II-salt. BP was recorded by telemetry. Gene expression was assessed by real-time qPCR. Plasma Ang II and tissue aldosterone were measured by RIA. Ang II alone caused a small increase in MAP (112±1 vs 99±1 mmHg), and BP was markedly increased by Ang II-salt (152±4 mmHg, P<0.05 vs. others). BP increases to Ang II-salt were largely inhibited by central infusion

of MR blockers (103±2mmHg), benzamil (100±3mmHg) or losartan (98±4mmHg). Ang II alone or together with salt decreased 11βHSD2 and MR mRNA expression but increased AT_1R and ENaC γ mRNA expression in the PVN, and increased AT_1R mRNA level in the RVLM. Sc Ang II or high salt diet had no effect on mRNA levels of CYP11B1, B2, ENaC α or β in the PVN or RVLM. Considering that AT_1R mRNA expression increases in both PVN and RVLM and central MR-ENaC-AT_1R blockade prevents the hypertension, these results suggest that activation of Ang II-AT_1R signaling and MR-ENaC pathway in the brain contribute to both Ang II and Ang II-salt hypertension.

Plasma Ang II, tissue aldo and mRNA expression

(n=6-8/group)		Control	Salt only	AngII only	AngII + salt
Plasma Ang II	(pg/ml)	14.4±2.3	7.2±1.9	12.2±3.0	13.6±2.0
Hypothalamic aldo	(pg/g)	102±23	79±15	-	153±37
mRNA levels/PGK1				PVN	
MR	(×10⁻³)	9.0±0.1	9.7±0.3	8.1±0.4*	7.7±0.5*
11βHSD2	(×10⁻⁴)	10.8±0.7	10.5±0.7	9.4±0.5*	9.2±0.4*
ENaC γ	(×10⁻⁴)	6.1±0.3	7.4±0.7	8.4±0.6*	8.2±0.5*
AT_1R	(×10⁻³)	5.4±0.2	5.5±0.3	6.9±0.9*	6.7±0.8*
				RVLM	
AT_1R	(×10⁻³)	5.8±0.3	5.9±0.2	7.4±0.6*	7.0±0.7*

*p<0.05 vs control or salt only

J. Lu: None. **H. Wang:** None. **M. Ahmad:** None. **F.H. Leenen:** None.

P251

3-hydroxy-3-methylglutaryl-coenzyme A reductase Inhibition Reduces Intimal Neovascularization and Plaque Growth in Apolipoprotein E-Deficient Mice

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Objective: The interactions between the renin-angiotensin system and neovascularization in atherosclerotic plaque development are unclear. We investigated the effects of 3-hydroxy-3-methylglutaryl-coenzyme a reductase inhibition with pitavastatin in the pathogenesis of atherosclerosis in ApoE-/- mice with special focus on plaque neovascularization. Methods and Results: Ten-week-old male ApoE-/- mice fed a high-fat diet were randomly assigned into two groups and administered vehicle (0.5% carboxymethyl cellulose) or pitavastatin (PiS, 1 mg/kg daily) for 12 weeks. Quantification of plaque areas at the aortic roots and in the thoracic and abdominal aortas revealed that, in all three regions, AT1R antagonism reduced the intimal neovessel density and the mRNA levels of toll-like receptor (TLR) 2 and TLR4. PiS increased the contents of collagen and elastin, lessened the macrophage component as well as the level of monocyte chemoattractant protein-1 and osteopontin protein in aortic roots, and reduced the mRNA and activity levels of matrix metalloproteinase (MMP)-2 and MMP-9 in aortic roots and thoracic aortas. Neointimal vessel density, the extent of atherosclerotic lesions, and the levels of TLR2 and TLR4 mRNA, were lower in ApoE-/-MMP-2-/- mice than in controls. The amounts of TNF-α and IL-1β protein as well as the levels of MMP2, MMP-9, TLR2, and TLR4 mRNA were increased by exposure to ox-LDL and lipopolysaccharide. These effects were diminished by PiS and small interfering RNAs targeting TLR2 or TLR4 in cultured macrophages. Conclusions: 3-hydroxy-3-methylglutaryl-coenzyme a reductase inhibition appears to inhibit intimal neovascularization in ApoE-/- mice, partly by reducing TLR2/TLR4-mediated inflammation and MMP activation, thus decreasing atherogenic plaque growth and instability.

X. Cheng: None. **L. Hu:** None. **A. Inoue:** None. **H. Wu:** None. **H. Jiang:** None. **X. Li:** None. **K. Okumura:** None. **M. Kuzuya:** None.

P252

Effect of Maternal Diabetes on Fetal Programming of Vascular Remodeling Mechanisms in Adult Rats

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Modifications of the intra-uterine environment are now recognized as an important cause of fetal stress. Leading to several responses such as loss of structure/function and pre-emptive adaptations to an adverse post-natal environment, and finally to adult diseases. It has been demonstrated that children of diabetic mothers have an increased risk of developing cardiovascular diseases (i.e. hypertension) during adulthood.
Blood vessels are able to reorganize their structure in response to physiological alteration of blood flow or pathological stimuli. Constrictive vascular remodeling is currently associated with the occurrence of cardiovascular diseases. Thus, our objective was to study vascular remodeling in case of in utero exposure to maternal diabetes. This is in order to investigate the effect of intra-uterine environment perturbations on vascular fetal programming. We have developed an animal model of rats exposed in utero to moderated maternal hyperglycemia (DMO), which developed an hypertension around 6 months of age.
We analyzed structure of elastic and resistance arteries (internal diameter, MCSA, intima-media thickness and remodeling parameters) in absence or presence of established hypertension in male DMO compared to controls (CMO) (at 3 and 18 months of age). Moreover, by in vivo sequential ligation of mesenteric arteries, we have studied vascular remodeling of resistance arteries in response to low (LF), normal (NF) or high flow (HF).
In old DMO, we did not observe any vascular remodeling induced by hypertension neither in aorta nor in mesenteric arteries. This could be related to the increase of smooth muscle cell attachments that we observed. Moreover, after 1 or 3 weeks of mesenteric arteries ligation, DMO did not exhibit any constrictive remodeling in LF although expansive remodeling in HF is maintained. Interestingly, we measured an increase of eNOS activity and GP91 in LF arteries of theses animals although transglutaminase-2 protein expression was not modified.
Our results demonstrate that in utero exposure to maternal diabetes induce modification of vascular remodeling mechanisms in adulthood. Absence of vascular response could be a pre-emptive adaptation to fetal programmed-hypertension in these animals.

A. Dib: None. **J. Bourreau:** None. **L. Loufrani:** None. **D. Henrion:** None. **C. Fassot:** None.

P253

Angiotensin Converting Enzyme Inhibition vs. Angiotensin II Receptor Blockade in Marfan Mouse: Role of Bradykinin

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Angiotensin receptor blockers (ARB) decrease aortic root aneurysm progression in a mouse model of Marfan syndrome, by interfering with angiotensin II (Ang II) activation of transforming growth factor beta (TGF-β) signaling. Angiotensin converting-enzyme inhibitors (ACEi)

decrease the formation of Ang II, but also increase bradykinin (BK) which can activate the TGF-β pathway. We tested the hypothesis that ACEi and ARBs differ in their effect on aortic aneurysm development in Fbn1C1039G/+ mice due to different effects on BK. We compared the effect of 12-week treatment with telmisartan (3 and 24 mg/L), ramipril (30mg/L) or placebo in the presence or absence of the BK B2 receptor antagonist HOE140 (0.1 mg/Kg/day). ARB and ACEi reduced blood pressure similarly. ARB and ACEi reduced aortic root diameter (by 17.3±3% and 19.1±5%) but only ARB prevented elastin disruption (Figures 1A and 1B), decreased mast cell infiltration and P-ERK immunoreactivity in the ascending aorta. HOE-140 alone did not reduce the aortic root diameter but diminished the aortic elastin disruption. In the lung, ARB compared to ACEi treatment, prevented the development of emphysematous changes (Figure 1C), decreased mast cell infiltration (4.87±0.98% vs. 9.12±1.23%, p=0.012), and decreased MMP12 (1.02±0.08 vs. 1.52±0.13 A.U, p=0.002) and MMP9 (1.32±0.08 vs. 1.69±0.11 A.U, p=0.006) activity. HOE-140 alone prevented emphysematous changes and decreased MMP12 activity. Endogenous BK contributes to elastin disruption in the aortic root and lung by promoting activation of P-ERK and increasing MMP12 activity, respectively. Clinical studies are required to compare the efficacy of ACEi and ARB in Marfan syndrome.

J.L. Gamboa: None. **K.D. Hill:** None. **S.M. Fowler:** None. **V. Kon:** None. **H.C. Dietz:** None. **N.J. Brown:** None.

P254

Hypertension: A proposed Mechanistic Pathway for Arterial System Remodeling

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Arterial properties change during hypertension (HTN) development. The time course of the changes can be used as a marker of vascular remodeling in the development of HTN.

Study Aim

To determine whether pressure induced changes in vascular properties result in alterations in pressure-flow patterns that impact the load for ventricular function: input & characteristic impedance, **A**rterial **C**ompliance (AC)= SV/PP (stroke volume)/(pulse pressure), AC=Tau/peripheral resistance (PR), and PP were investigated.

Methods

Pressure and flow relations were investigated in dogs during the development of renal-HTN over 4-weeks (Wks).

The sensitivity of hemodynamic markers of vascular remodeling was evaluated by changes in AC= SV/PP, and by AC= Tau/PR and PP.

Results

All BP components increased during the development of HTN.

Changes in Input Impedance, increased through Wks 2 to 4. Increases in the steady component of the cardiac cycle were reflected by peripheral PR, and changes in the pulsatile component were reflected by changes in AC=SV/PP, and AC=Tau /RP; and changes in characteristic impedance.

PP and PAP increased by Week 2, reflection of the cardiac and arterial workloads and vascular Tension RP that progressed through Wk 4

Conclusion: Reliable and accessible markers that indicate changes in arterial pressure, flow & volume, are: PP and AC (SV/PP), and AC (Tau

/TPR). These variables detected increases in the heart-load and alterations in pressure-flow patterns that impair cardiovascular-function resulting in HTN-CVD; input and characteristic impedance, also provides remodeling information about the arterial system but are not readily available to clinicians.

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P255

Age and Structural Alterations in Subcutaneous Small Resistance Arteries of Hypertensive Patients

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Background It was proposed that early vascular ageing may be an important mechanism of vascular damage in large conductance arteries. However it is not known whether aging may also affect small resistance artery morphology. Patients and Methods For this reason, we investigated 100 patients with essential hypertension. Secondary forms of hypertension were excluded according to standard clinical evaluations and biochemical or instrumental assessments. In all patients, an evaluation of small resistance arteries morphology was performed by a wire micromyographic approach (Mulvany's technique). A small amount of subcutaneous tissue was obtained by local biopsy or during election surgery and subcutaneous small resistance arteries were dissected and mounted on a myograph; the media to lumen ratio (M/L) was then measured. Results The age range of our populaton was 22-81 years, with a mean value of 57±12 years; 14% of them were current smokers, 32% had alterations in lipid patterns, none of them had diabetes mellitus, 58 were males and average blood pressure values were 156/95±19/12 mmHg.

We found a significant correlation between M/L and age (r=0.30, p=0.002): the statistical significance of the correlation persisted after correction for counfounding variables (gender, serum cholesterol, smoking status, serum glucose, systolic or diastolic blood pressure values). A statistically significant inverse correlation was also observed between internal diameter and age (r=-0.20, p=0.046), while the correlation beween age and media thickness did not reach statistical significance (r=0.09, p=0.37).

Conclusion Our data suggest that age may affect microvascular structure in hypertensive patients. It is also possible that hypertension may anticipate the effects of physiological

aging, and this should be explored in a relatively large population of normotensive subjects.

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P256

Structural Alterations of Subcutaneous Small Resistance Arteries in Resistant Hypertension

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Background It is was suggested that, in resistant hypertension, the presence of particularly pronounced microvascular alterations may contribute to explain the relative lack of response to treatment
Patients and Methods We investigated a population of 94 treated essential hypertensive patients. Secondary forms of hypertension were excluded, and in all patients a 24-hour blood pressure monitoring was performed in order to exclude a white coat effect. In all patients, we evaluated small resistance arteries morphology by a wire micromyographic approach. Subcutaneous tissue was obtained by local biopsy or during election surgery and subcutaneous small resistance arteries were dissected and mounted on a myograph; the media to lumen ratio (M/L) was then measured. We subdived our patents according to the presence or not of resistant hypertension (clinic blood pressure values above 140/90 mm Hg despite administration of three antihypertensive agent including a diuretic and 24-hour blood pressure values >130/80 mm Hg). Sixteen patients had true resistant hypertension, and were compared with the remaining 78 patients with non-resistant hypertension.
Results are summarized in the Table
The two groups were also different in terms of mean age (57±12 vs. 67±7 years, p=0.016 and pulse pressure/stroke volume, a rough index of large artery distensibility: 0.63±0.31 vs. 0.90±0.33, p=0.02).
Conclusion Hypertensive patients with true resistant hypertension have greater microvascular structural alterations compared with non-resistant hypertensive patients. This could partly explain the resistance to treatment and the high cardiovascular risk observed in these patients.

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P257

High Fat Diet Induces Metabolic Syndrome-Like Phenotype Associated with Adverse Micro- and Macro-Vascular Remodeling

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Metabolic diseases such as type 2 diabetes (T2DM), hypertension, and metabolic syndrome (MetS) have been associated with vascular disease, and we have previously demonstrated vascular-bed-specific remodeling in both mouse and porcine models of T2DM and MetS. The aim of this study was to determine whether high-fat

diet would induce MetS-associated adverse micro- and macro-vascular remodeling and mechanics in mice. Three week old male C57BL/6J mouse siblings were randomized to receive either a normal low-fat (LFD: 10% fat) or high-fat (HFD: 60% fat) diet for 20 weeks (n=7-10 per group). HFD induced a MetS-like phenotype characterized by increased body weight (LFD: 28.6±0.7 vs. HFD: 43.4±0.8 g, p<0.0001), increased mean arterial pressure (LFD: 65±3 vs. HFD: 91±2 mmHg, p<0.0001), increased plasma insulin (LFD: 106±39 vs. HFD: 368±54 pg/mL, p<0.001), and transient increases in fasting blood glucose. Passive pressure myography of septal coronary resistance microvessels (CRMs) revealed reduced internal (LFD: 151±11 vs. HFD: 113±7 µm at 125 mmHg, p<0.05) and external diameters, increased wall/lumen ratio (LFD: 5.5±0.6 vs. HFD: 7.8±0.5 at 125 mmHg, p<0.01) and reduced incremental modulus of elasticity (LFD: $7.9x10^6$±$1.7x10^6$ vs. HFD: $4.5x10^6$±$0.6x10^6$ dynes/cm^2 at 125 mmHg, p<0.01) in mice fed HFD. Adverse CRM remodeling was associated with reduced coronary flow at baseline and under hyperemic conditions, which reduced coronary flow reserve (LFD: 7.3±0.5 vs. HFD: 5.5±0.5, p<0.05). Aortic pulse wave velocity was increased (LFD: 0.31±0.02 vs. HFD: 0.36±0.01 cm/ms, p<0.05) and significantly correlated with the increased blood pressure (r=0.67, p<0.01). These data demonstrate that 20 weeks of a high-fat diet induces an early MetS-like pathophysiological state that is associated with vascular-bed-specific remodeling and alterations in vascular biomechanics. Furthermore, the presence of adverse vascular remodeling in the presence of an early MetS-like phenotype, but not overt MetS (i.e. in the presence of sustained elevation in fasting blood glucose), may suggest the presence of underlying sub-clinical disease during the early progression of metabolic syndrome.

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P258

Essential Hypertension Induces Early Functional and Structural Vascular Aging in Small Resistance Arteries

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We evaluated cross-sectionally whether vascular remodeling is physiologically present in normal aging, and whether hypertension causes an acceleration of the aging process for vascular function and structure.
40 essential hypertensive patients (EH, age 44.9±13.2 years; BP, 157±8/99±3 mmHg) and 36 normotensive control individuals (Ctrl, age 44.7±12.7 years; BP: 128±7/80±4 mmHg) underwent laparoscopic surgery with subcutaneous adipose tissue biopsy. Small resistance arteries were studied by pressure micromiography. Endothelium-dependent and -independent vasodilation were evaluated by dose-response curve to Acetylcholine (ACh) and sodium nitroprusside (SNP). Maximum %inhibition by L-NAME on response to Ach was calculated. Structural alterations were assessed by media-lumen ratio (M/L).
EH showed a reduced vasodilation to Ach (P<0.001), but not to SNP, compared to Ctrl. In both groups, %inhibition by L-NAME on response to ACh was inversely related to age (EH, r:-0.75; P<0.0001; Ctrl, r:-0.49; P<0.0001). NO availability was significantly reduced in EH as compared to Ctrl for each age group (<30

years: 22±6% vs 30±9%, P<0.05; 31-45 years: 17±3% vs 30±3%, P<0.0001; 46-60 years: 9±4% vs 21±6%, P60 years: 4±3% vs 13±3%, P<0.05). Age-hypertension interaction (Repeated measures ANOVA) was not significant (p= 0.25). EH showed an increased M/L (P<0.001) compared to Ctrl. In both groups, M/L was positively related to age. (EH, r:0.82; P<0.0001; Ctrl, r:0.50; P<0.0001). M/L was similar in EH and Ctrl for individuals <30 years, but greater in EH than Ctrl for the other age groups (31-45 years: 6.5±0.4% vs 5.6±0.4%, P<0.0001; 46-60 years: 7.4±0.5% vs 5.8±0.2%, P60 years: 7.9±0.3% vs 6.3±0.5%, P<0.0001). There was a significant age-hypertension interaction (Repeated measures ANOVA p= 0.0009).
In small resistance arteries, aging is characterized by progressive reduction in NO availability and increased M/L. In hypertensive patients, NO availability is early reduced in comparison to Ctrl, but the progression rate with age appears to be similar. Conversely, structural alterations are influenced by hypertension only after 30 years of age, but the progression rate with age is steeper in the presence of hypertension.

A. Virdis: None. **R. Bruno:** None. **E. Duranti:** None. **S. Taddei:** None.

P259

Endothelial Acid Sphingomyelinase Gene Determines Inflammasome Activation and Atherosclerotic Lesions in Carotid Arteries During Hypercholesterolemia

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Nucleotide oligomerization domain (NOD)-like receptor protein with pyrin domain containing 3 (Nlrp3) inflammasome has been reported to be activated by atherogenic factors, thereby triggering endothelial injury and consequent atherosclerotic lesions in the arterial wall. However, the mechanism activating and regulating Nlrp3 inflammasomes remains poorly understood. The present study tested whether membrane raft (MR) signaling platforms associated with acid sphingomyelinase (ASM) and its product ceramide (Ce) importantly contribute to the activation of Nlrp3 inflammasomes and atherosclerotic lesions during hypercholesterolemia (HC). By confocal microscopy and biochemical analyses, we demonstrated the formation and activation of Nlrp3 inflammasomes in the intima of the carotid arteries of $Asm^{+/+}$ mice with HC (as shown by a 2-fold increase in caspase-1 activity and a 6-fold enhancement of IL-1β positive stain areas), but not in $Asm^{-/-}$ mice. In endothelium-specific ASM transgenic mice (EC-Asm^{trg}), this inflammasome formation and activation were enhanced. Correspondingly, HC-induced increases in IL-1β production, ASM expression, Ce level and MR-$gp91^{phox}$ clustering in the carotid intima were abolished in $Asm^{-/-}$ mice, but enhanced in EC-Asm^{trg} mice. Functionally, endothelium-dependent vasodilation (EDVD) in carotid arteries *in vivo* (by ultrasound flowmetry) and *in vitro* (in perfused artery) was impaired by HC in $Asm^{+/+}$ mice by 33% and 54%, respectively. This endothelial dysfunction was not observed in $Asm^{-/-}$ mice. The endothelial tight junction protein, ZO-1 was reduced by HC in both $Asm^{+/+}$ and EC-Asm^{trg} mice, but not in $Asm^{-/-}$ mice. It was also found that HC-increased neointimal formation, T-cell infiltration, and fibrosis in 2-week partially ligated carotid arteries (PLCA) occurred in $Asm^{+/+}$ mice, but not in $Asm^{-/-}$ mice with HC. EC-Asm^{trg} mice even exhibited more severe inflammatory and atherosclerotic lesions. All these results suggest

that Asm gene and related MR clustering are essential to endothelial inflammasome activation and dysfunction in carotid arteries, ultimately determining the extent of atherosclerotic lesions.

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P260

Role of Primary Endothelial Cilia in Hypertension

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Abstract
Primary cilia are mechanosensory organelles that are projected into the lumen of blood vessels. It has been demonstrated that vascular endothelia require primary cilia to sense and transmit external mechanical stimuli into internal biochemical reactions. One of these reactions includes the biosynthesis and release of nitric oxide, which is one of the most potent endogenous vasodilators. This idea has *only* been investigated in cultured endothelial cells *in vitro*. Based on this finding, however, a very bold hypothesis is formed to test that abnormal cilia function results in vascular hypertension. Our laboratory has recently generated and obtained several conditional mouse models to specifically study the function and structure of primary cilia in vascular endothelia. These models include **1)** mice without cilia function (*Pkd1* or *Pkd2*); **2)** mice without cilia structure (*Tg737* or *Kif3a*). Our data indicate that mice with abnormal cilia function (*Pkd1*) or structure (*Tg737*) show significantly higher systolic (150±19 for *Pdgfbcre:Pkd1*$^{flox/flox}$ and 147±10 for *Tie2Cre:Tg737*$^{flox/flox}$ vs. 128±9 for wild-type) and diastolic (120±21 for *Pdgfbcre:Pkd1*$^{flox/flox}$ and 120±11 for *Tie2Cre:Tg737*$^{flox/flox}$ vs. 102±7 for wild-type) blood pressure than the corresponding wild-type mice. Because there is a positive and continuous correlation between blood pressure and cardiovascular diseases, satellite hypotheses are developed to look at the pathophysiological roles of endothelial cilia in cardiac functions and focal vascular diseases *in vivo*. Our data clearly point towards deteriorating phenotypes in the cardiac muscle, including cardiac fibrosis due to an increased cardiac workload. As a result, a heart-to-body weight ratio was significantly increased by 17 weeks old (0.008 *PdgfbCre;Pkd1*$^{f/f}$ vs. 0.006 *Pkd1*$^{f/f}$).The present study will very likely provide new insights for hypertension and offer advanced scientific understanding of vascular endothelial cilia in other cardiovascular diseases.

H. Saternos: None. **M. Hossain:** None. **W. AbouAlaiwi:** None.

P600

Conditional Knockout of AT1a Receptors Selectively in The Proximal Tubules Attenuates Renal Ischemia-Reperfusion Injury in Mice.

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The activation of the intrarenal renin-angiotensin system plays an important role in the pathogenesis of renal ischemia-reperfusion injury, but the underlying cellular and signaling mechanisms remain incompletely understood. In the present study, we tested the hypothesis that conditional knockout of AT1a receptors

selectively in the proximal tubules attenuates renal ischemia-reperfusion injury in mice. Three groups (n=5-8/per group) of adult male C57BL/6J (WT), global AT1a receptor-deficient (AT1a-KO), and conditional proximal tubule-specific AT1a-KO mice (PT-AT1a-KO) were subjected to sham surgery or 30 min unilateral left kidney ischemia, followed by reperfusion for 24 h or 7 days, respectively. Under basal conditions, systolic blood pressure was 25 ± 3 mmHg lower in global AT1a-KO ($p<0.01$) or 13 ± 3 mmHg lower in PT-AT1a-KO mice ($p<0.05$), respectively. Systolic blood pressure, 24 h urine and urinary sodium excretion were not significantly altered in all strains 24 h or 7 days after renal ischemia-reperfusion. Kidney wt. to body wt. ratio, but not heart wt. to body wt. ratio, was significantly increased in both AT1a-KO and PT-AT1a-KO mice ($p<0.05$). Renal ischemia-reperfusion injury was assessed by Masson Trichrome staining and compared between WT, AT1a-KO and PT-AT1a-KO mice. No significant glomerular, tubulointerstital, and peri-vascular fibrotic responses were observed in sham controls of all strains. However, significant fibrotic responses were observed in the glomeruli, cortical tubulointerstitial and peri-vascular tissues in WT mice 24 h or 7 days after renal ischemia-reperfusion ($p<0.01$). Surprisingly, however, glomerular, tubulointerstital, and peri-vascular fibrotic responses were significantly worsen, rather than improved, in global AT1a-KO mice ($p<0.01$). By comparison, conditional deletion of AT1a receptors selectively in the proximal tubules markedly attenuated glomerular, tubulointerstital, and peri-vascular fibrotic responses in PT-AT1a-KO mice 24 h or 7 days after renal ischemia-reperfusion ($p<0.01$). Our results suggest that AT1a receptors in the proximal tubule play an important role in the pathogenesis of renal ischemia-reperfusion injury, and thus may represent a therapeutic target in the future.

J. Zhang: None. **X.C. Li:** None. **F. Chen:** None. **M. Soleimani:** None. **J.L. Zhuo:** None.

P601

Matrix Metalloproteinase 2 Plays An Important Role In Angiotensin Ii-induced Vascular Injury Mediated In Part Through Epidermal Growth Factor Receptor Activation In Vascular Smooth Muscle Cells

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Background: Matrix metalloproteinase 2 (MMP2) is involved in cardiovascular disease. Whether MMP2 plays a role in hypertension and vascular damage is unknown. We hypothesized that Mmp2 knockout will prevent angiotensin (Ang) II-induced blood pressure (BP) rise and vascular injury.
Methods: Ten to 12-week-old male Mmp2 knockout (Mmp2-/-) and wild-type (WT) mice were infused with Ang II (1000 ng/kg/min, SC) for 14 days. Systolic BP was measured by telemetry, mesenteric arteries (MA) endothelial function and vascular remodeling by pressurized myography. In aortic wall or perivascular fat (PVAT), reactive oxygen species (ROS) generation was determined using dihydroethidium staining, and vascular cell adhesion protein 1 (VCAM-1), monocyte chemotactic protein-1 (MCP-1) expression and monocyte/macrophage infiltration by immunofluorescence. Spleen T cells and

monocyte profile were assessed by flow cytometry. Vascular smooth muscle cells (VSMCs) were isolated from MA of WT and Mmp2 knockout mice, stimulated 5 min with 100 nM Ang II and epidermal growth factor receptor (EGFR) phosphorylation measured by Western-Blotting.
Results: Ang II increased Systolic BP (172±7 vs 122±3, P<0.01), decreased MA vasodilatory responses to acetylcholine (33±5% vs 83±3%, P<0.01) and increases MA media-to-lumen ratio (5±0% vs 3±0%, P<0.01), media cross-sectional area (7224±467 vs 5345±336 µm2, P<0.05), and stiffness (P<0.01), as shown by a leftward shift of the stress/strain relationship, in WT. Furthermore, Ang II enhanced aortic ROS generation (73±11 vs 6±1 RFU/µm2, P<0.01), aortic VCAM-1 (17±3 vs 5±3 RFU/µm2, P<0.01) and MCP-1 expression (71±14 vs 11±3 RFU/µm2, P<0.01) and PVAT monocyte/macrophage infiltration (32±5 vs 4±0 RFU/µm2, P<0.05), and spleen activated CD4+CD69+ and CD8+CD69+ T cells and pro-inflammatory Ly-6Chi monocytes (17±2 vs 10±1%, 11±1 vs 5±1% and 53±6 vs 25±2%, respectively, P<0.05) in WT. Ang II increased EGFR phosphorylation in VSMCs in vitro (1.91±0.19 vs 1.0±0.0%, P<0.05). Mmp2 knockout prevented or reduced all of the above except BP elevation (P<0.05).
Conclusion: MMP2 plays an important role in Ang II-induced vascular injury, which could be mediated at least in part through EFGR activation in VSMCs.

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Patiromer Decreased Aldosterone, Urine Albumin/Creatinine Ratio, and Blood Pressure in Patients with Chronic Kidney Disease and Hyperkalemia on RAAS Inhibitors: Results from OPAL-HK

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Introduction: Elevated aldosterone (ALD) is associated with chronic kidney disease (CKD) and cardiovascular (CV) complications. Patiromer, a nonabsorbed potassium (K^+)-binding polymer, decreases serum K^+ (sK^+) and may allow increased use of RAAS inhibitors (RAASi) in patients (pts) with CKD and hyperkalemia (HK). This analysis examined the effect of patiromer on ALD, urinary albumin/creatinine ratio (ACR), and blood pressure (BP) in pts with CKD on RAASi.
Methods: OPAL-HK was a 2-part, phase 3 study in 243 CKD pts with sK^+ 5.1–<6.5 mEq/L on RAASi. Pts received patiromer for 4 wks (Part A); pts with moderate-severe HK at baseline (sK^+ ≥5.5 mEq/L) and sK^+ 3.8–<5.1 at Part A wk 4 continued on patiromer (n=55) or switched to placebo (n=52) in the 8-wk withdrawal phase (Part B). RAASi were stable prior to and during Part A. Changes in ALD, ACR, and systolic BP/diastolic BP (SBP/DBP) were analyzed.
Results: After 4 wks of patiromer sK^+, serum ALD and urine ALD/creatinine decreased, while plasma renin activity (PRA) was unchanged; SBP, DBP, and ACR also declined (Table). Mean±SE changes (ng/dL) in serum ALD from Part A wk 4 to Part B wk 4 and to Part B wk 8 were 4.6±1.6 (p<0.01) and 5.7±1.8 (p<0.01) in

the placebo and 0.9±1.0 (p=NS) and 0.2±0.8 (p=NS) in the patiromer groups, respectively. Compared with Part A wk 4, SBP (mm Hg) was further reduced at Part B wk 4 (3.1±2.1, p=NS) and Part B wk 8 (5.4±1.9, p<0.01) with maintained improvement in ACR in patiromer pts.

Conclusions: Patiromer reduced both sK^+ and ALD (independent of PRA) in CKD pts with HK on RAASi. ALD reductions correlated with lower BP and ACR. Reduction in sK^+ may have lowered ALD possibly improving BP and ACR.

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P603

Age and Angiotensin-(1-12) Expression in Human Atrial Tissue of Patients with Left Heart Disease

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In the human heart formation of angiotensin (Ang) II results from the hydrolysis of an alternate angiotensin substrate, -Ang-(1-12)-, by chymase rather than angiotensin converting enzyme. In our recent study a higher Ang-(1-12) expression and upregulation of chymase mRNA and enzymatic activity was found in left (LAA) versus right atrial appendages (RAA) of subjects with left heart disease. Since aging is associated with prominent changes in cardiac structure and function, we assessed the relationships among age, Ang-(1-12), and chymase gene expression and activity in both LAA and RAA in 44 patients undergoing cardiac surgery for the correction of valvular heart disease, resistant atrial fibrillation or ischemic heart disease. Immunohistochemistry for Ang-(1-12) detection was performed using an affinity purified polyclonal antibody directed to the COOH terminus of the full length of the sequence of human Ang-(1-12). Quantitative real-time polymerase chain reaction was used to detect chymase mRNA levels whereas chymase activity was assessed by HPLC. We report that Ang-(1-12) immunostaining in atrial appendages ($r=0.30$; $p<0.05$), but not chymase mRNA expression or activity, correlated directly with patients age. While a tendency for higher Ang-(1-12) expression in LAA (Intensity: 5.88 ± 0.91 units; n=11) when compared to RAA (Intensity: 3.948 ± 0.55 units; n=15) was noted in patients younger than 65 yrs, this difference was more prominent and statistically significant in patients older than 65 yrs of age (LAA Intensity: (n=12): 7.39 ± 1.06 units versus RAA Intensity (n=13): 4.74 ±0.54 units; $p < 0.05$). The results of the present study suggest an age-related increase in Ang-(1-12) expression in human atrial tissue that may be more prominent in the LAA of patients with left heart disease. We suggest that higher availability of Ang-(1-12) for direct Ang II formation may be an underlying

mechanism responsible, at least in part, for age- and disease-related atrial and ventricular remodeling and dysfunction.

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P604

Equivalency Validation of a Novel Freely Moving Animal Based Telemetry System versus a Stationary Cage Bound System for Blood Pressure Recordings

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Traditional rodent blood pressure (BP) monitoring required single housing, a known stressor for rodents. We developed a microprocessor based long range wireless telemetry system with large internal memory that allows rodents to live in groups, interact, exercise and be housed in large enriched environments. BP is recorded with a solid-state sensor along with core temperature and 3D axial activity within the implant. The measurement protocol is transmitted once to the programmable implant via a single room antenna mobile base station. The implant becomes an autonomous recording device until contacted again by the base station with an updated protocol. The implanted animals are thus allowed to undergo procedures, leave the room or be subject other tests while the recording is running. Data can be automatically downloaded periodically within the protocol. In order to objectively validate this system we contracted a study with the Michigan State University INVIVO facility. 8 male SD rats of 275g were implanted with a model PTA-M TSE Stellar telemetry implant. After 8 days of recovery the animals entered a crossover study and were dosed with vehicle, 3, 10, and 100 mg/kg L-NAME or Verapamil at t=0 hrs. BP was recorded for 10s hourly for 24hrs sampled at 200Hz following drug administration. The BP responses were compared to a control group of 8 animals implanted with a similar conventional implant from a competitor. Drug responses were qualitatively the same between the groups. Mean arterial pressures (MAP) at +12hrs in the 100 mg/kg LNAME group with the TSE implant increased by 26±11 versus 26±9 mmHg in the control group. MAP in the 100 mg/kg Verapamil group at +12 hrs dropped by 31±9 versus 20±6 mm Hg in the control group. Baseline MAP was significantly elevated to 118±5 versus 92±11 in the control group with the conventional implant which may account for the blunted verapamil response.
We conclude that there are no significant differences in quantitative blood pressure responses to L-NAME or Verapamil in animals implanted with a TSE Stellar device versus a leading competing device. We also conclude that the short hourly recordings used for the TSE device produced equivalent MAP values while significantly prolonging implant battery life, thus extending possible study length.

H. Knot: A. Employment; Significant; TSE Systems Inc. **G. Miller:** A. Employment; Significant; TSE Systems Inc..

P605

Liver-specific Antisense Inhibition Of Angiotensinogen Reduced BP In A Hypertensive Rat Model Resistant To Standard RAS Inhibitors

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Uncontrolled hypertension is an important contributor to cardiovascular disease. Despite the armamentarium of antihypertensive therapeutics, there remains a need for a novel agent effective in individuals with treatment-resistant hypertension who cannot reach their target BP. Pharmacological agents targeting the renin-angiotensin-aldosterone system (RAAS) are widely used but may not optimally inhibit RAAS. Experiments were conducted to characterize a series of angiotensinogen (Agt) oligonucleotides (ASOs) and compare their efficacy in a model of malignant hypertension. Agt ASOs which targeted all systemic sites of Agt (e.g. liver, fat and kidney) versus LICA-conjugated Agt ASOs (Agt-LICA ASOs) that preferentially target the liver were evaluated in normotensive and hypertensive rats. Liver, adipose and kidney Agt RT-PCR were performed and revealed Agt ASO treatment reduced liver, adipose and kidney Agt expression by 93%, 96% and 82%, respectively. Agt-LICA ASO treatment resulted in 93%, 10% and 11% reductions of Agt mRNA in liver, adipose and kidney, respectively, thus demonstrating specific liver targeting with LICA. Plasma Agt, measured by ELISA, revealed no significant differences between the Agt and Agt-LICA ASO treatments. For example, after a single administration of the ASOs, plasma Agt was reduced 15-18%, 41-48%, 84-89% and 92% at 12, 24, 48 and 72 hours, respectively, after ASO administrations. Radiotelemetry was used to compare the BP lowering efficacy in spontaneously hypertensive rats (SHRs) fed an 8% NaCl diet, a model of malignant hypertension resistant to standard RAAS inhibitors for BP control. Treatments of a control ASO or captopril or captopril plus losartan had a modest effect on BP whereas treatments with either the Agt or Agt-LICA ASOs resulted in similar MAP reductions of 30 - 40 mm Hg. These data demonstrate that the effects of global vs. liver-specific Agt inhibition result in similar reductions of plasma Agt or blood pressure. Additionally, ASO treatments produced superior BP efficacy relative to the standard of care in a model resistant to RAAS blockage. Such improvements could be desirable in individuals not at their BP goal with existing RAS therapeutics.

A.E. Mullick: A. Employment; Significant; Isis Pharmaceuticals. **S.T. Yeh:** A. Employment; Significant; Isis Pharmaceuticals. **M.J. Graham:** A. Employment; Significant; Isis Pharmaceuticals. **R.M. Crooke:** A. Employment; Significant; Isis Pharmaceuticals.

P606

Blood Pressure Regulation in Patients with Meniere's Disease

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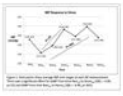
Background: The reason for inner ear fluid buildup in Meniere's disease patients is unidentified and current treatment is of limited effectiveness. Little is known about the regulation of blood pressure (BP) in this disease. We developed a protocol placing Meniere's patients under mild mental stress to induce changes in BP. The purpose of this study was to examine BP regulation in Meniere's patients and provide mechanistic insight into the disease. Methods: We measured BP in 9 individuals (5 males, 4 females; 8 Caucasians, 1 African-American) ranging from age 53 to 81.

Seven of these subjects were on a range of antihypertensive medications, including ACE inhibitors, aldosterone antagonists, alpha-blockers, beta-blockers, and diuretics. The protocol included 10 minutes of rest, 20 minutes of stress (competitive video game), and 10 minutes of recovery. BP was taken before and after each period and 10 minutes into the stressor. Results: Of the 9 subjects, 6 experienced a rise in BP during the stressor. There was a significant increase in systolic blood pressure (SBP) between rest (Rest10) and halfway through the stress protocol (Stress10) and between rest and the beginning of the recovery period (Recov0) as shown in Figure 1. Conclusions: According to our study, Meniere's disease patients exhibit dysregulation of BP that is exaggerated during stress, even while taking a range of antihypertensive medications. After the completion of the stressor, BP remained elevated and continued to rise in some cases. The degree to which these Meniere's patients are unable to control their BP is potentially clinically significant.



D.M. Kioseff: None. **D.L. Stewart:** None. **G.A. Harshfield:** None.

P607

Deletion of GPER Protects from Age-related Renovascular Dysfunction and Tubulo-interstitial Injury

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Aging is associated with reduced vasodilatory capacity in renal arteries. Activation of the G protein-coupled estrogen receptor (GPER) induces vasodilation and improves hypertensive renal injury in rats. Moreover, GPER, as observed with many G protein-coupled receptors, likely exhibits "basal activity" independent of ovarian estrogen production, which may become relevant in disease-like states such as aging. We hypothesized that deletion of basal GPER activity would further aggravate vasodilatory dysfunction in renal arteries and the associated tubulo-interstitial injury. In young (4 month-old) and senescent (24 month-old) male wild-type and GPER-deficient (*Gper* $^{-/-}$) mice, blood pressure and endothelium-dependent vasodilation to acetylcholine in renal arteries were determined. In addition, vascular injury (VIS score) and tubulo-interstitial injury (TIS score) including staining for amyloid were quantified in histologic sections. In wild-type mice, aging markedly reduced renal artery vasodilation to acetylcholine (from 77±5% to 28±4% of precontraction, n=4-5, $p<0.001$ vs. young mice), and increased vascular injury (VIS: 0.42±0.07 vs. 0.04±0.03), tubulo-interstitial injury (TIS: 2.9±0.6 vs. 0.3±0.1), and amyloid staining (2.8±0.7 vs. 0±0, all n=6-8, $p<0.001$ vs. young mice). Contrary to our hypothesis, vasodilatory responses to acetylcholine were largely preserved in renal arteries from senescent *Gper* $^{-/-}$ mice (63±4 vs. 28±4% of precontraction, n=4-5, $p<0.001$ vs. wild-type mice). Similarly, in senescent *Gper* $^{-/-}$ mice, vascular injury (VIS) was reduced by 46% (n=6-7, $p=0.09$ vs. wild-type mice), as were tubulo-interstitial injury (TIS) and

amyloid staining by 77% and 92%, respectively (both n=6-7, p<0.01 vs. wild-type mice). Deletion of *Gper* had no effect on blood pressure in either senescent (110±5/83±1 vs. 114±2/87±2 mmHg) or young animals (117±2/90±1 vs. 119±2/91±2 mmHg, both n=5-12, p=n.s. vs. wild-type mice). These results indicate a novel role for GPER expression in age-related impaired vasodilatory capacity in renal arteries and the associated tubulo-interstitial injury independent of blood pressure, as well as a pathophysiologically relevant activity of GPER in male mice, possibly independent of estrogen.

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P608

Role Of Nitric Oxide In The Pathophysiology Of Hypertension Associated With Low Nephron Endowment.

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A low nephron endowment is a strong predictor for future risk of hypertension, kidney and cardiovascular disease. Previously, we have shown that fetal uninephrectomy (uni-x) results in elevated arterial pressure, reduced renal function and low plasma renin activity in adulthood in sheep. However, the mechanisms via which a reduction in renal mass in early life contributes to the development of hypertension and renal disease are not well understood. Nitric oxide (NO) plays an important role in driving the fall in renal vascular resistance (RVR) and rise in glomerular filtration rate (GFR) that characterizes the normal postnatal maturation of renal function in the newborn. We hypothesized that the early onset of renal dysfunction in individuals with a congenital reduction in renal mass is due to a reduced renal contribution of NO. Fetal uni-x (n=9) or sham operation (n=7) was performed at 100d of gestation (term=150 days). In conscious female lambs at 6 months of age, mean arterial pressure (MAP) was measured via an indwelling carotid arterial catheter and GFR and renal blood flow (RBF) were measured via clearance methods before and after nitric oxide synthase inhibition (L-NAME; 20mg/kg/h iv). Basal MAP was elevated in uni-x as compared to the sham sheep (sham: 80 ± 2 mmHg, uni-x: 86 ± 1 mmHg; P < 0.01). In response to systemic administration of L-NAME MAP increased similarly in both groups (sham: 13 ± 3 mmHg, uni-x: 13 ± 4 mmHg). Basal RBF (sham: 933 ± 29 ml/kg/h; uni-x 757 ± 43 ml/kg/h; P<0.01) and GFR (sham: 144 ± 3 ml/kg/h; uni-x 107 ± 3 ml/kg/h; P<0.01) was lower in the uni-x as compared to the sham sheep. In response to L-NAME the decline in RBF (sham: 73 ± 3 %; uni-x: 47 ± 3%; P<0.001) and GFR (sham: 54 ± 4 %; uni-x: 43 ± 1%; P<0.03) was less in uni-x as compared to sham sheep. Filtration fraction decreased significantly in the sham (P<0.001) but not the uni-x sheep in response to L-NAME. Renal dysfunction and hypertension in young hypertensive sheep born with a low nephron endowment maybe underpinned by impaired nitric oxide bioavailability. Targeting the nitric oxide system may offer therapeutic potential in children born with a reduced renal mass, in terms of treatment interventions and as a biomarker of prognosis.

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P609

Estrogen Receptor ERα Plays a Major Role in Oxidative Stress Dependent Myocardial Dysfunction Caused by Ethanol in Conscious Female Rats

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Ethanol elicits estrogen (E_2)-dependent myocardial oxidative stress and dysfunction in female rats. The aim of this study was to elucidate the role of the individual E_2 receptor (ER), ERα, ERβ, and the G protein-coupled estrogen receptor-1 (GPER), in the ethanol-evoked myocardial dysfunction. To achieve this goal, female rats in proestrus phase (highest E_2 level) received selective antagonist (200 μg/kg; i.v) of ERα (MPP), ERβ (PHTPP) or GPER (G15) or saline 30 min before ethanol (1 g/kg; i.v) or saline infusion. ERα blockade virtually abrogated ethanol-evoked myocardial dysfunction and hypotension, while ERβ blockade had no effect on the hypotensive response, but caused delayed attenuation of the ethanol-evoked reductions in left ventricular developed pressure and the rate of left ventricle pressure rise. GPER blockade caused delayed attenuation of both cardiovascular effects of ethanol. While all 3 ERs subtype antagonists attenuated the ethanol-evoked increases in myocardial catalase and ALDH2 activities, ERα blockade also inhibited myocardial catalase activity in the absence of ethanol. Ethanol-evoked elevation in myocardial ROS and enhancement of myocardial Akt, ERK1/2, eNOS and nNOS phosphorylation were attenuated by the 3 ER subtype antagonists. In conclusion, the findings support a greater role for ERα signaling in the E_2-dependent myocardial oxidative stress and dysfunction caused by ethanol in proestrus rats.

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P610

Alterations in 20-HETE Production Contribute to End Organ Damage in Dahl S Rats

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It is well documented that a sexual dimorphism exists in the regulation of blood pressure in both the human population as well as experimental animal models, such that males have higher blood pressures than females of the same age. While there is a clear disparity in the development of hypertension and progression of renal injury in many rodent models, evidence of a sex difference is lacking in the Dahl S rat. While the current reports present conflicting data, we hypothesize that alterations in CYP450 expression and 20-HETE production contributes to the relative resistance of female Dahl S rats to target organ damage compared to males. Consistent to what

we have previously reported, the time course for the development of proteinuria and renal injury were significantly reduced in female Dahl SSJr rats challenged with a high salt diet relative to male rats. In addition, renal cortical 20-HETE production was elevated in female (120.8±4.2 pmol/min/mg) versus male rats (45.1±11.78 pmol/min/mg), while no difference was noted in the outer medulla (19.6±1.8 vs 17.15± 3.9 pmol/min/mg). Introgression of the CYP4A1 gene into the Dahl S genetic background, resulted in significant elevations in 20-HETE production both on low salt and high salt diets. Furthermore the rise in mean arterial pressure was attenuated in CYP4A1 overexpressing rats in both sexes (Δ19mmHg in CYP females vs 61mmg in Dahl S females; 20mmHg in CYP males vs 50mmHg in Dahl S males). Moreover, the degree of glomerular injury was reduced in CYP rats, both male and female, compared to Dahl S rats in response to a high salt diet. Therefore, increases in the CYP gene expression and 20-HETE production prevent the rise in mean arterial pressure and kidney injury in male Dahl S rats. AHA 14SDG20160020

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P611

Vascular-mediated Preterm Birth is Associated with Cardiovascular Risk after Pregnancy

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Objective*:* To investigate whether preterm birth and placental evidence of malperfusion is associated with subclinical atherosclerosis and a higher cardiovascular risk factor burden 4 to 12 years after pregnancy.
Methods: A cohort of women with preterm (n=119) and term births (n=242), mean age 38 years, was examined on average eight years after pregnancy for carotid artery intima-media thickness (IMT), fasting lipids, blood pressure and inflammatory markers (C-reactive protein [hsCRP] and Interleukin-6 [IL-6]). Pregnancy characteristics included placental pathology evidence of malperfusion (vasculopathy, infarct, advanced villous maturation, perivillous fibrin, fibrin deposition), infection (chorioamnionitis, funisitis, deciduitus), villitis (chronic inflammation), fetal thrombosis or chorangiosis. Vascular-mediated preterm births were those with malperfusion lesions, and by design, those with preeclampsia were excluded.
Results: Women with malperfusion lesions had a higher mean carotid IMT (+0.055 cm), total cholesterol (+17.49 mg/dl), LDL-C (+11.44), triglycerides (+17%), apolipoprotein-B (+8.95) and systolic and diastolic blood pressure (+4.58/+2.62 mmHg) compared to women with term births, independent of age, race, smoking and adiposity assessed before and after pregnancy (all $p<0.05$). Women with preterm birth and evidence of malperfusion accompanied by other lesions related to infection or chronic inflammation had the most atherogenic profile after pregnancy, and carotid IMT differences were

independent of traditional risk factors (+0.04 cm; p=0.027).

Conclusions:

Vascular-mediated preterm birth is associated with maternal subclinical atherosclerosis and a higher cardiovascular risk factor burden in the decade after pregnancy compared to term birth. The placenta may offer unique insight into how pregnancy complications can portend the emergence of maternal cardiovascular disease.

J.M. Catov: None. **S.E. Reis:** None. **M.F. Muldoon:** None. **R.B. Ness:** None. **L. Nguyen:** None. **W.T. Parks:** None.

P612

TLR3-Induced Placental miR-155 Contributes to Preeclampsia by Augmenting Inflammation

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Hypertensive disorders of pregnancy including preeclampsia (PE) affect ~15% of all pregnancies. We have previously demonstrated that excessive maternal immune system activation via Toll-like receptor 3 (TLR3) contributes to the development of PE-like symptoms such as hypertension, endothelial dysfunction, and proteinuria in mice only when pregnant. Recently, increased miR-155 (an inflammation-related miRNA) expression was reported in placentas from women with PE compared to normotensive women suggesting a pathophysiological role of miR-155 in PE. Whether miRNAs play a role in the etiology of PE by augmenting inflammation is unknown. We confirmed miR-155 expression was up-regulated in placentas of poly I:C (a TLR3 agonist) treated wild type mice by microarray (P-PIC: 1.56 fold, $p<0.5$ vs controls) and qRT-PCR analyses (P-PIC: 3.15 fold, $p<0.05$ vs. controls). Based on our data we hypothesized that TLR3 activation induces placental miR-155 expression that augments maternal inflammation leading to PE. Transcription factors NF-κB and AP-1 which are known to bind to the promoter of miR-155 were increased in placentas of P-PIC mice. TargetScan, a target identification algorithm predicted suppressor of cytokine signaling 1 (SOCS1) as a putative target of miR-155. SOCS1 prevents overactivation of immune responses but failed to increase in P-PIC mice. ICAM, a marker of inflammation increased significantly in P-PIC mice. P-PIC TLR3 KO mice did not develop hypertension and proteinuria as well as did not exhibit increased placental levels of NF-κB, AP-1, miR-155 expression and ICAM or decreased levels of SOCS1. To determine the placental etiology, human cytotrphoblasts (CTBs) were treated with poly I:C and NF-κB, AP-1 transcription factor levels and miR-155 expression were increased significantly while SOCS1 levels decreased. Together, these data suggest that TLR3 activation increases placental miR-155 expression which downregulates SOCS1 levels and increases inflammation which may contribute to the development of PE.

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P613

The Effect of Metabolic Factors on Hypoxia-Induced sFlt-1 Secretion in Rat Placental Villi

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Although the etiology of preeclampsia (PE) remains unclear, evidence indicates that impaired trophoblast invasion followed by placental ischemia/hypoxia promotes the release of placental anti-angiogenic factors, such as soluble fms-like tyrosine kinase-1 (sFlt-1), into the maternal circulation. sFlt-1 blocks the pro-angiogenic actions of vascular endothelial growth factor to elicit maternal endothelial dysfunction and ultimately hypertension. Obesity is a major risk factor for PE. In addition, increased circulating metabolic factors, such as leptin and insulin have been associated with PE. However, the mechanisms whereby obesity and its related metabolic factors increase the risk for the development of PE are unknown. The aim of this study was to evaluate whether chronic leptin or insulin exposure exacerbate hypoxia-induced sFlt-1 secretion from rat placental villi. In order to address this question, placental villous explants were isolated from placentas of normal pregnant rats (n=4, 3 placentas per rat) and pregnant rats treated with either leptin (0.5 mg/kg/min i.p.; n=3, 3 placentas per rat) or insulin (1.5 mU/kg/min s.c.) supplemented with 20% glucose in drinking water (n=3, 3 placentas per rat) from gestational day 14 to 19. Placental explants were then incubated for 48 h at 37 °C under normoxia (6% O2) or hypoxia (1% O2) and sFlt-1 secretion in cultured media was measured by ELISA. While hypoxia significantly enhanced sFlt-1 release of explants from normal pregnant rats compared with normoxia (3224±224 vs 4251±236 pg/mg; P<0.05), explants from chronic hyperleptinemic (3197±178 vs. 3762±317 pg/mg) or euglycemic hyperinsulinemic (4066±186 vs. 4251±213 pg/mg) pregnant rats secreted similar sFlt-1 levels under normoxic and hypoxic conditions, respectively. Additionally, chronic leptin or insulin treatments did not exacerbate the effect of hypoxia on sFlt-1 release. In conclusion, our in vitro studies with placental villi from chronic hyperleptinemic or euglycemic hyperinsulinemic pregnant rats showed no exacerbation of hypoxia-induced sFlt-1 secretion.

A.C. Palei: None. **F.T. Spradley:** None. **J.P. Granger:** None.

P614

Renin Angiotensin System: A Placental Perspective

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Renin Angiotensin System (RAS) is a major physiological regulator of Blood Pressure. In pregnancy the placental RAS plays a critical role in maintaining utero-placental blood circulation, necessary for proper fetal development. Proteinuria, a clinical implication of renal injury, is common in hypertensive disorders of pregnancy, including Preeclampsia. In the present study we tried to study whether Proteinuria is a manifestation of the RAS components in placenta. Placenta, collected from the pregnant women was divided in two categories- those having proteinuria and those not. The levels of the Ang II type 1 receptor (AT1), Ang II type 2 receptor (AT2) and ACE2 genes was determined by semi-quantitaive PCR while the Immunoblotting procedure was used to determine the protein levels of the AT1 and AT2 receptors, in the placenta of women of both categories. All the values were normalized with β-actin, used as an internal control. The AT1R gene expression was significantly

decreased in the placenta of women exhibiting proteinuria compared to those that did not (0.694±0.116, n=7 vs. 1.250±0.176,n=9 p<0.05), while the levels of AT2R gene expression also showed the same trend (0.9571±0.2206,n=7 vs. 1.960±0.329,n=9,p<0.05). Similarly the levels of ACE2 gene too was significantly decreased in placenta exhibiting proteinuria compared to those which did not exhibit proteinuria (0.2775 ± 0.05747, n=6 vs. 0.2343 ± 0.02277, n=7, p<0.05). The Immuno-blot analysis demonstrated that the levels of AT1R protein were significantly up-regulated in the placenta of the women exhibiting proteinuria compared to those that did not (0.600±0.125,n=5 vs. 0.330±0.046,n=8, p< 0.05), while the levels of AT2R protein was significantly decreased in the placenta of women exhibiting proteinuria compared to those that did not (0.493±0.028, n=5 vs. 0.9963±0.163,n=8, p< 0.05). A differential pattern of the receptor types of Ang II seems to be related with the presence of Proteinuria. The increased AT1 receptor protein function could be accounting for inducing mediators that cause Proteinuria. The effects of AT2 receptor Protein and gene and the ACE2 gene, having a protective role, needs to be elucidated further for mechanistic insights on the actual relationship between the RAS components.

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P615

Hypertension-Dependent Reprogramming of the Endothelial Transcriptome

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Hypertension has drastic consequences for the cardiovascular system and especially for endothelial cell health. Clinically this is observed through the repeated finding that hypertension is associated with endothelial dysfunction. Despite this strong evidence that hypertension leads to impairment of endothelial function, the molecular events that give rise to endothelial dysfunction are poorly characterized. To better understand how hypertension affects the health and function of endothelial cells at the molecular level, we utilized a mouse model of spontaneous hypertension (BPH/2J) with the overarching hypothesis that hypertension causes the transcriptional reprogramming of endothelial cells leading to endothelial dysfunction. First, we non-invasively quantified endothelial function from hypertensive and normotensive (BPN/3J) mice by measuring flow-mediated vasodilation with high-frequency ultrasound. We found that hypertensive mice failed to dilate their femoral artery while normotensive control mice dilated their femoral artery 17 ± 8% (Mean ± SEM). Next, we acutely

isolated cardiac endothelial cells by magnetic-assisted cell sorting for endothelial marker CD31. Through this technique, we enriched our sample for endothelial cells (as defined by expression of two endothelial-specific cell-surface markers CD31 and CD102) to 92 ± 1% compared to 9 ± 1% in pre-magnetically sorted cells. Finally, we isolated RNA and transcriptionally profiled the endothelial cells from normotensive and hypertensive mice (n=3 mice per group) with an average depth of 30 million reads per sample with RNAseq. We found that over 4000 genes were differentially expressed between groups (FDR cutoff of .01). Using Ingenuity Pathways Analysis (Qiagen), we identified multiple pathways that were dysregulated in endothelial cells exposed to hypertension including those related to immune function, cell morphology and cell-to-cell communication. This work represents one of the first studies to utilize cell-specific RNAseq to quantify how endothelial cells are transcriptionally reprogrammed in a pathological state. It is our hope that this work will lead to targeted therapeutics to improve endothelial function in patients with hypertension.

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P616

Renal Sympathetic Outflow And Beta-adrenergic Signaling Promote Dendritic Cell Activation In Hypertension

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Hypertension is associated with increased sympathetic outflow and activation of adaptive immunity. In hypertensive states, proteins that are oxidatively modified by highly reactive γ-ketoaldehydes (isoketals) accumulate in dendritic cells (DCs). These isoketal-protein adducts are immunogenic and lead to subsequent activation of T cells. We hypothesized that renal sympathetic nerves link the central nervous system to immune activation in hypertension. To test this, we performed bilateral renal denervation (RDN) in C57BL/6 mice by applying phenol to the renal artery. One week later, mice received an subcutaneous infusion of angiotensin II (490 ng/kg/min) for 14 days. RDN lowered the hypertensive response to angiotensin II infusion (130±3 vs. 161±3 mmHg) as measured by telemetry. By flow cytometry, we found that RDN reduced accumulation of T cells in the kidney. Ang II infusion caused resulted in 15-25% increases in expression of the maturation markers CD80 and CD86, as well as 2-fold increase in isoketal adducts in the DCs of spleen. It also increased IL-1α, IL-1β, and IL-6 production by splenic DCs by 4 to 6-fold. These increases were attenuated by RDN. Having confirmed that DCs express almost every subtype of adrenergic receptor by real time PCR, we further determined if sympathetic neurotransmitters contribute to DC activation by treating bone marrow-derived DCs with either norepinephrine (NE) or neuropeptide Y (NPY) in vitro. As measured by flow cytometry, NE dose-dependently increased isoketal-protein adducts in DCs (vehicle: 19 ± 3 vs. 3 µmol/L: 39 ± 4%). This was prevented by pretreating cells with the β-adrenergic antagonist propranolol (1 µmol/L), but not by blocking α_1 or α_2 adrenoreceptors with prazosin and yohimbine. In contrast, NPY did not affect DC isoketal-protein content. Therefore, our data indicate

that renal sympathetic nerves increase isoketal-adduct formation in DCs via β-adrenergic signaling and that this contributes to the activation of adaptive immunity in hypertension. These data suggest that beta blockade might have previously unappreciated anti-inflammatory effects in the treatment of hypertension.

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P617

Angiotensin II-induced Hypertension And Vascular Injury Is Mediated By Gamma/delta T Cells

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Objective: Both innate antigen presenting cells and the adaptive immune system have been shown to play a role in the development of hypertension. Nevertheless, the T cell subset involved in the pathophysiology of hypertension remains unclear. There is a small subset of "innate-like" T cells expressing gamma/delta T cell receptor (TCR) rather than the alpha/beta TCR that could play a role in bridging between the innate and adaptive immune systems. However, it is unknown whether gamma/delta T cells contribute to development of hypertension.
Method/Results: Thirteen to 15 week-old male C57BL/6 wild-type and Tcrd-/- mice, which are devoid of gamma/delta T cells, were infused or not with angiotensin (Ang) II (490 ng/kg/min, SC) for 7 or 14 days (n=4-9). Telemetric blood pressure, mesenteric artery endothelial function and vascular remodeling by pressurized myography and spleen T cell profile by flow cytometry were evaluated. Fourteen days of Ang II increased systolic blood pressure (167±4 vs 125±2 mmHg, P≤0.01) in wild-type compared to control mice. The frequency of gamma/delta T cells (6±1% vs 3±1%, P≤0.05) and activated (CD69+) gamma/delta T cells (11±1% vs 7±1%) was increased after 7 days of Ang II, and 7 days later were respectively unchanged or further increased (24±2% vs 10±1%) in wild-type compared to control mice. Ang II decreased mesenteric artery relaxation responses to acetylcholine (51±5% vs 88±3%, P≤0.01) and increased media/lumen (5±1 vs 3±0%, P≤0.01) in wild-type mice compare to controls. No gamma/delta T cells were detected in Tcrd-/- treated or not with Ang II. All the above Ang II effects were abrogated in Tcrd-/- mice.
Conclusion: These data suggest that gamma/delta T cells mediate Ang II-induced blood pressure rise and vascular injury. Gamma/delta T cells could be key immune cells bridging innate and adaptive immune responses during the development of hypertension.

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P618

Foxp3+ Regulatory T cell Depletion Eliminates Ang II-Induced Hypertension Resistance in Female Mice

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Compared to males, premenopausal females are resistant to the development of Ang II hypertension. In males, Ang II induces hypertension, in part, through mechanisms requiring T effector lymphocytes. Recently, our lab has demonstrated that females can prevent the T lymphocyte-dependent increase in blood pressure (SBP and MAP) and expression of pro-inflammatory cytokines in the kidney in response to Ang II infusion. Because $Foxp3^+$ T regulatory cells suppress the pro-inflammatory and hypertensive actions of T effector cells, we sought to determine whether $Foxp3^+$ T regulatory cells contribute to this resistance in females. Premenopausal (8 week old) 129SVE female mice were infused with Ang II (800ng/kg/min, 14d) and received 4 doses of the anti-CD25 antibody PC-61 to transiently deplete $Foxp3^+$ T regulatory cells (every 84 hours beginning 12 hours prior to Ang II infusion, 250µg/dose, i.p., vehicle control). Blood pressure was measured before and after Ang II infusion via non-invasive tail cuff. Ang II induced a significant increase in systolic blood pressure in $Foxp3^+$-depleted mice, while resistance was retained in vehicle-treated mice (Con Δ5 $\pm$ 5mmHg, Ang II Δ10 $\pm$ 7mmHg, PC-61 Δ28 $\pm$ 9*mmHg, *p<0.05 vs Con). Flow cytometric analysis demonstrated that PC-61-treatment significantly reduced the number of $Foxp3^+$ splenic T cells compared to control (Con $1.7x10^6$ cells, Ang II $2.3x10^6$ cells, PC-61 $8.3x10^{5*}$ cells, *P<0.05 vs Con) without changing $CD3^+$ and $CD4^+$ T cell counts. The number of $Foxp3^+$ T cells residing in the kidney was also significantly reduced by PC-61 (Con 1,152 $\pm$ 368 cells, Ang II 686 $\pm$ 389 cells, PC-61 210 $\pm$ 35* cells, *P<0.05 vs Con). Quantitative real-time PCR demonstrated that whole kidney expression of MCP-1 and ENaC alpha were significantly increased in $Foxp3^+$-depleted mice (MCP-1- Con 1.0 $\pm$ 0.1, Ang II 1.6 $\pm$ 0.4, PC-61 1.8 $\pm$ 0.2*; ENaC-α- Con 1.0 $\pm$ 0.1, Ang II 1.6 $\pm$ 0.2, PC-61 2.1 $\pm$ 0.1*, *P<0.05 vs Con). These data suggest that the anti-inflammatory $Foxp3^+$ T regulatory cells play a significant role in mediating the resistance to Ang II hypertension in premenopausal female mice, and may influence renal inflammation and sodium retention during chronic Ang II infusion.

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P619

Teen Obesity Leads To Changes In The Circulatory T Lymphocyte Profile

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Increasing evidence indicates that inflammatory markers exist in cardiovascular patients years prior to disease diagnosis. In addition, obesity is a major risk factor for cardiovascular disease, especially among African Americans. We hypothesized that obesity would lead to different circulating T lymphocyte profiles and activation status in Caucasian and African American teenagers. Lean (BMI < 60th percentile) and obese (BMI ≥ 95th percentile) teenagers (14-20 years-old; n=23-67/group) of both genders and races were recruited from public schools of Augusta, GA. Circulating immune cell phenotypes and activation status were analyzed by fluorescence-activated cell sorting (FACS), and total whole blood cell (WBC) counts were also determined. Obesity was associated with significant reduction of

circulating T lymphocyte percentages in white individuals independently of gender (lean vs. obese; CD3+, CD4+ and CD8+ cells, respectively: 29.40±8.14 vs. 25.58±7.53% (p=0.007), 28.51±7.29 vs. 25.95±6.04% (p=0.049) and 5.12±2.6.0 vs. 4.04±1.64% (p=0.013)). A significant decrease in percentages of circulating CD8+ and activated CD8+ cells was also observed in black males (lean vs. obese, CD8+ and CD8+/CD69+: 5.16±2.45 vs. 5.51±2.27% and 0.53±0.49 vs. 0.60±0.55%), but not in black females, indicating that activation of CD8+ cells may be important in the increased prevalence of cardiovascular disease in female African Americans. Interestingly, WBC counts were found to be increased in obese subjects (obese vs. lean: 6.67±2.16 vs. 5.67±1.62 x103/mm3), and, when results were normalized to these counts, obesity was associated with significantly elevated absolute values of circulating CD3+ and CD4+ cells, independently of gender and race. Our analysis also indicates a positive correlation between circulating CD4+ cell percentage and HDL cholesterol (HDLc) in obese whites (r=0.34), and a negative correlation between activated T cells (CD3+/CD69+) percentage and HDLc in obese black males (r=-0.42). In conclusion, teen obesity leads to changes in the circulatory T lymphocyte profile. Moreover, these changes could help to recognize at-risk cardiovascular patients and could be used to prevent the development of the disease in these individuals.

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P620

Renal Nerve Denervation Modulates GABA-ergic Input into Paraventricular Nucleus of the Hypothalamus and Exerts a Long Term Antihypertensive Effect in Hypertensive Mice Associated with Chronic Kidney Disease

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Background: Sympathoexcitation plays an important role in the pathogenesis of hypertension with chronic kidney disease (CKD). In hypertension, the paraventricular nucleus of the hypothalamus (PVN) in the brain controls the sympathetic outflow through GABA-ergic mechanisms. The renal nerve denervation (RDN) exerts a certain long term antihypertensive effect; however the precise mechanism is not fully elucidated. We aimed to clarify whether RDN modulates sympathetic outflow through GABA-ergic mechanisms in the PVN in hypertensive mice with CKD.
Methods and Results: In 5/6-nephrectomized ICR-mice (Nx) at 4-weeks after nephrectomy, systolic BP (SBP) was significantly increased (vs. Sham, 143±2 vs. 109±2mmHg, n=12-24, p<0.01), accompanied by sympathoexcitation (urinary norepinephrine (uNE): vs. Sham, 424±26 vs. 226±17μg/24hrs, n=12-24, p<0.01). We performed RDN or sham operation and divided into 3 groups (Sham-sham, Nx-sham, and Nx-RDN, n=12 for each). At 2-weeks after RDN, SBP was significantly decreased (123±2 vs. 136±2 mmHg, n=12 for each, p<0.01), and urinary sodium excretions were increased (0.64±0.04 vs. 0.47±0.03 mmol/24hrs, n=8 for each, p<0.01) in Nx-RDN compared with those in Nx-sham. The uNE levels were not different between two groups. At 6-weeks after RDN, SBP was kept decreasing (125±1 vs. 140±3 mmHg, n=12 for each, p<0.01) and uNE levels were also decreased (386±32 vs. 541±35 μg/24hrs, n=12 for each, p<0.01) in Nx-RDN compared with those in Nx-sham. The urinary sodium excretions were not different between two

groups. Bicuculline (GABA-A receptor antagonist, 50pmol) microinjection into PVN increased mean arterial pressure (MAP) and lumbar sympathetic nerve activity (LSNA) in all groups. The pressor responses and the change in LSNA were significantly attenuated in Nx-sham (vs. Sham-sham, ΔMAP/baseline MAP [%], 33±4 vs. 66±6 %; ΔLSNA (%baseline), 57±7 vs. 112±8 %, $p<0.01$, n=6 for each), but were enhanced in Nx-RDN at 6-weeks after RDN (vs. Nx-sham, ΔMAP/baseline MAP [%], 60±6 vs. 33±4 %; ΔLSNA (%baseline), 94±5 vs. 57±7 %, $p<0.05$, n=6 for each).
Conclusion: These data indicate that augmented GABA-ergic input into PVN induced by RDN, at least in the late phase, is involved in antihypertensive action in hypertensive mice with CKD.

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P621

Cellular Localization of Angiotensin Type 1a Receptor mRNA in the Paraventricular Nucleus of the Hypothalamus

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The chronic neurogenic hypertension that involves increased effects of angiotensin II (Ang II) via its type 1 receptor (AT1R) within brain cardiovascular control centers is associated with induction of microglial activation and neuroinflammation in the paraventricular nucleus of the hypothalamus (PVN). However, whether Ang II exerts *direct* effects via AT1R located on microglia is not established. Therefore, the objective of this study was to determine the cellular localization of AT1R in the PVN. Naïve twelve-week old normotensive (Sprague Dawley, SD; Wistar Kyoto, WKY) rats and spontaneously hypertensive rats (SHR) were euthanized and perfused with 4% paraformaldehyde. Brains were removed and sectioned coronally at the level of the PVN. Sections throughout the entire PVN underwent RNAscope fluorescence *in situ* hybridization to determine AT1aR mRNA expression, combined with immunohistochemistry using microglia (Ionized calcium binding adaptor molecule 1; Iba1)-, neuron (HuC/D)-, or astrocyte (Glial fibrillary acidic protein; GFAP)-specific markers. The results, obtained from at least 3 SD and 3 WKY rats indicate strong co-localization of AT1aR transcripts with neurons in the neuroendocrine and parvocellular PVN regions, as expected. By contrast, there was no detectable co-localization of AT1aR mRNA with either microglia or astrocytes throughout the PVN of these rats. Further, qRT-PCR revealed that while both AT1aR- and AT1bR mRNAs were detectable in SD rat hypothalamus (1.00±0.14; 0.40±0.12; n=7), neither transcript was detectable in microglia cultured from the hypothalamus of SD rats (n=6). The pattern of AT1aR mRNA expression in the PVN of SHR, a hypertensive model that exhibits over activity of Ang II/AT1R actions at the PVN, was similar to that observed in the SD and WKY rats, i.e. strong co-localization with HuC/D-positive cells, and no detectable co-localization with either Iba1 or GFAP-positive cells. Collectively, these findings indicate that AT1R are localized to neurons, not glia, in the PVN of normotensive rats or SHR *in situ*. Further, they suggest that it is unlikely that Ang II exerts direct effects at microglia in the PVN, and that induction of microglial activation at this site following Ang II infusion is likely an indirect action.

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P622

Regulation of Neuroprotective Myeloid Cell Leukemia 1 by Rapamycin and AT2R Agonists in Dopaminergic Neuronal Cell-line

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Myeloid Cell Leukemia I (MCL-1) is a critical protein for neuronal cell survival. MCL-1 is one of the anti-apoptotic proteins in the Bcl2 family. In neurons, MCL-1 regulates the rate of programmed cell death during development and after neuronal damage. It is now well established that without sufficient MCL-1 dopaminergic neuronal cells succumb to cell death under conditions of oxidative stress that result in neurodegenerative diseases such as Parkinsonism. Therefore, identifying drugs that can up-regulate the expression of MCL-1 in neuronal cells is critical for enhancing neuronal resistance to oxidative stress and improving neuronal survival. Aim of this study was to evaluate the effects of treatments with Rapamycin and a novel AT2R peptide agonist NP-6A4 on the MCL-1 expression in SH-SY5Y neuronal cell line. SH-SY5Y cells are a human-derived in vitro model of neuronal function and differentiation, expressing both adrenergic and dopaminergic markers. This cell line is a highly translational model for Parkinson's disease. Cells were maintained in a 1:1 mixture of DMEM and Ham's F-12 with 10% FBS. Cells were subjected to serum starvation and were treated with Rap (10nM), NP-6A4 (300nM) or their combination for 6 hours. MCL-1 protein expression was assessed by immunofluorescence using anti-MCL-1 antibody and a fluorophore-conjugated secondary antibody. Cells were imaged using a confocal microscope and fluorescence was quantified using Leica LAS AF software. It was observed that Rap treatment significantly suppressed MCL-1 expression in SH-SY5Y cells (~40% suppression, $p<0.001$), whereas Rap+NP-6A4 treatment reversed the Rap-mediated suppression of MCL-1 ($p<0.0002$). This data indicates that Rapamycin suppresses MCL-1 in dopaminergic neuronal cells and AT2R agonist, NP-6A4 is capable of reversing this effect.

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P623

Nutrient Stress Response of Cardiovascular Cells to β-Blockers, ARB and AT2R Agonists

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To determine how cardioprotective drugs modulate cell response to nutrient starvation, we investigated the effects of β-blockers (Nebivolol [Neb], Carvedilol [Car], Metoprolol [Met] and Atenolol [Aten] (3μM each), Angiotensin II (Ang II) (300nM), AT1R blocker (ARB-Losartan (1μM)) and AT2R agonists (CGP42112A [CGP] and a novel peptide agonist NP-6A4 (300nM each)) on serum-starved HL-1 cardiomyocytes. The Xcelligence Real-Time Cell Analyzer (RTCA), which measures area covered by cells in microtiter plates and displays it as a unitless quantity called Cell Index (CI), was used to assess cellular changes in response to drug treatment. To find whether changes in CI are due to altered proliferation or cell size, we utilized the MTS Proliferation Assay, a colorimetric assay that measures formazan dye

produced by viable cells, and fluorophore-conjugated Wheat Germ Agglutinin (WGA) labeling to measure cell size respectively. Difference in CI units is reported as % of CI units of cells treated with vehicle. CI units were suppressed by β-blockers (Aten≤15%; Met≤15%; Neb≤17%; Car≤8%), increased by Ang II (≥9.6%), CGP (≥14%) and NP-6A4 (≥25%), but not by losartan (n≥4 and p≤0.05 for all treatments). MTS assay revealed that only NP-6A4 increased (19%) formazan dye as measured at 490nm (n=3, p<0.05). Differences in cell size of WGA stained cells was calculated as % of size of cells treated with vehicle. Neb and Car decreased cell size (Neb≤14%, Car≤10%, p<0.05 and p<0.1 respectively), suggesting their mechanism for decrease in CI. Other treatments showed no significant change. Next, we determined changes in Myeloid cell leukemia 1 (MCL-1) levels, an essential protein for cardiomyocyte survival, in response to drug treatments by immunoblotting and immunofluorescence. β-Blockers suppressed MCL-1 expression in HL-1 cardiomyocytes (Neb≤26%, Car≤24%, Met≤24%, Aten≤16%) while AT2R agonists increased (CGP≥17%, NP-6A4≥28%, n=3; p<0.05 for all). Treatment of Female Human Coronary Artery Vascular Smooth Muscle Cells by these drugs also showed a similar expression pattern of expression for MCL-1 (CGP≥23%, NP-6A4≥43%). Therefore, AT2R agonist NP-6A4 is more effective in protecting serum starved cardiovascular cells.

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P624

Characterisation of the Chemerin Axis in the Human Vasculature

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Objective: The peptide chemerin is an emerging novel vasoconstrictor which has been implicated in altering vascular function in animal models. CMKLR1 is a known chemerin receptor which functions predominately in immune response. Chemerin has also been proposed as the ligand for orphan receptor GPR1, which our group has recently confirmed. We tested the hypothesis that chemerin was vasoactive in human vessels and identified which receptor mediated this action.
Method and Results: Immunohistochemistry revealed that chemerin was expressed in endothelial cells and receptors CMKLR1 and GPR1 were expressed in the smooth muscle cells of human vessels. Quantitative PCR analysis of human vessels supported this with CMKLR1 10 fold more abundant than GPR1. C-terminal fragment chemerin149-157 (C9) caused a concentration dependent contraction in isolated endothelium denuded human saphenous vein (hSV) with a pD2=7.4±0.3 (n=4), which was shifted to the right by antagonist CCX832 (100mM), pD2=6.1±0.2 (n=3). Full characterisation of the novel antagonist CCX832 with receptor-transfected cells confirmed it to be selective for the CMKLR1 receptor, and had no effect on GPR1. In β-arrestin recruitment assays, CCX832 was functionally active at the CMKLR1 receptor causing a rightward shift of the C9 response, logKD=-8.2±0.1 (n=3); however no effect was seen at the GPR1 receptor. Radiolabelled competition binding experiments showed that CCX832 competes for binding with [125I]-C9 at the CMKLR1 receptor, pIC50=9.0±0.1 (n=5), however it does not at the GPR1 receptor. In competition binding experiments on human saphenous vein

homogenate, CCX832 competes for binding with [125I]-C9, pIC50=8.6±0.4 (n=6). This is in agreement with the in vitro pharmacology data, that chemerin's vasoactivity is mediated by CMKLR1. Data is expressed as mean±SEM. Conclusion: These data support the emerging role of chemerin as an endogenous vasoconstrictor through its receptor CMKLR1. It identifies for the first time the components of the chemerin axis in the human vasculature, and confirms that chemerin causes vasoconstriction of human vessels. This study identifies a novel therapeutic drug target for hypertension in humans.

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Non-coding Rna Regulation Of Gene Expression In Angiotensin Ii-induced Vascular Damage

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Introduction: Non-coding RNAs (ncRNAs), including long ncRNAs (lncRNAs) and microRNAs (miRs), account for ~98% of the transcribed RNAs. They have been shown to play a role in cardiovascular disease. Vascular damage is an early manifestation and a cause of end-organ damage in hypertension. However, it is unknown whether ncRNAs are involved in the development of vascular injury in hypertension. We hypothesize that ncRNA regulation participates in mechanisms of vascular remodeling and plays an important role in the pathophysiology of hypertension.
Methods and Results: Ten-week old male C57BL/6 mice were infused or not with angiotensin (Ang) II for 14 days. Systolic blood pressure (BP) determined by telemetry was increased by Ang II infusion compared to control (146±8 vs 113±5 mmHg, P<0.001). Total RNA was extracted from mesenteric arteries for total and small RNA deep sequencing using Illumina HiSeq-2500. Sequences were aligned to the mm10 genome with STAR, annotated and counted using HTSeq-count or miRDeep2. Differential expression analysis was done in R. Differentially expressed (DE) mRNAs (550 up & 266 down), lncRNAs (7 up & 42 down), miRs (23 up & 12 down) were identified in the Ang II-treated group (1.5 fold change, q<0.05). Targetscan was used to predict interactions

between DE miRs and the inversely correlated DE mRNAs or DE lncRNAs. MEME Suite was used to predict DE transcription factor binding sites in the promoter region of genes encoding DE mRNAs, lncRNAs and miRs. Cytoscape was used to construct molecular networks integrating the above interactions and the gene expression profile and to perform functional enrichment analysis, which revealed enrichment of extracellular matrix and developmental processes in DE miR-targeting DE mRNAs (q<1E-20). Ten DE miRNAs whose expression levels correlated (P<0.05) with BP were identified, 9 of which are located in a single miRNA cluster that is conserved in humans.
Conclusions: We have identified a conserved miRNA cluster that may play a pivotal role in the regulation of vascular damage in hypertension. A sub-network of genes that participates in the interaction between the miRNA cluster and other BP-correlated RNAs was selected for future investigation to identify therapeutic targets.

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P626

Amine Oxidase Activity in Mesenteric Perivascular Adipose Tissue Adipocytes is Mediated by Semicarbazide Sensitive Amine Oxidase

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Perivascular adipose tissue (PVAT) is important in regulating vascular tone and reduces contraction of vessels to various agonists. PVAT is composed of adipocytes and the stromal vascular fraction (SVF), which contains immune cells, neurons, endothelial cells, fibroblasts and preadipocytes. Adipocytes, the main cell type in PVAT, contain various amine oxidases. We hypothesized that amine oxidases in mesenteric PVAT would metabolize vasoactive amines. To determine the primary enzyme(s) responsible for the amine oxidase activity in PVAT, an Amplex Red assay was performed. We added tyramine, a substrate for monoamine oxidase-A & B (MAO-A, MAO-B) and semicarbazide sensitive amine oxidase (SSAO), to cell and tissue fractions from male Sprague Dawley rats to test for H_2O_2 production as an indicator of oxidase activity. Oxidase activity in mesenteric PVAT increased from 7.92±2.27 (vehicle) to 20.83±5.30 with the addition of tyramine ($p<0.05$) and from 9.30±2.55 to 24.60±6.38 in adipocytes ($p<0.05$; pmol/min·mg±SEM; N=5). In both the mesenteric PVAT and isolated adipocytes, the oxidase activity was reduced to baseline levels (vehicle) by pre-incubating with the SSAO inhibitor semicarbazide (1 mM; PVAT: 8.49±2.56, adipocytes: 9.43±3.21; $p<0.05$; N=4-5). No reduction in oxidase activity in both the mesenteric PVAT and the isolated adipocytes was observed when the MAO-A inhibitor clorgyline or the MAO-B inhibitor pargyline (1 µM; a concentration at which each drug is specific for their respective enzyme) were used. Westerns revealed that mesenteric PVAT adipocytes had the highest expression of monomeric SSAO (~80 kDa; 124.75±30.71%) compared to the whole PVAT (66.15±9.92%), blood vessels (48.2±6.89%) and the SVF (38.76±11.65%) as quantified by percent density relative to β-actin using protein isolated from male Sprague Dawley rats ($p<0.05$; values given as %β-actin±SEM, N=4). Oxidase activity within the SVF remains to be determined. These

findings support that most of the oxidase activity in mesenteric PVAT can be attributed to SSAO and the adipocyte is an abundant source of SSAO. Due to the proximity of PVAT to the blood vessel, oxidase activity in PVAT through SSAO may alter vascular tone by metabolizing vasoactive amines and through the production of H_2O_2.

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P627

The β3 Adrenergic Receptor is Partially Responsible for Anti-contractile Effect of Perivascular Adipose Tissue in Rat Mesenteric Resistance Arteries

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In normal conditions, perivascular adipose tissue (PVAT) decreases contractile responses non-specifically in various vascular beds. This anti-contractile effect of PVAT is reduced in metabolic diseases and hypertension. The β3 adrenergic receptor (β3AR) is a G protein-coupled receptor expressed in adipocytes and involved in lipolysis and thermoregulation. We have previously demonstrated that chronic systemic infusion with a β3AR agonist induces white-to-brown adipose tissue remodeling and enhanced anti-contractile effects of PVAT via activation of cystathionine gamma lyase, enzyme involved in hydrogen sulfide synthesis. We hypothesized that the β3AR is directly mediating release of PVAT relaxing factors. Endothelium-intact mesenteric resistance arteries from adult male Wistar rats were used to measure contractile responses in the presence and absence of PVAT. In the absence of PVAT, the β3AR agonist CL316243 (1 nM-10 μM) did not directly induce relaxation of U46619-contracted arteries. In control conditions, norepinephrine (NE)-induced contraction was significantly reduced in the presence of PVAT. In contrast, incubation with the selective β3AR antagonist L-748337 (100 nM) led to a significant increase in NE-induced contraction in PVAT-intact arteries, while no change was observed in the absence of PVAT (figure). These data suggest that β3AR mediates the anti-contractile effect of PVAT on NE-induced contraction in resistance mesenteric arteries. Considering the structural and functional alterations of PVAT in hypertension, future studies may reveal a potential novel therapeutic approach via targeting of the PVAT β3AR pathway.

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P628

Angiotensin 1-7 Mas Receptor and Dopamine D1 receptor Interaction as a Novel Natriuretic Mechanism in Rat Kidneys

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Evidence to date suggests that a positive interaction between natriuretic factors promotes sodium excretion to maintain sodium homeostasis and blood pressure. Although the involvement of renal dopamine D1 receptor (D1R) in promoting sodium excretion is well established; the role of Angiotensin (Ang) 1-7 Mas receptor (MasR) is not clear. Here we

provide evidence for a functional interaction between these two renal G protein-coupled receptors which suggests that natriuretic response to Ang 1-7 via MasR is dependent on D1R activation. Male Sprague Dawley rats of comparable weight and age were infused with Ang 1-7, MasR antagonist DPro, D1R agonist SKF38393 and D1R antagonist SCH23390. Blood pressure was monitored throughout the experimental procedure and none of the infused drugs affected the pressure. Animals infused with saline alone served as controls. Infusion of Ang1-7 caused significant natriuresis and robust diuresis compared to saline. SKF38393 infusion also induced significant natriuresis and diuresis when compared to saline infusion. Both natriuretic and diuretic response to Ang 1-7 was blocked by Dpro and SCH23390. However, Dpro failed to block SKF38393 response. Concomitant infusion of SKF38393 and Ang 1-7 did not show a cumulative natriuretic or diuretic effect when compared to SKF38393 or Ang 1-7 infusion alone. FENa (%), control (saline): 0.30 ± 0.09; Ang 1-7: 1.03 ± 0.21; Ang 1-7 plus Dpro: 0.49 ± 0.11; Ang 1-7 plus SCH23390: 0.36 ± 0.10; SKF38393: 0.83 ± 0.16; SKF38393 plus SCH23390: 0.41 ± 0.09; SKF38393 plus Dpro: 0.82 ± 0.17; Ang 1-7 plus SKF38393: 1.06 ± 0.21. These data suggest that Ang 1-7 via MasR causes natriuresis which is dependent on D1R activation. On the other hand, renal D1R-mediated sodium excretion is independent of MasR. This study is a paradigm shift as these data identify a novel functional unidirectional interaction between renal MasR and D1R which deviates from commonly known receptor-receptor interaction.

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Effect of Circulating ACE2 Activity on plasma Angiotensin Peptides

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Circulating ACE2, an enzyme that degrades Ang II, has been reported to be increased in rodent models of diabetes. Upregulation of ACE2 by increasing hydrolysis of Ang II could help downregulate RAS overactivity in a compensatory way and/or reflect increased Ang II metabolism. ACE2 activity in plasma, however, is relatively low even in diabetic conditions. Therefore, further increases in ACE2 effected by exogenous ACE2 administration could have a therapeutic action as a result of enhanced Ang II degradation and formation of Ang 1-7.

We examined the effect of a massive increase in circulating ACE2 activity on plasma levels of Ang II and other Ang peptides in mice rendered diabetic by STZ. Circulating ACE2 was amplified using ACE2 mini-circle (ACE2MC) gene delivery. Multiple Ang peptides were evaluated in plasma from STZ-mice that received ACE2MC and sham STZ-mice (n=6/group).

Plasma Ang II in STZ-ACE2MC mice were markedly lower than in STZ sham mice (3±1 vs. 22±13, pg/mL, respectively), but the difference was not significant owing to high variability. Since the seemingly lower Ang II levels could be influenced by either lower levels of the precursor peptide Ang I, increased degradation of Ang II, or both, a ratio between Ang II and Ang I was calculated. Ang II/Ang I ratio was significantly lower in STZ-ACE2MC as compared to STZ sham mice (0.15±0.05 vs. 0.35±0.06,

p<0.05) indicating that per given amount of Ang I, there is significantly less Ang II in STZ-ACE2MC mice. This is consistent with enhanced Ang II degradation. Plasma levels of Ang 1-7 and Ang 1-9, the direct products of ACE2 cleavage of Ang I and Ang II, respectively, were very low and mostly below the level of detection. In plasma, Ang 1-7 is quickly converted to Ang 1-5, by ACE. Therefore, as an index of Ang II conversion to Ang 1-7, and as a surrogate for this conversion, we used Ang 1-5/Ang II ratio. This ratio was markedly higher in STZ-ACE2MC than in sham STZ-mice (3.15±1.5 vs. 0.38±0.07, p<0.05, respectively) suggesting an increased conversion of Ang II to Ang 1-7, and later to Ang 1-5 as a result of plasma ACE2 overexpression. We conclude that a massive increase in circulating ACE2 is associated with an accelerated metabolism of Ang II. The increase in Ang II degradation seems best reflected by an increase in Ang 1-5/Ang II ratio.

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P630

Captopril Reduces Cytopathic Effects in Herpes Simplex Virus 1 (HSV-1) Infected SH-SY5Y Cells

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Over 60% of the United States population is infected with HSV-1, a double-stranded DNA virus belonging to the Herpesviridae family. HSV-1 is continually documented as a leading infectious cause of corneal blindness and encephalitis. Members of the herpesviridae family have been implicated as cardiovascular pathogens and are independently associated with the future risk of cardiovascular death. Investigations have demonstrated that hypertension may be significantly related to inflammation, a major symptom of HSV-1 infection. Most HSV-1 antivirals are nucleoside analogs which are effective for individuals experiencing current outbreaks, but are limited by the development of antiviral resistance. Thus, there is a need for novel antiviral targets to decrease viral reactivation, a major player in viral associated neuropathologies. In addition to its well-known function of maintaining homeostatic control of arterial and osmotic pressure, components of the Renin Angiotensin System (RAS) have been demonstrated to exert antiviral functions. RAS targets, such as angiotensin peptides and angiotensin type 1 receptors, have been evaluated for their antiviral properties. Yet, the antiviral properties of other currently approved RAS inhibitors have not been investigated. To determine if other RAS components may inhibit viral activity, we tested the hypothesis that captopril, an inhibitor of angiotensin converting enzyme 1 (ACE), attenuates cytopathic effects of HSV-1 in SH-SY5Y, human neuroblastoma, cells. Photomicrographs of SH-SY5Y cells demonstrated that captopril protects cells from HSV-1-induced cytopathic effects. Additionally, cell viability assays revealed that captopril reduced HSV-1-induced cellular death by 18% (p-value<.05, compared to vehicle treated HSV-1 infected cells). Preliminary viral entry assay data suggests captopril may exert antiviral effects through entry inhibition as demonstrated by a 14.18% decrease in GFP immunofluorescence after captopril-treated cells were exposed to a GFP-expressing HSV-1 recombinant virus for 48h (compared to their untreated, uninfected counterparts). These

results support captopril as a therapeutic target for the treatment of HSV-1 and its associated pathologies.

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P631

Cholesterol Is Required To Maintain T-tubule Integrity And Intercellular Connections At Intercalated Discs In Cardiomyocytes

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Aims: Low serum cholesterol levels are associated with cardiac arrhythmias and poor prognosis in patients with chronic heart failure. However, the underlying mechanisms by which decreases in cholesterol content lead to cardiac dysfunction remain unclear. Multiple studies have implicated damage to cardiac transverse (T)-tubules as a key mediator of excitation-contraction (E-C) coupling dysfunction and heart failure. Since the T-tubule membrane system is enriched in cholesterol, we hypothesized that depletion of membrane cholesterol promotes T-tubule remodeling and E-C coupling dysfunction.

Methods and Results: We first examined the impact of membrane cholesterol depletion on T-tubule architecture by treating isolated C57BL/6 murine cardiomyocytes with methyl-β-cyclodextrin (MβCD). T-tubule structural integrity was progressively decreased by MβCD in a concentration- and time-dependent manner. Membrane cholesterol depletion also promoted in a severe decrease in the amplitude and frequency of Ca2+ transients and an increase in the number and amplitude of spontaneous Ca2+ sparks. Reintroduction of cholesterol restored T-tubule integrity and Ca2+ handling properties in acutely-treated myocytes and slowed down T-tubule deterioration in response to chronic MβCD exposure. Studies were extended to determine the impact of membrane cholesterol depletion on T-tubule structure in intact hearts. In addition to T-tubule remodeling, Langendorff perfusion of MβCD resulted in rapid and severe disruption of the intercellular connections between cardiomyocytes, in particular at intercalated disc regions in intact hearts.
Conclusions: These data provide the first evidence that cholesterol plays a critical role in maintaining cardiac T-tubule structure and the integrity of intercalated discs.

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P632

The Blood Pressure in Dialysis (BID) Pilot Study

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Pittsburgh, PA; Mahboob Rahman, Case Western Reserve Univ, Cleveland, OH; Lavinia Negrea, Univ Hosp Case Medical Ctr, Cleveland, OH; Cynthia Kendrick, Cleveland Clinic, Cleveland, OH

Background: The optimal blood pressure (BP) target for hypertensive hemodialysis (HD) patients is unknown. Current KDOQI guidelines have been extrapolated from data in the general population. The BID pilot, funded by NIDDK and DCI, is the first trial to randomize hypertensive HD patients to intensive (110-140 mm Hg) vs. usual (155-165 mm Hg) control of systolic blood pressure (SBP). The study's goal is to assess the feasibility of conducting a full-scale trial.
Methods: BID consortium consists of 5 clinical centers, a cardiac MRI reading center and a data coordinating center. Standardized predialysis SBP, measured in the dialysis unit in accord with AHA recommendations (SDUSBP), guide therapy. To be eligible for randomization patients needed a 2-week running mean SDUSBP ≥ 155 mm Hg. Home BP measurements (HBPM) are obtained twice on the day after the midweek dialysis. Ambulatory Blood Pressure Monitoring (ABPM) during a 44h interdialytic period is obtained quarterly. We compared SDUSBP, HBPM and ABPM.
Results: We enrolled 281 and randomized 126 participants. Major reasons for drop out during the baseline period were 2-week mean SDUSBP < 155 mm Hg (40.6%), no cardiac MRI (13.0%) and perception of protocol as burdensome (11.2%). Adherence with prescribed SDUSBP was satisfactory. The percent of patients with ≥ 4, ≥ 8 and ≥12 SBP per month were 96, 88 and 57% in month 1 and 78, 68 and 37% in month 12. In a constant cohort of participants followed ≥ 330 days 2-week mean SDUSBP were 144 ± 17.4 and 156 ± 15.2 mm Hg in the intensive and usual arms, respectively. Major reasons that participants in the intensive arm did not achieve target SBP included large interdialytic weight gain (27.2%), non-adherence with medications or dialysis prescription (40.9%), and intradialytic hypotension (31.9%). Differences between the SDUSBP and both HBPM and ABPM were often ≥ 10 mm Hg. Optimal control of BP requires measurements in and out of the dialysis unit. Rates of adverse events were similar to those in other NIDDK funded ESRD studies.
Conclusion: The difference in BP between arms was achieved and maintained throughout the study. It is feasible to conduct a full-scale clinical trial of intensive vs. usual treatment of hypertension in HD patients.

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P633

Low Serum Vitamin D is Associated with Greater Impairment in Autonomic Function upon Head Up Tilt in Children with Orthostatic Intolerance

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In previous work we identified a group of children (n = 48) between the ages of 10-18 years whose diagnostic workup for chronic nausea unexplained by conventional diagnostic tests revealed that 60% had underlying cardiovascular instability (n = 30) presenting as orthostatic intolerance (OI). The OI could be sub-classified based on head up tilt (HUT) testing into three groups: Postural Orthostatic Tachycardia Syndrome (POTS), orthostatic hypotension (OH) and syncope. Children with OI in all three groups had a greater reduction in autonomic control upon HUT manifested as greater loss of baroreflex sensitivity (BRS) and heart rate variability (HRV) and higher norepinephrine levels compared to those in the non OI group. Vitamin D deficiency is associated with impaired vascular responses to vasoconstrictors and alterations in autonomic control mechanisms in adults. In this study we sought to determine if vitamin D level is lower in these pediatric OI subjects and if it correlates with the hemodynamic responses to tilt. Serum 25(OH)D tended to be lower in OI vs non OI (18.6 ± 0.7 ng/ml, n = 25, vs 22.2 ± 2.4 ng/ml, n = 15; p = 0.16). Most importantly 25(OH)D showed a high positive correlation with supine measures of BRS (seq ALL, R = 0.51, p = 0.05), HRV (rMSSD, R = 0.44, p = 0.02) only in the OI group and there was a trend for a negative correlation with sympathovagal balance (LF/HF ratio: R =-0.35, p = 0.08). Low 25(OH)D correlated with greater loss of both BRS (R = 0.51, p = 0.01) and HRV (R = 0.44, p = 0.05) upon HUT. These findings support the concept that low vitamin D may contribute to impaired responses to tilt in OI subjects. Further work is needed to evaluate if vitamin D supplementation will improve the vascular and hemodynamic responses to tilt and help improve the OI symptoms. Our goal is to provide a safer therapeutic alternative for the treatment of OI in children.

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P634

The Influence Of Virtual Learning Environment On Therapy Adherence And On The White Coat Effect

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Systemic arterial hypertension is a risk factor for cardiovascular diseases and has become a common public health problem. Health education associated with educational technology may be used to encourage patients' adherence to treatment and enable them to adequately understand how harmful hypertension can be to health, thereby promoting their quality of life. OBJECTIVE: To evaluate the influence of a strategy in an individual orientation program using educational technology associated with virtual learning environment (VLE) of hypertension care on the reduction in the white coat effect and the improvement in blood pressure control to be promoted by a nurse in a hypertension unit in a government state hospital in São Paulo. METHODS AND MATERIALS:This was a randomized clinical education study conducted with two groups, the VLE group (study group, 10 patients) and the control group (16 patients). Both groups were interviewed 6 times by nurses during the 120-day follow-up at 20-day intervals. At baseline (randomization) and at the end of the study, the patients took Spielberg's State-Trait Anxiety Inventory (STAI), the Morisky test, and the WHOQOL, a quality of life instrument, and had their blood pressure taken (ambulatory blood pressure monitoring [ABPM]). Both groups had their blood pressure, weight, and abdominal circumference measured. Only the study group had remote access to the VLE. This consisted of 6 specific educational modules, each released according to the encounter number. RESULTS: At baseline, there were no statistical differences between the two groups with respect to the sociodemographic and hemodynamic variables. At the end of the study, there was a significant statistical difference between the groups on the Morisky test (p=0.001) and on the WHOQOL with respect to domain 3 social (p=0.001). There was no statistical difference with respect to the white coat effect between the groups. Nor was there any statistical difference between the groups with respect to the association of the anxiety degree measured by STAI and the white coat effect.CONCLUSION: In light of the results, our strategy improved the quality of life in the social domain and changed the adherence behavior of the study group in relation to the forgetfulness of medication schedules.

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P635

Improvement of Cardiovascular Outcomes in Diabetic Rats by a Novel Angiotensin II Receptor Peptide Agonist

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Diabetes Mellitus (DM) is an independent predictor of cardiovascular disease (CVD). Recent reports show that Angiotensin II type 2 receptor (AT2R) promotes cardiac repair after myocardial infarction. Therefore, we hypothesized that activation of AT2R would improve cardiac function in diabetic rats. Male Zucker obese (ZO) rats are leptin receptor−deficient and exhibit hyperphagia, obesity, insulin resistance and hyperlipidemia. They are a widely used rodent model for early stage Type 2 DM (T2DM). We and others have reported that male ZO rats exhibit diastolic and systolic dysfunction. Therefore we investigated whether a two week treatment with an AT2R agonist could improve cardiac functions of young male ZO rats. Thirteen-week old male ZO rats were subjected to daily intraperitoneal injections (0.9mg/kg/day) with a novel peptide AT2R agonist, NP-6A4 (from Novopyxis, Inc.) dissolved in saline (n=7) or saline only (n=6) for two weeks. Conventional echocardiography and speckle tracking strain analysis were performed using a Vevo 2100 (visualsonics) small animal ultrasound system. Fasting (6 hours) plasma analysis showed that triglycerides (mg/dL) were significantly reduced in response to treatment (ZO+ Saline=1229±164; ZO+NP-6A4= 610±109; $p<0.015$). Importantly, NP-6A4 treatment improved E/E' ratio (ZO+Saline= 32.3±2.06; ZO+NP-6A4= 26±2.1; $p<0.005$), which indicates a significant improvement in diastolic dysfunction. A unit rise in the E/E' ratio is associated with a 17% increment in risk of a cardiac event. Moreover, myocardial performance index of NP-6A4 treated rats was also reduced (ZO+ Saline= 0.516±0.03; ZO+ NP-6A4= 0.389±0.02; $p<0.006$). Finally, circumferential strain (deg/sec) of endocardium (short axis view) was also significantly improved in response to treatment, while no significant changes were observed in radial or longitudinal strains (ZO+ Saline= -20.56±1.65; ZO+ NP-6A4= -26.11±2.47; $p<0.024$). Collectively, these data suggest that activation of the AT2R by NP-6A4 had significant lipid lowering effect and improved diastolic and systolic functions in diabetic rats.

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P636

Sex Differences in Cardioprotective AT2R Expression in Diabetic Rats and Its Correlation with Myocardial Damage

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Diabetes mellitus (DM) is an independent risk factor for cardiovascular disease (CVD). Healthy, young women are protected from CVD, while diabetic women are more susceptible to CVD compared to age-matched diabetic men and non-diabetic women. Underlying mechanisms for this sex difference in CVD are not fully elucidated. The angiotensin II type 2 receptor (AT2R) is a member of the protective, vasodilative arm of the renin angiotensin

system. The Agtr2 gene that codes for AT2R is X-linked, and increased Agtr2 expression is reported in female vasculature of rodent models. We hypothesized that a sex difference might exist in DM-associated regulation of cardiac AT2R expression. To test this, we used hyperglycemic, male and female Zucker diabetic fatty (ZDF) rats and age- and sex-matched normoglycemic Zucker lean (ZL) rats. The male ZDF (ZDF-M) rat is an established model of type 2 DM. We have reported previously that hyperglycemic, female ZDF (ZDF-F) rats had the highest body fat and lowest lean muscle mass compared to male and female lean rats (ZL-M, ZL-F) and ZDF-M. Cardiac Agtr2 expression was measured by qRT-PCR at 5-months, cardiac function by echocardiography was compared at 3- and 5-months, and histopathology of cardiac tissue was assessed at 5-months. ZL-F had a nearly 2-fold increase of Agtr2 compared to ZL-M ($p<0.01$). Relative to lean controls, ZDF-M had no significant change in Agtr2, while ZDF-F exhibited ~60% suppression (Rq=0.42) of Agtr2 ($p<0.001$). Echocardiography data revealed evidence of compensated systolic function in all groups since fractional shortening was >50% at both ages, while heart rate and stroke volume were similar. However, diastolic dysfunction was observed in both ZDF-F and ZDF-M, relative to their lean counterparts, due to increased isovolumic relaxation time and decreased early:late ventricular filling ratio (E/A). ZDF-F exhibited the highest cardiomyocyte hypertrophy (≥35% over ZL-F, ZL-M and ZDF-M). Both ZDF and ZDF-M showed mitochondrial clustering and disrupted spatial orientation of mitochondria relative to the sarcomere (assessed by TEM). Based on our results, we propose that myocardial remodeling, diastolic dysfunction and loss of cardioprotective AT2R may underlie greater susceptibility of diabetic females to CVD.

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P637

Pigment Epithelium Derived Factor (PEDF) Deficiency Increases Blood Pressure And Accentuates Glomerular Pathology In Diabetic Mice

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Pigment Epithelium Derived Factor (PEDF) encoded by SERPINF1 gene has potent anti-angiogenic and cytoprotective activities. It has been reported that PEDF protein levels are reduced in the kidneys of rodents with experimentally induced diabetes. However the effect of PEDF on blood pressure has not been elucidated. Here, we used SERPINF1 KO mice to examine the impact of PEDF deficiency on kidney pathology and blood pressure in the STZ-induced mouse model of diabetes.
Twelve weeks after diabetes induction by STZ, SERPINF1 KO mice showed exacerbated glomerular damage with ~ 2-fold increase in mesangial matrix (2.8±0.14 vs. 1.2±0.14 arbitrary units, $p<0.01$) and ~ 20% decrease in podocyte counts (8.2±0.0.4 vs. 9.7±0.2 podocytes/glomerulus, $p<0.01$) compared to the wild type controls. Of note, STZ-treated SERPINF1-/- mice displayed elevated systolic blood pressure (SBP) (127±4 vs. 109±4 mmHg, $p<0.01$, $n=11$).
Our data indicate that global PEDF deficiency intensifies glomerular injury in STZ-induced mouse model of diabetes. An important drawback of most murine models of diabetic

kidney disease is the lack of hypertension, a known key factor that accelerates progression to CKD in humans. In contrast, we found that STZ-treated SERPINF1-/- mice become hypertensive. Together, our findings point to SERPINF1-/- mice as an attractive model to study diabetic kidney disease and hypertension in mice and suggest an important causative role of PEDF downregulation in glomerular pathology in diabetes.

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P638

Investigating Gene Pleiotropy in the Metabolic Syndrome in Lyon Hypertensive Rats

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The metabolic syndrome (MetS) - hypertension, obesity, dyslipidemia, and insulin resistance - is a major risk factor for cardiovascular disease and stroke. Our overall goal is to identify novel genes and pathways causing MetS. Our previous work determined that rat chromosome 17 (RNO17) contributes to several MetS-defining traits (including high blood pressure, obesity, and dyslipidemia) in the Lyon Hypertensive (LH) rat, a genetically determined MetS rat model. We hypothesized that at least some of the traits on RNO17 are controlled by a single gene with pleiotropic effects. To address this hypothesis, we generated congenic strains where a defined fragment of RNO17 from the LH rat was substituted by that of the control Lyon Normotensive (LN) rat, and measured MetS phenotypes. One congenic (LH-17^{LN}a), with the proximal 30 Mb of RNO17 from the LH genome substituted with that of the LN genome, did not show significant differences from the LH parental strain. However, another congenic strain (LH-17^{LN}c), with a substituted fragment at the distal end of RNO17 (84-97 Mb), showed significant differences from the LH rat in serum total cholesterol (3.15 $\pm$ 0.15 vs. 4.29 $\pm$ 0.17 mMol; $p<0.01$) and triglycerides (0.47 $\pm$ 0.06 vs. 1.27$\pm$ 0.13 mMol; $p<0.001$), and a trend for reduced blood pressure (SBP 150.8 $\pm$ 3.4 vs. 157.1 $\pm$ 1.7 mmHg; $p=0.1$). Interestingly, there was no difference in body weight between the LH-17^{LN}c and the parental LH rat (440 + 7.2 vs. 435 + 9.1 g). These data indicate that serum cholesterol and triglycerides, and possibly blood pressure are regulated by a gene(s) in the distal congenic interval, and could be due to pleiotropy. The data also indicate body weight is not determined by the same gene(s). Interestingly, only two small haplotypes spanning a total of 1 Mb differ between the LH and LN genomes in the congenic interval. Genes in these haplotypes are being studied as candidate genes for causing dyslipidemia in the LH rat. Overall MetS, even in a simplified genetic model such as the LH-17^{LN} rat, is likely due to both independent and pleiotropic gene effects.

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P639

Attenuation of Renal Inner Medullary Circadian Clock Gene Expression in Response to High Salt Intake is Dependent on the Endothelin B Receptor

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Our lab has recently shown that ETB deficient (ETB def) rats have a time of day dependent impairment in their ability to excrete a Na+ load. These observations suggest an interaction between renal ETB receptors and circadian mechanisms that regulate renal tubular Na+ transport and excretion. Given that knockout of the circadian clock gene Bmal1 reduces blood pressure in mice, we hypothesized that a high salt intake impairs the clock mechanism in the renal inner medulla in an ETB dependent manner. Transgenic control (Tg con) or ETB def rats were fed normal (NS, 0.8% NaCl) or high (HS, 4% NaCl) salt for two weeks. In one group, rats were euthanized every 4 hours beginning at zeitgeber time 0 (lights on) for tissue collection (and subsequent assessment of circadian clock genes), while in a second group of rats urine was collected in 12-hour intervals (active vs. inactive). Consistent with our hypothesis, we observed that HS abolished the normal oscillation in Bmal1 expression in the renal inner medulla of Tg con rats, and effect not observed in ETB def rats. Interestingly, renal production of ET-1, was significantly higher during the active period vs. inactive period in both NS (3.6±1.1 vs. 0.8±0.2 pg/12hr respectively) and HS (9.2±4.1 vs. 1.6±0.3 pg/12hr respectively) fed Tg con rats. There was no time-of-day-dependent difference in ET-1 excretion in ETB def rats on NS (6.6±2.2 vs. 4.6±1.7 pg/12hr respectively), although this pattern was restored in ETB def rats fed HS (2.2±1.0 vs. 9.2±2.5 pg/12hr inactive vs. active). Taken together, these data indicate that an increase in renal ET-1/ETB activation in response to HS modulates inner medullary clock gene expression to promote renal Na+ excretion.

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P640

Endogenous microRNAs in Human Microvascular Endothelial Cells Regulate mRNAs Encoded by Hypertension-Related Genes and Are Functionally Important

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Relatively few miRNA-target pairs have been shown to be involved in hypertension or physiological processes related to blood pressure regulation. The goal of the present study was to systematically identify endogenous microRNAs in endothelial cells that regulate mRNAs encoded by genes relevant to hypertension. Small RNA deep sequencing was performed in cultured human microvascular endothelial cells (HMVEC-D) to identify abundant miRNAs. Of the 50 most abundant microRNAs identified, 30 had predicted target mRNAs encoded by genes with known involvement in hypertension or blood pressure regulation. HMVEC-D were transfected with anti-miR oligonucleotides to inhibit each of the 30 microRNAs and the mRNA abundance of predicted targets was measured by qRT-PCR. Of 95 microRNA-target pairs examined, the target mRNAs were significantly up-regulated in 35 pairs and paradoxically down-regulated in 8 pairs. The functional relevance of miRNAs targeting *JAG1* (miR-21-5p) and *NOX4* (miR-92a-3p, miR-92b-3p, miR-100-5p, and miR-99b-5p), was tested in in vivo and in vitro models, respectively. Blood pressure was measured with radiotelemetry in C57BL/6J mice receiving LNA

anti-miR-21 or scrambled LNA anti-miR (n=4/group; 10mg/kg; i.p.). LNA anti-miR-21 induced a transient increase in arterial pressure in mice fed a 0.4% NaCl diet, but a significant and sustained reduction of 24 hour averaged mean arterial pressure on a 4.0% NaCl diet, reaching 96.3 ± 4.0 mmHg compared to 105.3 ± 1.6 mmHg (mean ± SEM) in mice treated with scrambled anti-miR ($p<0.05$). The release of H_2O_2 from HMVEC-D was measured by Amplex Red assay 48 hours after transfection with anti-miRs identified to increase *NOX4* mRNA abundance or scrambled anti-miR (n=6-12/group). The release of H_2O_2 from HMVEC-D was significantly increased following transfection of anti-miRs for miR-92a-3p, miR-92b-3p, miR-99b-5p, and miR-100b-5p by 82.2% to 139.6%. These findings indicate widespread, tonic control of mRNA abundance of genes relevant
to blood pressure regulation by endothelial microRNAs that are functionally significant.

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P641

Angiotensin Receptor Blocker Improves Pressure Natriuresis in Select African-Americans

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Objective
Hypertension in ~30-60% of African-Americans (AAs) is resistant to single or combination therapy, indicating the necessity for novel screening strategies in this population. Our research supports a strategy that identifies a subset of AAs (approximately 1 in 3) who increase their retained sodium load during mental stress (MS) with an accompanying volume-mediated increase in blood pressure (BP) that remains elevated until the volume expansion diminishes. We hypothesize is due to activation of the renin-angiotensin system (RAS).
Design and Method
We conducted a double blind placebo controlled crossover trial comparing the effects of irbesartan to placebo on sodium handling and consequent BP during MS. Subjects included 213 AA adults, of which 168 (78%) completed the study. After 7 days of vehicle (placebo or irbesartan) subjects underwent a 3 hr protocol comprised of 1 hr rest, 1 hr competitive video games, and 1 hr recovery. Blood and urine were obtained after each hour and BP every 15 mins. A 1 week washout ensued before subjects crossed over to repeat with the alternate vehicle. Subjects were divided into those who exhibited expected increases in urinary sodium excretion ($U_{Na}V$) (Excreters) or reduced $U_{Na}V$ (Retainers) during MS as defined by previous data. Statistical analysis was performed using crossover methods with a pooled analysis that assumes equal variances and the Satterthwaite test of treatment difference (Stest) that assumes unequal variances.
Results
Both statistical tests revealed that $U_{Na}V$

increased significantly for retainers (n=55) on irbesartan ($p<0.002$ and $p<0.015$, mean $\pm$ SD (pooled =7.15 $\pm$ 6.78, Stest =7.15)) normalizing the sodium response to MS. Irbesartan also enhanced $U_{Na}V$ during MS in excreters (n=113) with significant differences ($p<0.001$ and $p<0.001$, mean $\pm$ SD (pooled = 4.18 $\pm$ 9.03, Stest =4.18)). Overall SBP response was significantly reduced with treatment ($p< 0.001$ and $p< 0.001$, mean $\pm$ SD (pooled = -5.08 $\pm$ 3.19, Stest = -5.08)).

Conclusion

Agents that block the RAS are not usually considered effective in AAs. However, our results suggest identification and treatment of AAs who increase their retained sodium load during MS with these agents will reduce the percentage of AAs with hypertension resistant to standard therapy.

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P642

Impact of Urinary Endothelin-1 on Derangements in Stress-Induced Pressure Natriuresis

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Sodium retention during stress (retainer) is known to increase the risk of hypertension and other diseases. Our group has previously shown that angiotensin II type I receptor blockade improves natriuresis in retainers. Since our pre-clinical studies demonstrate that angiotensin II inhibits endothelin-1 (ET-1) dependent natriuresis, we predict that ET-1 may be linked to the response. We hypothesize that reduction in urinary (renal) ET-1 accounts for derangements in sodium handling under stress, a link never before explored in a large human cohort. We evaluated urinary ET-1 and albumin excretion in 4 studies of stress-induced pressure natriuresis, of which 3 were observational studies with 776 healthy youth (15 - 19 years) enrolled in a 5 hr protocol (1 hr of mental stress before and after 2 hrs of rest). The 4th study involved 213 African American adults (18 - 54 years) in a double blind crossover trial comparing irbesartan (angiotensin II type I receptor antagonist) to placebo. The protocol entailed 7 days of vehicle (placebo or irbesartan 150 mg P.O.) followed by a 3 hr protocol (1 hr of rest before and after 1 hr of mental stress). In all studies, 60 min urine samples were obtained. Subjects were grouped as retainers or excreters if they retained or excreted sodium under stress. In the observational studies, mean change in ET-1 between stress and baseline was significant ($p<0.001$), being negative (mean= -0.0154 pmol/min) in retainers but positive (mean= 0.0194 pmol/min) in excreters. ET-1 excretion was significantly higher ($p<0.028$) in retainers than excreters at baseline but significantly lower in retainers under stress ($p<0.0001$). ET-1 excretion continued to decline in retainers during recovery but returned to pre-stress levels in excreters. Albumin excretion and albumin to creatinine ratio were significantly higher in retainers ($p<0.046$, $p<0.008$, respectively). During stress, the irbesartan group had significantly higher ET-1 excretion than placebo ($p<0.001$). Thus loss of ET-1-dependent natriuresis may account for sodium retention during stress and correction of sodium handling re-establishes ET-1-mediated natriuresis. Retainers have both lower ET-1 excretion and increased albuminuria,

suggesting renal impairment and risk for future diseases.

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P643

Proliferative Potential of Perivascular Adipose Tissue Stromal Vascular Cells Is Dependent on Anatomical Site

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Perivascular adipose tissue (PVAT) is an important paracrine regulator of blood vessel function. Growth and pathological conditions such as obesity expand PVAT by hyperplasia and hypertrophy, however, these remodeling processes may differ depending on the PVAT anatomical site leading to diverse effects on the vasculature. A higher proliferative capacity of PVAT localized around mesenteric arteries may contribute to increased visceral fat mass and therefore intensify CVD risk. We hypothesize that PVAT proliferative potential is dependent on PVAT anatomical localization. PVATs from aorta (aPVAT) and mesenteric arteries (mPVAT) were collected from male Sprague Dawley rats at 10 weeks of age (n=5). Visceral depots including gonadal (GON) and retroperitoneal (RP), and the subcutaneous inguinal pad (SC) were collected as non-perivascular adipose controls. Stromal vascular fraction cells (SVF) from each adipose site were harvested and flow cytometry was performed to assess their expression of surface markers for committed preadipocyte precursors including CD34, CD44, and CD140a. Cells co-expressing these markers have high adipogenic capacity and are highly proliferative in visceral adipose tissues during obesity. No differences among sites were observed in the percentage of SVF cells expressing CD34 (aPVAT 39.3%±9.1; mPVAT 46.4%±11.16; GON 51.15%±14.11; RP 37.68%±5.6; SC 29.74%±2.5) and CD140a (aPVAT 1.43%±0.65; mPVAT 3.54%±1.33; GON 3.53%±0.36; RP 1.95%±0.91; SC 2.42%±0.81). There was a higher number of $CD44^{+}$ SVF in mPVAT (3.93%±0.8) and GON (4.4%±1.11) compared to aPVAT, RP, and SC (1.4%±0.13; 1.53%±0.53; 2.08±0.37. $P<0.05$). Proliferation capacity of SVF was evaluated by plating $2x10^5$ cells/cm^2 of PVATs and GON, as highly proliferative and adipogenic control site, supplemented with DMEM:F12 media (10%FBS). At 11 days in culture, the number of SVF/cm^2 was significantly lower in aPVAT ($7.04x10^5$ cells/cm^2) vs. mPVAT and GON ($13.75 x10^5$ and $12.62 x10^5$ cells/cm^2; $P<0.05$). These data demonstrate a site dependent proliferation capacity of SVF cells from PVATs that may be explained in part by differences in the cellular distribution of adipocyte progenitors.

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P644

Mineralocorticoid receptor activation may contribute to supine hypertension in patients with primary autonomic failure

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Primary autonomic failure is characterized by disabling orthostatic hypotension; but at least half of these patients have paradoxical supine hypertension. Renin-angiotensin (Ang)

mechanisms were not initially thought to contribute to this hypertension as autonomic failure patients often have undetectable plasma renin activity. Despite suppressed renin, plasma aldosterone levels are normal and we recently showed that plasma Ang II is elevated and acts at AT_1 receptors to contribute to supine hypertension in these patients. Since aldosterone and Ang II also bind mineralocorticoid receptors (MR) to elevate blood pressure, we tested the hypothesis that MR activation plays a role in the hypertension of autonomic failure. To test this, we determined the acute effects of the MR antagonist eplerenone (50 mg, PO) versus placebo on supine blood pressure in a randomized, double blind, crossover study. Medications were given at 8:00 PM and blood pressure was recorded q2 hours for 12 hours. Seven autonomic failure patients with supine hypertension completed this study (5 pure autonomic failure, 1 Parkinson's, 1 multiple system atrophy; 5 male; 68±2 years of age). Eplerenone maximally reduced supine systolic blood pressure by 34±6 mmHg at 8 hours after administration (vs. 6±12 mmHg for placebo, p=0.047), with no effect on volume measures (12-hour urine volume: 927±205 placebo vs. 1247±151 ml eplerenone, p=0.438; nocturnal weight loss: -1.1±0.2 placebo vs. -1.2±0.2 kg eplerenone, p=0.813). These findings suggest that inappropriate MR activation may contribute to hypertension in autonomic failure, and provide rationale for use of eplerenone in treatment of these patients. While still under investigation, the lack of effect on volume measures combined with the rapid time course for blood pressure lowering following eplerenone may suggest extra-renal mechanisms are involved in MR antagonism in autonomic failure.

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P645

Chronic Angiotensin-(1-7) Improves Whole-Body Insulin Sensitivity in High-Fat Fed Mice by Enhancing Skeletal Muscle Glucose Uptake

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Angiotensin (Ang)-(1-7) is a vasodilatory peptide implicated in the pathophysiology of hypertension, in part by opposing deleterious Ang II cardiovascular actions. Recent studies show that Ang-(1-7) restoration lowers blood pressure and improves glycemic control in animal models of cardiometabolic syndrome. The tissue-specific sites of action and blood pressure dependence for these metabolic effects, however, remain unclear. We hypothesized that Ang-(1-7) improves insulin sensitivity by enhancing peripheral glucose delivery. To test this hypothesis, adult male C57BL/6 mice were placed on standard chow or 60% high-fat diet for 11 weeks, with Ang-(1-7) [400 ng/kg/min] or saline given during the last 3 weeks of diet by subcutaneous osmotic mini-pump. Hyperinsulinemic (4 mU/kg/min) euglycemic clamps were performed in conscious, unrestrained mice at the end of the treatment period. High-fat fed mice exhibited

modest hypertension (systolic blood pressure: 137±3 high-fat vs. 123±5 mmHg chow; p=0.043), which was not altered by Ang-(1-7) infusion (141±4 mmHg; p=0.516). Body weight, body composition, and fasting plasma glucose and insulin levels were not significantly different following Ang-(1-7) treatment in chow or high-fat fed mice. Ang-(1-7) increased the glucose infusion rate (GIR) needed to maintain euglycemia in high-fat fed mice (steady-state GIR: 31±5 Ang-(1-7) vs. 16±1 mg/kg/min vehicle; p=0.017) indicating enhanced whole-body insulin sensitivity, with no significant effect in chow fed mice. The improvement in insulin sensitivity in high-fat fed mice was due to an enhanced rate of whole-body glucose disappearance (R_d: 34±5 Ang-(1-7) vs. 20±2 mg/kg/min vehicle; p=0.049), with increased rates of glucose uptake in gastrocnemius, vastus, and soleus muscle. There was no effect of Ang-(1-7) on insulin-mediated suppression of hepatic glucose production. Our data shows that Ang-(1-7) has direct insulin-sensitizing effects on skeletal muscle, which are independent of changes in body weight or systemic blood pressure. These overall findings provide new insight into mechanisms by which Ang-(1-7) improves insulin action, and provide further support to targeting this peptide for treatment of cardiometabolic disease.

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P646

Vascular AT1 Angiotensin Receptors Regulate Sodium Transporter Abundance in Kidney Epithelium

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Vasoconstriction is a signature physiological action of angiotensin II (AngII) acting via AT1 receptors (AT1R). In order to define the contribution of AT1R in vascular smooth muscle cells (VSMCs) to BP control, we generated mice with cell-specific deletion of AT1AR from VSMCs (SMKOs) using Cre-loxp technology. Baseline BP was reduced by ~7 mmHg and responses to AngII-induced hypertension were significantly blunted by in SMKO mice compared to controls (16 vs. 30 mm Hg change in BP from baseline after 4 wks AngII, P<0.02). Baseline renal blood flow (RBF) was higher, and renal vasoconstriction after Ang II was impaired in SMKOs. Moreover, SMKO mice displayed Na+ sensitivity and exaggerated natriuresis during chronic AngII infusion. To investigate the mechanism of the lower baseline BP and the enhanced natriuresis during AngII infusion (1000ng/kg/min for 5 days), we measured a panel of key Na+ transporters in the kidney by immunoblot. Baseline measurements in SMKO vs. controls detected reductions in NKCC2 in both cortex (0.8±0.03 vs. 1±0.03; P=0.0002) and medulla (0.6±0.02 vs. 1±0.05; P<0.0001); medullary NHE3 was similarly reduced (0.6±0.07 vs. 1±0.07; P=0.002). In controls, AngII infusion was associated with reduced levels of cortical and medullary NHE3 and medullary NKCC, consistent with the pressure-natriuresis response, whereas cortical NKCC, NCC and ENaC were all significantly activated. By contrast, in SMKOs, there was no AngII infusion dependent depression in cortical or medullary NHE3, nor medullary NKCC. However, the extent of increase in activated (cleaved) αENaC was significantly less than controls (cortex:

1.46±0.16 vs. 2.58±0.17, P=0.002; medulla: 1.49±0.09 vs. 2.22±0.31, P=0.01). Yet, 24 hr urinary aldosterone excretion was not different between the groups (18.6±2.7 vs. 15.8±4.5 ng/24hrs). Our studies indicate that the lower baseline BP in SMKO mice is associated with reduced Na+ transporter abundance along the loop of Henle, and that attenuated hypertension and improved natriuresis during AngII infusion are associated with diminished ENaC activation. In conclusion, we suggest that vascular-epithelial cross-talk modulates renal Na+ handling and thereby contributes to control of BP at baseline and during hypertension.

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