Hypertension 2017 Scientific Sessions Abstracts

001

Sympathetic Innervation Promotes Bone Marrow Homing of Hypertension-specific $CD8^+$ Effector Memory T Cells

PrimaryAuthor.AuthorBlock:**Liang Xiao**, Hana A Itani, Jason D Foss, David G Harrison, Vanderbilt Univ Medical Ctr, Nashville, TN

We have recently identified a critical role of hypertension-specific effector memory T lymphocytes (T_{EM} cells) in the blood pressure elevation and renal dysfunction caused by repeated hypertensive stimuli. Formed during an initial immune challenge, T_{EM} cells reside in the bone marrow (BM) in a quiescent state for prolonged periods, and can be reactivated upon re-exposure to the hypertensive stimulus. Hypertension is associated with increased sympathetic outflow. We therefore hypothesized that sympathetic nerves regulate accumulation and reactivation of hypertension-specific T_{EM} cells in the BM. We performed unilateral superior cervical ganglionectomy (SCGx) in wild-type C57BL/6 mice, causing selectively sympathectomy of the forelimb on the surgical side. After recovery, mice received Ang II infusion for two weeks. To determine the changes of T cells in the BM that were specific to hypertension, 5×10^6 BM cells were isolated from either the SCGx or control limbs, loaded with proliferation marker CFSE, and co-cultured with 0.5×10^6 splenic dendritic cells isolated from another Ang II-infused mouse. We found 30% less $CD8^+$ T cell proliferation in the SCGx BM compared to control side (1.8 ± 0.1 vs. $2.6\pm0.3\times10^4$), but no difference in $CD4^+$ T cells. To further study the effect of sympathetic nerves on BM T cell homing, 1×10^7 pan T cells were isolated from $CD45.2^+$ wild-type mice after Ang II infusion and adoptively transferred to CD45.1 mice that had previously undergone unilateral SCGx. Flow cytometry indicated that 7 days after transfer, 25% fewer $CD8^+$ T_{EM} cells from the hypertensive donors homed to the SCGx BM compared to the innervated BM (27.8 ± 2.6 vs. 20.8 ± 2.5 per 10^3 total BM cells). This effect was specific for hypertension as homing of OT-I T_{EM} cells, which are responsive to ovalbumin, was not influenced by sympathectomy. Further adoptive transfer studies using mice lacking beta 2 adrenergic receptors (β2AR) indicate that β2AR in the bone marrow niche, rather than T cell β2AR is critical for T_{EM} cell homing. These data define a novel role of sympathetic nerves in regulation of memory T cell trafficking, and this likely contributes to the predisposition to hypertension and end-organ damage for prolonged periods following an initial episode of hypertension.

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002

Neuropilin1: New Splenic Mechanisms Mediating the Guidance of Neural Cues in Immune Cells Relevant for Hypertension

PrimaryAuthor.AuthorBlock:**Daniela Carnevale**, Sapienza Univ/IRCCS Neuromed, Pozzilli, Italy;

Marialuisa Perrotta, Maria Piacenti, Roberta Iacobucci, Giuseppe Cifelli, IRCCS Neuromed, Pozzilli, Italy; Giuseppe Lembo, Sapienza Univ/IRCCS Neuromed, Pozzilli, Italy

Investigation of immune mechanisms involved in hypertension (HTN) is one of the most emerging research area in the field. On this note, we found that hypertensive hits activate nervous system in splenic marginal zone (MZ) that represents the boundary between lymphoid white pulp and innate red pulp. The fact that both innate and adaptive immunity contribute to HTN strongly suggests that neuroimmune mechanisms couple myeloid and lymphoid cells in transducing hypertensive hits into increased blood pressure (BP). We previously showed that Placental Growth Factor (PlGF), a VEGF homolog, released in the splenic MZ upon Angiotensin II (AngII), mediates this interaction. Here we dissect the downstream pathway conveying nervous outflow into immune activation. PlGF is expected to act through its major receptor VEGFR1, its only known tyrosine kinase receptor. First we found that it is mainly expressed in splenic innate immune cells and vasculature, both in basal conditions and upon AngII. To test VEGFR1 in HTN, we challenged transgenic mice with defective VEGFR1 signaling (Flt1-TK) with AngII. To our surprise, they raised BP upon 28-days AngII as WT mice did (SBP: Flt1-TK 139±2; WT 138±2; ***p< 0.001), thus suggesting the existence of alternative pathways. PlGF may also act on a co-receptor, Neuropilin1 (Nrp1), known to enhance responses to ligands. In addition, Nrp1 has unique functions in neurons' guidance during development and in the maturation of dendritic cells from monocytes. We found Nrp1 basally expressed and strongly increased upon AngII, especially in MZ macrophages. To test the hypothesis that PlGF/Nrp1 pathway could mediate a neuroimmune activation of dendritic cells relevant for HTN, we selectively deleted the receptor in myeloid lineage, generating $Nrp1^{fl;fl}$;LysM-Cre transgenic mice. After assessing Nrp1deletion in splenic monocyte/macrophages by FACS, we infused AngII and found a protection from T cells costimulation and egression from the spleen. More important, they were protected from HTN (SBP: $Nrp1^{fl;fl}$;LysM-$Cre^{+/-}$ 140±3; $Nrp1^{fl;fl}$;LysM-$Cre^{-/-}$ 105±2; ***p< 0.001). Our data indicate a crucial role of Nrp1 in HTN, mediating non-canonical PlGF signaling in innate immunity that serves as a guidance of nerves priming adaptive immune responses.

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003

Role of Transient Receptor Potential Ankyrin 1 in Monocyte Activation and Hypertensive Kidney Injury

PrimaryAuthor.AuthorBlock:**Shuangtao Ma**, Michigan State Univ, East Lansing, MI; Kecheng He, Shujie Wei, Chengdu Military General Hosp, Chengdu, China; Peijian Wang, Chengdu Medical Coll, Chengdu, China; Yongjian Yang, Chengdu Military General Hosp, Chengdu, China; Donna H. Wang, Michigan State Univ, East Lansing, MI

It has been shown that monocyte activation and infiltration occur during the development of hypertensive kidney disease, a condition newly recognized as an inflammatory disorder. Indeed, monocytosis is linked to chronic kidney disease. Transient receptor potential ankyrin 1 (TRPA1), a Ca^{2+}-permeable non-selective cation channel, has been shown to be an oxidative

stress sensor that regulates neurogenic inflammation due to its abundant expression in sensory nerves. With the use of qPCR and immunostaining, we identified that the TRPA1 is also expressed in human and murine monocytes. The gene expression of TRPA1 is decreased in peripheral monocytes in hypertensive patients compared to normotensive subjects (-6-fold, $p<0.05$) and in kidney tissues in angiotensin II (1000ng/kg/min, s.c., 14 days)-induced hypertensive compared to control mice (-9-fold, $p<0.01$). The differentiation of human monocytic leukemia cells (THP-1) into macrophages induced by phorbol 12-myristate 13-acetate (PMA, 100nM, 48h) was accompanied with decreases in TRPA1 expression (-8-fold, $p<0.05$) and increases in pro-inflammatory cytokines, including interleukin (IL)-1β (+705-fold, $p<0.01$), monocyte chemotactic protein (MCP)-1 (+16-fold, $p<0.01$), and CD36 (+7-fold, $p<0.01$). Treatment with supercinnamaldehyde (SCA), a TRPA1 agonist, decreased the expression of IL-1β (-12-fold, $p<0.01$), MCP-1 (-3.5-fold, $p<0.01$), and CD36 (-2-fold, $p<0.05$) in THP-1-derived macrophages, whereas these effects were abolished by pre-treatment with the selective TRPA1 antagonist, HC-030031 (HC). Additionally, HC promoted the proliferation of THP-1 cells while SCA suppressed THP-1 cell growth and variability. SCA induced the apoptosis of THP-1 cells (Annexin V positive cells: 8.5±1.4% *vs.* 30.1±2.4%, $p<0.01$), which can be blocked by HC (30.1±2.4% *vs.* 15.6±1.9%, $p<0.01$). These results show that TRPA1 activation inhibits monocyte activation and differentiation, and that suppressed expression of TRPA1 occurs in monocytes and kidneys in hypertensive patients and animals, respectively. These findings indicate that impaired expression and function of TRPA1 may contribute to monocytosis and pro-inflammatory state during hypertension, leading to hypertension related kidney injury.

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004

Plasma Zonulin, Along With a Unique Gut Microbiome Profile, Are Potential Predictors of Systolic Blood Pressure in Humans

PrimaryAuthor.AuthorBlock:**Seungbum Kim**, Ruby Goel, Yanfei Qi, Elaine M Richards, Mohammed Mohammed, Eileen M Handberg, Carl J Pepine, Mohan K Raizada, Univ of Florida, Gainesville, FL

Objectives: We have previously shown that gut pathophysiology and dysbiosis are closely associated with hypertension (HTN) in animal models. However, whether this association also occurs in human HTN is unknown. To address this knowledge gap, we tested two hypotheses. 1. Hypertensive patients (HTN) have gut barrier dysfunction. 2. Prediction of blood pressure will be possible from circulating markers of gut health and gut microbiome composition. **Design and Method:** Plasma and fecal samples were collected from HTN (n=22, mean SBP 155.8±3.4mmHg) and reference subjects without HTN (REF) (n=18, mean SBP 121.1±1.5mmHg) (see ClinicalTrials.gov, NCT02188381 for protocol). Gut microbiomes were analyzed using shotgun metagenomic sequencing and Qiime. Plasma analytes were measured by ELISA. **Results:** Plasma intestinal fatty acid binding protein (REF;1.2 ± 0.1 ng/ml, HTN;1.9 ± 0.2 ng/ml, $p=0.0097$) and lipopolysaccharide (REF; 39.0 ± 9.5 pg/ml, HTN; 98.0 ± 26.2 pg/ml, $p=0.0423$) were increased in HTN, suggesting increased intestinal inflammation and permeability. Additionally,

the soluble form of zonulin (regulator of gut tight junction proteins) was markedly elevated in the plasma of HTN (REF; 28.4 ± 2.0 ng/ml, HTN; 42.6 ± 2.7 ng/ml, P=0.0002) further supporting gut barrier dysfunction. Plasma zonulin was correlated with SBP (R^2=0.5301, p<0.0001). Two models predicting SBP were built using stepwise linear regression analysis of microbiome shotgun metagenomics data and circulating markers of gut health. The first model used plasma zonulin as a single predictor, and the second zonulin plus butyrate producing bacteria (adjusted R^2 values of 0.506 and 0.554 respectively, p<0.001 for both). Our first model was validated by prediction of SBP in a separate validation cohort (n=36) from zonulin plasma levels (R^2=0.4608, p<0.0001). **Conclusions:** Markers of increased gut permeability, particularly zonulin, and abundance of butyrate producing bacteria predicted SBP. These results support the hypothesis that gut barrier dysfunction and gut microbiome composition are directly linked with HTN in humans.

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005

Loss of Interleukin 21 Protects Against Hypertension and Associated Inflammation and End Organ Damage

PrimaryAuthor.AuthorBlock:**Bethany L Dale**, Vanderbilt Univ, Dept of Molecular Physiology and Biophysics, Nashville, TN; Arvind Pandey, Fanny Laroumanie, Hana Itani, Liang Xiao, Vanderbilt Univ, Div of Clinical Pharmacology, Dept of Med, Nashville, TN; Allison Norlander, Vanderbilt Univ, Dept of Molecular Physiology and Biophysics, Nashville, TN; Jason Foss, Vanderbilt Univ, Div of Clinical Pharmacology, Dept of Med, Nashville, TN; Holly Algood, Vanderbilt Univ, Div of Infectious Diseases, Dept of Med, Nashville, TN; Meena Madhur, Vanderbilt Univ, Div of Clinical Pharmacology, Dept of Med, Nashville, TN

T cell derived cytokines such as interferon gamma (IFNγ) and interleukin 17A (IL17A) are upregulated by and can promote angiotensin II-induced hypertension and end-organ damage. Interleukin 21 (IL21) is produced primarily by T follicular helper (Tfh) cells and has the potential to promote IL17A and IFNγ production from T effector cells while inhibiting T regulatory cells. IL21 is also a potent activator of germinal center (GC) B cells. *Thus, we hypothesize that IL21 promotes hypertension and hypertensive end-organ damage through its effect on T cell polarization and GC B cell activation.* The data indicate that indeed $IL21^{-/-}$ mice exhibit a 30 mmHg reduction in blood pressure in response to 4 weeks of angiotensin II (Ang II) infusion compared to age-matched wild type (WT) mice (p=0.0275). IL21 mRNA expression increases 1.5 fold in splenic T cells in response to Ang II infusion (p=0.015). Moreover, Tfh cells and GC B cells are increased in the aortas of WT mice after Ang II infusion (p=0.0136 and p=0.0067, respectively). Further, IL17A production from splenic CD4+ T cells and IFNγ production from splenic CD8+ T cells was reduced in $IL21^{-/-}$ mice compared to WT mice (p=0.0025 and p=0.0237, respectively) following Ang II infusion. Renal function was assessed by measuring albuminuria. WT mice developed 2-fold more albuminuria compared to $IL21^{-/-}$ mice in response to Ang II infusion (p=0.0349). Lastly, Ang II infusion impaired endothelium-dependent relaxation to acetylcholine in mesenteric vessels from WT mice (52.9% vs 17.5%; p=<0.0001). $IL21^{-/-}$ mice demonstrated moderately impaired endothelium-dependent

relaxation at baseline (34.9%) but interestingly, there was no further impairment in these vessels following Ang II infusion. Taken together, these studies suggest that IL21 and the Tfh cell–GC B cell axis may play a key role in hypertension and hypertensive end-organ damage.

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006

Hypertension is Accompanied With Over Activity of the Adaptive Immune System

PrimaryAuthor.AuthorBlock:**Tuantuan Zhao**, Xiao Z Shen, Zhejiang Univ, Hangzhou, Zhejiang, China; Kenneth E Bernstein, Cedars-Sinai Medical Ctr, Los Angeles, CA

Adaptive immune response plays an important role in the pathogenesis of hypertension, but how does hypertension influence on the immune system is still poorly understood. To understand this, we studied antigen presenting cells (APCs) isolated from normotensive and experimental hypertensive mice induced by angiotensin II (490 ng/kg/min, 2 weeks). The APCs were loaded with ovalbumin(OVA) or its MHC class I epitope SIINFEKL (SKL) for 3h. Then, splenocytes from OT-I mice, containing OVA-specific, $CD8^+$ T cells (OT-I cells), were added for 4h. Significantly more activated T cells expressing surface marker CD69 and cytokine IFN-γ were detected by flow cytometry when stimulated with APCs from hypertensive mice than equivalent cells from normotensive mice ($P<0.05$). Thus, *in vitro,* APCs derived from hypertensive mice more effectively present antigen to T cells. To study antigen presentation *in vivo*, we immunized AngII and sham treated mice with OVA and adjuvant. Consistently more OVA-specific $CD8^+$ T cells were induced in the blood (2.7 ± 0.23% vs. 1.2 ± 0.47%, $P<0.05$) and the spleen (1.51 ± 0.17% vs. 2.62 ± 0.31%, $P<0.05$) of AngII mice vs sham when measured by flow using an H-$2k^b$-SKL tetramer. We also evaluated the APC and T cell activation signals before and after hypertension. Flow analysis showed that splenic DCs divided from AngII treated mice resulted in a substantially increased surface expression of co-stimulatory factor CD86 compare with DCs from normotensive controls (MFI: 131±7.8 vs. 97±5.3). RIP-mOVA mice were used to study if hypertension affects APC cross-presentation of self-antigens. This transgenic mouse line expresses membrane-bound OVA in pancreatic islet β cells. When OT-I cells ($5x10^6$) are injected into RIP-mOVA mice, they activate by cross-presentation of OVA and cause insulitis and diabetes. RIP-mOVA mice were made hypertensive with either AngII or L-NAME (1.5 mg/ml, 4 weeks). When OT-I cells were *i.v.* infused into RIP-mOVA mice, the hypertensive mice had more OT-I cells infiltration into the pancreas (AngII 75%, L-NAME 73%, control 13%) and developed severer diabetes (Blood glucose: AngII 331, L-NAME 315, control 168 mg/dl). In conclusion, high blood pressure itself is associated with over activity of the adaptive immune system.

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007

Obstructive Sleep Apnea Induced Hypertension Involves Gut Dysbiosis and Neuroinflammation

PrimaryAuthor.AuthorBlock:**David J. Durgan**, Bhanu P. Ganesh, James W. Nelson, Robert M. Bryan, Baylor Coll of Med, Houston, TX

Obstructive sleep apnea (OSA) is an independent risk factor for systemic hypertension, and the most common underlying cause of resistant hypertension. The importance of a healthy gut microbiota on host physiology is becoming increasingly evident. We have shown that gut dysbiosis plays a causal role in the development of OSA-induced hypertension. The mechanisms linking gut dysbiosis to hypertension are unknown. We tested the hypothesis that OSA-induced dysbiosis leads to gut barrier dysfunction, systemic inflammation, and neuroinflammation, which is linked to hypertension. We exposed rats to 2 weeks of sham or OSA (60 apneas/hr). OSA led to a >100-fold increase in TNFα expression in the cecum wall (n=5, $p<0.001$), and decreased goblet cells/crypt (8.4 vs 11.4; n=6, $p<0.05$). Consistent with gut barrier dysfunction and bacterial translocation, we found bacterial 16S rRNA in adipose tissue, as well as a 4-fold increase in adipose IL-6 mRNA expression following OSA (n=4-7, $p<0.05$). Flow cytometric analysis revealed a decrease in the percentage of T-reg cells in the brain of OSA vs. sham rats (0.08% vs. 0.25%; n=3, $p<0.05$). In addition, the percentage of activated microglia was increased following OSA (20% vs. 10%; n=3, $p<0.05$). Next, we treated sham and OSA rats with a prebiotic (20% resistant starch diet) or probiotic (*C. butyricum*; 10^9 CFU gavage every three days) to increase short chain fatty acids, important in maintaining gut barrier integrity and regulating immune responses. Pre- and probiotic prevented OSA-induced loss of goblet cells and TNFα expression in the cecum. Compared to control rats, pre- and probiotic increased the percentage of T-reg cells in the brain of OSA rats by 10- and 5-fold, respectively (n=3-6, $p<0.05$ for each). Additionally, pre- and probiotic prevented OSA-induced activation of microglia (n=3-6). Importantly, pre- and probiotic prevented OSA-induced hypertension (prebiotic sham=158.5 vs. OSA=160.3 mmHg, probiotic sham=147.4 vs. OSA=145.6 mmHg; n=6-7, NS). These data demonstrate a causal role for gut dysbiosis in the development of hypertension that involves gut barrier disruption, bacterial translocation, and neuroinflammation. Manipulation of the gut microbiota may serve as a novel therapy in the prevention of hypertension.

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008

Leptin Treatment Attenuates Vascular Dysfunction and Inflammation in Mouse Model(s) of Acquired Lipodystrophy via Reducing Nox1-derived Ros

PrimaryAuthor.AuthorBlock:**Thiago Bruder-Nascimento**, Weiqin Chen, David Stepp, Eric J Belin de Chantemèle, Augusta Univ, Augusta, GA

Although extremely efficient at suppressing HIV replication, highly active antiretroviral therapy (HAART) induces lipodystrophy, a metabolic disorder characterized by an abnormal adipose tissue distribution, reduced leptin levels and vascular dysfunction. Leptin replacement therapy (LRT) is currently used to improve metabolic function in patients suffering from

congenital lipodystrophy. Here, we analyzed whether LRT restores vascular function and inflammation in mice treated with the antiretroviral agent, ritonavir (Rit). Four weeks of Rit reduced body weight [control (C): 28.4±0.5 vs. Rit: 24.4±0.2g*, *P<0.05] and fat mass (C: 10±1 vs. Rit: 6.5±1 %*) confirming that it induces lipodystrophy. Rit impaired aortic endothelial function [Relaxation to acetylcholine: C: 74±4 vs. Rit: 21±15%*), increased ROS producing enzymes (NOX1 and NOXA1), induced vascular inflammation (increased IL-1β, MCP-1, GATA Binding Protein 3 and INF-γ gene expression) and increased TBARS levels. ROS scavenging via tempol or GKT137117, (Nox1/4 inhibitor) pre-incubation blunted endothelial dysfunction. LRT (10µg/day/7 days, osmotic mini-pump), at the end of the 3-week Rit, restored endothelial function, reduced Nox1 and NOXA1 gene expression and vascular inflammation. NOX1 deficiency in Nox1 KO mice protected mice from Rit-induced endothelial dysfunction and vascular inflammation. Increasing endothelial leptin sensitivity via specific deletion of protein tyrosine phosphatase 1B (Ptp1b) in endothelial cells (*Ptp1b-/-EC* mice) protected mice from Rit-induced endothelial dysfunction and reduced Nox1 and NOXA1 gene expression, and vascular inflammation. To address the relevance of these observations to other forms of acquired lipodystrophy, experiments were repeated in mice in which lipodystrophy was induced at 8 week of age, by the deletion of Bscl2, a gene involved in adipocyte maturation. Bscl2 deletion reduced fat mass, and induced endothelial dysfunction via ROS-mediated mechanisms. Again, LRT reverted endothelial dysfunction by downregulating Nox1 expression. All together, these data presents leptin as a key regulator of endothelial oxidative stress level and as a potential avenue for the treatment vascular disease.

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009

Mitochondria-targeting Overexpression of AT_2 Receptors Inhibits Intracellular Angiotensin II-induced Respiratory and Glycolytic Stress Responses in Mouse Proximal Tubule Cells

PrimaryAuthor.AuthorBlock:**Xiao C Li**, Jia L Zhuo, Univ of Mississippi Medical Ctr, Jackson, MS

Angiotensin II (ANG II) plays an important role in mitochondrial dysfunction associated with cardiovascular, hypertensive and kidney diseases, but it is not known whether extracellular or intracellular ANG II mediates this effect via activation of cell surface or mitochondrial receptors. As a proof of concept study, we overexpressed an intracellular cyan fluorescent ANG II fusion protein, mito-ANG II, with or without a GFP-tagged full length AT_2 receptor, mito-AT_2R, selectively in the mitochondria of mouse proximal tubule (mPCT) cells. The mitochondrial respiratory and glycolytic stress responses were measured using Seahorse XF Cell Mito and XF Glycolysis Stress Test Kits, respectively. Live cell fluorescent imaging confirmed the expression and colocalization of mito-ANG II and mito-AT_2R with a mitochondrial marker MitoTracker®. Overexpression of mito-ANG II for 48 h significantly increased mitochondrial oxygen consumption rate (OCR) by 30% (Control: 239.4 ± 9.2 vs. mito-ANG II: 310.4 ± 12.6 pmol/min; $p<0.01$, n=5) and extracellular acidification rate (ECAR) by 33% (Control: 6.3 ± 0.3 vs. mito-ANG

II: 8.1 ± 0.5 mpH/min; $p<0.01$, n=5). The effects of mito-ANG II on OCR and ECAR responses were associated with significant increases in phosphorylated MAP kinase ERK1/2, Na^+/K^+-ATPase, and mitochondrial redox carries, Complex I (NADH coenzyme Q reductase), Complex II (succinate dehydrogenase), Complex III (cytochrome bc_1 complex) and Complex IV (cytochrome c oxidase) ($p<0.01$, n=6). The mito-ANG II-induced OCR and ECAR responses were blocked by the AT_1 blocker losartan (10 μM, $p<0.01$, n=5), but not by the AT_2 receptor blocker PD123319 (10 μM, *n.s.*, n=5). However, concurrent overexpression of mito-AT_2R with mito-ANG II in the mitochondria of mPCT cells significantly attenuated the effects of mito-ANG II on OCR and ECAR responses ($p<0.01$, n=6), while the effects of mito-AT_2R overexpression were completely blocked by PD123319 ($p<0.01$, n=5). Taken together, our results provide strong evidence that activation of mitochondrial AT_1 receptors by intracellular ANG II stimulates, whereas activation of mitochondrial AT_2 receptors by intracellular ANG II inhibits, mitochondrial respiratory and glycolytic responses in mouse proximal tubule cells.

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010

Renal Proximal Tubule-derived Angiotensin Converting Enzyme 2 (ACE2) in Blood Pressure Regulation

PrimaryAuthor.AuthorBlock:**Zhidan Xiang**, Katharine Bendt, Robert Griffiths, Duke Univ Sch of Med, Durham, NC; Jorge F Giani, Cedars Sinai Medical Ctr, Los Angeles, CA; David I Ortiz Melo, Susan B Gurley, Duke Univ Sch of Med, Durham, NC

Angiotensin Converting Enzyme 2 (ACE2) is a carboxypeptidase that metabolizes angiotensin II (AngII) to Ang1-7, playing a critical role in blood pressure (BP) regulation. Previous studies from our lab demonstrated that loss of ACE2 resulted in enhanced susceptibility to AngII hypertension and this was associated with elevated renal AngII levels. ACE2 is highly expressed in kidney, particularly in proximal tubule epithelia, and kidney cross-transplant experiments performed by our group revealed a novel mechanism by which ACE2 may be functioning to regulate BP. However, the exact cellular sources and sites of action where ACE2 regulates BP remain unclear. The objective of this study is to assess proximal tubule-derived ACE2 in BP regulation by studying a new mouse model generated in our laboratory: proximal tubule-specific ACE2 knock-out mice (referred to as ACE2 PTKO mice). ACE2 PTKO mice were generated by crossing the *Ace2*$^{-flox/flox}$ conditional mouse line with the phosphoenolpyruvate carboxykinase (PEPCK)-Cre mouse (V. Haase, Vanderbilt). ACE2 PTKO mice and their littermate control mice were inbred 129/SvEv, co-caged, and only males were studied. ACE2 PTKO mice exhibited reduced ACE2 mRNA and protein in renal cortex compared to controls (*Ace2* mRNA relative expression: 0.47 ± 0.1 vs. 1.08 ± 0.1, N=3 vs 3, $P<0.05$; relative ACE2 protein expression: 0.38 ± 0.04 vs. 1.15 ± 0.12, N=3 vs 3, $P<0.01$). Measured with radiotelemetry, there were no differences in BPs between ACE2 PTKO mice and control mice (119 ± 1 vs 117 ± 2 mmHg, N=16 vs 16, p=NS) at baseline. However, ACE2 PTKO mice exhibited an enhanced hypertensive response during the first week of AngII infusion compared to control mice (139.9 ± 4.8 vs 152.8 ± 3.7 mmHg at day 7, N=16 vs 16, $P=0.038$). Furthermore, the ACE2 KO kidneys accumulated significantly higher renal AngII than control kidneys, measured at day4 (897±104 vs 536±115 fmol/g, $P<0.05$). Our data demonstrate that proximal tubule-derived ACE2 plays a

critical role in BP regulation during AngII infusion. The enhanced hypertensive response in ACE2KO mice is consistent with their intra-renal AngII levels, suggesting that that proximal tubule-derived ACE2 regulates BP via metabolizing AngII within the kidney.

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011

ACE2 Gene Transfer Ameliorates Dysfunctions in Hematopoietic Stem/Progenitor Cells of Diabetic Patients

PrimaryAuthor.AuthorBlock:**Yagna P Jarajapu**, Shrinidh Joshi, NDSU, Fargo, ND; Julio C Quiroz-Olvera, Miguel Duran-Mendez, Wendy Cantu-Delgado, Samuel C Gomez, Museum District Eye Ctr, Houston, TX; Stephen H Bartelmez, BetaStem Therapeutics, Inc., Sanfrancisco, CA; Mohan K Raizada, Univ of Florida, Gainesville, FL; Charles Garcia, Univ of Texas Health Science Ctr, Houston, TX

Hematopoietic stem/progenitor cells (HS/PCs) have the propensity of ischemic vascular repair, and this innate vasoprotective function is impaired in diabetes. Angiotensin-converting enzyme (ACE) and ACE-2 are primary enzymes of the cardiovascular-detrimental and protective axes of the renin-angiotensin system, respectively. In this study, we tested the hypothesis that diabetic dysfunction in HS/PCs is due to ACE2/ACE-imbalance, and that increasing ACE2 expression will restore the vasoreparative potential. Lineage- (Lin^-) or $CD34^+$ cells were isolated from the peripheral blood mononuclear cells (MNCs) of nondiabetic subjects (ND), and type 1 or type 2 diabetic (DB) patients (male or female, age 48-76 years, HbA1C 6.5-11.2). ACE and ACE2 activities were measured in lysates of MNCs, Lin^- and $CD34^+$ cells by enzyme-selective fluorogenic substrates and inhibitors. Lentiviral ACE2 (LV-ACE2) gene transfer was carried out by spinoculation. Reparative function of Lin^- cells was evaluated in $Foxn1^{nu}$ mice undergoing hind limb ischemia (HLI). Lin^- cells with or without ACE2-overexpression were administered (i.m) in the peri-ischemic region, and the blood flow recovery was monitored. Circulating Lin^- or $CD34^+$ cells are lower in DB (Lin^- ($(2\pm0.2)\times10^5$, $P<0.04$) and $CD34^+$ ($(0.6\pm0.1)\times10^3$, $P<0.02$), n=8) compared to ND group (Lin^- $(25\pm4)\times10^5$; $CD34^+$ $(2\pm0.4)\times10^3$ per 10^6 MNCs, n=8). This was associated with decrease in ACE2 and increase in ACE activity, resulting in 4.5-fold decrease in ACE2/ACE ratio in DB-$CD34^+$ cells (0.4±0.07 vs ND 1.8±0.2, $P<0.01$, n=6). This was negatively correlated with HbA1C ($r^2=0.89$, $P<0.01$). Administration of ND-Lin^- cells has no effect on the blood flow recovery of mice following HLI. In contrast, administration of DB-Lin^- cells decreased this recovery (48±6%, n=5, $P<0.001$ vs ND-Lin^- 105±7%). However, treatment with ACE2-overexpressing DB-Lin^- cells robustly enhanced blood flow recovery (112±8%, n=4, $P<0.001$ vs DB). These observations suggest that ACE2/ACE imbalance is correlated with diabetic vasoreparative dysfunction in HS/PCs, and that increasing ACE2 expression reverses the dysfunction. ACE2 gene transfer is a promising approach for enhancing vascularization outcomes of cell-based therapies in diabetic individuals.

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012

Vascular Smooth Muscle ADAM17 Contributes to Angiotensin II-induced Abdominal Aortic Aneurysm Formation but Not Hypertension in Mice

PrimaryAuthor.AuthorBlock:**Tatsuo Kawai**, Takehiko Takayanagi, Kyle J Preston, Steven J Forrester, Rosario Scalia, Victor Rizzo, Satoru Eguchi, Temple Univ Sch of Med, Philadelphia, PA

Over activation of the renin angiotensin II (AngII) system has been implicated in abdominal aortic aneurysm (AAA). However, the precise molecular mechanism(s) by which AngII promotes AAA remain insufficiently understood, which hinders pharmacological treatment. At a past AHA conference, we reported critical roles for the metalloprotease, ADAM17, and downstream EGFR transactivation, in the development of AngII-induced AAA in mice treated with a lysyl oxidase inhibitor beta-aminopropionitrile (BAPN). Specifically, we demonstrated that in the mouse AngII-induced AAA was attenuated by either deletion of ADAM17 in VSMC or treatment with an EGFR inhibitor erlotinib. To better understand the clinical relevance of these findings, we studied the effect of an ADAM17 inhibitory antibody (A9B8), which recognizes both human and mouse ADAM17. Eight-week-old male C57BL6 mice were given AngII 1000 ng/kg/min via osmotic minipumps for 4 consecutive weeks and BAPN 150 mg/kg/day in drinking water for the initial 2 weeks. Additionally, mice were given 10 mg/kg/day A9B8 or control human IgG2 i.p. injection at day 1, 7, 14 and 21. As an index for AAA formation, abdominal aortic internal diameter was measured by ultrasound at day 0, 14, 21 and 28. Aortic external diameter was also measurement at day 28. AngII/BAPN treated mice given control IgG2 have a mortality rate of 46.2% due to aortic rupture/dissection. Mortality rate in mice given A9B8 was reduced to 23.1% (n=13). Surviving IgG2 treated mice developed AAA with max external/internal diameter (mm) of 2.55±0.43/1.79±0.34 (age matched control mice values were 1.10±0.13/1.01±0.09; $p<0.01$; n=6). Following A9B8 treatment, aortic maximal external/internal diameter (mm) were reduced to 1.84±0.32/1.45±0.32 ($p<0.05$ vs IgG2; n=6). Pathologically, less extravascular fibrosis, medial layer disruption, and matrix deposition were observed. Interestingly, both groups treated with AII/BAPN/A9B8 or AII/BAPN/IgG2 developed hypertension as assessed by telemetry (MAP mmHg: 181±5 vs 174±7; n=5). In conclusion, immunomodulation of ADAM17 function appears to be a potential therapeutic target to slow AAA development and rupture.

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013

The Molecular Program of Renin^{Null} Cells

PrimaryAuthor.AuthorBlock:**Evan Brown**, Masafumi Oka, Maria F Martinez, Silvia Medrano, R Ariel Gomez, Univ of Virginia, Charlottesville, VA

Mice homozygous for the *Ren1c* gene disruption (*Ren1c*$^{-/-}$) display structural and functional defects characterized by a poorly developed renal medulla, urine concentration failure, polydipsia, polyuria, hydronephrosis, renal failure and anemia. Underlying this complex phenotype, mice exhibit unique renal vascular abnormalities, including concentric arteriolar hypertrophy. Using lineage and promoter activity tracking, we showed that renin^{null} cells contribute directly to blood vessel thickening and distribute throughout renal arterial trees. We hypothesize that renin^{null} cells synthesize factors that lead to arterial thickening and maintain an active molecular memory of the renin phenotype.

To test this hypothesis, we performed RNA-seq of YFP sorted single cells from kidneys of *Ren1c*$^{-/-}$*; Ren1c*-YFP (KO) and *Ren1c*$^{+/+}$*; Ren1c*-YFP (wildtype, WT) adult mice. We captured individual cells using a microfluidic C1 system and sequenced cDNA from cellular mRNA. We also performed ATAC-seq of KO cells to identify open chromatin regions available for transcription factor binding.

KO and WT cell populations were distinct, with the average Euclidean distance between genotypes 1.9x greater than within genotypes. Differential expression analysis revealed that KO cells upregulated 1395 genes and downregulated 364 compared to WT (log fold change > 1, $p < 0.05$). Among the upregulated genes were 107 potentially secreted proteins and 64 putative transcription factors. Secreted protein genes were enriched for GO terms such as angiogenesis and cell proliferation ($p < 0.01$), suggesting a possible cause of arteriolar abnormalities in KO kidneys. We identified several upregulated transcription factors, including Foxp1, Stat1, and KLF family genes, that had predicted binding motifs in open chromatin regions, such as upstream the Ren1 gene ($p < 1.0E-10$). These factors are key candidates for regulating the molecular memory of the renin cell.

This study shows that over activation of the renin program due to lack of renin causes expression of a distinct suite of genes that may be responsible for vascular pathologies observed in KO mice. These data also provide insight into how the cell regulates the renin cell program in response to chronic stimuli that jeopardize homeostasis.

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014

B1 Integrin is Essential for Kidney Structure, Function and Vascular Development

PrimaryAuthor.AuthorBlock:**Tahagod Mohamed**, Rajwinderjit Kaur, Maria Luisa S. Sequeira-Lopez, Univ of Virginia, Charlottesville, VA

Integrins are the largest family of cell adhesion molecules. β1-Integrin (Itgb1) is the most abundantly expressed β subunit and is present in almost every cell type. Previous studies showed that Itgb1 is required for normal development of the ureteric bud and podocytes and for the function of the proximal tubule. However, its role in the kidney vasculature has not been explored. Renin cells are crucial for blood pressure homeostasis and for normal nephrovascular development. The mechanisms involved in their morphogenetic functions are not well understood. We found that Itgb1 is highly expressed in renin expressing cells throughout development. Therefore, to study

the role of Itgb1 in renin cells we generated a conditional deletion (cKO) of Itgb1 in cells of the renin lineage by crossing floxed Itgb1 mice with mice expressing *cre recombinase* driven by the *renin* locus. Itgb1 cKO mice were smaller in size (20.32 ± 4.35 g *vs* 29.55 ± 7.39 g, *p=0.016*), had smaller kidney to body weight ratio (0.92 ± 0.30 *vs* 1.31 ± 0.30, *p=0.017*), hypotension (MABP 82.33 ± 4.00 mmHg *vs* 92.55 ± 7.72 mmHg, *p=0.02*), anemia (hemoglobin 11.26 ± 1.48 g/dL *vs* 15.07 ± 1.31 d/dL, *p=0.003*; hematocrit 40.39 ± 5.48% *vs* 53.8 ± 4.21%, *p=0.002*), renal failure (BUN 71.44 ± 32.81 mg/dL *vs* 29.4 ± 7.67mg/dL, *p=0.004),* and lower plasma renin levels (6664.58 ± 3251.92 *vs* 43357.09 ± 17032.63 pg/ml, *p*=0.001*).* Mutants also developed hyposthenuria (urine osmolality 452 ± 138.98 mOsm/kg *vs* 1460.5 ± 482.09 mOsm/kg, *p<0.02*). Histological analysis revealed excessive collagen deposition in the interstitium and peri-glomerular areas; fibrocystic glomeruli; tubular dilatation and protein casts in the tubules. Immunostaining for renin and α-smooth muscle actin showed a marked decrease in renin protein expression and abundant α-smooth muscle actin expression in the interstitium. Microdissection of the renal arterial tree combined with renin immunostaining confirmed the marked decrease in renin and evidenced the overall vascular abnormalities including fewer and shorter arterial and arteriolar branches. Overall, this study shows that β1-Integrin in cells of the renin lineage is crucial for renin expression, morphogenesis of the renal vasculature and maintenance of the normal kidney architecture and function.

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015

Ctcf is Required for Renin Expression and Maintenance of the Structural Integrity of the Kidney

PrimaryAuthor.AuthorBlock:**Maria Florencia Martinez**, Minghong Li, Silvia Medrano, R Ariel Gomez, Dept of Pediatrics, Child Health Res Ctr, Univ of Virginia, Sch of Med, Charlottesville, VA

Renin is crucial for the regulation of blood pressure and fluid electrolyte homeostasis. The transcriptional machinery that regulates renin expression and thus determines the identity of renin cells is not completely understood. CCCTC-binding factor (*Ctcf*), is an important chromatin organizer of genes that confer cell identity and tissue-specificity. Because *Ctcf* binds to several sites in the neighborhood of the renin locus, we hypothesized that *Ctcf* may regulate renin expression. To test this hypothesis, we generated mice with conditional deletion of *Ctcf* in cells of the renin lineage (*Ctcf* cKO). *Ctcf* cKO mice showed fewer renin-positive cells as shown by immunostaining, and a 70% reduction of renin mRNA levels when compared to control mice (0.292 ± 0.246 *vs* 1.003 ± 0.097, *p<0.001*). In addition, plasma renin levels were significantly decreased in *Ctcf* cKO versus control mice (15276.544 ± 6778.735 pg/ml *vs* 62321.62 ± 21881.99 pg/ml, *p<0.001*). Consistent with reduced renin levels, *Ctcf* cKO mice had lower mean arterial pressures (60.09 ± 3.12 mmHg *vs* 75.07 ± 3.06 mmHg, *p<0.001*). The kidney/body weight ratio in *Ctcf* cKO mice was markedly reduced (1.05 ± 0.228 % *vs* 1.29 ± 0.074 %, *p<0.05*), indicating a more pronounced effect in kidney than in somatic growth (20.09 ± 1.47 g *vs* 22.95 ± 2.95 g, *p<0.05*). Masson's trichrome staining revealed interstitial fibrosis coinciding with cortical depressions in *Ctcf* cKO kidneys. Moreover, PAS staining of *Ctcf* cKO

kidneys showed dilated tubules with intraluminal casts, and areas with crowded sclerotic and crescent glomeruli surrounded by disorganized packed cells. Finally, *Ctcf* cKO mice exhibited renal failure evidenced by increased BUN (44.29 ± 17.62 mg/dL *vs* 26 ± 3.39 mg/dL, *p<0.05)*, and inability to concentrate their urine (448 ± 85.57 mOsm/kg *vs* 1519.33 ± 382.39 mOsm/kg, *p<0.001*). In summary, deletion of *Ctcf* in cells from the renin lineage leads to decreased endowment of renin-expressing cells accompanied by decreased circulating renin, hypotension, severe morphological abnormalities of the kidney and ultimately renal failure. We conclude that *Ctcf* is necessary for the appropriate expression of renin, control of renin cell number and structural integrity of the kidney.

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016

Combined AT_2 Receptor Stimulation/ AT_1 Receptor Blockade Does Not Cause Renal Dysfunction

PrimaryAuthor.AuthorBlock:Dhaniel Baraldi, Lucinda M Hilliard, Tracey A Gaspari, Kate M Denton, **Robert E Widdop**, Monash Univ, Melbourne, Australia

Combined ACE inhibition and angiotensin type 1 receptor (AT_1R) blockade (dual RAS) blockade) causes renal failure which limits their combined use. Angiotensin type 2 receptor (AT_2R) stimulation evokes renoprotective effects but potential adverse renal effects in drug combination are not known. We examined the effects of the AT_2R agonist compound 21 (C21) combined with either candesartan cilexetil or perindopril on renal function in a preclinical model that is predictive of clinical outcome. Adult (~25 week-old) male SHR were placed on a normal salt (0.35%, n=8) or a low salt (LS; 0.05%) diet for 17 days with rats on a LS diet randomised to receive one of the following treatments for the final 10 days: untreated (n=8); C21 (0.3mg/kg/d s.c., n=5); candesartan (2 mg/kg/d, n=6); perindopril (0.5mg/kg/d, n=5); C21+candesartan (n=8); C21+perindopril (n=5); or candesartan+perindopril (dual RAS blockade, n=7). Systolic arterial pressure (SBP) was measured via tail cuff at days 0, 7 and 17. At the end of the treatment, renal function was assessed by measuring plasma creatinine, urea, K+ and glomerular filtration rate (GFR) in conscious rats via transdermal assessment of elimination half-life kinetics of FITC-sinistrin (3-5mg/100g i.v.). Candesartan (-43±10 mmHg) or perindopril (-49±12 mmHg) reduced SBP to a similar extent alone or combined with C21, whereas dual RAS blockade markedly reduced SBP (-115±14 mmHg; P<0.01 versus all groups). Plasma creatinine (424 ±65 µmol/L), urea (>50 mmol/L cut-off) and K+ (5.94 ±0.82 mmol/L) levels were all significantly elevated by dual RAS blockade compared with untreated SHR on normal diet (creatinine 39 ±2 µmol/L; urea 7.08±0.22 mmol/L; K+ 4.23±0.13 mmol/L; all P<0.01) or LS diet (creatinine 29 ±2 µmol/L; urea 5.2±0.57 mmol/L; K+ 4.29±0.17 mmol/L; all P<0.01), whereas plasma measurements for C21+candesartan and C21+perindopril were similar to control groups. Estimated GFR in LS group (1.18±0.06 ml/min/100g BW) was similar to other groups except during dual RAS blockade where GFR was markedly impaired (0.32±0.10 ml/min/100g BW; P<0.01 versus LS). Collectively, these data suggest that, unlike dual RAS blockade, an AT_2R agonist combined with

either ACE inhibition or AT_1R blockade is not likely to cause renal dysfunction.

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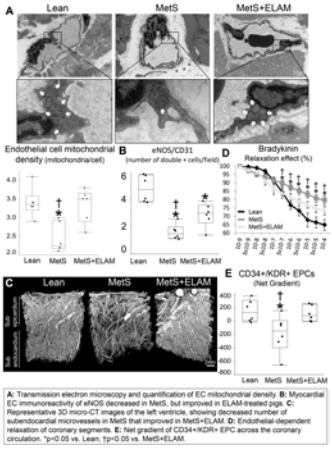
Mitoprotection Attenuates Myocardial Vascular Impairment and Decreases Coronary Retention of Endothelial Progenitor Cells in Swine Metabolic Syndrome

PrimaryAuthor.AuthorBlock:Fang Yuan, Henan Provincial People's Hosp, Henan, China; Ahmad F Hedayat, Christopher M Ferguson, Hui Tang, Amir Lerman, Lilach O Lerman, **Alfonso Eirin**, Mayo Clinic, Rochester, MN

Introduction: The metabolic syndrome (MetS) leads to cardiac vascular injury, which may reflect in increased retention of endothelial progenitor cells (EPC). Coronary endothelial cell (EC) mitochondria partly regulate vascular function and structure. We hypothesized that chronic mitoprotection would preserve EC mitochondria and attenuate coronary vascular injury and dysfunction in swine MetS. Methods: Pigs were studied after 16 weeks of diet-induced MetS, MetS treated for the last 4 weeks with the mitochondria-targeted peptide elamipretide (ELAM, 0.1mg/kg SC q.d), and lean controls (n=6 each). Coronary artery and sinus blood samples were collected. EC mitochondrial density (electron microscopy), endothelial nitric oxide (eNOS) immunoreactivity (staining), myocardial microvascular density (3D micro-CT), and coronary endothelial function (organ bath) were assessed ex-vivo. The number and arteriovenous gradient of CD34+/KDR+ EPC was calculated by FACS (a negative gradient indicating EPC retention). Results: MetS and MetS+ELAM pigs developed similar MetS (obesity, hyperlipidemia, insulin resistance, and hypertension). EC mitochondrial density decreased in MetS compared to lean, but normalized in MetS+ELAM. ELAM also improved eNOS immunoreactivity, subendocardial microvascular density, and coronary endothelial function. ELAM-induced vasculoprotection was reflected in decreased coronary retention of EPCs. Conclusions: These observations underscore the role of mitochondria in regulating the cardiac circulation in experimental MetS, and the benefits of mitoprotection to preserve EC myocardial microvascular integrity.



A: Transmission electron microscopy and quantification of EC mitochondrial density. B: Myocardial EC immunoreactivity of eNOS decreased in MetS, but improved in ELAM-treated pigs. C: Representative 3D micro-CT images of the left ventricle, showing decreased number of subendocardial microvessels in MetS that improved in MetS+ELAM. D: Endothelial-dependent relaxation of coronary segments. E: Net gradient of CD34+/KDR+ EPC across the coronary circulation. *p<0.05 vs. Lean; †p<0.05 vs. MetS+ELAM.

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018

Neuronal (Pro)renin Receptor Deletion Prevents High-fat Diet Induced High Blood Pressure and Type II Diabetes

PrimaryAuthor.AuthorBlock:**Caleb J Worker**, Univ of Nevada, Reno Medical, Reno, NV;

Wencheng Li, Wake Forest Univ, Winston-Salem, NC; Yumei Feng, Univ of Nevada, Reno, Reno, NV

We recently reported that the (pro)renin receptor (PRR) is a key component of the brain renin-angiotensin system, mediating the majority of Ang II formation, and plays a pivotal role in the development of hypertension. Its importance in obesity-related metabolic syndrome is, however, unknown. We hypothesize that brain PRR plays a regulatory role in high-fat diet (HFD) induced metabolic syndrome. To test our hypothesis, neuron-specific PRR knockout (PRRKO) mice and wildtype (WT) littermates were fed with either HFD (60% calories from fat) or normal fat chow (NFD, 10% calories from fat) with matching calories for 16 weeks. Weekly body weight (BW) and monthly fasting blood glucose (FBG) measurements were recorded and end point glucose tolerance (GTT) and insulin sensitivity tests (IST) were performed. Blood pressure (BP) was recorded using radiotelemetry in conscious free moving mice. We observed no difference in BW or food intake between genotypes in either HFD or NFD. The baseline BP and heart rate (HR) were similar between PRRKO and WT mice; however, following 16 weeks HFD the BP (101±6 vs. 111±3 mmHg, P=0.035) and HR (536±12 vs. 578±4 BPM, P=0.046) were significantly lower in PRRKO compared with WT mice. Interestingly, neuronal PRR deletion attenuated the elevation of FBG (127.12±10.46 vs. 167.77±16.57 mg/dl, P=0.039) induced by HFD. Glucose tolerance was significantly improved in PRRKO compared with WT following 16 weeks of HFD (AUC: 20557±894 vs. 29994±2976, P=0.006), while there was no difference in the IST between the groups. We also found that HFD mice had higher levels of plasma (pro)renin (9.95±1.83 vs. 2.74± 0.47 ng/ml, P=0.005) and brain angiotensin II (656.8±94.9 vs. 375.3±32.0 pg/g, P=0.02), as well as higher cardiac (ΔHR to propranolol: -150±6 vs. -82±15 bpm , P=0.0054) and vasomotor (ΔBP to chlorisondamine: -44±3 vs. -22±3 mmHg, P=0.0004) sympathetic tone, suggesting that the HFD-induced rise in BP is sympathetically mediated and associated with elevation of brain angiotensin II. Our data indicates that PRR deletion in the neurons protects against glucose intolerance and BP elevation in HFD mice with no effect on insulin sensitivity or body weight. We conclude that neuronal PRR plays a role in the development of obesity-related metabolic syndrome.

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019

Effect of CPAP on Blood Pressure and Central Sympathetic Outflow in Diabetic Patients with OSA, Resistant HTN, and CKD

PrimaryAuthor.AuthorBlock:**Marcel Ruzicka**, Swapnil Hiremath, Ottawa Hosp, Ottawa, ON, Canada; Frans HH Leenens, Univ of Ottawa Heart Inst, Ottawa, ON, Canada; Judith Leech, Shawn Aaron, Ottawa Hosp, Ottawa, ON, Canada

Introduction: Central sympathetic hyperactivity as assessed by muscle sympathetic nerve activity recordings is thought to play a crucial role in the development and maintenance of hypertension (HTN) in patients with obstructive sleep apnea (OSA). Decreases in daytime and nocturnal blood pressure (BP) in response to treatment with continuous positive airway pressure (CPAP) are paralleled by decreases in muscle sympathetic nerve activity (MSNA). Patients with chronic kidney disease (CKD) have high MSNA. Bilateral nephrectomy, but not

renal transplantation normalizes MSNA indicating central sympathoexcitatory effects by renal afferents. The objective of this study was to assess to what extent is HTN driven by central sympathetic hyperactivity in patients with diabetic CKD, OSA, and resistant HTN. Thirteen patients (age 62.2±7.4 years) with diabetic CKD, resistant HTN defined as SBP on 24-hr ABPM above 135 mmHg while on 3 or more BP lowering drugs (including diuretic) with OSA, were randomized to therapeutic CPAP or non-therapeutic CPAP for one months. 24-hr ABPM, plasma catecholamines, aldosterone, and renin, and MSNA were assessed before and 1 months after randomization. Our results show (Table) that in contrast to sham CPAP, therapeutic CPAP decreased daytime and nighttime BP. In contrast, neither therapeutic nor sham CPAP caused any changes in MSNA and plasma catecholamines. In conclusion, decreases in BP in response to CPAP in patients with in diabetic CKD, despite maintained high MSNA, indicate other mechanism contributing to HTN in these patients as well as other central sympathoexcitatory pathways activated.

Variable	CPAP	Sham CPAP	p value
ABPM SBP Overall (median, IQR in mm Hg)	133 (130.3, 136.8)	153 (148, 161)	0.008
ABPM DBP Overall (median, IQR in mm Hg)	72.5 (66.5, 76.3)	84 (72, 94)	0.07
ABPM SBP night (median, IQR in mm Hg)	119 (115.8, 134)	146 (137, 167)	0.008
ABPM DBP night (median, IQR in mm Hg)	65 (56.5, 73.8)	81 (65, 87)	0.12
MSNA (median, IQR in burst/min)	49 (35.4, 52.4)	46 (38.5, 53.8)	0.99

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Heterozygote Knockout of TMEM16B in Intestinal Vagal Afferents Causes Cholecystokinin Insensitivity and Obesity in Male but Not Female Mice

PrimaryAuthor.AuthorBlock:**Runping Wang**, Yongjun Lu, Michael Z Cicha, Christopher J Benson, Mark W Chapleau, Francois M Abboud, Univ of Iowa, Iowa City, IA

Cholecystokinin (CCK) is a well-known satiety peptide that inhibits food intake. The CCK induced satiety signal in intestinal vagal afferents is attenuated in mice on a High Fat Diet (HFD). We have shown that down regulation of a Ca^{2+}-activated Cl^- channel (CaCC) downstream of CCK receptors is responsible for the neuronal insensitivity to CCK. We also found that the CaCC subunit Ano2/TMEM16B is essential for the CCK-induced current in nodose neurons. In this study we tested the hypothesis that reduction of this subunit *in vivo* contributes to weight gain. One allele of the Ano2/TMEM16B was knocked out in sensory neurons by crossing the $ano2^{fl/fl}$ mice with Nav1.8Cre mice to generate the Nav1.8Cre;$ano2^{fl/wt}$ mice. The Cre negative littermates were used as control. We found that CCK-induced suppression of food intake is eliminated in male Nav1.8Cre;$ano2^{fl/wt}$ mice. Food intake measured over 4 hours was 1.21±0.11g (n=5) in male wild type (wt) mice injected with saline and was reduced to 0.77±0.18 g (n=7, $p<0.05$) in mice injected with CCK-8 (3µg/kg). Those values were 0.63±0.15 g (n=6) in saline injected and 0.91±0.13 g (n=7, $p>0.05$) in CCK injected male Nav1.8Cre;$ano2^{fl/wt}$ mice. However, the CCK injection did not affect food intake in either female wt or Nav1.8Cre;$ano2^{fl/wt}$ mice. The male Nav1.8/$ano2^{fl/wt}$ mice were on the average 5.5g heavier than wt mice at 40 weeks of age (39.8±1.4 g, n=13 vs 34.3±1.4 g, n=14, $p<0.01$). Body weight of females was significantly lower than in males but was not different between wt and Nav1.8Cre;$ano2^{fl/wt}$ mice (30.4±1.0 g, n=14 vs 28.5±1.0 g, n=11, p=0.20 respectively). Single cell mRNA level of Ano2 and CCK-induced TMEM16 currents in nodose neurons were reduced significantly in male Nav1.8Cre;$ano2^{fl/wt}$

mice compared to Cre negative controls, but such changes were not seen in female mice. We conclude that heterozygote knockout of Ano2/TMEM16B specifically in sensory neurons causes neuronal insensitivity to CCK and excessive weight gain in male but not female mice. Reduction of this subunit may contribute to the HFD induced obesity. The reason for the phenotype and allele expression variability between sexes is unclear.

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021

Role of Central Nervous System Suppressor of Cytokine Signaling 3 (socs3) in Regulating Metabolic and Cardiovascular Function in Obesity

PrimaryAuthor.AuthorBlock:**Jussara M do Carmo**, John Nathan Freeman, Alexandre A. da Silva, Sydney P. Moak, John E. Hall, UMC, Jackson, MS

Suppressor of cytokine signaling 3 (SOCS3), a negative regulator of leptin signaling, is upregulated in obesity and may contribute to development of leptin resistance. We determined whether deletion of SOCS3 in the entire central nervous system (CNS) would protect mice from adverse metabolic and cardiovascular effects of a high fat diet (HFD). $SOCS3^{flox/flox}$/Nestin-cre mice were generated by breeding $SOCS3^{flox/flox}$ with Nestin-Cre mice. Male and female mice with CNS deletion of SOCS3 ($SOCS3^{flox/flox}$/Nestin-Cre, n=5-10) or control ($SOCS3^{flox/flox}$, n=6-10) were fed a HFD plus sucrose from 6 until 22 weeks of age. Mean arterial pressure (MAP) and heart rate (HR) were recorded by telemetry and oxygen consumption (VO_2) was monitored by indirect calorimetry in 22-week-old mice. Compared to control mice, $SOCS3^{flox/flox}$/Nestin-Cre mice were lighter (male: 34±3 vs. 45±3 and female: 27±1 vs. 37±2 g) and had elevated VO_2 (94±12 vs. 69±6 ml/kg/min) but there were no significant differences in food intake (male: 3.3±0.7 vs. 3.8±0.5 and female: 3.0±0.6 vs. 3.0±0.5 g/day) or plasma glucose (male: 148±8 vs. 198±31 and female 149±12 vs. 164±12 mg/dl). Male $SOCS3^{flox/flox}$/Nestin-Cre mice had similar MAP (115±2 vs. 116±1 mmHg) but higher HR (657±3 vs. 592±3 bpm) compared to control mice. However, female $SOCS3^{flox/flox}$/Nestin-cre mice had higher MAP (121±1 vs. 108±1 mmHg) and HR (655±2 vs. 606±5 bpm) compared to control mice. No significant differences were observed in glucose tolerance in $SOCS3^{flox/flox}$/Nestin-Cre vs. control mice (AUC: 391±36 vs. 429±54 mg/dL x 120 min in males and 459±70 vs. 372±51 mg/dL x 120 min in females). These results indicate that CNS SOCS3 deletion reduced body weight and increased energy expenditure and HR but did not improve glucose tolerance in male or female mice fed a HFD. However, HFD significantly increased BP in female $SOCS3^{flox/flox}$/Nestin-Cre mice compared to control mice fed a HFD, suggesting a sex difference in the role of CNS SOCS3 signaling in BP regulation in obesity. (NHLBI PO1HL51971and NIGMS P20GM104357)

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022

mTORC1 is Required for the Cardiovascular but Not the Metabolic Actions of Leptin

PrimaryAuthor.AuthorBlock:**Balyssa Bell**, Donald Morgan, Kamal Rahmouni, Univ of Iowa, Iowa City, IA

Obesity represents a major risk factor for the development of hypertension, and inappropriate leptin action has been implicated as an essential mediator of obesity-associated hypertension. Leptin plays a critical role in energy homeostasis, acting through the brain to stimulate energy expenditure and suppress food intake. Leptin also increases sympathetic outflow to a variety of target organs, including those involved in blood pressure regulation. Here, we investigate the role of mTORC1 as a potential mediator of leptin's cardiovascular and metabolic actions. For this, we generated conditional knockout mice that lack the critical mTORC1 subunit, Raptor, specifically in leptin receptor (LRb) expressing cells ($LRb^{Cre}/Rap^{fl/fl}$). $LRb^{Cre}/Rap^{fl/fl}$ displayed similar body weight, food intake and body composition as compared to littermate controls when fed a normal chow diet (body weight=29.6±0.8 g vs 31.0±0.8g at 14 weeks of age). Control and $LRb^{Cre}/Rap^{fl/fl}$ mice also developed diet-induced obesity to a similar extent when fed either a 45% high-fat (37.2±3.1g g vs 40.9±2.2) or high-fat/high-sucrose diet (35.4±1.1g vs 35.2±2.7g). Additionally, fasting blood glucose (77.3±6.7mg/dL vs. 71.8±4.3mg/dL) as well as insulin (AUC=7788 ±1013, n=3 vs. 8964±884, n=4) and glucose (AUC=39750±2075, n=3 vs. 44259±1948, n=4) tolerance in high fat/high-sucrose diet fed mice were not changed in $LRb^{Cre}/Rap^{fl/fl}$ mice as compared to littermate controls. Conversely, while baseline mean arterial pressure (MAP) was comparable between $LRb^{Cre}/Rap^{fl/fl}$ mice (108±9 mmHg) and controls (103±7 mmHg), intracerebroventricular administration of leptin significantly increased MAP in control mice (30±14 mmHg), but not in $LRb^{Cre}/Rap^{fl/fl}$ mice (1±9 mmHg, $P<0.05$ vs controls). Consistent with this, $LRb^{Cre}/Rap^{fl/fl}$ mice displayed a blunted renal sympathetic nerve response to leptin (-4±15%, n=9 vs. 127±16%, n=9, $P<0.05$) but a preserved increase in sympathetic outflow to brown adipose tissue (109±27%, n=5 vs. 173±52%, n=4). Together, our data indicate a critical role for mTORC1 in mediating the cardiovascular but not the metabolic effects of leptin.

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023

Inducible Deletion of Adipocyte Prorenin Receptor Reverses Obesity Related Hypertension

PrimaryAuthor.AuthorBlock:**Eva Gatineau**, Dianne Cohn, Ming Gong, Frédérique Yiannikouris, Univ of Kentucky, Lexington, KY

Obesity contributes to approximatively 2.5 million deaths every year and is associated with life threatening conditions including hypertension. Recently, we found that constitutive deletion of adipocyte (pro)renin-receptor (PRR) prevented high-fat diet-induced obesity through a drastic decrease in fat mass. However, adipocyte PRR deficient mice were characterized by a fatty liver and by an elevated systolic blood pressure (SBP), classic features of models of lipodystrophy. The purpose of this study was to investigate whether the temporally-controlled deletion of adipocyte PRR in obese mice reverses obesity related hypertension. After 18 weeks of high fat diet, inducible adipocyte-PRR deficient (PRR^{ERT}) and control ($PRR^{fl/Y}$) male mice (n=7-11 mice/ group)

were injected intraperitoneally with tamoxifen (TMX) for 5 consecutive days. Body weight, body composition and blood pressure, measured by radiotelemetry in a subgroup of mice (n=2-4 mice/ group), were recorded before and after TMX injection. The inducible deletion of adipocyte PRR in PRR^{ERT} mice decreased significantly body weights ($PRR^{fl/fl}$, 46.6 ± 1.3 g; PRR^{ERT}, 42.1 ± 1.4 g, P<0.05) and fat mass ($PRR^{fl/fl}$, 15.8 ± 1.0 g; PRR^{ERT}, 8.1 ± 0.7 g, P<0.05) compared to control mice. PPARγ, FABP4 and FAS mRNA levels were significantly decreased by 68% (6.8 out 10), 80% (8 out 10) and 68% (6.8 out 10) respectively in white adipose tissues of PRR^{ERT} mice suggesting that PRR positively regulated adipogenesis and lipid metabolism in adipose tissue. In addition, the inducible deletion of adipocyte PRR in PRR^{ERT} mice decreased significantly SBP compared to control mice ($PRR^{fl/fl}$, -4.3 ± 3.2 g; PRR^{ERT}, -10.2 ± 2.4 g, P<0.05). Interestingly, adipocyte angiotensinogen mRNA abundance was significantly decreased in adipose tissue of PRR^{ERT} mice fed a standard diet suggesting that the decrease in blood pressure might be mediated by a local renin angiotensin system (RAS). The measurement of local (liver, kidney, adipose tissue and brain) and systemic RAS in HF-fed mice is under investigation. Taken together, our results highlight a new signaling pathway in which PRR regulates adipogenesis, lipid metabolism and blood pressure. PRR could represent a new potential therapeutic target for obesity and hypertension.

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024

Angiotensin-(1-7) Attenuates Diet-induced Obesity in Mice by Browning White Adipose Tissue to Enhance Energy Expenditure

PrimaryAuthor.AuthorBlock:Traci A Czyzyk, Timothy K Cooper, Sarah S Bingaman, **Amy C Arnold**, Penn State Coll of Med, Hershey, PA

Overactivation of the renin-angiotensin (Ang) system, and in particular Ang II, contributes to obesity-induced insulin resistance via multiple mechanisms. Obesity is also associated with deficiency of Ang-(1-7), a beneficial hormone that opposes Ang II actions. In this study, we tested the hypothesis that Ang-(1-7) could prevent development of diet-induced obesity in mice. To test this hypothesis, adult male C57BL/6J mice received 12-week Ang-(1-7) (400 ng/kg/min; n=7) or saline (n=6) infusion via subcutaneous osmotic mini-pumps. Mice were placed on 60% high fat diet (HFD) immediately following mini-pump implantation. Body composition, energy balance, and insulin sensitivity were measured during the last week of treatment. Ang-(1-7) attenuated HFD-induced weight gain (39.7±1.3 vs. 43.9±0.7 g saline; p=0.023) and adiposity (32±1 vs. 29±1% saline; p=0.050). This weight-reducing effect was due to enhanced average energy expenditure [0.55±0.02 vs. 0.42±0.06 kcal/hour saline, p=0.038], with no changes in locomotor activity or food intake. Ang-(1-7) attenuated HFD-induced hyperinsulinemia (1.4±0.4 vs. 5.3±1.6-ng/mL saline; p=0.032) and improved the ability of intraperitoneal insulin to decrease blood glucose levels (20% reduction in area under the curve vs. saline; p=0.015), consistent with insulin-sensitizing effects. Given effects of Ang-(1-7) on energy expenditure, we performed blinded histological and semi-quantitative real-time PCR analysis of brown and white adipose tissue. Ang-(1-7) reduced HFD-induced lipid droplet accumulation in brown adipose, but did

not improve thermogenesis markers (e.g. uncoupling protein-1). Ang-(1-7) improved inflammation in visceral white adipose (mean number crown-like structures: 3±1 vs. 8±2 saline; p=0.046). Ang-(1-7) reduced adipocyte size and increased gene expression of the browning marker PRDM16 (1.7±0.2 vs. 1.1±0.1; p=0.024) in subcutaneous white adipose. These data suggest that Ang-(1-7) attenuates diet-induced obesity in mice by enhancing energy expenditure, in part by browning of white adipose tissue. These overall findings provide rationale for targeting Ang-(1-7) to prevent development of obesity and related pathology such as insulin resistance and adipose inflammation.

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025

Lacking of Gamma Delta T Cells Exaggerate Cardiac Dysfunction in Angiotensin II-induced Hypertension in Balb/cByJ Mice

PrimaryAuthor.AuthorBlock:**Tang-Dong Liao**, Jiang Xu, Oscar A Carretero, Henry Ford Hosp, Detroit, MI

Gamma Delta T lymphocytes are important innate immune component which express γδ T cell receptor (TCR). These cells are capable of spontaneous secretion of IL-17 and IFN gamma proinflammatory cytokines. Recently, new evidence suggests that the innate and adaptive immune system is involved in the hypertension and end-organ damage. We tested the hypothesis whether deficiency in γδ TCR has a beneficial effect on cardiac function in Angiotension II (Ang II)-induced hypertension. Male Balb/cByj wild-type (WT) and Tcrγδ knockout (Tcrγδ-/-) mice were infused with vehicle or Ang II at dosage of 400ng/kg/min for 4 weeks. Our results showed Systolic blood pressure (SBP) was increased significantly after 1 week of Ang II infusion, and the increase was sustained 4 weeks in WT mice, however in Tcrγδ-/- mice, SBP dropped significantly at 4 weeks compared to WT (table1). Echocardiography data showed that ejection fraction (EF) and shortening fraction (SF) were decreased significantly after Ang II infusion; these effects were exacerbated in Tcrγδ-/- mice given Ang II. Also both mass and chamber dimension increased greater in Tcrγδ-/- mice given Ang II compared to WT (table1). The results indicated Tcrγδ-/- mice given Ang II develop eccentric hypertrophy. We conclude that lacking of γδ T cells has a detrimental effect on cardiac function in Ang II-induced hypertension in Balb/cByJ mice.
table1:

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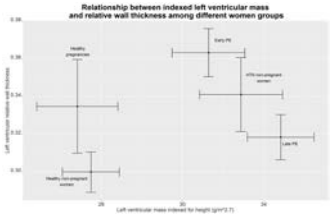
026

Early-onset Preeclampsia is Associated With Left Ventricular Concentric Remodeling at 1-month Post-partum Follow-up

PrimaryAuthor.AuthorBlock:GianLuca Colussi, Cristiana Catena, Div of Internal Med, Dept of Med, Univ of Udine, Udine, Italy; Lorenza Driul, Div of Obstetrics and Gynecology, Dept of Med, Univ of Udine, Udine, Italy; Francesca Pezzutto, Valentina Fagotto, **Leonardo A Sechi**, Div of Internal Med, Dept of Med, Univ of Udine, Udine, Italy

Preeclampsia (PE) is associated with persistent cardiac abnormalities and a high cardiovascular risk during the woman life. Early onset PE presenting before the 34th gestational week is a more severe form of disease, but its relevance on cardiac abnormalities is unknown. The aim of this study was to assess cardiac structure and function in women with PE without preexistent hypertension. One month after delivery, the clinical and echocardiographic variables of 65 preeclamptic women (age 36±6 y), 37% of which with early PE, was compared with a group of 30 normotensive (age 37±6 y), 16 hypertensive (age 40±5 y) nonpregnant women, and 6 women with normal pregnancy (age 36±5 y) who were matched for age and height. Despite comparable antihypertensive treatment, women with early PE had lower 24-hour average systolic and diastolic blood pressure than those with late PE (125±11 Vs 136±15). Left ventricle (LV) relative wall thickness was significantly greater in women with early PE (0.36±0.06) than in those with late PE (0.31±0.07; p<0.05) and normotensive (0.30±0.06; p<0.05) women, but not hypertensive women (figure). Women with early and late PE had comparably greater LV mass and worse diastolic function as assessed by the E/A ratio (PE 1.6±0.4 Vs 1.8±0.4 P<0.01) and isovolumic relaxation time (PE 96±28 Vs 73±12 msec, P<0.001) than normotensive women. In conclusion, women with early onset PE have more pronounced LV concentric remodeling than women with late onset PE. This observation could account for the greater cardiovascular risk of these patients and might prompt the use of antihypertensive drugs specifically acting on LV remodeling.



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027

ProANP 31-67 and Renal Function in Heart Failure

PrimaryAuthor.AuthorBlock:**Robin A. Felder**, Peng Xu, Katie Schiermeyer, John J Gildea, The Univ of Virginia, Charlottesville, VA; T R Geimer, Larry Mahan, Madeleine Pharmaceuticals, Madison, WI

The physiologic interplay between heart and kidney is best understood in the context of heart failure (HF). Two-thirds of HF patients suffer from cardiorenal syndrome (CRS) - heart failure with renal insufficiency wherein both organs contribute to their reciprocal decline. Four peptides are released from an atrial natriuretic prohormone in response to atrial-ventricular wall stress in HF. One in particular, a well characterized 28 a.a cyclic peptide from the C-terminus termed ANP elicits systemic vasodilation via cGMP release and was developed as Natrecor® to treat acute HF. Limited preclinical data exists regarding the remaining three N-terminal peptides although all three have been explored for clinical utility in a limited series of Phase 1 and 2a clinical trials in the U.S. and in Australia. We have examined

a 37 a.a. linear peptide, mid-sequence in the prohormone termed proANP 31-67 (MP3167) to better understand its role in CRS. MP3167 is unique among the four as it has the longest half-life of all in humans (1-1/2 hours vs. minutes), is highly acidic (pI=3.2; -5 net charge), appears resistant to degradation by neprilysin and has been reported to act through PGE2 although the tissue source in humans is unknown. We show for the first time that MP3167 elicits a 3-fold increase in PGE2 release over basal levels predominantly in human renal medullary tissue (from 2401 ± 97.2 to 8437 ± 62.9 pg/ml, N=3, p<0.001). The positive control, beta hydroxyl butyric acid (BOH) also increased PGe2 (from 2401 ± 97.2 to 7749 ±120 pg/ml, N=3, p<0.01). In 3D cultured cells isolated from digested tissue, we also observed an increase in MP3167 stimulated PGE2 (From 41.1 ± 1.3 to 62.9 ± 3.19 pg/ml, N=3, p<0.05). The PGE2 signal co-localizes with cells stained by L1-CAM antibody implicating collecting tubules/ducts at the cortical-medullary interface. As the receptor and second messenger pathway for MP3167 is unknown, we have isolated L1-CAM positive cells and created immortalized cell lines of human origin. Initial findings reiterate MP3167 stimulated PGE2 release, reaching a maximum by ~10 min. These human studies will enable better understanding of MP3167 as a regulator of renal function and the paracrine actions of PGE2 in rebalancing heart and renal function in CRS associated HF.

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028

Endothelial Nogo-B Controls Sphingolipid de novo Biosynthesis to Impact Coronary Artery Atherosclerosis

PrimaryAuthor.AuthorBlock:Yi Zhang, Linda Sasset, Ren Xu, Matthew Blake Greenblatt, **Annarita Di Lorenzo**, Weill Cornell Medical Coll, New York, NY

Coronary artery disease is a leading cause of myocardial infarction (MI) worldwide. Alterations in sphingolipid levels have been linked to atherosclerosis although specific molecular mechanisms are poorly understood. Recently, we discovered that endothelial Nogo-B, a membrane protein of the ER, regulates vascular functions by inhibiting serine palmitoyltransferase (SPT), the rate-limiting enzyme of the *de novo* sphingolipid biosynthesis. Mice lacking Nogo-B are resistant to hypertension and heart failure. Here, we employed a novel model of coronary atherosclerotic lesions induced by hypercholesterolemia and hypertension, well-known risk factors for atherosclerosis. To this aim, transverse aortic constriction (TAC) surgery was performed in mice lacking endothelial Nogo-B in $ApoE^{-/-}$ background and $ApoE^{-/-}$ mice

as control. ApoE$^{-/-}$ mice developed coronary atherosclerotic lesions within 6 weeks following TAC, (without the need of long-term high-cholesterol diet) and ~70% of the mice died of MI at 6-week post-TAC. On the contrary, mice lacking Nogo-B specifically in endothelial cells were markedly resistant to the development of coronary atherosclerotic lesions and MI (~20%). Mechanistically, in the absence of endothelial Nogo-B, the biosynthesis of sphingolipids, particularly S1P, is upregulated, protecting the endothelium from hypertension and hypercholesterolemia-triggered vascular inflammation and atherogenesis. This study identifies an important and novel role of endothelial Nogo-B-dependent regulation of sphingolipid *de novo* biosynthesis in the coronary atherosclerosis, a primary cause of myocardial infarction.

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029

Nox5 is a Pro-contractile Nox Isoform - Implications in Vascular Contraction and Cardiac Fibrosis

PrimaryAuthor.AuthorBlock:**Augusto C Montezano**, Livia L Camargo, Adam P Harvey, Francisco J Rios, ICAMS-Univ of Glasgow, Glasgow, United Kingdom; Chet E Holterman, Christopher R Kennedy, OHRI - Univ of Ottawa, Ottawa, ON, Canada; Rhian M Touyz, ICAMS-Univ of Glasgow, Glasgow, United Kingdom

The functional significance of Nox5 is unknown. Considering the fact that Nox5 is closely associated with changes in [Ca^{2+}] and that it generates ROS, both of which are important in contraction, we questioned whether Nox5 plays a role in pro-contractile signaling and whether it influences vascular function. We generated humanised Nox5 mice with Nox5 expressed in a VSMC-specific manner (Nox5+SM22+). Vascular contraction was measured by myography. ROS production was assessed by HPLC, amplex red and ELISA. Protein levels were evaluated by immunoblotting. Fibrosis was assessed by Picro Sirius red staining and polarized microscopy. Contraction to U46619 was increased in Nox5+/SM22+ mice (5.8±0.3 mN vs WT: 4.2±0.2 mN, $p<0.05$), an effect blocked by a NAC (ROS scavenger), calmidazolium (calmodulin inhibitor), dantrolene (ryanodine receptor Ca^{2+} channel inhibitors) and CDN1163 (SERCA channel activator). $ONOO^-$ levels were increased in vessels from Nox5+/SM22+ (5.8±0.9 vs WT 3.4±0.1 AU/mg, $p<0.05$). ZIPK is an important regulator of MYPT1 inactivation. In vessels from Nox5+/SM22+ mice, ZIPK activation was increased (58.6±3.64 vs 27.73±7.64 AU, $p<0.05$). VSMC-Nox5 exhibited increased cardiac levels of superoxide (WT: 606.3±78.5 vs 1456.0±184.8 nmol/mg of protein), H2O2 (WT: 11.1±1.3 vs 23.88±5.1 µM/µg of protein) lipid peroxidation (WT: 0.70±0.09 vs 1.18±0.18 nmol/ µg of protein), cardiac fibrosis (WT: 3.46±1.71 vs 4.39±0.04 AU), p38 MAPK activation (WT:0.98±0.04 vs 1.61±0.12 AU) and fibronectin expression (WT:1.23±0.07 vs 2.31±0.29 AU) ($p<0.05$). Moreover, peroxiredoxin oxidation was increased (WT: 1.43±0.4 vs 6.28±2.0 AU, $p<0.05$). In VSMCs, downregulation of Nox5, but not Nox1,2,4, by siRNA was associated with reduced phosphorylation of MLC20 and MYPT1. In conclusion, our results demonstrate that Nox5 regulates vascular contraction through processes that involve , ROS, calmodulin, ryanodine and ER-Ca^{2+} channels. Nox5 may be an important regulator of the contractile machinery in VSMCs. In addition, VSMC-Nox5 induces oxidative stress in the heart, leading to fibrosis. Our study defines a novel role for Nox5

as a pro-contractile Nox isoform that may have important implications in conditions associated with vascular hypercontractility.

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030

Angiotensin II-induced Hypertension and Cardiac Hypertrophy are Mediated Differentially by TLR3- and TLR4-dependent Pathways

PrimaryAuthor.AuthorBlock:**Madhu V Singh**, Michael Z Cicha, Mark W Chapleau, François M Abboud, Univ of Iowa, Iowa City, IA

The innate immune system plays a key role in onset and maintenance of hypertension. We showed that the toll-like receptor (TLR) adaptor protein TRIF (toll interleukin receptor domain-containing adaptor-inducing interferon-b), but not MyD88 (myeloid differentiation 88), is required for angiotensin II (Ang II) induced hypertension in mice. TRIF transduces signals from TLR3 and TLR4, to induce inflammatory gene expression. In this study, we sought to determine which TLR is responsible for TRIF-mediated hypertensive effects of Ang II. We used a TLR4 deficient mice (TLR4-del) that have TLR4 gene deleted and the wild type (WT) control strain (C57BL/10). We also used a TLR3-knockout mice (TLR3ko) that have the exon 1 of the *Tlr3* gene deleted. Saline or Ang II (1000 ng/kg/min) was infused subcutaneously for 3 weeks using mini-osmotic pumps and systolic blood pressure (SBP) was measured by tail cuff. Ang II increased peak SBP in WT (from 108 ± 1.3 mmHg to 136.0 ± 12.7 mmHg, n= 3) and TLR4-del (from 115.6 ± 2.7 mmHg to 154.4 ± 3.3 mmHg, n=3) mice. In contrast, peak SBP was not increased significantly with Ang II infusion in TLR3ko mice (from 111.3 ± 4.5 to 122.2 ± 18.6 mmHg, n=6) nor was it increased in WT mice after selective inhibition of TLR3 signaling with CU CPT 4a. Thus, the TLR3/TRIF, but not TLR4, pathway is essential for Ang II hypertension. In parallel with the pressure responses to Ang II, renal expression of *Nox4* was significantly decreased in TLR3ko mice but was unchanged in TLR4-del and WT mice.
In contrast to Ang II-induced hypertension, AngII-induced cardiac hypertrophy, measured as heart weight to body weight ratio, after 3 weeks of Ang II infusion was reduced in both TLR4-del (5.38 ± 0.19 mg/g) and TLR3ko (4.99 ± 0.15 mg/g) compared to WT (7.14 ± 0.49 mg/g). Thus, cardiac hypertrophy is abrogated in TLR4-del despite a robust pressor response to Ang II. The cardiac pro-inflammatory gene expression of tumor necrosis factor alpha (*Tnfa*), NADPH oxidase 4 (*Nox4*), and matrix metalloproteinase 9 (*Mmp9*) was increased in WT heart but attenuated in both TLR4-del and TLR3ko hearts. The results indicate a selective dependence of Ang II hypertension on TLR3/TRIF pathway whereas Ang II-induced cardiac hypertrophy depends on both TLR3 and TLR4, likely through the common TRIF adaptor protein.

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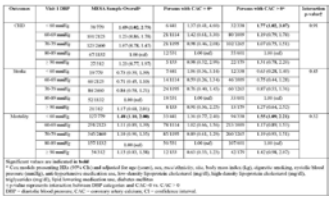
031

Diastolic Blood Pressure, Coronary Artery Calcium, and Cardiac Outcomes in the Multi-ethnic Study of Atherosclerosis

PrimaryAuthor.AuthorBlock:**John W McEvoy**, Faisal Rahman, Mahmoud Al Rifai, Michael Blaha, Khurram Nasir, Johns Hopkins Univ SOM, Baltimore, MD; Matthew Budoff, UCLA, Los Angelos, CA; Bruce Psaty, Depts of Med, Epidemiology and Health Services, Univ of Washington, Seattle, WA; Wendy Post, Roger Blumenthal, Johns Hopkins Univ SOM, Baltimore, MD

Diastolic blood pressure (BP) has a J-curve relationship with coronary heart disease and death. Because this association is thought to reflect reduced coronary perfusion at low diastolic BP, our objective was to test whether the J-curve is most pronounced among persons with coronary artery calcium. Among 6,811 participants from the Multi-Ethnic Study of Atherosclerosis, we used Cox models to examine if diastolic BP category is associated with coronary heart disease events, stroke, and mortality. Analyses were conducted in the sample overall and after stratification by coronary artery calcium score. In multivariable-adjusted analyses, compared with diastolic BP of 80 to 89 mmHg (reference), persons with diastolic BP <60 mmHg had increased risk of coronary heart disease events (HR 1.69 [95% confidence interval 1.02-2.79]) and all-cause mortality (HR 1.48 [95% confidence interval 1.10-2.00]), but not stroke. After stratification, associations of diastolic BP <60 mmHg with events were present only among participants with coronary artery calcium >0. Diastolic BP <60 mmHg was not associated with events when coronary artery calcium was zero. We also found no interaction in the association between low diastolic BP and events based on race. In conclusion, diastolic blood pressure <60 mmHg was associated with increased risk of coronary heart disease events and all-cause mortality in the sample overall, but this association appeared strongest among individuals with elevated CAC; suggesting that added caution may be needed when pursuing intensive BP treatment targets among persons with subclinical atherosclerosis.



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032

Acute Inhibition of HDACs After MI Leads to Suppression of Angiogenesis

PrimaryAuthor.AuthorBlock:**Sudhakar - Veeranki**, Suresh C Tyagi, Univ of Louisville, Louisville, KY

Aim: Although histone deacetylase inhibitors (HDACi) confer protection after myocardial injury (MI), the changes in the profiles of the angiogenic regulators are unknown after the usage of clinically approved HDAC inhibitors. **Methods:** All the mice underwent permanent LAD ligation. The mice were treated with Belinostat (PXD101), a clinically approved HDACi, for a period of 3 days at a dose of 100 mg/kg, I.P., daily after the creation of MI. Mice were sacrificed after 72 hrs of MI and the ischemic and non-ischemic portions of heart tissue were collected. Relative levels of 53

angiogenesis-related proteins were quantified by using angiogenic arrays. Heart function was evaluated before and after 72 hrs of MI injury. Markers of inflammatory cells (macrophages and neutrophils) and endothelial progenitor cells (EPCs) were assayed in the ischemic tissues. Levels of different cytokines were measured in the plasma. **Results:** Belinostat (PXD101) treatment failed to ameliorate heart function after the 72 hrs of treatment. There were specific changes in the levels of EPC chemokines such as CXCL1 and MCP1 after the treatment. Further assaying of various endothelial markers revealed diminished endothelial migration and differentiation in the ischemic tissues after the treatment, which was coincided with reduced macrophage presence. The transcriptional ability of the two major regulators of CXCL1 and MCP1, STAT3 and NFκB, was suppressed. Reduced IL-6 signaling was found to be the major cause behind the low inflammatory profile, diminished inflammatory cell presence and declined EPC presence. **Conclusions:** Acute inhibition of HDACs through Belinostat (PXD101) led to lower inflammatory profile and neoangiogenesis after MI. Our study underscored the complex role of HDACi in the regulation of myocardial angiogenic responses after MI injury and suggested careful reexamination before their translational use. It was also noted that the timing of HDACi use after the MI is crucial in the successful translation of HDACi into the clinic.

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033

Depressor Mechanisms of Nicotinamide in a Murine Model of Preeclampsia

PrimaryAuthor.AuthorBlock:Tomofumi Fushima, Hiroshi Sato, Sadayoshi Ito, **Nobuyuki Takahashi**, Tohoku Univ, Sendai, Japan

Introduction: We have reported that upregulation of the endothelin (ET) system plays a pivotal role in a mouse model of preeclampsia. Because ETA receptor antagonists are teratogenic, we cannot use them to treat pregnant women. Nicotinamide (Nam) inhibits ADP ribosyl cyclase (ADPRC), diminishes a release of Ca^{2+} from intracellular stores, and dilates vessels constricted by ET. We have recently reported that Nam ameliorates the high BP induced by excessive sFlt-1. Nam also shows beneficial effects on pregnancy maintenance and fetal growth. **Objective:** The aim of the present study is to clarify the depressor mechanisms of Nam. **Methods:** We measured tail-cuff BP before and 5 days after administering $1x10^9$ pfu sFlt-1 in non-pregnant WT mice and mice lacking CD38, the major form of ADPRC. We next measured SBP using radiotelemetry to investigate effects of Nam 500 mg/kg BW by using DAB (2,2'-dihydroxyazobenzene, a specific inhibitor of ADPRC) 0.1 mmol/kg BW, CrMP (chromium mesoporphyrin, a specific inhibitor of heme oxygenase) 2 μmol/kg, or fasudil (a Rho kinase inhibitor) 40 mg/kg. **Results:** Lack of CD38 prevented sFlt-1 induced hypertension (WT 106.0 ± 2.1 mmHg, CD38-/- 105.0 ± 2.1 mmHg, WT sFlt-1 126.3 ± 2.0 mmHg, CD38-/- sFlt-1 110.2 ± 1.2 mmHg), and diminished the decrease in SBP by Nam. DAB transiently decreased SBP, whereas Nam has a depressor effect lasting more than an hour. Pretreatment with CrMP for 30 min reversed the hypotensive effect of Nam within 60min. Both Nam and fasudil decreased elevated BP by sFlt-1, and the

combination of them additively decreased BP (sFlt-1 145.9 ± 3.6 mmHg, sFlt-1+Nam 122.9 ± 7.1 mmHg, sFlt-1+fasudil 93.6 ± 9.7 mmHg, sFlt-1+Nam+fasudil 81.9 ± 8.9 mmHg). **Conclusions:** Nam decreases BP in mice by inhibiting ADPRC and by increasing the production of HO-1. Fasudil explains why depressor effect of Nam is smaller than that of inhibiting ETAR. However, because fasudil is teratogenic, Nam is a promising drug to treat hypertension in preeclampsia.

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034

Remote Patient Monitoring for Postpartum Hypertension: A Pilot Study

PrimaryAuthor.AuthorBlock:**Kara K Hoppe**, Julia B Zella, Univ of Wisconsin-Madison, Madison, WI; Nicole A Thomas, UnityPoint Health-Meriter, Madison, WI; Heather M Johnson, Univ of Wisconsin-Madison, Madison, WI

Background: Postpartum hypertension is a leading indication for hospital readmission and maternal mortality. Objective: To conduct a pilot intervention to establish the feasibility of telehealth monitoring/treatment after hospital discharge for women with or at risk of developing severe postpartum hypertension (SBP≥150 or DBP≥105 mmHg). Methods: This prospective intervention at a Midwestern academic group practice included adult women with a hypertension-related diagnosis in the antenatal and/or postpartum period. Prior to discharge, patients received a tablet computer and blue tooth equipment (scale, blood pressure, oxygen saturation and heart rate monitor) to transmit home vital signs daily to a nurse. Patients had a nurse telehealth visit 48 hours and 7 days post-discharge. A nurse-driven outpatient treatment algorithm was developed and utilized for initiation and/or cessation of antihypertensive medications. Study follow-up was 6 weeks postpartum, followed by transfer to routine clinical care. Results: Among the 121 women with a hypertension-related disorder of pregnancy; 32 of the intended 55 have been enrolled to date (26%). [HJ2] Among the study patients, 6/32 (19%) were discharged on antihypertensive medication, of which 4/6 (67%) required an increase in the dose after discharge. After discharge, 10/32 (31%) required antihypertensive initiation after discharge due to development of severe hypertension. Overall, postpartum blood pressures became severe on day 4.9 (mean SBP was 158mmHg and DBP was 96mmHg). Among enrolled participants, there were two emergency room visits, however no hospital readmissions; 3/32 (9%) discontinued the study prior to study completion due to social situations. Conclusions: The preliminary results of our pilot intervention demonstrate feasibility and patient acceptability with telehealth monitoring for postpartum hypertension-related disorders. Furthermore, we demonstrated important temporal trends in the natural history of postpartum hypertension from discharge through 6 weeks postpartum which will guide larger clinical trials. Telehealth monitoring is a promising outpatient treatment strategy for postpartum hypertension to reduce readmissions and decrease maternal morbidity.

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Fetal Growth Restriction is Associated with Spontaneous Gestational Hypertension in African Green Monkeys

PrimaryAuthor.AuthorBlock:**Chelsea C Weaver**, Univ of Kentucky, Lexington, KY; Justin L Grobe, Mark K Santillan, Univ of Iowa, Iowa City, IA; Jeffrey L Osborn, Univ of Kentucky, Lexington, KY

Hypertensive pregnancy disorders, including gestational hypertension (GH) and preeclampsia (PE), are a major cause of worldwide maternal and fetal mortality. The only treatment for PE is delivery of the infant and placenta. Elucidating the cause of GH and PE is critical for improving diagnosis and therapy. This study provides the pregnant African Green Monkey (AGM; *Chlorocebus aethiops sabeus*) as the first known nonhuman model of spontaneous GH with pathophysiological characteristics of PE. Here, maternal blood pressures, water balance, renal electrolyte excretion, and fetal birth weight are characterized in AGMs. Following ketamine sedation (15 mg/kg i.m.), systolic blood pressures (SBP) were obtained in AGMs using forearm plethysmography. Animals were defined as normotensive (NT; SBP < 120 mmHg), hypertensive (HT; SBP ≥ 140 mmHg), or GH (SBP increased from non-HT to ≥15 mmHg in 3rd trimester). Nonpregnant SBP for NT AGMs was 98.7 ± 3.7 mmHg (n = 18), for HT AGMs was 154.7 ± 5.7 mmHg (n = 6), and for GH AGMs was 111.3 ± 6.1 mmHg (n = 7). Third trimester SBP for NT AGMs was 102.9 ± 4.0 mmHg (n = 13), and for GH AGMs was 146.3 ± 4.6 mmHg (n = 7). SBP decreased by 5.2 ± 8.1 mmHg in NT pregnancies (n = 5) and increased 35.0 ± 8.1 mmHg in GH pregnancies (n = 6).There were no differences in water intake or 24-hour urinary Na^+/K^+ excretions among AGMs regardless of group. Infant birth weight was lower in GH AGMs than that of NT AGMs (GH 233.1 ± 27.0 g, n = 5; NT 314.5 ± 16.7 g, n = 7; $p<0.05$). These data show GH in the AGM is associated with fetal growth restriction, decreased late pregnancy SBP in controls, and increased SBP in GH, similar to human pregnancies. Future studies include examination of renal glomerular structure, placental histopathology and transcriptomics, protein excretions, and plasma PE biomarkers to further establish this animal as a spontaneous model of GH and possibly PE. The AGM, having diverged from the human lineage approximately 29 million years ago, has close genetic homology to humans. This evolutionary history has led to a shared organ physiology, circadian rhythmicity, upright posture, and complex familial and behavior systems with humans, making it a highly translational animal model of hypertensive pregnancy disorders critical to cardiovascular research.

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036

Regulation of sFlt-1 Splicing by U2AF and JMJD

PrimaryAuthor.AuthorBlock:**Adrian C Eddy**, Heather Chapman, Eric M George, Univ of MS Medical Ctr, Jackson, MS

Preeclampsia (PE) is a gestational disorder defined by new-onset hypertension and maternal endothelial dysfunction. Though its

origins are unclear, placental ischemia is thought to be the central cause. In response, the placenta releases pathogenic factors, such as the anti-angiogenic soluble form of the VEGF receptor Flt-1 (sFlt-1), into the maternal circulation. sFlt-1 is a soluble splice variant of the VEGF receptor Flt-1, acting as a VEGF sink in the circulation. The regulation of sFlt-1 splicing is not completely understood, but it is proposed that the splicing factor U2AF65 promotes splicing, while JMJD6 opposes it by degrading U2AF65. Here, we test the hypothesis that decreased expression of U2AF65 will decrease sFlt-1 release, while decreased JMJD6 will increase sFlt-1 release. Using immortalized placental trophoblasts (BeWo), siRNA was used to knock down the expression of U2AF65, confirmed by RT-qPCR (1.014 $\pm$ 0.12 vs 0.379 $\pm$ 0.131; $p<0.05$). After transfection, cells were incubated at 8% and 1% oxygen for 24 hours, mimicking the conditions found in the healthy and ischemic placenta respectively. As hypothesized, U2AF65 knockdown caused a 52% decrease in released sFlt-1 compared to controls in hypoxia (56.8 $\pm$ 9.4 pg/mL vs 118.9 $\pm$ 9.2 pg/mL; $p<0.05$). siRNA successfully reduced JMDJ6 mRNA (1.008 $\pm$ 0.09 vs 0.3698 $\pm$ 0.04; $p<0.05$) and demonstrated undetectable protein levels. Unexpectedly, this resulted in a 33.8% decrease (342.7 $\pm$ 35.8 pg/mL vs 517.8 $\pm$ 15.4 pg/mL; $p<0.005$) in the amount of sFlt-1 release from cells in hypoxia and a 49% decrease (246.2 $\pm$ 23.6 pg/mL vs 482.8 $\pm$ 39.4 pg/mL; $p<0.001$) in normoxia compared to their respective controls. We also measured both U2AF65 and JMJD6 in the placentas of control rats and those that had chronic placental ischemia due to the Reduced Uterine Perfusion Pressure (RUPP) procedure. RUPP placentas showed a 61.6% increase in U2AF65 compared to controls (161.6 $\pm$ 11.6 AU vs 100 $\pm$ 9.8 AU; $p<0.005$) and a 121% increase in JMJD6 in RUPP placentas (221.1 $\pm$ 30.4 AU vs 100 $\pm$ 40.9 AU; $p= 0.0549$). Based on these data, we speculate that both JMJD6 and U2AF65 are crucial for the production of sFlt-1 by affecting alternative splicing. Targeting these factors could prove a useful approach for reducing sFlt-1 production in the preeclampsia patient.

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037

Exaggerated Placental Ischemia-induced Hypertension in Endothelin Receptor Type B (ETB)-deficient Pregnant Rats s Independent of Increased sFlt-1 or ROS Levels

PrimaryAuthor.AuthorBlock:**Frank T Spradley**, U of Mississippi Medical Ctr, Jackson, MS

While the pathogenesis of preeclampsia is not fully understood, studies implicate placental ischemia. Reduced uterine perfusion pressure (RUPP)-induced placental ischemia/hypoxia in animal models stimulates release of factors like antiangiogenic sFlt-1 into the maternal circulation increasing vascular-renal ET-1. ET-1 promotes hypertension via reactive oxygen species (ROS). Blockade of vasoconstrictive ETA abolishes RUPP hypertension. Deficiency of vasodilatory ETB in rats leads to increased blood pressure in pregnancy. While ETB deficiency markedly enhances RUPP hypertension, it is unknown if there is exaggerated RUPP-induced sFlt-1, ET-1 or ROS levels in ETB-def rats. The hypothesis was tested that placental ischemia/hypoxia-induced release of sFlt-1 and circulating ET-1 and ROS are greater in ETB-def rats. Eighteen-week-old ETB-def and transgenic (Tg) control pregnant rats were generated with Wistar Hannover males. RUPP or Sham

surgeries were on gestational day 14 and assessment of plasmas and placentas at day 19. RUPP increased placental sFlt-1 (pg/mg) similarly in RUPP ETB-def (781±113, N=5) vs Sham ETB-def (573±54, N=12) and RUPP Tg (631±62, N=5) vs Sham Tg (547±31, N=12) (P<0.05). In placental explant cultures, acute hypoxia (48 h 1% O2 vs normoxia 6% O2) stimulated a comparable release of sFlt-1 (pg/mg) in Sham ETB-def (2577±135 vs 2070±78) and Sham Tg (3208±318 vs 2553±107) (P<0.05). Unexpectedly, plasma sFlt-1 (pg/mL) was lower in RUPP ETB-def (153±48) vs Sham ETB-def (476±125) and RUPP Tg (238±32) vs Sham Tg (463±102) (P<0.05). Plasma ET-1 (fmol/L) was exaggerated in RUPP ETB-def (954±70) and greater in Sham ETB-def (735±43) vs RUPP Tg (122±14) or Sham Tg (142±41) (P<0.05). Plasma H2O2 (umol/L) was not exaggerated in RUPP ETB-def (5.4±1.2) or RUPP Tg (4.0±0.5) but was greater (P<0.05) in Sham ETB-def (6.2±0.3) vs Sham Tg (3.6±0.3). In conclusion, these data suggest in 1) normal pregnancy, ETB is crucial for blood pressure control by regulating bioavailable ET-1 to prevent ROS production and 2) placental ischemia, ETB reduces excess ET-1 to buffer hypertension independently of sFlt-1 or ROS. These data support ETB physiology as important in controlling blood pressure in pregnancy and its loss in mediating hypertension in preeclampsia.

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038

MiR-210 Overexpression Induces sFlt1 Production and Contributes to Preeclampsia-like Symptoms in Pregnant Mice

PrimaryAuthor.AuthorBlock:**Valorie L. Chiasson**, Kelsey R. Bounds, Lu J. Pan, Rachel M. Engelbrecht, Baylor Scott & White HL, Temple, TX; Piyali Chatterjee, Baylor Scott & White HL/Texas A&M HSC, Temple, TX

Preeclampsia (PE) is an obstetric complication that is diagnosed by hypertension and proteinuria at or after 20 weeks of gestation. PE is a multifactorial disease, but it is widely accepted that impaired proangiogenic factors such as vascular endothelial growth factor (VEGF), placental growth factor (PlGF) and antiangiogenic factor such as soluble FMS-like tyrosine kinase-1 (sFlt1) or VEGF receptor-1 balance during pregnancy is linked to PE. sFlt1, a splice variant of Flt1 binds to either VEGF or PlGF and blocks their action. In addition, placental angiogenesis is known to be regulated by specific miRNAs that are typically dysregulated during PE highlighting the importance of these miRNAs in pregnancy disorders. miR-210 is upregulated in placentas and serum/plasma of PE women and is also known to modulate angiogenic pathways. Therefore, we hypothesized that miR-210 overexpression contributes to the development of PE-like symptoms by altering the angiogenic/antiangiogenic pathway. To this end, we demonstrate increased systolic blood pressure (SBP: miR-210 TG P = 115 ± 2 mm Hg vs. WT P = 95 ± 2 mm Hg, p<0.05), endothelial dysfunction and proteinuria in pregnant miR-210 TG mice as compared to control WT mice. Placental sFlt-1 levels increased and PlGF levels decreased in miR-210 TG mice as determined by immunoblotting. Immunohistochemistry (IHC) analysis demonstrated an increase in sFlt1 and a decrease in PlGF immunoreactivity in miR-210

TG placentas. Pregnant miR-210 TG mice exhibited increased serum levels of sFlt-1 as well as decreased PlGF as compared to pregnant WT mice. Furthermore, immunohistological analysis of stained human placental sections revealed an increase in sFlt-1 and a decrease in PlGF in pregnant miR-210 TG as compared to pregnant WT mice. To determine the placental etiology, human cytotrophoblasts (CTBs) overexpressing miR-210 demonstrated a decrease in PlGF while inhibition of miR-210 in CTBs using anti-miR-210 oligos increased the PlGF expression as determined by immunoblotting. These data taken together suggest that miR-210 modulates the angiogenic/antiangiogenic pathway and contributes to PE-like features in mice. Thus, targeting miR-210 by delivering anti-miR-210 oligos may prevent PE-like symptoms in pregnant women.

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039

Blockade of Macula Densa NOS1 Decreases GFR and Promotes the Development of Hypertension During Pregnancy

PrimaryAuthor.AuthorBlock:**Jin Wei**, Jie Zhang, Univ of South Florida, Tampa, FL; Suttira Intapad, Tulane Univ Sch of Med, New Orleans, LA; Lei Wang, Shan Jiang, Univ of South Florida, Tampa, FL; Joey Granger, Univ of Mississippi Medical Ctr, Jackson, MS; Ruisheng Liu, Univ of South Florida, Tampa, FL

We hypothesized that neuronal nitric oxide synthesis (NOS1) β in the macula densa promotes the renal hemodynamic changes during normal pregnancy and inhibition of macula densa NOS1 induces hypertension during pregnancy. First, we measured the protein levels of NOS1β in the renal cortex, which increased by 11.5±1.7 folds in pregnant mice (n=5) compared with virgin mice (n=7, $p<0.01$ vs virgin), while there was no change in NOS1α. Then, in isolated and perfused juxtaglomerular apparatus, we measured tubuloglomerular feedback (TGF)-induced NO generation by the macula densa, which increased by 66.7±6.1% in pregnant mice (n=5) compared with the virgin mice (n=5, $p<0.01$ vs virgin). We next measured TGF *in vivo* using micropuncture, which was 3.6±0.3 mmHg in mice at day 19 of pregnancy (n=3) and 5.5±0.7 mmHg in virgin mice (n=7, $p<0.05$ vs virgin). To determine the significance of the macula densa NOS1, we measured glomerular filtration rate (GFR) and mean arterial pressure (MAP) with telemetry in macula densa specific NOS1 knockout (KO) mice ($NKCC2^{cre}$; $NOS1^{flox/flox}$) and WT ($NOS1^{flox/flox}$) mice. In the WT mice, GFR raised by 25.7±1.1% while the MAP decreased by 6.1±2.7 mmHg at day 18 of pregnancy (n=7, $p<0.01$). The elevations in GFR were largely blunted in KO mice with only a 13.7±2.8% increase while MAP gradually rose by 28.2±3.3 mmHg above basal level at day 18 of pregnancy (n=5, $p<0.05$ vs baseline). Finally, we examined macula densa NOS1 in a mouse model of preeclampsia, a reduced uterine perfusion pressure (RUPP) model. At day 19 of pregnancy, the protein levels of NOS1β decreased 71.4±9.6% (n=5), NO generation reduced 26.7±5.8% (n=5) and TGF *in vivo* increased 41.6±12.2% (n=3) in RUPP mice, compared with normal pregnant mice ($p<0.05$). Moreover, the MAP or GFR was no significant different between the pregnant KO mice with RUPP and the pregnant KO mice. In conclusion, upregulated macula densa NOS1β blunts TGF response and promotes elevation of GFR during normal pregnancy. In contrast, downregulation

of NOS1β in the macula densa enhances the TGF response, decreases GFR and promotes hypertension during pregnancy. These results suggest that inhibition of macula densa NOS1β could be an important mechanism mediating the decrease in GFR and elevation in MAP in preeclampsia.

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040

Low Soluble Fms-like Tyrosine Kinase-1, Endoglin, and Endothelin-1 Levels in Women With Confirmed or Suspected Preeclampsia Using Proton Pump Inhibitors

PrimaryAuthor.AuthorBlock:**Alexander H Danser**, Langeza Saleh, Willy Visser, Anton H van den Meiracker, ERASMUS MC, Rotterdam, Netherlands

Preeclamptic patients display elevated placenta-derived soluble Fms-like tyrosine kinase-1 (sFlt-1) and endoglin levels, and decreased placental growth factor (PlGF) levels. Proton pump inhibitors (PPIs) decrease trophoblast sFlt-1 and endoglin secretion in vitro. PPIs are used during pregnancy, to combat reflux disease. Here we investigated whether PPIs affect sFlt-1 in women with confirmed/suspected preeclampsia, making use of a prospective cohort study involving 430 women. Of these women, 40 took PPIs (6 esomeprazole, 32 omeprazole and 2 pantoprazole) for 8-45 (median 29) days before sFlt-1 measurement. Measurements were only made once, at study entry between weeks 20-41 (median 33 weeks). PPI use associated with lower sFlt-1 levels, with no change in PlGF levels, both when compared to all non-PPI users, and to 80 gestational age-matched controls selected from the non-PPI users. No sFlt-1/PlGF alterations were observed in women using ferrous fumarate or macrogol, while, as expected, women using antihypertensive medication displayed higher sFlt-1 levels and/or lower PlGF levels. The PPI use-associated decrease in sFlt-1 was independent of the application of antihypertensive drugs and also occurred when restricting our analysis to patients with hypertensive disease of pregnancy at study entry. PPI users displayed more cases with preexisting proteinuria, less gestational hypertension, and a lower number of neonatal sepsis cases. Finally, their plasma endoglin and endothelin-1 levels were lower, while sFlt-1 levels correlated positively with both. In conclusion, PPI use associates with low sFlt-1, endoglin, and endothelin-1 levels, warranting prospective trials to investigate the therapeutic potential of PPIs in preeclampsia.

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041

New Evidence for G Protein-coupled Estrogen Receptor as a Natriuretic Factor

PrimaryAuthor.AuthorBlock:**Eman Y Gohar**, Ijeoma E Obi, Carmen De Miguel, Jennifer S Pollock, David M Pollock, Univ of Alabama at Birmingham, Birmingham, AL

Premenopausal women have a lower risk of cardiovascular and renal diseases compared to

age-matched men. This protection includes fewer salt-dependent complications. We have shown that female rats have a more robust natriuretic capacity compared to males in response to increased dietary salt. The novel estrogen receptor, G protein-coupled estrogen receptor (GPER), is a membrane-bound receptor linked to acute signaling pathways. GPER activation elicits protective effects throughout the cardiovascular system. However, its role in sodium handling is not defined. We hypothesized that activation of GPER in the renal medulla stimulates sodium excretion. In female Sprague Dawley (SD) rats, isosmotic saline was infused into the renal medullary interstitium (500 µl/h) during a 60-80 min equilibration period and 20 min baseline urine collection period. This was followed by infusion of the GPER agonist G1 (50 pmol/kg/min) or vehicle into the renal medulla for two further 20 min periods. Compared with vehicle, G1 significantly increased urinary sodium excretion and urine flow (from 0.5 ± 0.1 to 0.9 ± 0.2 µmol/min and from 5.3±1.1 to 8.3±1.6 µl/min, respectively, n=6, $p<0.05$). Urinary potassium excretion and mean arterial pressure remained unchanged during the experiments (0.5 ± 0.1 vs. 0.4 ± 0.1 µmol/min and 108.6 ± 3.4 vs. 107.2 ± 5.1 mmHg, respectively, n=6). Immunohistochemical analysis of GPER revealed more predominant staining in both inner and outer renal medulla in female SD rats, compared to males. GPER appears to be predominantly expressed in interstitial cells. These data reveal that renal medullary GPER plays an important role in renal sodium handling and may account for the enhanced ability to handle increased dietary salt in females.
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042

Evidence of Angiotensin II (AngII)-dependent Obesity-induced Hypertension in Female Mice Exposed to Postnatal Neglect

PrimaryAuthor.AuthorBlock:Kierrariel Mitchell, Dianne Cohn, Univ of Kentucky, Lexington, KY; **Analia S Loria**, Univ of Kentucky, Lexington, KY

Previously, we have shown that female mice subjected to maternal separation with early weaning (MSEW), a model of postnatal neglect, display exacerbated diet-induced obesity and high blood pressure (BP) compared with control mice. Female MSEW mice show activated renin-angiotensin system components, including increased plasma renin activity and adipose tissue-derived angiotensinogen secretion. The goal of this study was to test whether augmented obesity-induced hypertension in female MSEW mice is AngII-dependent. Mouse MSEW was achieved by repeated, daily separations from the dam and 4-day early weaning. Normally reared controls (C) were weaned at postnatal day 21. Each experimental group of female weanlings was comprised of 6 mice each and derived from 3 different litters, that were placed on high fat diet (HFD, 60% kcal from fat). After 18 weeks, mice were implanted with radiotelemetry for BP measurement. At week 20, average 24-hr systolic blood pressure (SBP) was 134±2 mmHg in MSEW mice and 126±2 in C ($P<0.05$). No significant changes in mean arterial pressure, diastolic blood pressure or heart rate were observed between groups. Next, we also determined the BP sensitivity to the acute administration of AngII (1, 10 and 50 ug/kg, s.c.). AngII-induced BP changes, assessed

by BP area under the curve, were similar between MSEW and C mice at all doses (50 ug/kg dose:145±10 vs. 132±15 mmHgx30 min, respectively). Chronic enalapril treatment (2.5 mg/kg/day, drinking water, 7 days) was conducted to block endogenous AngII synthesis. Enalapril reduced SBP 15±2 mmHg in MSEW mice but only 6±1 mmHg in C mice ($p<0.05$). The BP response to acute AngII doses increased similarly in MSEW and C enalapril-treated mice, (50 ug/kg dose: 200±13 vs. 207±22 mmHgx30 min, respectively). In addition, BP and HR responses to acute injections (i.p) of mecamylamine (5 mg/kg), propranolol (5 mg/kg) or atropine (1 mg/kg) were similar between untreated MSEW and C mice, suggesting that exacerbated BP in female MSEW mice is independent of sympathetic or parasympathetic dysfunction. Taken together, these data provide evidence that increased BP in female MSEW mice results from elevated circulating AngII rather than enhanced AngII sensitivity or sympathetic nerve activity.

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043

Influence of Sex, Hormones and Age on Cerebrovascular Function: Role of Large Conductance Potassium Channel Subunits

PrimaryAuthor.AuthorBlock:**Mallikarjuna R Pabbidi**, Maniselvan Kuppusamy, Tanya Pareek, Barbara T Alexander, Ashley D Newsome, UMMC, Jackson, MS

Although adult females are protected, the cerebrovascular disease is greater in aged post-menopause females than in age matched males, but the role of vascular function and large conductance potassium (BK) channel is not clear. We hypothesize that "compared to adult males, cerebral vasculature of intact adult female rats displays an attenuated myogenic response and greater intrinsic tone due to differential expression and function of BK channel subunits. Aging or menopause exaggerates myogenic response, diminishes intrinsic tone and thus, vasodilatory capacity in females more than males due to a reduction in the BK subunit function." Our results suggest that middle cerebral arteries (MCA) of intact adult females had attenuated myogenic response compared to adult males and ovariectomy (OVX) females (% change in diameter: 16±8, -25±4 and -49±7). Development of intrinsic tone was greater in intact adult females than adult males and OVX females (% intrinsic tone: 47±5, 27±4 and 15±5). The ratio of BK β1 to α subunits was less in intact adult females than adult males and OVX females (0.4±0.07, 2±0.3 and 0.6±0.1). Spontaneous transient outward currents (STOC) were higher in smooth muscle cells of intact adult females compared to adult males (pA: 204±27 and 128±7). While aging exaggerated the myogenic response in females, it had no effect in males (fold change 4±1 Vs 0.8±0.5). However, intrinsic tone was decreased in aged females but increased in aged males (% change -43±9 Vs 37±6). Aging decreased the amplitude of STOCs but with a greater magnitude of effect in females (fold change females: -4.9±1; males: -0.8±0.2). While aging increased BKα subunit, it decreased β1 subunit protein (β1 change: females -52±8; males -37±13). Acute activation of BKα subunit by NS1619, while had no effect in intact adult females (-5±3%), it dilated the MCA of adult males (32±9%). However, acute activation of BKβ1 by estradiol while relaxed MCA of adult females (35±7%) and males (22±2%), it had less effect in OVX females (-7±8%) and aged females (4±9%). In conclusion, a higher incidence of cerebrovascular disease in aged and

menopause female may be due to sex-specific changes in vascular properties and BK channels that could result in diminished estradiol-mediated vasodilation.

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044

Sex Differences in Relative Contributions of Hemodynamic Parameters to Blood Pressure: A Population-based Study of Adolescents and Middle-aged Adults

PrimaryAuthor.AuthorBlock:**Catriona A Syme**, Jean Shin, The Hosp for Sick Children, Toronto, ON, Canada; Michal Abrahamowicz, Gabriel Leonard, McGill Univ, Montreal, QC, Canada; Michel Perron, Louis Richer, Suzanne Veillette, Univ du Québec à Chicoutimi, Chicoutimi, QC, Canada; Daniel Gaudet, Univ de Montréal, Chicoutimi, QC, Canada; Tomas Paus, Rotman Res Inst, Toronto, ON, Canada; Zdenka Pausova, The Hosp for Sick Children, Toronto, ON, Canada

Hypertension is a leading cause of death. Sex differences in blood pressure (BP) and hypertension prevalence emerge during adolescence and remain throughout reproductive age. Sex differences in BP-underlying hemodynamics, i.e., in the relative contributions of stroke volume (SV), total peripheral resistance (TPR) and heart rate (HR) to BP, have not been investigated in these age categories in a population-based setting. We studied a cohort of 1,347 individuals, including 911 adolescents (12-18 years, 52 % female) and 426 young to middle-aged adults (36-65 years, 56 % female). Beat-by-beat systolic BP (SBP) and diastolic BP (DBP), together with HR, SV, and TPR, were measured with a Finometer throughout a 52-min protocol; the protocol was intended to “mimic” daily-life activities, such as changes in posture and mental stress. It is well established that BP during regular daily activities (ambulatory BP monitoring) is a better predictor of target-organ damage than standard office BP. The relative contributions of HR, SV and TPR to SBP and DBP were determined by decomposing the model-explained variance into non-negative contributions. The relative contributions of SV, TPR and HR to SBP and DBP showed marked sex differences in young and middle-aged adults. The main determinant of higher SBP was SV in females (55 [50-60] % in females vs. only 35 [30-40] % in males), whereas it was TPR in males (47 [41-52] % in males vs. only 30 [26-34] % in females). The main determinant of higher DBP was TPR in both sexes, but its contribution was higher in males than females (58 [52-63] % vs. 41 [36-45] %, respectively). These sex differences were seen across most of the 52-min protocol, being most prominent during standing and least evident during mental stress. Similar sex differences were observed in adolescents, but they were less pronounced, being significant only during standing. The present population-based study of adolescents and young to middle-aged adults, suggest that marked sex differences exist in BP-underlying hemodynamics, with BP being driven mainly by SV in females and by TPR in males. These results underscore the need for sex-specific treatments of hypertension (not recommended currently), which affects 3-5 % of adolescents and 23-58% of young to middle-aged adults.

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045

The Enhanced Pressor Response to AngII in Aged Females is Attenuated by Estrogen Replacement via an AT_2R-mediated Mechanism

PrimaryAuthor.AuthorBlock:**Giannie Barsha**, Katrina M Mirabito Colafella, Tracey Gaspari, Iresha Spizzo, Lucinda M Hilliard, Robert E Widdop, Chrishan S Samuel, Kate M Denton, Monash Univ, Melbourne, Australia

Loss of estrogen (E_2) following menopause contributes to the sharp rise in cardiovascular risk with age. E_2 is postulated to play a protective role against hypertension and end-organ damage by counterbalancing the pressor actions of the RAS and enhancing the depressor RAS pathways. The aim was to determine whether E_2 replacement in aged females can lower arterial pressure and improve endothelial function via an AT_2R-mediated mechanism. MAP was measured via telemetry in ovary-intact adult (4-month old), aged (17-month old) and aged+E_2 (3 µg/day sc) FVB/N female mice, which were co-treated with vehicle, AngII (600 ng/kg/min sc) or AngII+PD (PD123319, AT_2R antagonist; 3 mg/kg/day sc). On day 21 of treatment, endothelium-dependent relaxation in response to acetylcholine was assessed in aortic vessels. Cardiac and renal, tissue fibrosis and gene expression of E_2 receptors and RAS components were also analysed. Basal MAP was lower in E_2-treated aged mice (90±1 mmHg, n=20) relative to adult (94±1 mmHg, n=10) and aged controls (94±1 mmHg; n=21, both P<0.05). Similar to previous studies, the AngII pressor response was enhanced in aged compared to adult females (Figure). E_2 treatment reduced the AngII pressor response in aged females (Figure). Moreover, the attenuated pressor response observed in the aged E_2+AngII group was abolished by co-infusion with PD (Figure). Endothelial function was markedly impaired with age (~23% reduced maximal vasodilation, P=0.03) and worsened by AngII infusion (~38%, P=0.001), however E_2 did not significantly improve the response in any group. In conclusion, E_2 replacement may reinstate cardio-protection in aged females via an AT_2R-mediated mechanism.

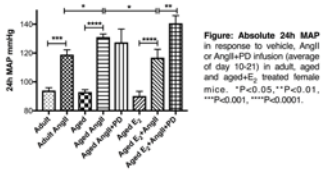

Figure: Absolute 24h MAP in response to vehicle, AngII or AngII+PD infusion (average of day 10-21) in adult, aged and aged+E_2 treated female mice. *P<0.05,**P<0.01, ***P<0.001, ****P<0.0001.

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046

Chronic Estrogen Replacement Prevents the Increase in Blood Pressure in Female IUGR Offspring at 12 Months of Age

PrimaryAuthor.AuthorBlock:**Gwendolyn K. Davis**, Ashley D. Newsome, Norma B. Ojeda, Barbara T. Alexander, Univ of MS Medical Ctr, Jackson, MS

Low birth weight **(LBW**) is associated with an earlier age at menopause and a greater prevalence of hypertension in women in later life. Using a rodent model of LBW induced by placental insufficiency, previous studies from our lab indicate that intrauterine growth restriction (**IUGR**) programs a significant increase in blood pressure (**BP**) by 12 months of age in female IUGR offspring. Female IUGR are also acyclic by 12 months of age, or 6 months prior to control indicative of early reproductive senescence. The mechanisms involved in menopause related cardiovascular risk are not clear. Furthermore, the link between birth weight and the greater prevalence for hypertension in LBW women in later life is not known. Female IUGR exhibit a shift in the testosterone to estradiol ratio at 12 months of age. Thus, this study tested the hypothesis that a loss of estradiol (**E2**) contributes to the increase in BP in female IUGR offspring that develops by 12 months of age. Female rats received vehicle or 17β-Estradiol valerate minipellets (1.5mg for 60-day release) for 6 weeks starting at 12 months of age with BP measured in conscious, chronically catheterized rats following treatment (2way ANOVA; Tukey's multiple comparison). BP was significantly increased in vehicle-treated IUGR relative to vehicle-treated Control (*, $p<0.05$); an increase that was abolished by chronic E2 in IUGR (**, $p<0.05$). Uterine weight, a crude marker of E2, was significantly increased by chronic E2 in both Control and IUGR E2-treated offspring (**, $p<0.05$). Therefore, this study indicates that E2 withdrawal after early reproductive senescence contributes to the increase in BP that develops in later life in female IUGR offspring.

Blood Pressure (mmHg)		
	Vehicle	E2-Treated
Control	109±7	119±2
IUGR	139±6*	118±4**
	Vehicle	E2-Treated
Control	0.59±0.05	0.97±0.03**
IUGR	0.53±0.07	0.82±0.06**

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047

Clinical Outcomes by Race and Ethnicity in the Systolic Blood Pressure Intervention Trials (SPRINT): A Randomized Control Trial

PrimaryAuthor.AuthorBlock:**Carolyn H Still**, Case Western Reserve Univ, FPB Sch of Nursing, Cleveland, OH; Carlos J Rodriguez, Wake Forest Sch of Med, Winston-Salem, NC; Jackson T Wright Jr, Case Western Reserve Univ, Sch of Med, Cleveland, OH; Timothy E Craven, Wake Forest Sch of Med, Winston-Salem, NC; Adam P Bress, Univ of Utah, Sch of Med, Div of Health System Innovation and Res, Dept of Public Health Sciences, Salt Lake City, UT; Glenn M Chertow, Stanford Univ Sch of Med, Div of Nephrology, Palto Alto, CA; Paul K Whelton, Tulane Univ Sch of Public Health and Tropical Med, New Orleans, LA; Jeffrey C Whittle, Dept of Med, medical Coll of Wisconsin, Milwaukee and Primary Care, Clememt J. Zablocki Veterans Affairs Medical Ctr, Milwaukee, WI; Barry I Freeman, Wake Forest Sch of Med, Winston-Salem, NC; Karen C Johnson, Dept of Preventive Med, Univ of Tennessee Health Science Ctr, Memphis, TN; Capri G Foy, Wake Forest Sch of Med, Dept of Social Science and Health Policy, Winston-Salem, NC; Jiang He MD, Dept of Epidemiology, Tulane Univ Sch of Public Health and Tropical Med, New Orleans, LA; John B Kostis, Cardiovascular Inst, Rutgers Robert Wood Johnson Medical Sch, New Brunswick, NJ; Jame P Lash, Dept of Med, Univ of Illinois

Chicago, Chicago, IL; Carolyn F Pedley, Wake Forest Baptist Health, Winston-Salem, NC; Roberto Pisoni, Dept of Med, Univ of South Carolina, Charleston, SC; James R Powell, Div of Med, Brody Sch of Med, East Carolina Univ, Greenville, NC; Barry M Wall, Memphis Veterans Affairs Medical Ctr, Memphis, TN

Background: Lowering systolic blood pressure (SBP) reduces cardiovascular disease morbidity and mortality; however, appropriate SBP targets, especially by race/ethnicity remain uncertain.
Methods and Results: We examined the effects of an intensive SBP goal (<120 mm Hg) compared to the current recommendation (< 140 mmHg) on cardiovascular disease (CVD) outcomes in racial-ethnic groups in SPRINT (Systolic Blood Pressure Intervention Trial). High-risk non-diabetic patients with hypertension (N = 9,361; 30% Black; 11% Hispanic), 50 years and older were enrolled at 102 clinical sites across the U.S. and Puerto Rico. Primary outcome was a composite of the first occurrence of a myocardial infarction, acute coronary syndrome, stroke, decompensated heart failure, or CVD death. Average ± SD post-baseline SBP across race/ethnic groups ranged from 134.7±0.1 to 135.5±0.2 mmHg in the standard arm compared to 119.9±0.4 to 122.6±0.2 in the intensive arm. Intensive vs. standard arm hazard ratios [HRs] (95% CI) for the primary outcome were 0.70 (0.57-0.86), 0.71 (0.51-0.98), 0.62 (0.33-1.15) in Non-Hispanic Whites, Non-Hispanic Blacks, and Hispanics respectively. CVD mortality HRs were 0.49 (0.29-0.81), 0.77 (0.37-1.57), and 0.17 (0.01-1.08) with all-cause mortality HRs 0.61 (0.47-0.80), 0.92 (0.63-1.35), and 1.58 (0.73-3.62). Tests for interaction were not statistically significant after adjustment for multiple comparisons.
Conclusion: Regardless of racial/ethnic origin, there are cardiovascular benefits from treating to a SBP target of < 120 mmHg compared to <140 mmHg.

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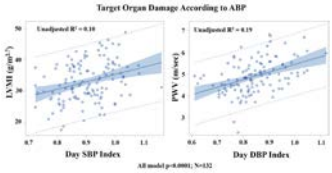
048

Systolic and Diastolic Ambulatory Blood Pressure Affect Target Organ Damage Differently in Adolescents: The SHIP AHOY Study

PrimaryAuthor.AuthorBlock:**Gilad Hamdani**, Elaine M Urbina, Cincinnati Children's Hosp Medical Ctr, Cincinnati, OH; Marc Lande, Univ of Rochester Medical Ctr, Rochester, NY; Kevin Meyers, Children's Hosp of Philadelphia, Phildelphia, PA; Joshua Samuels, Univ of Texas Health Sciences Ctr, Houston, TX; Joseph T Flynn, Seattle Children's Hosp, Seattle, WA

Hypertensive target organ damage (TOD) is associated with increased risk for CV events. Ambulatory BP (ABP) measures are more strongly related to TOD than casual BP in adults but data in youth are lacking. Our objective was to determine which ABP parameters associated with TOD in adolescents. We evaluated casual BP (mean of 6 measures by auscultation), ABP (Spacelabs OnTrak), anthropometrics, labs, LVM, pulse wave velocity (PWV), diastolic function (E/E' ratio), and systolic function (global longitudinal strain, GLS) in 132 adolescents (mean 15.8±1.4 yrs, 66% white,

57% male). Day, night and 24H SBP and DBP index (mean/95th %ile for sex and height) and loads (%readings above the 95th %ile) were defined according to sex and height-specific pediatric cut-points. General linear models were used to determine independent associations between ABP and TOD. Only systolic ABP means and loads were associated with LVMI and diastolic function, while both systolic and diastolic ABP means and loads were associated with PWV. There was a weak association between systolic and diastolic loads and GLS. In multiple regression analysis (full model: demographics, age, BMI, HR, ABP, metabolic profile, CRP) day SBP index was the strongest predictor of LVMI (β=15.2, R^2 0.4, p=0.006) and E/E' (β=5.2, R^2 0.23, all p=0.007), while day DBP index was the strongest predictor of PWV (β=3.0, R^2 0.37, p<0.0001). Day DBP load was the sole independent ABP predictor of GLS (β=0.05, R^2 0.25, p=0.02). We conclude that during adolescence, systolic and diastolic ABP parameters are differentially associated with TOD: SBP predicted LVMI, while DBP predicted PWV. ABP parameters may be used to evaluate risk for BP-related TOD.



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049

Prevention of Glomerular Hyperfiltration by Enhancement of Tubuloglomerular Feedback Response Induces Hypertension and Exacerbates Diabetic Kidney Injury

PrimaryAuthor.AuthorBlock:**Jie Zhang**, Jin Wei, Shan Jiang, Lei Wang, Shaohui Wang, Ruisheng Liu, The Univ of South Florida, Tampa, FL

The mechanism underlying the higher prevalence of hypertension in diabetes remains unclear. We hypothesized that blunted tubuloglomerular feedback (TGF) mediated by the neuronal nitric oxide synthesis (NOS1) β in the macula densa promotes glomerular hyperfiltration in diabetes, whereas inadequate NOS1β in the macula densa restricts glomerular hyperfiltration, induces hypertension and exacerbates diabetic kidney injury. First, we measured the protein levels of NOS1β in the renal cortex, which was 6.5±1.2 fold higher in db/db mice than in db/+ mice (n=5, p<0.01). Then, in isolated and perfused juxtaglomerular

apparatus, we measured TGF-induced NO generation by the macula densa, which was 115.4±9.8 Units/min in db/+ mice and 161.5±12.7 Units/min in db/db mice (n=5, p<0.01). We next measured TGF *in vivo* using micropuncture, which was 3.7±0.4 mmHg in db/db mice and 5.3±0.3 mmHg in db/+ mice (n=5, p<0.05). Then we developed a macula densa specific NOS1 knockout db/db mouse line (KO) ($Lepr^{db/db}$; $NKCC2^{cre}$; $NOS1^{flox/flox}$). Littermate db/db ($Lepr^{db/db}$; $NOS1^{flox/flox}$) and db/+ ($Lepr^{db/+}$; $NOS1^{flox/flox}$) mice serve as diabetic and non-diabetic controls. Glomerular filtration rate (GFR) was 236±18 µl/min in db/+ mice, and significantly increased to 375±12 and 284±11 µl/min in the db/db and KO mice (n=4-5, p<0.01). Telemetric mean arterial pressure (MAP) was 97.1±3.5 and 103.6±5.2 mmHg in db/+ and db/db mice, and significantly raised to 124.4±8.1 mmHg in the KO mice (n=4-5, p<0.01). Proteinuria increased by 70 and 120 folds in the db/db and KO mice compared with db/+ mice (n=4, p<0.01). The kidney histology was assessed with light and transmission electron microscopy in the mice at age of 24 weeks. The diabetic kidney injury score was 0.37±0.16 in the db/+ mice, and significantly increased to 1.14±0.2 and 2.37±0.67 in the db/db and KO mice (n=5, p<0.01). In conclusion, upregulation of the macula densa NOS1 blunts TGF response and promotes elevation of GFR in diabetes. Contrary to popular belief about glomerular hyperfiltration in diabetes, our data suggest that directly limiting the increase in GFR by enhancement of TGF response is detrimental, which induces hypertension and exacerbates diabetic kidney injury.

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050

TLR4 Antagonism Prevents Early Left Ventricular Hypertrophy and Dysfunction Associated With Neonatal Hyperoxia Exposure

PrimaryAuthor.AuthorBlock:**Muhammad Oneeb Rehman Mian**, Ying He, Rafael Fernandes, Mariane Bertagnolli, Anik Cloutier, Thuy Mai Luu, Anne Monique Nuyt, CHU Sainte-Justine, Montreal, QC, Canada

OBJECTIVE: Preterm birth is associated with proinflammatory conditions early in life. Our lab has shown in rats that transient neonatal exposure to high O_2, an established model of prematurity-related conditions, leads to early CV inflammation and remodeling. TLR4 signaling is a critical link between inflammation and the pathogenesis of CVD. Whether programmed innate immune activation, via TLR4 signaling, impacts long term CVD is unknown. In our rat model of prematurity we investigated whether neonatal TLR4 antagonism will prevent the development of CV dysfunction. **METHODS:** Male Sprague-Dawley pups were kept with their mother in 80% O_2 or room-air from day (P)3 to 10 of life. At P10, cardiac TLR4 protein expression was assessed. In other experiments, pups were treated i.p. with TLR4 antagonist LPSRS (100 µg/kg) or vehicle (0.9% NaCl) at P3, P6 and P9 (concomitant to O_2 exposure; n=6-9/group, max 3 animals/group/liter). At 4, 7 and 12 wks, body weights were measured and left ventricular (LV) echocardiography was performed under isoflurane using VEVO 3100 (VisualSonics). At 12 wks, central mean BP was measured under isoflurane by catheterism (BIOPAC, n=4-6). Comparisons were made using T-test or one-way ANOVA. **RESULTS:** At P10, cardiac TLR4 protein expression was increased

~2 fold in O_2-exposed pups compared to room-air controls (P<0.05). At 4 wks, body weight in vehicle- or LPSRS-treated O_2-exposed animals (101±2 g and 106±2 g) was lower compared to room-air vehicles (117±2 g, P<0.01). Compared to room-air vehicles, vehicle- but not LPSRS-treated O_2-exposed animals exhibited increased LV mass index (3.8±0.1 and 3.4±0.1 vs 3.3±0.1 mg/g, P<0.05 for O_2-exposed vehicles vs controls), reduced ejection fraction (74±2 and 79±2 vs 82±1 %, P<0.05) and fractional shortening (43±2 and 48±2 vs 52±2 %, P<0.01), reduced cardiac output index (0.43±0.02 and 0.48±0.02 vs 0.56±0.03 ml/min/g, P<0.01), and decreased mitral E/A ratio (1.3±0.1 and 1.7±0.1 vs 1.7±0.1 mg/g, P<0.01). Findings were similar at 7 and 12 wks. At 12 wks, mean BP was higher in vehicle- but not LPSRS-treated O_2-exposed animals, compared to controls (95±4 and 89±4 vs 81±4 mmHg, P<0.05). **CONCLUSION:** TLR4 antagonism prevents BP rise and LV hypertrophy and dysfunction associated to neonatal exposure to hyperoxia.

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051

Influence of Sex and Obesity on the Effect of Preterm Birth on the Renin-angiotensin System in Adolescents

PrimaryAuthor.AuthorBlock:**Andrew Michael South**, Patricia A. Nixon, Mark C. Chappell, Debra I. Diz, Gregory B. Russell, Elizabeth T. Jensen, Hossam A. Shaltout, Lisa K. Washburn, Wake Forest Sch of Med, Winston Salem, NC

Background:
Preterm birth increases the risk of cardiovascular disease, but the underlying mechanisms are not known. Prematurity may induce programming effects that differentially influence the renin-angiotensin (Ang) system (RAS), particularly suppression of the beneficial Ang-(1-7) axis. Patient factors such as sex and obesity may influence the degree of RAS programming. Therefore, we hypothesize that preterm birth is associated with alterations in the RAS in adolescence in a sex and adiposity-dependent manner.
Methods:
We evaluated a cohort of 175 adolescents born preterm and 51 term controls at age 14 years. We recorded systolic and diastolic BP z-scores, measured Ang II and Ang-(1-7) levels in plasma and urine, and calculated the peptide ratios. We applied generalized linear models to estimate the association between preterm birth and the RAS, adjusting for race, socioeconomic status, and maternal hypertension and smoking; the models were stratified by sex and overweight/obesity (body mass index ≥85th %ile for age and sex).
Results:
Mean systolic and diastolic BP z-scores were higher among those born preterm (p<0.001 and p=0.03, respectively). Relative to term birth, preterm birth was associated with an increased plasma ratio of Ang II to Ang-(1-7) (β: 4.42, 95% CI 1.52 to 7.32), decreased Ang II (β: -5.16 pmol/L, -10.28 to -0.04), decreased Ang-(1-7) (β: -5.38 pmol/L, -8.66 to -2.09), and a decreased urinary ratio of Ang II to Ang-(1-7) (β: -0.13, -0.26 to -0.003). In stratified analyses, female sex (β: -7.14 pmol/L, -11.03 to -3.24) and overweight/obesity (β: -8.21 pmol/L, -12.51 to -3.91) were associated with greater reductions in plasma Ang-(1-7). Overweight/obesity was associated with a greater increase in the ratio of plasma Ang II to Ang-(1-7) (β: +6.13, 0.58 to 11.68).
Conclusions:

Circulating Ang-(1-7) was lower relative to Ang II in adolescents born preterm. This suggests fetal RAS programming may contribute to the increased risk of cardiovascular disease in those born preterm. We note an important influence of sex in that the decrease in Ang-(1-7) is intensified in girls. Moreover, obesity may confer a second physiologic insult through a higher Ang II/Ang-(1-7) that exacerbates the risk of cardiovascular disease, including hypertension.

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052

Chronic Angiotensin Type 2 Receptor Stimulation Abolishes the Sex-difference in Ang II-induced Hypertension

PrimaryAuthor.AuthorBlock:**Katrina M Mirabito Colafella**, Lucinda M Hilliard, Monash Univ, Melbourne, Australia; Donna L Ralph, Jasmine S Manwani, Keck Sch of Med of USC, Los Angeles, CA; Tracey Gaspari, Robert E Widdop, Monash Univ, Melbourne, Australia; Thomas Unger, CARIM, Maastricht Univ, Maastricht, Netherlands; U. Muscha Steckelings, Univ of Southern Denmark, Odense, Denmark; Kate M Denton, Monash Univ, Melbourne, Australia

Arterial pressure is age and sex dependent. The angiotensin type 2 receptor (AT_2R) plays a greater role in the regulation of arterial pressure and renal function in adult females than age-matched males and aging females. In the present study, we investigated the effect of chronic AT_2R stimulation using the AT_2R agonist, compound 21 (C21), to attenuate the hypertensive effects of AngII. Mean arterial pressure (MAP) was measured via radiotelemetry in 14 week old male and female mice treated with vehicle, AngII (36 µg/kg/hr), C21 (18 µg/kg/hr), AngII+C21 or AngII+C21 plus PD123319 (AT_2R antagonist; 125 µg/kg/hr) for 21 days. At the end of the treatment, renal excretory function, angiotensin receptor expression and sodium transporter profiles were assessed. In agreement with previous studies, the pressor response to AngII was greater in male than female mice (35±3 vs 20±4 mmHg respectively, $P<0.05$). There was no effect of C21 treatment alone on MAP nor did C21 alter the pressor response to AngII in females. However, in males, C21 blunted the pressor response to AngII such that MAP was similar between AngII+C21 males, AngII females and AngII+C21 females (18±2, 20±4 and 17±4 mmHg on day 21, respectively). Co-infusion of

PD123319 restored the normal pressor response to AngII in males. Conversely, in females, treatment with AngII+PD123319 or AngII+C21+PD123319 enhanced the pressor response to AngII by ~20 mmHg, to a level seen in AngII males. The renal AT_2R/AT_1R ratio was greater in female than male mice and was not affected by treatment. Vehicle treated females had lower proximal versus distal Na+ transporter abundance than males. AngII-treatment increased NCC in both sexes, while proximal and loop transporters (NHE, NHE3-P, NaPi2 and NKCC2) decreased in males only. Treatment with C21 alone did not significantly affect sodium transporters. In males, AngII+C21treatment increased distal transporters and this effect was reversed by PD123319. Conversely, in females, C21 blunted the AngII effect on sodium transporters (claudin-2, NKCC-P, NCC-P and ENaC). Our novel data demonstrate that chronic AT_2R stimulation attenuates AngII-induced hypertension in adult males, but not females. Thus, AT_2R agonists may be a novel antihypertensive therapy for males and ageing females.

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053

Urinary Extracellular Messenger RNA Transcripts as Biomarkers of Mineralocorticoid Receptor Activity

PrimaryAuthor.AuthorBlock:**J. Brian Byrd**, Brian G. Bazzell, William E. Rainey, Richard J. Auchus, Scott L. Hummel, Univ of Michigan, Ann Arbor, MI

Objectives: Mineralocorticoid receptor (MR) activation regulates sodium homeostasis & blood pressure. Our objective was to identify a non-invasive biomarker of MR activation in humans.
Background: MR is a ligand-activated transcription factor that alters expression of target genes in the distal renal tubule, as shown in cell & animal models. Renal tubular cells secrete extracellular vesicles which carry RNA into urine. Whether human urinary extracellular mRNA reflects changes in gene expression in the renal tubule & thus might provide insight into MR activation is unknown.
Methods: Pre-hypertensive participants consumed a low-sodium diet followed by sodium loading. We designed qPCR assays for MR gene targets & control genes not expected to respond to MR activation. Using samples collected during low-sodium diet & after sodium infusion, we assayed plasma renin activity (PRA), serum aldosterone, urinary sodium & urinary MR-responsive & control extracellular mRNA.
Results: Eighteen participants' low-sodium & 17 participants' sodium-loaded urine samples were available. PRA & serum aldosterone were higher (P<0.001) & urinary sodium excretion was lower (P<0.001) during low-sodium diet. Four of 14 target gene qPCR assays (29%) changed after sodium loading, including assays for *SCNN1A*, *SCNN1G* & *TSC22D3* (P=0.006 to 0.01), whereas an assay for *SGK1* approached significance (P=0.07). In contrast, only 1 out of 10 control gene assays (for *NR3C2*, encoding MR itself) was significantly different after sodium loading (P=0.003). Log serum aldosterone inversely associated with C_t values for 7 of 14 target gene assays (including assays for *SCNN1A*, *SCNN1G*, *SGK1* & *TSC22D3* [r= -0.46 to -0.52, P=0.003 to

0.048]) & for control gene assays for *NR3C2* & *UMOD*. C_t values for 6 of 14 target gene qPCR assays associated with log urinary sodium/creatinine ratio including assays for *SCNN1A*, *SCNN1G*, *SGK1* & *TSC22D3* associated with log urinary sodium/creatinine ratio ([r= 0.36 to 0.52, *P*=0.003 to 0.048]) but not with control gene assays.
Conclusions: Perturbations in human endocrine physiology can be detected as changes in urinary extracellular mRNA. Our findings suggest a new strategy to screen for mineralocorticoid excess & to assess pharmacologic or dietary changes in MR activity.

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054

Visinin Like Protein 1 Regulation of Aldosterone Biosynthesis

PrimaryAuthor.AuthorBlock:**Maniselvan Kuppusamy**, Jeanne Ishimwe, Univ of Mississippi Medical Ctr, Jackson, MS; Tracy A Williams, Paolo Mulatero, Univ of Turin, Turin, Italy; Elise P Gomez-Sanchez, Celso E Gomez-Sanchez, Univ of Mississippi Medical Ctr, Jackson, MS

Visinin-Like Protein-1(Vsnl1) is a member of the EF-hand calcium sensor proteins. Its mRNA was found to be upregulated in aldosterone-producing adenomas and to be expressed in the zona glomerulosa (ZG) of the rat and human adrenal. We studied the expression pattern of Vsnl1 and its co-localization with the CYP11B2 enzyme and ZG marker Dlk1 (ZOG) using double and triple immunofluorescence in adrenal glands of rats on normal, high and low sodium diets and the effect of Vsnl1 on calcium mobilization and aldosterone biosynthesis in the human adrenocarcinoma cell line, the HAC15 cell. In rat adrenals, Vsnl1 was expressed only in ZG, in the same cells as Dlk1, in both functional and undifferentiated ZG cells. CYP11B2 co-localized with Vsnl1 in nearly all ZG cells of rats on a low sodium diet. Co-expression of CYP11B2 with Vsnl1 in adrenals of rats on normal and high sodium diets was significantly less. HAC15 were transduced with a lentivirus carrying Vsnl1 or an empty virus. Three days later cells were incubated with vehicle, A-II 10

nM, Forskolin 10µM, or potassium 16 mM. Aldosterone and cortisol were measured after 24 hr. Basal secretion of aldosterone was not increased; cortisol was. Aldosterone and cortisol secretion were greater after A-II, forskolin or potassium stimulation in cells overexpressing Vsnl1. Intracellular calcium measured by Fluo-4 AM dye and proliferation were increased by Vsnl1 overexpression. HAC15 cells were transduced with 2 different shRNA for Vsnl1. Aldosterone synthesis was reduced in HAC15 cells transduced with the shRNA for Vsnl1 stimulated with A-II. Vsnl1 has a myristoylation consensus sequence at the N-terminal domain. Site directed mutagenesis of Vsnl1 at the 2 position (G2A) resulted in a significant decrease in the aldosterone response to A-II. In summary, the calcium sensor protein Vsnl1 is ZG specific and detected in both steroidogenically active and inactive cells, as well as undifferentiated cells in the subcapsular area of the rat adrenal. Vsnl1 expression and activity in HAC15 cell correlate positively with calcium mobilization, proliferation and aldosterone synthesis.

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055

Systemic Effect of Renal 11β-HSD2 Deficiency on Blood Pressure Regulation

PrimaryAuthor.AuthorBlock:**Kohei Ueda**, Mitsuhiro Nishimoto, Daigoro Hirohama, Nobuhiro Ayuzawa, Wakako Kawarazaki, Atsushi Watanabe, RCAST, The Univ of Tokyo, Tokyo, Japan; Tatsuo Shimosawa, Dept of Clinical Lab, Intl Univ of Health and Welfare, Sch of Med, Tokyo, Japan; Johannes Loffing, Natl Ctr of Competence in Res 'Kidney Control of Homeostasis', Zurich, Switzerland; Ming-Zhi Zhang, Vanderbilt Univ Sch of Med, Nashville, TN; Takeshi Marumo, Toshiro Fujita, RCAST, The Univ of Tokyo, Tokyo, Japan

Background:
Renal mechanism of 11β-HSD2 deficiency for developing hypertension is to be evaluated because vascular mechanism associated with sympathetic nervous activity was prevailing in global *Hsd11b2* knockout (KO) mice although brain-specific KO mice needed high salt intake to develop hypertension. We have demonstrated the importance of renal 11β-HSD2 deficiency on developing hypertension by using kidney-specific *Hsd11b2* knockout (*Hsd11b2*$^{Ksp-/-}$; KS-KO) mice (*Hypertension*, in press) and have continued the analysis of the systemic effect of renal 11β-HSD2 deficiency.
Method:
Blood pressure (BP) and heart rate (HR) was measured by using 24h telemetry. Amiloride (25 mg/L) and hydrochlorothiazide (HCTZ, 300 mg/L) were administered through drinking water. Pellet containing MR antagonist spironolactone (MRA; 50 mg/KgBW/day) was administered subcutaneously. Corticosterone concentration was determined by ELISA. Data are presented as mean±SE.
Result:
Systolic and diastolic BPs of KS-KO mice were significantly higher, although the HR was lower, than those of WT mice: SBP, 142.4±1.0 vs 122.4±0.8 mmHg; DBP, 103.9±0.8 vs 94.4±0.8 mmHg; HR, 492.5±2.7 vs 555.4±2.4 (n=7). Mean BP was decreased to the level of WT mice by reducing dietary sodium content from 0.3 % to 0.01 %. Plasma [K^+] was significantly lower in KS-KO mice: 2.9±0.2 vs 4.2±0.2 mEq/L (n=5). Renal membrane expressions of NCC, T53-phosphorylated NCC (pNCC), cleaved ENaCα and full-length ENaCα were upregulated in KS-

KO mice. Correction of plasma [K^+] of KS-KO mice by using high KCl diet or amiloride downregulated the renal membrane expression of pNCC and decreased the MBP to the level of WT mice as well as chronic HCTZ-treated KS-KO mice. Subcutaneous administration of MRA decreased MBP of KS-KO mice and the renal membrane expressions of pNCC and cleaved ENaCα. Diurnal variation of plasma corticosterone concentration was diminished in KS-KO mice and the urinary excretion of corticosterone was higher compared to that in WT mice.
Conclusion: Renal 11β-HSD2 deficiency is sufficient in developing hypertension via MR activation induced by excessive corticosterone, the systemic effect of which are also suggested.

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056

Differential Roles of GRK2 and GRK5 in Cardiac Aldosterone Signaling Suggest GRK5-Mediated Cardio-protection Against Mineralocorticoids

PrimaryAuthor.AuthorBlock:**Anastasios Lymperopoulos**, Katie A McCrink, Ava Brill, Jennifer Maning, Victoria L Desimine, Beatrix Aukszi, Nova Southeastern Univ, Fort Lauderdale, FL

Background: Aldosterone (Aldo) contributes significantly to the morbidity & mortality of heart failure (HF). In the heart, Aldo binds the mineralocorticoid receptor (MR) to exert damaging effects, e.g. fibrosis, apoptosis, oxidative stress, etc. Additionally, Aldo can activate the G protein-coupled estrogen receptor (GPER), which may have beneficial, anti-apoptotic effects for cardiomyocytes, e.g. in ischemia/reperfusion injury. GRK2 and GRK5 are the most abundant G protein-coupled receptor (GPCR)-kinases (GRKs) in the heart and both phosphorylate GPCRs but also non-GPCR substrates. The human MR is known to undergo inhibitory phosphorylation at Ser-843, inside its ligand binding domain, which blocks its transcriptional activity. Hypothesis: In the heart, GRK5 phosphorylates and inhibits the MR, whereas GRK2 phosphorylates and desensitizes GPER.
Methods: We used the cardiomyocyte cell line H9c2 and isolated adult rat ventricular myocytes (ARVMs). We performed co-immunoprecipitation experiments for GRK interactions with MR or GPER. We measured MR phosphorylation via immunoblotting and MR transcriptional activity via the luciferase reporter assay. We also measured apoptosis and oxidative stress in ARVMs.
Results: GRK5, but not GRK2, phosphorylates the MR in H9c2 cardiomyocytes. $Beta_2$-adrenoceptor activation stimulates this non-canonical effect of GRK5. In contrast, GRK2, but not GRK5, phosphorylates and desensitizes agonist-activated GPER. The GRK5-phosphorylated MR is incapable of activating gene transcription, since MR transcriptional activity is markedly suppressed upon GRK5 overexpression. Conversely, CRISPR-mediated GRK5 gene deletion augments cardiac MR transcriptional activity. Importantly, GRK5 is necessary for the protective effects of the MR antagonist drug eplerenone against Aldo-induced apoptosis/oxidative stress in ARVMs. Finally, $beta_2$-adrenoceptor-stimulated GRK5 phosphorylates and inhibits the MR in ARVMs.
Conclusions: GRK5 blocks the cardio-toxic MR-dependent effects of Aldo in the heart, whereas GRK2 may hinder GPER`s cardio-protective effects. Thus, cardiac GRK5 stimulation (e.g. via

beta$_2$-adrenoceptor activation) enhances the cardio-protection exerted by Aldo inhibitors in HF.

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057

Cellular and Genetic Causes of Idiopathic Hyperaldosteronism

PrimaryAuthor.AuthorBlock:**Kei Omata**, Univ of Michigan, Ann Arbor, MI; Fumitoshi Satoh, Ryo Morimoto, Sadayoshi Ito, Yuto Yamazaki, Tohoku Univ, Sendai, Japan; Yasuhiro Nakamura, Tohoku Medical and Pharmaceutical Univ, Sendai, Japan; Sharath Anand, Univ of Michigan, Ann Arbor, MI; Zeng Guo, Michael Stowasser, Univ of Queensland Diamantina Inst, Brisbane, Australia; Hironobu Sasano, Tohoku Univ, Sendai, Japan; Scott Tomlins, William Rainey, Univ of Michigan, Ann Arbor, MI

Background. Primary aldosteronism (PA) affects ~10% of hypertensive patients and has unilateral and bilateral forms (30%:70%). Unilateral PA is caused by aldosterone-producing adenomas (APA), which express CYP11B2 (aldosterone synthase) and frequently harbor somatic mutations in aldosterone regulating genes (*KCNJ5>>CACNA1D*). We recently demonstrated that adrenals from normotensive patients present with pockets of cell expressing aldosterone synthase (CYP11B2). These aldosterone-producing foci (APF) have somatic gene mutations similar to those found in APA (*CACNA1D>>KCNJ5*). Bilateral PA, which is typically treated by mineralocorticoid receptor (MR) blockade rather than surgery, is termed idiopathic hyperaldosteronism (IHA). Its pathobiology is largely unknown but has been thought to be due to zona glomerulosa (ZG) hyperplasia. *Methods*. We studied 11 IHA patients (7 males, 4 females) who had unilateral adrenalectomy. Immunohistochemistry for CYP11B2 and next generation sequencing (NGS) targeting genes found in APA were performed on formalin fixed paraffin embedded adrenal tissue. Results were compared to previously described cohorts of 53 age-matched normotensive patients (29 males, 24 females) which were evaluated similarly. *Results*. CYP11B2 expression was absent from intervening ZG cells in 8/11 (73%) IHA adrenals, but all adrenals harbored at least one APF. The median number and size of APF per case were significantly larger in IHA than normotensive controls (6.1 vs 0 APF/cm^2 of adrenal cortex and 0.25 vs. 0.16 mm^2, respectively; $p<0.0001$ and $p<0.006$). In this IHA cohort, NGS identified *CACNA1D* and *KCNJ5* somatic mutations in 44/71 (62%) and 1/71 (1%) of APF, respectively. *Interpretations.* Diffuse CYP11B2 expression in adrenal ZG cells was only observed in 3/11 IHA cases, arguing against ZG hyperplasia as the major underlying pathobiology. Rather, we demonstrated increased and enlarged APF in IHA adrenals compared to normotensive controls, supporting potential contribution to the clinical manifestations of hyperaldosteronism. The frequent occurrence of aldosterone-dysregulating *CACNA1D* somatic mutations in APF support *CACNA1D* as a potential therapeutic target in IHA to complement current MR blockade approaches.

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058

Deoxycorticosterone-induced Hypertension Caused by Fluconazole

PrimaryAuthor.AuthorBlock:Matthew Hakimi, Sandeep Krishnan, O'Neil Mason, Joshua Trabulus, Phillip Zakowski, Cedars Sinai Medical Ctr, Los Angeles, CA; Michael McPhaul, Quest Labs, San Juan Capistrano, CA; Richard Auchus, Univ of Michigan, Ann Arbor, MI; **Ronald G Victor**, Cedars Sinai Medical Ctr, Los Angeles, CA

Deoxycorticosterone (DOC), a steroid precursor of aldosterone, constitutes an endogenous mineralocorticoid receptor agonist. Exogenous DOC produces a well-established animal model of mineralocorticoid-induced salt-dependent hypertension (HTN), but, except for rare cases of congenital adrenal hyperplasia with pediatric HTN due to *CYP11β1* (11*β* hydroxylase, P450c11*β)* deficiency, DOC is rarely considered a cause of clinical hypertension (HTN). We recently suspected DOC as the novel mechanism of severe low-renin HTN (blood pressure, BP 220/150 mmHg, plasma renin activity 0.21 ng/mL/hour) that developed *de novo* in a 47- year old African American man with previously normal BP (118/74) during treatment of disseminated coccidiodomycosis with high-dose fluconazole, which blocks P450 enzymes in the fungal cell wall. To determine if the fluconazole also inhibited human CYP11β1, the adrenal P450 enzyme that converts DOC to corticosterone, we performed a comprehensive adrenal steroid panel on the patient's serum using mass spectrometry. During the HTN, his serum [DOC] was 24-times the upper limit of normal (354 vs. <15 ng/dL), while corticosterone was low and aldosterone was undetectable. Both the HTN and the high DOC were caused by fluconazole, because they normalized after treatment ended. We then proved that fluconazole causes dose-dependent inhibition of CYP11β1 *in vitro* by transfecting a mammalian (hamster V79) cell line with human *CYP11β1* and incubating the cells with DOC. The observed IC50—10 μM— is certainly less than the plasma [fluconazole] during oral high-dose therapy. From these data, we conclude that high-dose fluconazole inhibits DOC conversion by human CYP11β1, causing in this adult patient an acquired form of severe DOC-induced HTN. Given the recent 10-fold increase in coccidiodomycosis cases in the United States and the marked propensity for disseminated disease in African American patients, BP should be monitored carefully during treatment with high-dose fluconazole. Additional case finding studies are warranted.

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059

miR-29 is Required for Normal Endothelium-dependent Vasodilation and Protects Against Hypertension

PrimaryAuthor.AuthorBlock:**David M Jensen**, Michael E Widlansky, Mingyu Liang, Jingli Wang, Yong Liu, Aaron Geurts, Alison Kriegel, Pengyuan Liu, Rong Ying, Mobin Malik, Amberly Branum, Michael J Tanner, Kristie Usa, Marc

Casati, Guangyuan Zhang, Chen Chu, Medical Coll of Wisconsin, Milwaukee, WI

MicroRNA miR-29 is down-regulated in Dahl salt-sensitive (SS) rats compared to salt-insensitive SS-13^{BN} rats. We investigated the role of miR-29 in endothelial function and the development of hypertension. Using TALENs (Transcriptional Activator-Like Effector Nucleases) we deleted 4 nucleotides from *Mir29b-1* gene in SS-13^{BN} rats. The targeted mutation resulted in reduced abundance of miR-29b-3p and the co-transcribed miR-29a-3p in arteriolar endothelial cells. When stimulated with acetylcholine to induce endothelium-dependent vasodilation (EDVD), gluteal arterioles from *Mir29b-1*$^{-/-}$ rats were only able to dilate to an average 47% of maximum diameter while wild-type littermates dilated to 77% (N=7 and 9, respectively, p<0.05). The development of hypertension was significantly exacerbated in *Mir29b-1*$^{-/-}$ compared to *Mir29b-1*$^{+/+}$ littermates. Mean arterial blood pressure was 129 ± 2 mmHg in *Mir29b-1*$^{+/+}$ rats after 2 weeks of 4% NaCl diet and 140 ± 5 mmHg in *Mir29b-1*$^{-/-}$ rats (N=6, 10, respectively, p<0.05). Gluteal arterioles from *Mir29b-1*$^{-/-}$ rats exhibited significantly reduced nitric oxide (NO) levels compared to *Mir29b-1*$^{+/+}$ littermates as measured by DAF2-DA intensity (N=6, 11, respectively). Mutation of the *Mir29b-1* gene resulted in preferential differential expression of genes in arterioles related to the regulation of NO levels including Lypla1. Lypla1 is a direct target of miR-29 and could abrogate the effect of miR-29 in promoting NO production. Reduction of LYPLA1 by transfection of pre-miR-29b or a Lypla1 siRNA resulted in a significant increase in NO as measured by DAF2-DA in cultured human dermal microvascular endothelial cells. Taken together, we have shown that targeted mutation of *Mir29b-1* reduces EDVD and exacerbates the development of hypertension. We have identified a mechanism by which miR-29 affects NO production. These findings indicate miR-29 is required for normal endothelial function in rats and has therapeutic potential for hypertension.

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060

Role of Mir-214 in the Regulation of Perivascular Fibrosis in Angiotensin II Induced Hypertension

PrimaryAuthor.AuthorBlock:**Ryszard Nosalski**, Jagiellonian Univ Collegium Medicum, Krakow, Poland; Mateusz Siedliński, Mateusz Siedliński, Krakow, Poland; Aurelie Nguyen Dinh Cat, Cordeliers Res Ctr, INSERM, Paris, France; Dominik Skiba, Eilidh Mcginnigle, Univ of Glasgow, Glasgow, United Kingdom; Andrew Baker, Laura Denby, Univ of Edinburgh, Edinburgh, United Kingdom; Tomasz J. Guzik, Univ of Glasgow, Glasgow, United Kingdom

Objective: Hypertension (HT) is associated with perivascular inflammation and increased vascular fibrosis. MicroRNAs (miR) are a novel gene expression regulation mechanism and play a pivotal role in a range of pathological processes. The role and mechanism of miR214 in vascular fibrosis is unknown. **Methods:** 3-month-old C57BL/6, miR214KO and wild-type littermates were treated with angiotensin II (AngII, 490ng/kg/min; n=6-10) or control buffer for 14 days. PVATs from C57BL/6 animals were analysed using

TaqMan_Rodent_microRNA_Arrays. Histological studies, wire myography, lucigenin-enhanced luminometry and cytometrical analysis was conducted, followed by statistical analysis with ANOVA or t-test. Data are expressed as a mean±SEM. **Results:** Out of 381 miRs, 16 were significantly overexpressed in C57BL/6 AngII animals, with only miR214 showing 8-fold induction ($p<0.01$) after Bonferroni correction. Also, 3-fold elevation of pri-miR-214 was observed. Interestingly, hydralazine treatment prevented both these changes ($p<0.01$). AngII infusion in miR214 KO animals did not alter blood pressure when compared to WT mice. Mir214 KOs exhibited diminished peri-aortic fibrosis (44779±2491 vs 78805±8696µm, $p<0.01$), upon AngII hypertension. This was associated with a significantly reduced induction of COL1A1, COL3A1 and TGFβ1 mRNA expression in PVAT and aortas ($p<0.05$). Vascular studies revealed improved endothelial function (69±10 vs. 22±4%, $p<0.01$), protection against oxidative stress (66±7 vs 118±19 RLU/sec/mg, $p<0.001$) and NOX2 mRNA expression (1.9±0.2 vs1.1±0.1, $p<0.05$) in AngII miR-214-KO aortas, while these parameters were not altered in mesenteric arteries. Recruitment of T cells into aortic PVAT was abolished in KO HT animals in comparison to control group (192±65 vs. 603±164 cell/mg; $p<0.05$). AngII HT was associated with 4-fold increase of miR-214 expression in the circulating peripheral blood T cells and 2-fold in the spleen. Moreover, AngII infusion increased TNFα mRNA expression in WT T cells (1±0.1 vs 1.6±0, $p<0.01$) whereas this effect was not seen in miR214 KO T cells (0.9±0.3 vs 0.9±0.1). **Conclusions:** MiR-214 plays a major role in modulation of aortic fibrosis, vascular function, oxidative stress and perivascular inflammation.

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061

Loss of Lymphocyte Adaptor Protein LNK Promotes Acute Aortic Dissection

PrimaryAuthor.AuthorBlock:**Fanny C Laroumanie**, Vanderbilt Univ Medical Ctr, Nashville, TN; Mohamed A Saleh, Dept of Pharmacology and Toxicology, Mansoura University, Egypt; Bethany L Dale, Vanderbilt Univ Medical Ctr, Nashville, TN; Kyle A Gavulic, Vanderbilt Univ, Nashville, TN; William G McMaster, Meena S Madhur, Vanderbilt Univ Medical Ctr, Nashville, TN

Background: Acute aortic dissection (AD) is a life-threatening vascular disease associated with an inflammatory response. A polymorphism in the gene *SH2B3* that encodes LNK has been associated with several cardiovascular and autoimmune diseases in humans. LNK is an adaptor protein expressed in hematopoietic and endothelial cells that serves as a brake to cellular proliferation and cytokine production. We hypothesize that loss of LNK promotes AD through an exacerbation of the acute immune response.
Methods: Angiotensin II (Ang II) was infused for 3 or 14 days into wild type (WT) and $LNK^{-/-}$ mice. Kaplan-Meier survival curves were generated. After 3 days, the aortic remodeling was accessed by standard histological staining methods and microscopy. The aortic inflammation was characterized by flow cytometry and immunohistochemistry.
Results: Ang II infusion induces a rapid and drastic mortality in $LNK^{-/-}$ mice compared to WT mice (66% vs 8%, $P<0.001$). Necropsies revealed that deaths are due to the development of AD or rupture, localized primarily in the abdominal aorta. Interestingly, during the phase that

precedes AD development (day 3 of Ang II infusion), the aortas of $LNK^{-/-}$ mice show significant remodeling with more elastin fragmentation than WT mice (10.5 vs 4.6 breaks, *P*<0.01) and less adventitial collagen deposition (3.3 vs 4.6 $x10^4$ μm^2, *P*=0.057). Strikingly, collagen qualitative analysis reveals thinner collagen fibers and several areas of disruption and disorganization in the aorta of $LNK^{-/-}$ mice prior to AD development. In parallel, the aorta of $LNK^{-/-}$ mice show an increase in the number of neutrophils and macrophages but not in T cells compared to WT mice.
Conclusion: In our model, LNK seems to play a key role in maintaining the aortic wall integrity. Loss of LNK promotes acute inflammation and matrix degradation, leading to the development of AD. Targeting LNK could be a potential therapeutic strategy for the management of aortic dissection.

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062

Vascular Dysfunction and Hypertension are Prevented by a Novel PPARγ Target Gene, RhoBTB1

PrimaryAuthor.AuthorBlock:**Masashi Mukohda**, Stella-Rita C Ibeawuchi, Chunyan Hu, Ko-Ting Lu, Anand R Nair, Larry N Agbor, Jing Wu, Frederick W Quelle, Curt D Sigmund, Univ of Iowa, Iowa City, IA

RhoBTB1 is a novel peroxisome proliferator-activated receptor gamma (PPARγ) target gene expressed in smooth muscle cells (SMC) which may mediate some of the vascular protective and antihypertensive benefits of PPARγ. Here, we tested the hypothesis that RhoBTB1 can prevent angiotensin II (ANG)-induced hypertension. RhoBTB1 expression in aorta from C57BL/6 mice was decreased by 54±9% (n=16) in response to ANG infusion (490 ng/min/kg, 2 weeks). To test if RhoBTB1 expression is protective, we generated double transgenic mice with tamoxifen-inducible, Cre-dependent overexpression of RhoBTB1 specifically in SMC (S-RhoBTB1). S-RhoBTB1 and non-transgenic (NT) mice were treated with tamoxifen (Tx; 75 mg/kg, ip, 5 days) or vehicle (corn oil) and then ANG was infused. Although RhoBTB1 expression was decreased in ANG-infused control mice (p<0.01, n=8-10), RhoBTB1 expression in Tx-treated S-RhoBTB1 mice infused with ANG was restored to a level similar to NT treated with saline (n=11). Overexpression of RhoBTB1 did not alter baseline blood pressure (BP) in the absence of ANG (n=7-8). However, the increase in BP induced by ANG was significantly attenuated by RhoBTB1 restoration in S-RhoBTB1 mice with Tx compared to ANG-infused control mice (either NT with Tx, NT with corn oil, or S-RhoBTB1 with corn oil) in which RhoBTB1 was not restored (Systolic BP, 159±5 in control mice vs 132±6 mmHg in S-RhoBTB1 mice with Tx, p<0.01, n=7-8). We also observed increased heart weight in ANG-infused control mice, which was prevented in S-RhoBTB1 mice treated with Tx (p<0.05, n=8). Thoracic aorta and basilar artery from ANG-infused control mice exhibited impaired acetylcholine-induced endothelial-dependent relaxation (Aorta, 48±2%, p<0.01, n=6-8), which was prevented by restoration of RhoBTB1 in SMC (Aorta, 76±5%, p<0.01, n=6-8). Thoracic aorta from ANG-infused control mice also displayed decreased sodium nitroprusside-induced endothelial-independent relaxation with a right-shifted dose-response (76±9%, p<0.01, n=8), which was also prevented in

tamoxifen-treated S-RhoBTB1 mice (95±10%, $p<0.01$, n=8). We conclude that the novel PPARγ target gene, RhoBTB1, functions in SMC to specifically facilitate vasodilation and mediates a protective anti-hypertensive effect.

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063

Mechanical Stretch on Endothelial Cells Promotes Monocyte Activation and Differentiation into Immunogenic Dendritic Cells via STAT3

PrimaryAuthor.AuthorBlock:**Roxana Loperena**, Hana A Itani, Jose A Gomez, Noah Engel, Annet Kirabo, David G Harrison, Vanderbilt Univ, Nashville, TN

Mechanical stretch activates the endothelium to produce reactive oxygen species and expression of adhesion molecules and cytokines. Human monocytes that traverse the endothelium differentiate into dendritic cells (DCs) upon exposure to a pro-inflammatory state. We hypothesized that human endothelial cells exposed to mechanical stretch will promote conversion of human monocytes into activated DCs. We co-cultured human aortic endothelial cells (HAECs) with monocytes from normotensive human donors and exposed the endothelial cells to either normal (5%) or hypertensive (10%) uniaxial cyclical stretch for 48 hours. Co-culturing monocytes with HAECs exposed to 10% stretch showed a marked increase in pro-inflammatory cytokines such as IL-6, IL-23A and IL-1β compared to 5% stretch. HAECs exposed to 10% stretch promoted monocytes in culture to differentiate into DCs. We have shown that DCs from hypertensive mice accumulate isolevuglandins (IsoLGs) that adduct to proteins and promote T cell activation. Thus, we performed intracellular staining and flow cytometry and found that these monocytes significantly accumulate higher levels of IsoLG-adducted proteins compared to 5% stretch (69.7 ± 5.8 vs 10.8 ± 1.85, respectively). In addition, monocytes co-cultured with endothelial cells exposed to 10% stretch expressed phosphorylated STAT3 (pSTAT3), which was blocked by stattic, a STAT3 inhibitor. Similarly, monocytes co-cultured with HAECs exposed to 10% stretch induced a 1,500-fold and a 1,300-fold increase in $CD4^+$ and $CD8^+$ T cell proliferation; while, inhibition of STAT3 prevented this T cell proliferation. To test if this is due to cell-cell contact, we seeded monocytes in a transwell with endothelial cells exposed to either 5% or 10% stretch. We found that these monocytes expressed DC markers, pSTAT3, and accumulated IsoLG-peptides when exposed to hypertensive mechanical stretch. In addition, angiotensin II (490ng/kg/min) infusion of C57Bl/6 mice increased pSTAT3 in monocytes, macrophages, and DCs in the renal and vascular tissue compared to sham controls. Thus, our data indicate that endothelial cells exposed to mechanical stretch cross-talk with monocytes to promote differentiation into DCs known to be immunogenic via STAT3.

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Transactivation of Egfr by Tgfβ Induces Hypertension by Increasing Arteriolar Myogenic Response

PrimaryAuthor.AuthorBlock:**Daniela Carnevale**, Sapienza Univ/IRCCS Neuromed, Pozzilli, Italy; Manuel Casaburo, Stefania Fardella, Giuseppe Cifelli, Gualtiero Innocenzi, IRCCS Neuromed, Pozzilli, Italy; Giorgio Bressan, Univ of Padova, Pozzilli, Italy; Giuseppe Lembo, Sapienza Univ/IRCCS Neuromed, Pozzilli, Italy

Emilin1 (E1) is a protein of the extracellular matrix regulating TGFβ bioavailability through proTGFβ proteolysis. E1 KO mice are hypertensive with increased TGFβ activation. As E1 is expressed in vessels from embryonic life to adulthood, is still unknown whether the E1 KO phenotype results from a developmental defect or lack of homeostatic role in the adult. To dissect this issue, we used a conditional gene targeting inactivating E1 in smooth muscle cells (SMCs) of adult mice, by the use of E1flox/flox and tamoxifen (TAM) inducible Cre recombinase specific for SMCs. When E1fl/fl mice carrying the Smmhc-CreERT2 were given TAM blood pressure significantly increased (SBP: 123±2 vs basal condition 104±3;***p< 0.001) as well as myogenic tone (MT) of resistance arteries (16.3±0.7 vs basal condition 11.4±0.1 % at 125 mmHg). How increased TGFβ signaling in SMCs could determine an increased MT is still unknown. Relevant to this, we found that the higher TGFβ signaling in E1 KO SMCs stimulates heparin binding epidermal growth factor (HB-EGF) and subsequent transactivation of the EGF receptor, a mechanism typically implied in potentiating MT. When mesenteric resistance arteries from E1 were subjected to step-increases in intraluminal pressure, EGFR inhibition rescued the increased MT. At the molecular level, TGFβ-induced EGFR transactivation resulted into the activation of transient receptor potential classical type 6 (TRPC6) and melastatin type 4 (TRPM4) channels. To put our data into translational perspective, we measured MT of resistance arteries isolated from hypertensive patients and normotensive subjects, finding increased MT (HT 16.4±0.7; NT 11±0.4;***p< 0.001) and TGFβ signaling in the former group. By using a neutralizing anti-TGFβ or an anti-EGFR we found a normalization of the increased MT (HT+anti-TGFβ 11±1.3; NT+anti-TGFβ 11.2±1.2;***p< 0.001 and HT+anti-EGFR 9±0.6; NT+anti-EGFR 10.8±1.1;***p< 0.001), thus confirming the relevance of TGFβ-EGFR pathway in humans. Taken together these data suggest that primary increase of MT induced by TGFβ-EGFR transactivation can cause hypertension and that higher TGFβ signaling and MT are common alterations of resistance arteries of hypertensive patients.

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Induction of Human Endothelin-1 Overexpression for 3 Months Causes Blood Pressure Rise and Renal Injury

PrimaryAuthor.AuthorBlock:**Pierre Paradis**, Suellen C. Coelho, Olga Berillo, Sofiane Ouerd,

Júlio C. Fraulob-Aquino, Antoine Caillon, Tlili Barhoumi, Lady Davis Inst for Medical Res, SMBD-Jewish General Hosp, McGill Univ, Montreal, QC, Canada; Stefan Offermanns, Max-Planck-Inst for Heart and Lung Res, Bad Nauheim, Germany; Ernesto L. Schiffrin, Lady Davis Inst for Medical Res, SMBD-Jewish General Hosp, McGill Univ, Montreal, QC, Canada

Introduction: Endothelium-derived endothelin (ET)-1 has been implicated in hypertension and renal disease but the mechanisms are complex and remain unclear. We have shown that tamoxifen-inducible endothelium-restricted human ET-1 overexpressing (ieET-1) mice exhibited BP rise after 3 weeks of induction in an ET type A receptor (ET_AR)-dependent manner, in absence of renal injury. It is unknown whether long-term exposure to ET-1 overexpression results in sustained BP elevation and renal injury.
Methods: Adult male ieET-1 and control tamoxifen-inducible endothelium-restricted Cre recombinase (ieCre) mice were treated with tamoxifen (1 mg/kg/day, SC) for 5 days and 2.5 months later were treated or not with an ET_AR blocker, atrasentan (10 mg/kg/day, PO) for 2 weeks. Blood pressure (BP) by telemetry, renal artery flow (RAF) by ultrasonography, immune cell infiltration by flow cytometry, kidney injury molecule (KIM)-1 expression by immunofluorescence, 24h urinary albumin by ELISA and creatinine by alkaline picrate method were determined at the end of the study.
Results: Induction of ET-1 overexpression for 3 months resulted in greater systolic BP (135±4 vs 114±2 mmHg, *P*<0.001) and reduced RAF (1.7±0.2 vs 3±0.3 mL/min, *P*<0.05). ieET-1 mice presented increased myeloid (21255±5294 vs 5146±1987 $CD11b^+$ cells/kidney, *P*<0.001) and myeloid-derived suppressor cells (5332±1463 vs 1126±507 $CD11b^+Gr\text{-}1^+$ cells/kidney, *P*<0.01) renal infiltration associated with greater frequency of $CD11b^+$ (23.2±1.8 vs 7.5±1.6 % of $CD45^+$ cells, *P*<0.001) and non-immune renal cells ($CD45^-$, 5.7±0.8 vs 3.2±0.6 % of $CD45^-$ cells, *P*<0.001) expressing a pro-inflammatory maker, CD36. Early renal injury was demonstrated in ieET-1 by increased KIM-1 expression in proximal tubules (4.0±0.7 vs 1.0±0.2 % of renal cortex, *P*<0.05) and unchanged albumin/creatinine ratio (327±74 vs 173±36 µg/mg, P=0.215). Atrasentan reversed or reduced all of the above except the decreased RAF (*P*<0.05).
Conclusions: Long-term exposure to endothelial ET-1 overexpression caused sustained BP elevation and renal injury via ET_AR.

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Regulation of Nephron Afferent Arteriole Resistance in Obesity: Role of Insulin and Connecting Tubule Glomerular Feedback

PrimaryAuthor.AuthorBlock:**Sumit R Monu**, Yilin Ren, Mani Maheshwari, Ed Peterson, Oscar A Carretero, Henry Ford Health System, Detroit, MI

Introduction: In obesity, increased glomerular capillary pressure (P_{GC}) may participate in renal damage.P_{GC} is controlled in part by afferent arteriolar (*Af-Art*) resistance that in turn is regulated by two renal intrinsic feedback mechanisms, the vasoconstrictor Tubulo-Glomerular Feedback (TGF), and vasodilator Connecting Tubule Glomerular Feedback (CTGF). CTGF is initiated by an increase in NaCl transport by the epithelial sodium channel (ENaC) in the connecting tubule (CNT). Interestingly, obesity is strongly associated with hyperinsulinemia and insulin is a potent ENaC activator.
Hypothesis: In obesity, hyperinsulinemia increases CTGF *via* activation of the ENaC, this increase, in turn, contributes to TGF attenuation leading to increased P_{GC} and renal damage.
Methods: *In vivo:* In zucker obese rats (ZOR) and zucker lean rats (ZLR), we measured TGF and CTGF using renal micropuncture at 9-10 weeks of age. We quantify stop-flow pressure (P_{SF}) as an index of P_{GC}. We measured proteinuria as a marker of renal damage in both ZOR and ZLR.
In vitro: Microdissected rabbit Af-Arts and their adherent CNTs were perfused with NaCl, insulin or ENaC inhibitor (Benzamil; BZ) to investigate the role of insulin on TGF and CTGF.
Results: *In-vivo*: Maximal TGF response was significantly less in ZOR (6.11 ± 0.75 mmHg) in comparison to the ZLR (9.5 ± 1.1 mmHg, *p<0.05).* CTGF inhibition by BZ normalized the TGF response in ZOR similar to ZLR (ZOR, 14.01±1.70 mmHg *vs* ZLR, 11.46± 2.25 mmHg) suggesting CTGF playing a key role in TGF resetting in ZOR. Additionally, ZOR develops proteinuria (mg/24h) at 12 weeks of age (ZOR; 24.85±3.02 *vs* ZLR; 7.21±1.09, *p<0.05*).
In-vitro microperfusion of NaCl in the CNT that elicited a half-maximal response (EC_{50}, mmol/L) of *Af-Art* dilation was 25.0±0.8; an addition of insulin 10^{-7} mol/l to the CNT lumen decreased the EC_{50} to 8.1±0.8 (*P*<0.05) suggesting insulin potentiates CTGF. BZ blocked the insulin-mediated CTGF (Insulin EC_{50}: 7.8±0.9 *vs*. Insulin+BZ, EC_{50}: 19.7±5.5; *P*<0.05).
Conclusion: *In-vivo*: TGF is reset in ZOR due to enhanced CTGF before they develop proteinuria. *In-vitro*: Insulin increased CTGF during microperfusion experiments.
Perspective: Insulin-induced increased in CTGF may explain higher P_{GC} and renal damage in obesity.

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Genetic Complementation Establishes That a K572Q Mutation in Gamma-adducin Plays a Causal Role in Renal Microvascular Dysfunction in FHH and MNS Rats

PrimaryAuthor.AuthorBlock:**Chao Zhang**, Ying Ge, Paige N Mims, Fan Fan, Richard J Roman, Univ of Mississippi Medical Ctr, Jackson, MS

The Fawn-Hooded hypertensive (FHH) rat is a genetic model of hypertension-induced renal disease. However, the causal genes and pathways involved are unclear. We previously reported that the transfer of a small region in Chr. 1 of Brown-Norway (BN) rats which contains 15 genes, including gamma-Adducin (Add3), into the FHH background restores renal microvascular function and attenuates the development of proteinuria in FHH rats. Our further work identified a K572Q mutation in Add3 in FHH rats as a potential candidate variant in the pathogenesis of renal disease. The present study examined the role of Add3 in the impaired myogenic response of the afferent artery (Af-art) and autoregulation of renal blood flow (RBF) using transgenic and KO rats. RBF

increased by 21.5 ± 3.0% in SD. Add3 KO rats (n=7) when mean arterial pressure (MAP) was increased from 100 to 150 mmHg. In contrast, RBF only increased by 3.5 ± 0.9% in wildtype SD rats (n=13). The diameters of the renal Af-art decreased by 12.9 ± 0.8% in SD rats when perfusion pressure was increased from 60 to 120 mmHg, but it increased in SD.Add3 KO rats. The myogenic response of the Af-art in FHH rats was markedly impaired and increased by 8 ± 1.2% when the pressure was increased by from 60 to120 mmHg. The myogenic response was restored, and the diameters of the Af-art decreased by 12 ± 0.7% and 7 ± 1.0% in FHH. 1^{BN} congenic rats (n=27) and FHH.Add3 transgenic rats that express wt-Add3. RBF increased by 35.1 ± 3.0% when MAP was increased from 100 to 150 mmHg in FHH rats (n=15) versus 7.5 ± 1.7% and 6.0+ 1.3% in FHH.1^{BN} or a F1 cross of FHH and FHH.1^{BN} rats (n=8) and FHH.Add3 transgenic rats. The myogenic response of Af-art and autoregulation of RBF were also impaired in MNS rats (n=6) that carry the same K572Q mutation in Add3 as FHH rats. These phenotypes were complemented in a F1 cross of FHH and MNS rats (n=7), but the myogenic response and autoregulation of RBF were restored in an F1 cross of FHH and FHH.1^{BN} rats with one copy of wt-Add3. These results indicate that the recessive K572Q mutation of Add3 in FHH and MNS rats plays a causal role in renal microvascular dysfunction, which may contribute to the development of chronic kidney disease induced by hypertension in these models.

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068

Impact of Renal Outer Medullary Potassium Channel Inhibition on Afferent Arteriolar Tone in Rats With Type 1 Diabetes

PrimaryAuthor.AuthorBlock:**Pamela K Carmines**, Univ of Nebraska Medical Ctr, Omaha, NE; Xiaoyan Zhou, Maya Dajee, Kathleen A. Sullivan, Shawn P. Walsh, Alexander Pasternak, Merck & Co, Inc., Kenilworth, NJ

The bee venom toxin tertiapin Q (TPQ) evokes tubuloglomerular feedback-independent afferent arteriolar constriction in kidneys from rats with streptozotocin-induced type 1 diabetes (STZ rats). Because TPQ inhibits both Kir1.1 and Kir3.x channels, the contribution of Kir1.1 (renal outer medullary potassium channel; ROMK) to afferent arteriolar tone in diabetes remains uncertain. To test the hypothesis that Kir1.1 exerts a tonic dilator influence on the afferent arteriole during diabetes, we compared afferent arteriolar responses to the novel, small molecule Kir1.1-selective inhibitor Compound C (CmpdC) to those evoked by TPQ. The *ex vivo* blood-perfused juxtamedullary nephron technique was used to monitor afferent arteriolar lumen diameter before and during exposure to TPQ (1-100 nM) or CmpdC (10 nM-10 μM). Neither agent altered afferent arteriolar diameter in

kidneys from normal rats. In kidneys from STZ rats (blood glucose concentration = 465 ± 12 mg/dl), baseline afferent arteriolar diameter averaged 22.9 ± 0.6 µm (*n* = 30). Both TPQ and CmpdC evoked concentration-dependent arteriolar constriction, with 100 nM TPQ decreasing diameter by 2.5 ± 0.6 µm (*n* = 6; *P* < 0.001 vs baseline) and 100 nM CmpdC reducing diameter by 1.7 ± 0.6 µm (*n* = 10; *P* = 0.004 vs baseline; *P* = 0.42 vs 100 nM TPQ). During subsequent exposure to 10 µM CmpdC, arteriolar diameter was 2.1 ± 0.8 µm below baseline. The similar effects of the Kir1.1/3.x inhibitor TPQ and the Kir1.1-selective inhibitor CmpdC on arteriolar diameter in kidneys from STZ rats support the contention that Kir1.1 exerts a tonic afferent arteriolar dilator influence during type 1 diabetes. In follow-up studies, the NKCC inhibitor furosemide (30-300 µM) administered via the bath did not alter afferent arteriolar diameter in kidneys from STZ rats; however, prior exposure to furosemide prevented the vasoconstrictor response to 100 nM CmpdC (Δ diameter = -0.4 ± 0.3 µm; *n* = 8) although the response to 100 nM TPQ remained intact (Δ diameter = -2.6 ± 1.3 µm; *n* = 6). The differential impact of furosemide pretreatment on arteriolar responses to TPQ and CmpdC suggests a complex interplay between the furosemide-sensitive vascular NKCC1 and tubular NKCC2, and the TPQ- and CmpdC-sensitive Kir1.1 expressed in both the vasculature and tubule.

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Increased Stretch-induced O_2^- Production in Dahl Salt-sensitive Rats is Mediated by TRPV4

PrimaryAuthor.AuthorBlock:Fara Saez, Nancy J Hong, **Jeffrey L Garvin**, Case Western Reserve Univ, Cleveland, OH

Medullary O_2^- production is elevated in Dahl salt-sensitive (SS) when compared to Dahl salt-resistant rats (SR), and this is one of the main contributors to salt-sensitive hypertension in this model. In thick ascending limbs (TALs), flow-induced O_2^- is caused by elevated ion delivery and cellular stretch. Mechanical stimulation by cellular stretch, in turn, leads to increases in intracellular calcium (Cai). We hypothesized that the elevated O_2^- production by SS TALs is due to greater stretch-induced increases in Cai mediated by Transient Receptor Potential Vanilloid (TRPV4). To test our hypothesis, we measured O_2^- and Cai in isolated, perfused TALs using the ratiometric dyes dihydroethidium and Fura2, respectively. Stretch led to a greater increase in Cai in SS (243±51 nM; n=9) compared to SR (124±27 nM; n=10; $p<0.05$ vs. SS). The increase in Cai and the difference between strains were blunted when tubules were treated with RN1734, a TRPV4 inhibitor (SS: 59±10 nM; SR: 24±3 nM; n=5 in each group). TRPV4 facilitates Ca influx into the cell. Thus we tested the effect of removing extracellular Ca on the response to stretch. When tubules were perfused and bathed with in Ca-free solutions stretch-induced increases in Cai and the difference between SS and SR TALs were completed eliminated (SS: 10±6 nM; SR: 8±4 nM; n=5 in each group). Transfecting SS TALs with an adenovirus expressing a TRPV4-small hairpin RNA abolished the difference in the stretch-induced Cai response between SS and SR tubules (SS: 75±15nM; SR: 56±28 nM; n=4 for each group). Stretch-induced O_2^- production was greater in SS TALs compared to SR tubules (SS: 59±10 AU/min; SR: 24±3 AU/min; $p<0.02$; n=5 for each group). The increase in O_2^- production caused by stretch and the difference between SS and SR TALs were eliminated when tubules were treated with the TRPV4 inhibitor RN1734 (SS: 15±7 AU/min; SR: 10±4 AU/min). Our results indicate that: 1) stretch increases Cai in SS and SR TALs and this is mediated by activation of TRPV4; 2) stretch raises Cai more in SS than SR tubules; 3) stretch-induced O_2^- production is elevated in SS TALs compared to those from SR and this is due to greater increases in Cai; and 4) differences in TRPV4 activation likely explain, in part, both the differences in stretch-induced Cai and O_2^- production.

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Canonical Wnt Signaling Mediates Enhanced Renal Afferent Arteriolar Reactive Oxygen Species and Contractility in Diabetic Mice

PrimaryAuthor.AuthorBlock:**En Yin Lai**, Dept of Physiology, Zhejiang Univ Sch of Med, Div of Nephrology and Hypertension, and Hypertension Ctr, Georgetown Univ, Washington, DC; Suping Zhang, Qian Huang, Qiaoling Wang, Liang Zhao, Dept of Physiology, Zhejiang Univ Sch of Med, Hangzhou, China; Andreas Patzak, Inst of Vegetative Physiology, Charité-Univsmedizin Berlin, Berlin, Germany; Lingli Li, Christopher S. Wilcox, Div of Nephrology and Hypertension, and Hypertension Ctr, Georgetown Univ, Washington, DC

Background: Canonical Wnt signaling is involved in oxidative stress and diabetes but its role in diabetic renal microvascular dysfunction is unclear. We tested the hypothesis that enhanced canonical Wnt signaling in renal afferent arterioles from diabetic mice increases

reactive oxygen species (ROS) and contractions to endothelin-1 (ET-1). **Methods**: Diabetic or control C57Bl/6 mice received vehicle or sulindac (40 $mg \cdot kg^{-1} \cdot day^{-1}$) to block canonical Wnt signaling for 4 weeks. ET-1 contractions were measured in diameter changes and H_2O_2 and $O_2^{\cdot -}$ by fluorescence microscopy. Arteriolar protein expression and enzymatic activity were examined by standard methods. **Results**: Compared to control, diabetic mouse afferent arteriole had significantly increased $O_2^{\cdot -}$ (+84%) and H_2O_2 (+91%) and enhanced sensitivity to ET-1 at 10^{-8} $mol \cdot l^{-1}$ (-72±4% versus -43±4%, P<0.05) accompanied by significantly (P<0.005) reduced protein expressions and activities for catalase and superoxide dismutase 2 (SOD2). Incubation of afferent arterioles from normal or diabetic mice with PEG-SOD reduced responses to ET-1 whereas incubation with PEG-catalase reduced sensitivity to ET-1 selectively in arterioles from diabetic mice. The arteriolar protein expressions for canonical Wnt signaling indicated overactivation of this pathway in diabetic mice (2.6-fold increase in p-GSK-3β/GSK-3β and 3.3-fold decrease in p-β-catenin/β-catenin). Sulindac given to diabetic mice normalized the canonical Wnt signaling protein and arteriolar $O_2^{\cdot -}$, H_2O_2 and ET-1 contractions while doubling (P<0.05) microvascular catalase and SOD2. C**onclusions:** Increased ROS, notably H_2O_2, mediated by canonical Wnt signaling contributes to enhanced afferent arteriolar sensitivity to ET-1 in diabetes. Thus, antioxidant pharmacological strategies targeting canonical Wnt signaling may improve vascular function in diabetic nephropathy.

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An Attenuated Nocturnal Dip in Systemic Vascular Resistance is Associated With Increased Left Ventricular Mass

PrimaryAuthor.AuthorBlock:**Alan Hinderliter**, Univ of North Carolina, Chapel Hill, NC; LaBarron Hill, James Blumenthal, Andrew Sherwood, Duke Univ, Durham, NC

Introduction: Previous work from our laboratory has demonstrated that the nocturnal "dip" in blood pressure (BP) is due primarily to a decrease in systemic vascular resistance (SVR), and that nondipping is associated with a blunted nighttime reduction in SVR. In this analysis, we examined the hypothesis that a blunted nocturnal SVR fall is associated with increased LV mass.
Methods: 24-hour ambulatory hemodynamics were assessed in a biracial sample of 116 men and women with elevated clinic BP (130-159/80-99 mmHg). Ambulatory BP monitoring was coupled with synchronized measurements of cardiac output by ambulatory impedance cardiography. Values for SVR were derived for each ambulatory BP measurement. Left ventricular (LV) mass was measured by echocardiography and indexed by $height^{2.7}$ to adjust for differences in body size. Multivariable regression models were used to examine the relationships between LV mass index and demographic characteristics, anthropomorphic variables, daytime ambulatory systolic BP, and dipping of SVR and systolic BP.
Results: The study cohort averaged 45.3 ± 8.3 years of age; 42% (49 of 116) of the subjects were female and 47% (54 of 116) were African American. The mean body mass index (BMI) was 28.3 ± 3.8 kg/m^2. The mean daytime ambulatory systolic BP was 137 ± 11 mmHg; the systolic BP dip averaged 13 ± 5%, and the nocturnal fall in SVR averaged 13 ± 15%. In a model that included age, sex, race, BMI, and

daytime systolic BP, daytime systolic BP was significantly associated with LV mass index (b=0.33, p=0.0004). The SVR percent dip, (b=-0.21, p=0.02) but not the percent dip in systolic BP (b=-0.15, p=0.11), was an independent predictor LV mass index when added to this model.
Conclusions: A blunted nocturnal dip in SVR is associated with increased LV mass index, independent of daytime systolic BP. This finding suggests that an attenuated nighttime fall in SVR may contribute to left ventricular hypertrophy.

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Paradoxical Blood Pressure Increase After Brief Patient Rest Period

PrimaryAuthor.AuthorBlock:**Abimbola Shofu**, Timothy Kennedy, Bassam Omar, Christopher Malozzi, G. Mustafa Awan, Univ of South Alabama, Mobile, AL

BACKGROUND: Office-based blood pressure (BP) measurement is subject to variations which may influence management. OBJECTIVE: To assess the effect of rest period on repeat BP measurement. METHODS: Clinic charts were review identified 200 encounters with BP re-measurement due to initial BP of > 130/80 mmHg. BP was measured initially by a nurse, with the patient in a sitting position and the arm resting at the level of the heart. If BP was > 130/80 mmHg, it was repeated by physician after resting the patient for 15 minutes. Mean age was 64 ± 12 years. RESULTS: Among encounters with BP re-measurement, initial systolic BP (SBP) was 154 ± 25 mmHg, and diastolic BP 87 ± 15 mmHg. Upon re-measurement, 135 of 200 patients (68%) had lower SBP of 144 ± 21 mmHg compared with initial SBP of 161 ± 25 mmHg; a 17 mmHg drop ($P < 0.01$). However, 53 of 200 patients (27%) had higher SBP of 149 ± 17 mmHg compared with initial SBP of 138 ± 14 mmHg; an 11 mmHg increase ($P < 0.01$). Twelve patients (6%) had no BP change. In 47% (93/200) of encounters, BP re-measurement necessitated medication changes. Compared with the remaining patients, those with paradoxical increase in BP were younger (60 ± 9 years versus 66 ± 12 years; $p < 0.01$), and with lower initial SBP (138 ± 14 versus 161 ± 25, $p < 0.01$). DISCUSSION: Hypertension is a major challenging public health problem. JNC 8 guidelines recommend that prior to BP measurement, patients should be seated quietly for at least 5 minutes in a chair, with feet on the floor, and arm supported at heart level; this may decrease initially elevated BP. However, 27% of our patients exhibited a paradoxical response, with elevation of the SBP after a 15 minute period of rest. The cause of this paradox is not clear, but may have resulted from white-coat hypertension during the rest period, which may be more common in younger patients, as noted in our study. This underscores the importance of ambulatory BP monitoring, especially in subsets of patients prone to having labile or white coat hypertension, to avoid the cost and side effects of BP overtreatment. Studying larger number of patients, and including patient with normal initial BP, may help clarify the mechanism and clinical significance of this observation.

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Blood Pressure Variability and Control by 24-hour Ambulatory Blood Pressure Monitoring Before and After Resection of Catecholamine-secreting Neuroendocrine Tumors

PrimaryAuthor.AuthorBlock:**Jordana B. Cohen**, Univ of Pennsylvania, Philadelphia, PA; Anirban Ganguli, Georgetown Univ, Washington Hosp Ctr, Washington, DC; Bonita J. Bennett, Raymond R. Townsend, Debbie L. Cohen, Univ of Pennsylvania, Philadelphia, PA

Background: Measurements obtained by 24-hour ambulatory blood pressure monitoring (ABPM), such as nocturnal dipping and 24-hour blood pressure variability, are strongly associated with long-term renal and cardiovascular outcomes. Sparse data exist about how these parameters change after resection of catecholamine-secreting neuroendocrine tumors (NETs). **Methods:** We performed a prospective observational study of patients who underwent resection of catecholamine-secreting NETs at Penn between January 2014 and December 2016. Patients underwent 24-hour ABPM 1-3 weeks prior to and 6-8 weeks following resection. **Results:** Of the 32 patients who underwent resection, median age was 56 years, with 44% males (14 of 32), 78% Caucasians (25 of 32), and 84% (27 of 32) on at least one antihypertensive medication pre-operatively. Following resection, 50% (16 of 32) were on no antihypertensive medications and 74% (20 of 27) were on less medications than at baseline. There was a significant decline in clinic systolic blood pressure ([SBP] 134.6 vs. 122.5 mmHg, p<0.001), clinic pulse pressure (56.0 vs. 50.7 mmHg, p=0.037), clinic heart rate (80.8 vs. 75.5 mmHg, p=0.029), 24-hour mean SBP (133.1 vs. 127.4 mmHg, p=0.036), 24-hour SBP average real variability (10.0 vs. 9.0, p=0.031), 24-hour mean pulse pressure (54.5 vs. 51.6 mmHg, p=0.012), and 24-hour mean heart rate (78.5 vs. 74.0 bpm, p=0.023). Although the decline in nocturnal mean SBP (125.2 vs. 116.3 mmHg, p=0.007) was greater than the decline in daytime mean SBP (135.2 vs. 128.4, p=0.035), there was no reduction in non-dipping (38% vs. 44%, 12 vs. 14 of 32, p=0.854). Among patients who had masked (7 of 32), white coat (4 of 32), or sustained hypertension (9 of 32) at baseline, 60% (12 of 20, p=0.002) had controlled hypertension upon follow up. **Conclusion:** Following resection of catecholamine-secreting NETs, patients had a significant decline in 24-hour blood pressure variability and many experienced resolution of white coat, masked, and sustained hypertension, on less antihypertensive medications compared to baseline. There was no significant decline in non-dipping. NET resection may have greater prognostic significance than appreciated by changes in in-office blood pressure measurements and related parameters.

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074

Use of Antihypertensive Drugs, Drug Classes, and Combinations in Resistant versus Non-resistant Hypertension, and in True versus White-coat Resistant Hypertension

PrimaryAuthor.AuthorBlock:**Alejandro De La Sierra**, Hosp Mutua Terrassa, Terrassa, Spain; Pedro Armario, Hosp Moises Broggi, Sant Joan Despi, Spain; Anna Oliveras, Hosp delMar, Barcelona, Spain; Jose R Banegas, Univ Autonoma, Madrid, Spain; Manuel Gorostidi, Hosp General de Asturias, Oviedo, Spain; Ernest

Vinyoles, Cap La Mina, Barcelona, Spain; Julian Segura, Hosp 12 de Octubre, Madrid, Spain; Juan J De la Cruz, Univ Autonoma, Madrid, Spain; Luis M Ruilope, Hosp 12 de Octubre, Madrid, Spain

We aimed to evaluate the use of specific antihypertensive drugs and drug classes, as well as combinations in patients treated with 3 or more drugs classified as having or not resistant hypertension (RH), controlled or uncontrolled RH and true versus white-coat RH. From the Spanish ABPM Registry, we identified 21238 patients treated with 3 (14264) or more (6974) antihypertensive drugs of different classes. Among patients treated with 3 drugs we compared those with controlled (<140/90 mmHg; No RH) or uncontrolled (RH) office BP. In patients treated with 4 or more drugs we compared controlled versus uncontrolled RH. Moreover in uncontrolled RH patients, we compared those with white-coat (normal ABPM) versus true RH. We evaluated the use of different antihypertensive drug classes, specific antihypertensive drugs inside each class, and types of combinations. Results were adjusted for age, gender, and previous history of cardiovascular disease. With respect to RH treated with 3 drugs, those No RH used more frequently aldosterone antagonists (AA; Relative risk: 1.82; 1.40-2.37. They also used more frequently clorthalidone (CTL) among diuretics (RR: 1.54; 1.24-1.91), amlodipine (AML) among calcium channel blockers (CCB; RR: 1.12; 1.00-1.24) and bisoprolol (BIS) among beta blockers (RR: 1.18; 1.03-1.35). In patients treated with 4 or more drugs, controlled RH was also associated with the use of AA (RR: 1.41; 1.14-1.73) and AML (RR: 1.42; 1.25-1.62). No differences were observed in the type of combination used. When comparing patients with true versus white-coat RH, the latter group used more frequently diuretics (RR: 1.31; 1.16-1.47), CTL (RR: 1.79; 1.48-2.16) among diuretics, and AML (RR: 1.44; 1.32-1.57) among CCB. The triple combination of RAS blockers, CCB, and diuretics was also more frequently used in those with white-coat RH (RR: 1.09; 1.00-1.19). In conclusion, controlled office BP among patients treated with 3 or more drugs is associated with AA, CTL, and AML use. White-coat RH is associated with more diuretic use, especially CTL, AML, and the combination of RAS blockers, CCB, and diuretics. These results support the use of such triple combination, preferably including CTL and AML, and the addition of AA in order to reduce the prevalence of RH and true RH.

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Seasonal Variation and Day-by-day Variability in Nocturnal Blood Pressure Fall: The Nagahama Study

PrimaryAuthor.AuthorBlock:**Yasuharu Tabara**, Takeshi Matsumoto, Kimihiko Murase, Shunsuke Nagashima, Toyohiro Hirai, Shinji Kosugi, Takeo Nakayama, Tomoko Wakamura, Kazuo Chin, Fumihiko Matsuda, Kyoto Univ, Kyoto, Japan

Backgrounds: Abnormalities in circadian blood pressure (BP) variation have been suggested to be associated with cardiovascular diseases and mortality. Factors affecting the variability need to be clarified to precisely evaluate the risk of circadian BP abnormalities. Given the seasonal differences in casual BP, it was hypothesized that nocturnal BP may also differ by season. Here we aimed to clarify seasonal and day-by-

day variability of circadian BP variation in a large-scale general population. **Methods:** We analyzed a dataset describing in the Nagahama study. Study participants were 4,792 community residents. Evening, sleep, and morning BP values were measured for 5 days at home using an automatic cuff-oscillometric device (HEM-7080IC). Participants were required to sleep with putting a cuff on the upper arm. The BP monitor was programmed to automatically measure BP at 0, 2, and 4 o'clock, and all readings were recorded in a built-in memory of the device. Sleeping period was objectively defined by actigraphy. **Results:** Overall frequency of extreme-dipper, dipper, non-dipper, and riser were 6.5, 34.9, 45.1, and 13.5%, respectively. Nocturnal systolic BP fall was significantly smaller in individuals whose BP was measured during the summer season (summer, −5.8 ± 7.8%; middle, −8.2 ± 7.5%; winter, −11.1 ± 7.7%; p <0.001), resulting in higher frequencies of riser (summer, 19.9; middle, 12.8; winter, 7.5%) and non-dipper (summer, 51.5; middle, 46.2; winter, 36.7%) in summer season (p <0.001). The mean ambient temperature showed significant association with nocturnal SBP dipping level (r = 0.259, p <0.001). Results of linear regression analysis identified middle (beta = 0.156, p <0.001) and summer season (beta = 0.263, p <0.001) as strong positive determinants for decreasing nocturnal SBP fall, independently of other possible covariates. No marked seasonality was observed in day-by-day variability of the dipping pattern (Kendall's coefficient: winter, 0.590; middle, 0.602; summer, 0.581). **Conclusion:** Nocturnal BP fall was largely different by season with higher frequency of riser and non-dipper in summer. The seasonality might not be due to the seasonal difference in day-by-day variability of nocturnal BP changes.

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Factors Associated With Home Blood Pressure Monitoring Among US Adults: The National Health and Nutrition Examination Survey (NHANES), 2013-2014

PrimaryAuthor.AuthorBlock:**Joel C Marrs**, Sarah L Anderson, Univ Colorado Anschutz Medical Campus, Aurora, CO

Introduction: National prevalence data on home blood pressure monitoring (HBPM) has previously been reported from 2009-2010, but no recent data has been reported. **Methods:** This report is based on national-level, cross-sectional data for noninstitutionalized US adults aged ≥18 years (n = 6,113 participants) from the National Health and Nutrition Examination Survey (NHANES), 2013-2014. **Results:** Overall, 25.5% (1560 of 6113) of the adults engaged in HBPM monitoring in the 2013-2014 survey year. This is an increase from the previous NHANES 2009-2010 analysis reporting a 21.7% (1302 of 6001) rate. The frequency of HBPM increased with higher age, higher education level, having a partner, hypertensive, hypertensive aware, and hypertensive treated. Both SBP and DBP were higher in the HBPM group and were both statistically significant. The groups of hypertensive, hypertensive-aware, and hypertensive-treated patients all showed higher rates of HBPM use than nonuse. The frequency of HBPM monitoring in the categories of less than monthly, monthly, and weekly was reported at rates of 7.8% (476 of 6113), 6.6% (401 of 6113), and 11.2% (683 of 6113), respectively. These reported rates are

consistent and slightly increased in the weekly HBPM reported use compared with the NHANEs 2009-2010 analysis (7.2% [432 of 6001], 6.6% [396 of 6001], and 7.9% [474 of 6001), respectively). Adjusting for covariables, those who were aware of, treated for, and had known hypertension were more likely to have a higher frequency of HBPM than the reference: unaware, untreated, and no known hypertension (odds ratio (OR) = 1.98; OR = 2.13; and OR = 1.64, respectively). Individuals with less than a high school diploma and having no partner were less likely to perform HBPM than the reference: high school graduate or greater and having a partner (OR = 0.73; OR = 0.65, respectively). **Conclusions:** Approximately 21.0% (1084 of 6113) of adults engaged in monthly or more frequent HBPM which is an increase from the reported 14.5%(870 of 6001) rate in the 2009-2010 analysis. Having hypertension, being aware of hypertension, and being treated for hypertension were associated with an increased frequency of HBPM. Having no partner and less than a high school diploma were associated with lower frequency of HBPM.

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077

Angiotensinogen in Neurons of the Arcuate Nucleus May Regulate Metabolic Rate in Mice

PrimaryAuthor.AuthorBlock:**Sarah A Sapouckey**, Guorui Deng, Kristin E Claflin, Katherine N Gibson-Corley, Justin L Grobe, Univ of Iowa, Iowa City, IA

Recent studies from our laboratory have demonstrated that angiotensin II (ANG) type 1A receptors (AT1A) within the arcuate nucleus, expressed in the subset of neurons which express both the leptin receptor (LepR) and agouti-related peptide (AgRP), are critically involved in the control of thermogenic adipose sympathetic nerve activity (SNA) and resting metabolic rate (RMR) by leptin. This mechanism appears to involve AT1A-mediated suppression of gamma-aminobutyric acid (GABA) synthesis and packaging in AgRP neurons of the arcuate nucleus (ARC). It remains unclear, however, how leptin/LepR signaling results in AT1A activation within AgRP neurons. We hypothesize that LepR signaling results in increased transcription and local release of angiotensinogen (AGT) within the ARC, and consequently increased autocrine or paracrine ANG signaling within the ARC. Fluorescent in situ hybridization (RNAscope) uncovered expression of AGT mRNA in various cells of the ARC of C57BL/6J mice, including cells expressing mRNA for LepR, AgRP, proopiomelanocortin (POMC), insulin II, or glial fibrillary acidic protein, supporting local generation of AGT within the ARC. In silico re-analysis of a publically-available gene expression dataset interrogating individual cell types of the mouse hypothalamus (GSE74672) similarly uncovered expression of AGT in astrocytes, microglia, and many neuronal cell types of the ARC, including those expressing AgRP and POMC. Stimulation of immortalized mouse hypothalamic AgRP-like cell cultures (N47) with leptin may increase AGT mRNA (100 nM, 4 hrs; n=4 passages, 1.0 (0.6-1.8) vs 2.0 (1.2-3.6) fold, p=0.19). Further, using Cre-lox technology, mice lacking AGT in LepR- or AgRP-expressing cells ($AGT^{LepR\text{-}KO}$ or $AGT^{AgRP\text{-}KO}$ mice) were generated. In females at 8 weeks of age fed a chow diet (Teklad 7013), trends toward increased fat mass were noted (control n=28, 0.7±0.1; $AGT^{LepR\text{-}KO}$ n=2, 1.0±0.5; $AGT^{AgRP\text{-}KO}$ n=3, 1.0±0.2 g), despite normal food intake (13.5±0.6, 16.3±0.0, 12.4±0.8 kcal/d) and digestive efficiency (79±1, 83±0, 80±1 %). Collectively, these findings support local

production of ANG peptides within the ARC, and may indicate transcriptional regulation of AGT by leptin and a role for ARC AGT in the control of energy expenditure.

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078

Ox-LDL Leads to Increases of Endothelial Lysine Acetylation Linking Metabolic Memory to Diabetic Vascular Dysfunction

PrimaryAuthor.AuthorBlock:**Maria Alicia Carrillo-Sepulveda**, NYIT Coll of Osteopathic Medicin, Old Westbury, NY

Around 70% of type 2 diabetic (T2DM) patients will die due to sustained vascular complications, including hypertension. Evidence from clinical trials has shown that even under proper glycemic control, diabetic patients exhibit persistent vascular dysfunction. This phenomenon referred as “metabolic memory” (MM) is a major problem in treating diabetic patients. A current gap in knowledge of how MM contributes to diabetic vascular dysfunction needs to be addressed. Elevated serum ox-LDL levels are frequently found in T2DM. Whether ox-LDL is linked with MM remains undefined. We hypothesize that transient ox-LDL exposure increases endothelial lysine acetylation which in turn may contribute to persistent vascular dysfunction. Using a model of MM in cultured primary human aortic endothelial cells (HAEC), we found that 16h stimulation with ox-LDL (40 μg/mL) significantly increased lysine acetylation in the total cellular proteins (2.2 fold of increase vs unstimulated cells, $p<0.01$, $n=4$). Moreover, ox-LDL increased vascular cellular adhesion molecule-1 protein (VCAM-1, 1.8-fold increase, $p<0.05$, $n=3$), and superoxide production detected by dihydroethidium (2.5-fold increase, $p<0.001$, $n=4$) in comparison to unstimulated HAEC. When HAEC stimulated with ox-LDL were returned to a regular medium for 48h, VCAM-1 expression markedly decreased, whereas superoxide and lysine acetylation levels remained unchanged, suggesting that transient ox-LDL exposure generates MM. To determine whether increased endothelial lysine acetylation correlates with vascular dysfunction, we assessed endothelium-depend relaxation through concentration-dependent responses to acetylcholine using wire myograph in freshly isolated mice superior mesenteric arteries in organ culture. Vasorelaxation was impaired in vessels incubated with 40 μg/mL ox-LDL for 8h (66.3 ± 3.4 vs 90.1 ± 2.2 % control, $p<0.05$, $n=4$). This impairment was ameliorated when vessels exposed to ox-LDL were returned to regular medium for 12h (75.5 ± 2.0 vs 66.3 ± 2.4 %, $p<0.01$, $n=4$). These results indicate that transient ox-LDL causes persistent endothelial dysfunction and augmented endothelial lysine acetylation which may be an epigenetic modification driving MM and diabetic vascular dysfunction.

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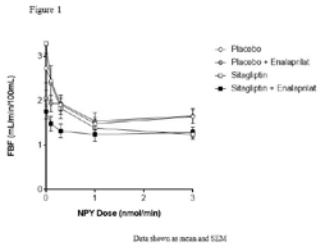
Dipeptidyl Peptidase IV (DPP4) Inhibition Enhances the Vasoconstrictor Response to Neuropeptide Y During Angiotensin-converting Enzyme Inhibition

PrimaryAuthor.AuthorBlock:**Scott Hubers**, Jessica Wilson, Nancy Brown, Vanderbilt Univ Medical Ctr, Nashville, TN

Background: Dipeptidyl peptidase IV (DPP4) inhibitors are antidiabetic medications that may increase risk of heart failure. Neuropeptide Y (NPY), a substrate of DPP4, is co-released with norepinephrine (NE) and causes vasoconstriction via the Y1 receptor. We tested the hypothesis that DPP4 inhibition would potentiate the effect of exogenous NPY on forearm blood flow (FBF). **Methods:** Seven healthy non-smokers participated in a randomized, double-blinded, placebo-controlled crossover study. Subjects underwent two study days and received sitagliptin 100 mg daily or placebo for seven days before each study day. On each study day, NPY was infused at 0.1, 0.3, 1.0, and 3.0 nmol/min through the brachial artery and FBF was measured using plethysmography. Following 90-minute washout, subjects received intra-arterial enalaprilat and NPY infusion was repeated. Venous and arterial samples were obtained for NE and NPY. **Results:** FBF decreased to a similar extent with increasing doses of NPY during sitagliptin and placebo (Figure 1). During enalaprilat, sitagliptin potentiated the vasoconstrictor effect of NPY compared to placebo (FBF 1.32 vs 1.94 mL/min/100mL at 0.3 nmol/min NPY during sitagliptin and placebo, respectively, P=0.039, Figure 1). NE decreased with ACE inhibition during NPY infusion, but this effect was not altered by DPP4 inhibition (Change in NE -47 vs -66 pg/mL with sitagliptin and placebo, respectively, P=0.92). **Conclusion:** DPP4 inhibition potentiates the vasoconstrictor effect of NPY in the forearm vasculature in the setting of ACE inhibition. These findings have implications for the cardiovascular effects of DPP4 inhibitors in patients receiving ACE inhibitors.



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Endothelin-1 Exaggerates Type-1 Diabetes-Accelerated Atherosclerosis Through NADPH Oxidase 1

PrimaryAuthor.AuthorBlock:**Pierre Paradis**, Sofiane Ouerd, Noureddine Idris-Khodja, Lady Davis Inst for Medical Res, SMBD-Jewish General Hosp, McGill Univ, Montreal, QC, Canada; Michelle Trindade, State Univ of Rio de Janeiro, Rio de Janeiro, Brazil; Suellen C. Coelho, Lady Davis Inst for Medical Res, SMBD-Jewish General Hosp, McGill Univ, Montreal, QC, Canada; Mario F. Neves, State Univ of Rio de Janeiro, Rio de Janeiro, Brazil; Karin A. Jandeleit-Dahm, Baker IDI Heart & Diabetes Res Inst, Melbourne, Australia; Ernesto L. Schiffrin, Lady Davis Inst for Medical Res, SMBD-Jewish General Hosp, McGill Univ, Montreal, QC, Canada

Introduction: NADPH oxidase (NOX) 1 but not NOX4-dependent oxidative stress plays a role in diabetic vascular disease, including atherosclerosis. Endothelin (ET)-1 has been implicated in diabetes-induced vascular complications. We showed that crossing mice overexpressing ET-1 selectively in endothelium (eET-1) with apolipoprotein E knockout (*Apoe*$^{-/-}$) mice exaggerated high-fat diet-induced atherosclerosis in part by increasing oxidative stress. We hypothesized that ET-1 overexpression in the endothelium would exaggerate diabetes-accelerated atherosclerosis through a mechanism involving NOX1 but not NOX4.
Methods: Six-week-old male *Apoe*$^{-/-}$ mice, eET-1/*Apoe*$^{-/-}$ and eET-1/*Apoe*$^{-/-}$ mice deficient in *Nox1* (eET-1/*Apoe*$^{-/-}$/*Nox1*$^{y/-}$) or *Nox4* (eET-1/*Apoe*$^{-/-}$/*Nox4*$^{-/-}$) were rendered diabetic with 55 mg/kg/day streptozotocin (STZ) IP for 5 days and studied 14 weeks later. Aortic atherosclerotic lesions were quantified using Oil Red O staining. Monocyte/macrophage infiltration and alpha-smooth muscle actin area were determined by immunofluorescence in aortic atherosclerotic lesions. Plasma cholesterol, HDL and triglycerides were measured.
Results: ET-1 overexpression exaggerated 2.5-fold the atherosclerotic lesion area of the aortic sinus in diabetic *Apoe*$^{-/-}$ mice (plaque area [x10^5 μm^2]: 5.3±0.2 vs 2.1±0.4, *P*<0.05), which was reduced ~35% by *Nox1* (3.5±0.4 x10^5 μm^2, *P*<0.05) but not *Nox4* knockout (5.0±0.7 x10^5 μm^2). Monocyte/macrophage infiltration was reduced ~30% in diabetic eET-1/*Apoe*$^{-/-}$ and eET-1/*Apoe*$^{-/-}$/*Nox4*$^{-/-}$ mice (31±1 and 35±2 vs 48±5% of lesion area, *P*<0.05) but not eET-1/*Apoe*$^{-/-}$/*Nox1*$^{y/-}$ mice (35±2%). ET-1 overexpression decreased alpha-smooth muscle actin content by ~35% (9±1 vs 14±2% of lesion area, *P*<0.05), which was blunted by *Nox1* (15±2%, *P*<0.05) but not *Nox4* knockout (9±1%). Plasma triglycerides were unaffected by ET-1 overexpression (3.4±0.3 vs 3.6±0.5 mmol/L) but reduced by *Nox1* and *Nox4* knockout (2.2±0.4 and 1.8±0.4 mmol/L, *P*<0.05). Plasma HDL and cholesterol were similar between groups.
Conclusions: Endothelium ET-1 overexpression exaggerates diabetes-accelerated atherosclerosis and reduces plaque stability through NOX1.

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081

Pathogenic Role of Ubiquitin Ligase Klhl3 in Diabetic Nephropathy

PrimaryAuthor.AuthorBlock:**Kenichi Ishizawa**, Shunya Uchida, Shigeru Shibata, Div of Nephrology, Dept of Internal Med, Teikyo Univ Sch of Med, Tokyo, Japan

<Background and aim> Kelch-like 3 (KLHL3) is a component of an E3 ubiquitin ligase complex that regulates blood pressure by targeting With-No-Lyisne (WNK) kinases for degradation. Mutations and inactivation of KLHL3 cause hypertension resulting from increased Na-Cl cotransporter (NCC) activity in the kidney.

Previously, we have reported that angiotensin II (Shibata et al. PNAS 2014) and potassium deficiency (Ishizawa et al. BBRC 2016) inactivate KLHL3 by protein kinase C (PKC)-mediated phosphorylation at S433 in the Kelch-domain, thereby contributing to increased blood pressure. Although clinical studies have shown that diabetic patients display salt-sensitive hypertension, its pathogenesis remains unclear. In this study, we examined the possible involvement of KLHL3 in the diabetic kidney, using a model of type 2 diabetes. <Methods> We examined the expression levels of total KLHL3, KLHL3 phosphorylated at S433 (inactive form; $KLHL3^{S433\text{-}P}$), and NCC in the kidney of Db/+ and Db/Db mice by Western blot. Distribution of $KLHL3^{S433\text{-}P}$ and NCC was analyzed by immunofluorescent microscopy. In some experiments, bisindolylmaleimide (BIM; the PKC inhibitor) was administered intraperitoneally. <Results> We found that $KLHL3^{S433\text{-}P}$ levels were significantly increased in the kidneys of Db/Db mice (2.2-fold increase versus Db/+ mice; $P < 0.01$), which was associated with the increased levels of WNK1/4. Moreover, NCC levels in the membrane fraction were significantly higher in Db/Db mice than Db/+ mice **(**2.3-fold increase, $P < 0.01$**)**. Immunofluorescent study indicated that $KLHL3^{S433\text{-}P}$ is increased in the distal convoluted tubules (where NCC is present). Of note, active, phosphorylated PKC was increased in the kidney of Db/Db mice (1.8-fold increase; $P < 0.01$), explaining the $KLHL3^{S433\text{-}P}$ induction in this model. To investigate the causal role of PKC and $KLHL3^{S433\text{-}P}$, we administered BIM to Db/Db mice. Importantly, $KLHL3^{S433\text{-}P}$ levels were significantly decreased by BIM (29% decrease vs Db/Db mice; $P < 0.05$). Furthermore, the increased NCC at the plasma membrane in Db/Db mice was also ameliorated by the PKC inhibitor**.** <Conclusion> These data indicate that the inactivation of KLHL3 is involved in the aberrant NCC activity in the kidney of Db/Db mice.

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082

On the Significance of Urinary Renin in Diabetic Kidney Disease: A Critical Role of Impaired Proximal Tubular Reabsorption

PrimaryAuthor.AuthorBlock:**Jeannette Tang**, Jan Wysocki, Johannes Rein, Minghao Ye, Northwestern Univ, Chicago, IL; Maryam Afkarian, Univ of California, Davis, CA; Daniel Batlle, Northwestern Univ, Chicago, IL

Increased expression of renin in the kidney collecting tubule of rodents made diabetic by streptozotocin (STZ) has been well demonstrated but whether this site is the main source of urinary renin is unknown. We wanted to examine the origin and significance of urinary renin in diabetic kidney disease (DKD).
Total and active renin was evaluated in urines from people with longstanding type 1 diabetes of more than 25 years, with (n=36) or without DKD (n=38) (eGFR 101 vs. 39 mL/min/1.73m^2; $p<0.001$). Mice given STZ (n=15) or vehicle (n=8) 20 weeks prior to study were also studied. In people with DKD, total renin was markedly increased compared to people without DKD (82 vs. 49 pg/mg Cr; $p=0.023$). Active renin was also significantly increased in people with DKD compared to people without DKD (3.2 vs. 1.3 pg/mg Cr; $p<0.001$). In mice with STZ-induced DKD a significant increase in renin was found compared to controls (1093±319 vs. 64±18 pg/mg Cr; $p=0.0001$). To examine the role of filtration and tubular reabsorption on urinary renin, human active renin was measured in urines from non-diabetic mice infused with human recombinant renin (hrRenin) (n=8), a

combination of lysine and hrRenin (n=5) and non-infused controls (n=15). Urines of mice infused with a combination of lysine (a blocker of proximal tubular protein reabsorption) and hrRenin had markedly higher urinary human active renin than those of controls (179±129 vs. 1.6±0.4 pg/mg Cr; p=0.001). The values were also markedly higher than those of mice infused with hrRenin only (4.4±1.1 pg/mg Cr; p=0.003). The effect of lysine was also evaluated in regard to endogenous mouse renin. Urinary mouse renin in mice infused with lysine (n=5) was markedly increased compared to non-infused controls (n=18) (22360±8673 vs. 346±82 pg/mg Cr; p=0.001).
In conclusion, in humans with DKD, urine concentrations of both total and active renin are increased. In mice with STZ-induced DKD, urine total renin is also markedly increased. The data further demonstrate that 1) renin is both filterable and reabsorbable in normal mice and 2) the increase of urinary renin in DKD can be attributed largely to impaired reabsorption mainly in the proximal tubule.

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083

Neurons in the Organum Vasculosum of the Lamina Terminalis Contribute to Salt-sensitive Hypertension

PrimaryAuthor.AuthorBlock:**Sean D Stocker**, Univ of Pittsburgh, Pittsburgh, PA

Excess dietary salt intake raises plasma and cerebrospinal fluid NaCl concentrations to elevate sympathetic nerve activity (SNA) and arterial blood pressure (ABP). Changes in extracellular NaCl concentrations are sensed by neurons in the organum vasculosum of the lamina terminalis (OVLT) - a circumventricular organ that lacks a complete blood-brain barrier. The purpose of the present study was to investigate the hypothesis that salt-sensitive hypertension was mediated, in part, by an elevated activity of OVLT neurons. Dahl-Salt-Sensitive or Sprague-Dawley rats (8-10 weeks) were fed 0.5% or 4.0% NaCl diets for 3-4 weeks. First, in vivo single-unit recordings demonstrate the discharge of OVLT neurons in Dahl-Salt-Sensitive rats was higher after a 4.0% versus 0.5% NaCl diet (4.1±0.4 Hz vs 1.9±0.3 Hz, n=6 per group, P<0.05). OVLT neuronal discharge of Sprague-Dawley rats was not different after a 4.0% or 0.5% NaCl diet (2.1±0.4 Hz vs 1.7±0.3 Hz, n=6-9 per group, P>0.5). In a second set of experiments, injection of hypertonic NaCl (1.0M NaCl, 20nL) into the OVLT produced significantly greater increases in lumbar SNA (131±6% vs 116±3%, n=4 per group, P<0.05) and mean ABP (14±2 vs 8±2 mmHg, n=4 per group, P<0.05) of Dahl-Salt-Sensitive rats fed 4.0% versus 0.5% NaCl respectively. Sprague-Dawley rats fed 4.0% versus 0.5% NaCl exhibited responses of smaller magnitude for both lumbar SNA (115±4 vs 108±3%, n=4 per group, P<0.05) and mean ABP (9±2 vs 6±2 mmHg, n=4 per group, P<0.05). Interestingly, the duration of the response was much longer in Dahl-Salt-Sensitive versus Sprague-Dawley rats (data not shown). Finally, inhibition of neuronal activity by injection of the GABA agonist muscimol (5mM, 20nL) into the OVLT produced a significantly greater fall in lumbar SNA (-25±4% vs -11±3%, n=4 per group, P<0.05) and mean ABP (-19±4 vs -6±2 mmHg, n=4 per group, P<0.05) of Dahl-Salt-Sensitive rats fed 4.0% versus 0.5% NaCl, respectively. Injection of muscimol into the OVLT of Sprague-Dawley rats did not significantly affect SNA or

mean ABP. Collectively, these findings suggest a high salt diet increases the activity of OVLT neurons to elevate SNA and ABP in salt-sensitive hypertension.

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084

Neurons in the Nodose Ganglion that Express Angiotensin Type 1a Receptors Function as Primary Baroreceptor Afferents: An *in vitro* and *in vivo* Optogenetic Study

PrimaryAuthor.AuthorBlock:**Erin B Bruce**, Gean Domingos-Souza, Michael D Smeltzer, Yalun Tan, Karlena Cahill, Scott W Harden, Charles J Frazier, Colin Sumners, Mohan K Raizada, Eric G Krause, Annette D de Kloet, Univ Florida, Gainesville, FL

The baroreflex is an essential regulator of blood pressure (BP); whereby, baroreceptors sense acute changes in BP and convey this information, via the nodose ganglia (NG), to the nucleus of the solitary tract (NTS). Manipulation of the baroreflex may provide a novel strategy in the treatment of hypertension. To that end, our neuroanatomical studies revealed a dense localization of angiotensin type 1a receptor (AT1a)-containing neuronal cell bodies in the NG and terminals in the NTS, thereby positioning them to play a role in the baroreflex. We, therefore, hypothesized that AT1a neurons residing in the NG potently influence cardiovascular function. Male and female mice expressing channelrhodopsin-2 (ChR2) and yellow fluorescent protein (eYFP) specifically in AT1a-expressing neurons (AT1aR-ChR2-eYFP) were used to determine the function of these neurons. *In vitro* patch clamp electrophysiological recordings from neurons in the NTS receiving axons expressing ChR2-eYFP revealed that optogenetic stimulation (473nm) reliably evoked excitatory postsynaptic currents (EPSCs). In concurrence with studies demonstrating that baroreceptor afferents utilize glutamate in the NTS, these EPSCs were eliminated by the presence of glutamate receptor antagonists. Next, the NG of anesthetized mice were subjected to optogenetic stimulation of varying frequencies (1, 15, 30Hz) for 1 min, and BP and HR responses were assessed. Optogenetic stimulation of these AT1a-expressing neurons led to significant decreases in mean arterial pressure (ΔMAP= -16±3*, -36±4*, -44±7* mmHg) and HR (ΔHR= -13±7, -104±44*, -163±59* bpm) in AT1a-ChR2-eYFP mice (n=6), but there was no effect on control mice harboring only the stop-FLOX-ChR2-eYFP gene (n=5; ΔMAP= 0±2, -2±1, 5±5 mmHg; ΔHR= 4±4, 3±4, -13±21 bpm). Additionally, AT1a-ChR2-eYFP mice rendered hypertensive via DOCA-salt (n=7) exhibited a dampened response to optogenetic stimulation (ΔMAP= -7±1#, -14±3*#, -30±6*# mmHg; ΔHR= -29±34, -71±37*, -178±50* bpm). Collectively, these data suggest that AT1a neurons in the NG are key regulators of the baroreflex, and may serve as a target for antihypertensive therapeutics. ($p<0.05$, *significantly different from control, #significantly different from normotensive AT1a-ChR2-eYFP)

Disclosures:**E.B. Bruce:** None. **G. Domingos-Souza:** None. **M.D. Smeltzer:** None. **Y. Tan:** None. **K. Cahill:** None. **S.W. Harden:** None. **C.J. Frazier:** None. **C. Sumners:** None. **M.K. Raizada:** None. **E.G. Krause:** None. **A.D. de Kloet:** None.

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085

CHIP: E3 Ubiquitin Ligase Mediates Proteasomal Degradation of Neuronal Nitric Oxide Synthase in the Paraventricular Nucleus of Rats with Heart Failure

PrimaryAuthor.AuthorBlock:**Neeru M Sharma**, Kenichi Katsurada, Xuefei Liu, Kaushik P Patel, UNMC, Omaha, NE

The exaggerated sympathetic drive is a characteristic of heart failure (HF) due to reduced neuronal nitric oxide synthase (nNOS) within the paraventricular nucleus (PVN). Previously we have shown that there were increased accumulation of nNOS-ubiquitin (nNOS-Ub) conjugates in the PVN of rats with HF (1.0±0.05 Sham vs. 1.29±0.06 HF) due to the increased levels of PIN (a protein inhibitor of nNOS, known to dissociate nNOS dimers into monomers) (0.76±0.10 Sham vs. 1.12±0.09 HF) and decreased levels of tetrahydrobiopterin (BH4): a cofactor required for stabilization of nNOS dimers (0.62±0.02 Sham vs. 0.44±0.03 HF). We also showed that there is blunted nitric oxide-mediated inhibition of sympathetic tone via the PVN in HF. Here we examined whether CHIP(C-terminus of Hsp70 -interacting protein), a chaperone-dependent E3 ubiquitin-protein isopeptide ligase known to ubiquitylate Hsp90-chaperoned proteins could act as an ubiquitin ligase for nNOS in the PVN. Immunofluorescence studies revealed colocalization of nNOS and CHIP in the PVN indicating their possible interaction. CHIP expression was increased by 50% in the PVN of rats with HF(0.96±0.08 Sham vs.1.44±0.10* HF). It is shown that Hsp90 protects nNOS from ubiquitination while Hsp70 promotes the ubiquitination and degradation. We observed significant upregulation of Hsp70 (0.49±0.03 Sham vs. 0.65±0.02* HF) with a trend toward the decrease in Hsp90 expression (0.90±0.07 Sham vs. 0.71±0.06 HF). The opposing effects of the two chaperones could account for the increased CHIP-mediated ubiquitination and degradation of dysfunctional nNOS monomers in the PVN of rats with HF. Furthermore, neuronal NG108-15 cell line transfected with the pCMV3-CHIP-GFP spark (CHIP overexpression plasmid) showed approximately 74% increase in CHIP with concomitant 49% decrease in nNOS expression. *In vitro* ubiquitination assay in NG108 cells transfected with pCMV-$(HA\text{-}Ub)_8$ and pCMV3-CHIP-GFP spark plasmid reveal increased HA-Ub-nNOS conjugates (1.13 ± 0.09 Scramble vs. 1.65 ± 0.12* CHIP plasmid). Taken together, our results identify CHIP as an E3 ligase for ubiquitination of dysfunctional nNOS and CHIP expression is augmented during HF leading to increased proteasomal degradation of nNOS in the PVN.

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086

Knockdown of the Neuronal (Pro)renin Receptor in the Paraventricular Nucleus of the Hypothalamus Attenuates ERK1/2 Activation and the Development of Salt Sensitive Hypertension

PrimaryAuthor.AuthorBlock:**Dane D Jensen**, Univ of Nevada Reno, Dept. Pharmacology and Physiology & Cell Biology; Ctr for Cardiovascular Res, Reno, NV; Wencheng Li, Wake Forest Univ, Winston-Salem, NC; Yumei Feng, Univ of Nevada Reno, Dept. Pharmacology and Physiology & Cell Biology; Ctr for Cardiovascular Res, Reno, NV

The (Pro)renin (PRR) receptor is a key player of the brain renin-angiotensin system. We

previously reported that neuron-specific knockout of the PRR attenuated the development of salt-sensitive hypertension (SSH). However, the brain region responsible for this effect is unknown. To test our hypothesis that PRR plays a regulatory role in the paraventricular nucleus of the hypothalamus (PVN) during SSH development, we knocked down PRR using adeno-associated virus (AAV2)-mediated Cre recombinase micro-injection into the PVN of PRR-LoxP mice. Blood pressure (BP, mmHg), heart rate (HR, bpm), and autonomic function were monitored with radio telemetry. Immunofluorescence was used to determine cell localization of AAV2 in the PVN and ERK1/2 activation (pERK1/2). We found that AAV2-eGFP positive cells co-localized with the neuronal marker NeuN, but not with astrocytes (GFAP) or microglia (IBa1). PRR-LoxP mice were injected with either AAV2-eGPF (GFP) or AAV2-Cre-eGFP (Cre) bilaterally into the PVN ($1.8x10^8$ Vg). Three days post viral injection, mice received either DOCA-salt (50mg DOCA pellet + 0.9%NaCl drinking solution) or sham (sham pellet + tap water) treatment for 21 days. PVN injection of Cre or GFP had no effect on baseline BP. After 21 days of DOCA-salt treatment, the BP (119 ± 3 vs. 139 ± 7) was significantly lower in mice that received Cre compared with GFP. The cardiac sympathetic tone (ΔHR to propranolol: -123 ± 46 vs. -181 ± 16) and vasomotor sympathetic tone (ΔBP to chlorisondamine: -47 ± 9 vs. -69 ± 5) were also lowered in Cre compared GFP treated mice following DOCA-salt treatment. The pERK1/2 levels in the PVN were increased in the DOCA-salt compared to sham (fold change: 3.29 ± 0.62, P<0.0001) treated mice. Importantly, the pERK1/2 elevation in the PVN was blocked by Cre (0.98 ± 0.15) compared with GFP with DOCA-salt treatment. PVN-targeted PRR knockdown had not effect on pERK1/2 in the subfornical organ compared to GFP (0.74 ± 0.05 vs. 1.0 ± 0.14); while, it reduced pERK1/2 in the rostral ventrolateral medulla (RVLM) (0.77 ± 0.06 vs. 1.23 ± 0.11, P<0.001). In summary, PVN-targeted PRR deletion attenuates SSH and ERK1/2 activation in the PVN and RVLM, and is associated with reduction in sympathetic tone in mice indicating an important role of PVN PRR in BP regulation.

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087

Deletion of Brain-specific Isoform of Renin (renin-b) Increases Resting Metabolic Rate by Stimulating Thermogenic Sympathetic Nerve Activity

PrimaryAuthor.AuthorBlock:**Javier Gomez**, Pablo Nakagawa, Keisuke Shinohara, Donald A Morgan, Benjamin J Weidemann, Matthew D Folchert, Kamal Rahmouni, Justin L Grobe, Curt D Sigmund, Univ of Iowa, Iowa City, IA

The brain specific isoform of renin, Renin-b (Ren-b), has been proposed as a negative regulator of the brain renin-angiotensin system (RAS). We generated mice with a selective deletion of Ren-b (Ren-b KO) while preserving expression of the classical Renin-a isoform. Deletion of Ren-b induced central RAS activation and hypertension through increases in brain Renin-a. However the metabolic effects of Ren-b deletion have yet to be described. Under basal conditions, Ren-b KO mice do not show differences in body weight, food consumption or physical activity. On a high fat diet, Ren-b KO male mice gained significantly less weight than the control mice (Ren-b KO: 36.8g ± 1.2 vs control: 41.9g ± 1.4; p<0.03, mean ± SEM). All subsequent experiments were performed on male mice. The analysis of the area under the curve (AUC) in a glucose

tolerance test revealed a mild but significant glucose intolerance in Ren-b KO mice (Ren-b KO: 152.5 mg/dL*hr ± 20.9 vs control: 93.0 mg/dL*hr ± 14.3; p<0.03). However, there were no significant differences in the AUC in the insulin tolerance test (Ren-b KO: 174.3 mg/dL*hr ± 12.8 vs control: 207.0 mg/dL*hr ± 12.8; p=0.077). Ren-b KO mice exhibited increased resting metabolic rate (Ren-b KO: 0.156 ± 0.005 kcal/h vs control: 0.145 ± 0.003 kcal/h; p<0.015). Ren-b KO mice also exhibited an increase in sympathetic nerve activity (SNA) to the interscapular brown adipose tissue (BAT) (Ren-b KO: 40.8 ± 3.1 spikes/sec vs control: 27.2 ± 3.1 spikes/sec; p<0.003). This was associated with an increase in BAT uncoupled protein 1 (UCP1) protein expression (Ren-b KO: 2.1 ± 0.5 fold increase vs control, p<0.05). Altogether, these data indicate that the brain RAS regulates energy homeostasis. The changes noted in Ren-b KO mice may be due to activation of the brain RAS. The activation of BAT SNA and the subsequent increase in UCP1 levels likely contributes to the metabolic phenotype observed in Ren-b KO mice. Dysregulation of Ren-b may be an important pathophysiological mechanism involved not only in the development of neurogenic hypertension, but also in obesity and other metabolic diseases.

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088

At_1 Receptor on Glutamatergic Neurons Regulate Autonomic Function Through Modulation of Neuronal Excitability and Sympathetic Outflow

PrimaryAuthor.AuthorBlock:**Jiaxi Xu**, LSU Health Sciences Ctr-NO, New Orleans, LA; Adrien Molinas, Andrea Zsombok, Tulane Univ Sch of Med, New Orleans, LA; Eric Lazartigues, LSU Health Sciences Ctr-NO, New Orleans, LA

Using a mouse model with Angiotensin II type 1 receptor ($AT_{1a}R$) deletion from the central nervous system neurons, we previously reported attenuated hypertension, improved autonomic function, and importantly, blunted ADAM17 (A Disintegrin And Metalloprotease 17) activation after deoxycorticosterone-salt treatment (DOCA 1mg/g body weight sc + 1% saline po for 3 weeks) compared to their control littermates. Since neuronal $AT_{1a}R$ has a pivotal role in ADAM17-mediated ACE2 shedding and the maintenance of neurogenic hypertension, we further investigated the role of central $AT_{1a}R$ and ADAM17 in excitatory neurons. New mouse models were generated with deletion of $AT_{1a}R$ (AT1G) or ADAM17 (A17G) specifically from glutamatergic neurons. AT1G (n=9), A17G (n=9), and their control littermates (n=10) were implanted with telemetry probes for continuous recording of blood pressure. Following DOCA-salt treatment, both strains showed increased mean arterial pressure, however the pressor responses were significantly lower in both transgenic lines (AT1G: +16 ±3 mmHg, A17G: +21 ±3 mmHg), compared to the controls (+31 ±2 mmHg). Meanwhile, the pulse pressure in the AT1G and A17G mice were not significantly increased compared to their baseline, while it was elevated in the control littermates by around 20 mmHg (*P<0.01*). In addition to a

reduced hypertension, AT1G mice exhibited preserved baroreflex sensitivity and autonomic function at the end of the DOCA-salt protocol. The DOCA-salt-induced dysautonomia was also attenuated in A17G mice but still exhibited decreased cardiac parasympathetic tone and increased vascular sympathetic tone, indicating that glutamatergic AT_1R can modulate autonomic regulation independently of ADAM17. To further dissect this mechanism, patch-clamp recording was performed in AT1G and control mice. Interestingly, lower action potential frequency was recorded in kidney-projecting PVN neurons of AT1G mice (0.43 ±0.06 *vs.* 2.04 ±0.96 Hz, n=3, *P=0.01*), suggesting that $AT_{1a}R$-knockdown neurons have a lower excitability compared to controls. Altogether, our data provide evidence that $AT_{1a}R$ located on glutamatergic neurons regulate autonomic function and the development of hypertension through ADAM17-dependent and independent pathways.

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089

A Simple Algorithm Identifies Hypertensive Patients Who Benefit From Intensive Blood Pressure Lowering

PrimaryAuthor.AuthorBlock:**Shidan Wang**, Rohan Khera, Sandeep Das, Rebecca Vigen, Tao Wang, Xin Luo, Rong Lu, Xiaowei Zhan, Guanghua Xiao, Wanpen Vongpatanasin, Yang Xie, Univ of Texas Southwestern Medical Ctr, Dallas, TX

Background Large randomized trials have provided inconsistent evidence regarding the benefit of intensive BP lowering in hypertensive patients. Identifying which patients derive a higher net benefit is essential in informing clinical decision-making. **Objectives** To assess whether stratification by cardiovascular disease (CVD) risk will identify patients with a more favorable risk/benefit profile for intensive BP lowering. **Methods** We used patient-level data from two trials that tested intensive vs. standard BP lowering: SPRINT and ACCORD. Within SPRINT, we selected a subset of patients at extremes of major adverse cardiovascular event (MACE) rates to develop a decision-tree using recursive partitioning modeling. We then validated its predictive effects in the remaining 'intermediate' SPRINT subset (n=8,357) and externally in ACCORD (n=2,258). **Results** Recursive partitioning produced a three-variable decision-tree model consisting of age≥74 years, urinary albumin/creatinine ratio (UACR) ≥34, and history of clinical CVD. It classified 48.6% of SPRINT and 55.3% of ACCORD patients as "high-risk". Compared with standard treatment, intensive BP lowering was associated with lower rates of MACE in this high-risk population in both SPRINT cross-validation data (HR=0.66, 95% CI 0.52-0.85) and ACCORD (HR=0.67, 95% CI 0.50-0.90), but not in the remaining low-risk patients (SPRINT: HR=0.83, 95% CI 0.56-1.25; ACCORD: HR=1.09, 95% CI 0.64-1.83). Additionally, intensive BP lowering did not confer an excess risk of serious adverse events in the high-risk group. **Conclusions** A simple risk prediction model consisting of age, UACR, and clinical CVD history successfully identified a subset of hypertensive patients who derived a more favorable risk/benefit profile for intensive BP lowering.

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090

Nocturia as an Unrecognized Symptom of Uncontrolled Hypertension in Middle-age Black Men

PrimaryAuthor.AuthorBlock:**Ronald G Victor**, Cedars Sinai Medical Ctr, Los Angeles, CA; Ning Li, Univ of California-Los Angeles, Los Angeles, CA; O'Neil Mason, Kathleen Lynch, Mohamad Rashid, Cedars Sinai Medical Ctr, Los Angeles, CA; Robert Elashoff, Univ of California-Los Angeles, Los Angeles, CA

Hypertension (HTN) is assumed to be asymptomatic ("the silent killer"). Yet nocturia - awakening at night to urinate - is bothersome if occurring $\geq$ 2 times/night and constitutes a putative but understudied symptom of HTN. Hypertensive black men may be especially prone to nocturia due to high sodium diet, blunted nocturnal blood pressure (BP) dipping (driving pressure-natriuresis), diuretic BP drugs, and common comorbid determinants of nocturia (prostate disease, diabetes, sleep apnea). To test if uncontrolled HTN is an independent, common, and potentially reversible determinant of nocturia in black men, we conducted in-person structured interviews and measured BP with a highly-rated automated monitor (that took 5 readings and averaged the last 3) in a large community-based sample of black men in their barbershops, a uniquely relaxed social setting to obtain high participation and accurate out-of-office BP. As nocturia is steeply age-dependent, we studied younger men ages 35 to 49 Y in whom nocturia would be unexpected. Among 1,748 black men, mean age 43±4 (SD) years, 45% (782 of 1748) had HTN; of these, HTN was: controlled with drugs (barbershop BP < 135/85 mmHg) in only 16% (123 of 782). Nocturia prevalence ranged from 24% (232 of 966) of normotensive men to 50% (96 of 191) of men whose HTN was drug-treated but still uncontrolled. Using normotensive men (BP 120±9/71±7) as the reference group and adjusting for all known nocturia determinants, the odds of having nocturia ($\geq$ 2 episodes/night vs. 0-1) was: (1) 34% higher in men with untreated HTN (BP 143±11/87±10): adjusted odds ratio (aOR) 1.34 (95% confidence intervals [CI]: 1.04-1.71, p=.02); (2) 174% higher in men with treated but uncontrolled HTN (BP 148±14/91±11): aOR 2.74 (95% CI:1.97-3.82, p<.001); but (3) not increased in men whose HTN was both treated and controlled (BP 123±8/74±7): aOR 1.24 (95% CI: 0.81-1.89, p=.32). Thus, in the largest study of nocturia in black men to date and the only one to measure BP, the data show that nocturia—a bothersome symptom—often signifies uncontrolled HTN in black men ages 35 to 49 Y. Because nocturia is far more likely when HTN is inadequately treated than when untreated, the data also suggest that nocturia may be a side-effect of BP drugs unless strict BP control is achieved.

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091

Improved Blood Pressure Control and High Adherence to a Novel Management Model of

Hypertension Care in a West African Population

PrimaryAuthor.AuthorBlock:Isaac Owusu, Kwame Nkrumah Univ of Sciences and Technology, Kumasi, Ghana; Fred Adomako-Boateng, Suntreso District Hosp, Kumasi, Ghana; Fred Kueffer, Molly Guy, Chemuttaai Lang'at, Daniel Grossman, **Douglas Hettrick**, Teri Whitman, Keith Holloman, Medtronic, St Paul, MN; O. Opare-Sem, Kwame Nkrumah Univ of Sciences and Technology, Kumasi, Ghana

Background: Logistic and socioeconomic barriers limit effective blood pressure (BP) control in many parts of Sub-Saharan Africa, including the Republic of Ghana. We tested a novel hypertension management model of care designed for resource-limited settings. **Methods and Results:** The "Akoma Pa" model was developed using human-centered design methodology involving patients, physicians, and nurses. The model consisted of a mobile tablet, BP machine and a novel software application in a unique platform to allow for longitudinal patient management. Patients were provided with a tailored hypertension management plan based on their enrollment comorbidities and risk factors. A cohort of 150 hypertensive patients (57±8 years; 73% female) accessed regular blood pressure assessments at a local pharmacy and received real-time automated feedback based on their individualized plan. On the mobile application, clinicians were able to view patient data, provide patients with feedback via SMS on their condition, and write electronic prescriptions which could be accessed by participating pharmacies. Average baseline BP was 135±18/84±10 mmHg in the overall cohort and 153±13/90±11 mmHg in the subgroup with uncontrolled hypertension (n=58). After 6 months of voluntary weekly monitoring, systolic blood pressure decreased significantly ($p<0.01$) in the overall cohort (-4.7±18.7 mmHg) and in the uncontrolled subgroup (-15.2±17.6mmHg). Systolic blood pressure remained constant in the sub group with controlled pressure at baseline. The proportion of the population with uncontrolled hypertension decreased from 39% to 27% ($p=0.01$). Patient compliance with weekly BP assessments was 61% and 2,855 BP assessments were conducted. During 33 of the 2,855 BP assessments (1% of pharmacy visits), the software application directly referred patients to a health facility (33 visits in 25 patients). Improvement in overall health awareness was reported in 82% of the participants and 95% of participants indicated a desire to continue using this model in the future. **Conclusions:** Compliance and satisfaction with this multifaceted hypertension care model were high and led to significant and sustained decreases in blood pressure in this West African hypertensive population.

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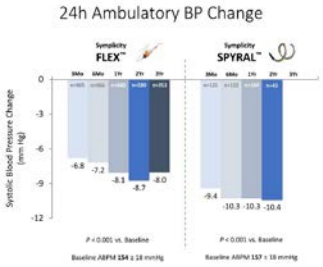
092

Global SYMPLICITY Registry: 3 Year Safety and Efficacy Data

PrimaryAuthor.AuthorBlock:**Michael Böhm**, Univskliniken des Saarlandes, Klinik für Innere Med III, Homburg/Saar, Germany; Giuseppe Mancia, Univ of Milano-Bicocca, Monza, Italy; Roland Schmieder, Univ Hosp Erlangen, Erlangen, Germany; Markus Schlaich, Royal Perth Hosp Unit, The Univ of Western Australia, Perth, Australia; Felix Mahfoud, Univskliniken des Saarlandes, Klinik für Innere Med III, Homburg/Saar, Germany

The objective of the Global SYMPLICITY Registry (GSR) is to collect and analyze real-world data on the safety and efficacy of renal denervation (RDN) using either the original Symplicity Flex™ renal denervation catheter or the newer-generation Symplicity Spyral™ catheter, which simultaneously applies energy to each renal artery quadrant.
GSR is a prospective, multi-center, non-randomized international RDN registry for patients with uncontrolled hypertension. In-office and ambulatory blood pressure measurements, clinical assessment and blood tests are among the data collected. To date, there are 2237 patients treated with the Symplicity Flex™ catheter with 6 month follow up and 1199 patients with 36 month follow up. Baseline demographics for this cohort included a mean age of 60.8 ± 11.9 years, 58.0% male and baseline eGFR of 76.3 ± 25.0 mL/min/1.73 m^2. At 36 months, reduction in office systolic blood pressure (OSBP) was -16.5 mm Hg (N=872). A smaller subset of patients was treated with the Symplicity Spyral™ catheter, and 174 of these patients had blood pressure measurements at 12 months, with a reduction in OSBP of -16.3 mm Hg. Reductions in ambulatory blood pressure measurements (ABPM) were sustained for both cohorts as shown in Figure 1. Rate of renal artery re-intervention at 36 months was 0.8% (10/1199) for Symplicity Flex™ and 0.0% (0/211) for Symplicity Spyral™.
Reductions in OSBP and ABPM were sustained to 36 months for patients who had RDN treatment with the Flex catheter. A subset of patients that received treatment with the Spyral catheter also saw reductions in blood pressure measurements. Rate of renal artery re-intervention was low for both groups.



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093

Can Hypertension Control be Improved Within a Short Time Frame? - Results From Implementing the Measure Accurately, Act Rapidly and Partner With Patients (MAP) Program

PrimaryAuthor.AuthorBlock:**Brent Egan**, Univ of South Carolina Sch of Med-Greenville, Greenville, SC; Michael Rakotz, American Medical Association, Chicago, IL; R. Bruce Hanlin, Irfan Asif, Dept of Family Med, Greenville Health System, Greenville, SC; Jianing Yang, American Medical Association, Chicago, IL; Susan Sutherland, Univ of South Carolina Sch of Med-Greenville, Greenville, SC; Robert Davis, Care Coordination Inst, Greenville, SC; Gregory Wozniak, American Medical Association, Chicago, IL

Objective: Evaluate a multifaceted quality improvement program with evidenced-based interventions for Measuring blood pressure (BP, mm Hg) accurately, Acting rapidly to manage uncontrolled BP, and Partnering with patients to promote BP self-management (MAP) in primary care. **Methods**: *Study design*: Quasi-experimental, pre-post intervention, design. BP control and BPs of uncontrolled patients were compared at baseline, February 2015[A1] to May 2016, at the last visit of the next 6 months.. Measure accurately included training staff in BP measurement. If attended BP was ≥140/90, unattended, automated office (AO) BP was obtained. Act rapidly included intensification of BP meds when unattended AOBP was ≥140/90 assessed by percent of visits with uncontrolled BP and no treatment change (therapeutic inertia). Partner with patients including BP self-monitoring and using low-priced generic BP meds assessed indirectly by the fall in systolic BP (SBP) per therapeutic intensification. *Population Studied:* Hypertensive patients (21,035) from 16 practices who had a visit during the baseline period and either no visit (4,691) or at least one visit (16,344) during the program. **Results**: BP control rose from 65.6% (13,790 of 21,035) to 74.8% (12,234 of 16,344) (p<.001); 12 of 16 practices had significant increases in BP control. In uncontrolled patients at baseline, mean SBP/DBP fell from 149/85 to 139/80 (p<.001/p<.001). Measure accurately lowered SBP 12.8 mm Hg (p<.001) in uncontrolled patients with better technique in attended BP reducing SBP ≥6.5 mm Hg per practice; while unattended AOBP lowered SBP 8.6 mm Hg (p<.001). Therapeutic inertia was unchanged (50.2% vs. 48.4%; p=.10); the mean fall in SBP per therapeutic change increased from 5.4 to 14.0 mm Hg (p<.001). **Conclusions**: MAP was associated with significant improvement in hypertension control in primary care during a six-month period. The decrease in SBP and improved control were largely explained by Measure accurately and Partner with patients as therapeutic inertia (Act rapidly) did not change. Evidence-based strategies in MAP provide opportunities for primary care practices to quickly improve hypertension control toward the national goals of 80%, and importantly to reduce cardiovascular risk.

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094

Predicting the Risk of Apparent Treatment Resistant Hypertension: A Retrospective, Longitudinal, Cohort Study in an Urban Hypertension Referral Clinic Setting

PrimaryAuthor.AuthorBlock:**Michael G Buhnerkempe**, Albert Botchway, Carlos Nolasco-Morales, Vivek Prakash, Lowell Hedquist, John M Flack, Southern Illinois Univ Sch of Med, Springfield, IL

Background: Apparent treatment resistant hypertension (aTRH) is associated with increased prevalence of secondary hypertension and adverse pressure-related clinical outcomes. We previously showed that cross-sectional prevalence estimates of aTRH are lower than its true prevalence as patients with uncontrolled hypertension undergoing intensification/optimization of therapy will, over time, increasingly satisfy diagnostic criteria for aTRH.
Methods: aTRH (SBP and/or DBP at or above a clinically defined goal BP [140/90, 130/85, 130/80, or 125/75 mmHg] over two consecutive office visits when on ≥ 3 antihypertensive drug classes, including a diuretic; or SBP and DBP below goal when on ≥ 4 drug classes, including a diuretic) was assessed in an urban referral hypertension clinic in 924 patients ≥ 30 years old (57.7 ± 12.6) with at least two follow-up visits over 240 days. Patients were mostly African-American (86%; 795/924) and female (65%; 601/924). A minority (28.7%; 265/924) were taking diuretics at their index visit, and analyses were stratified according to this use. Risk for aTRH was estimated using logistic regression with patient characteristics at index visit as predictors. Performance of this risk score at discriminating aTRH status over follow-up was assessed using AUC and was internally validated using bootstrapping.
Results: Amongst those on diuretics, 80/265 (30.2%) developed aTRH; the risk score discriminated well (AUC = 0.79, bootstrapped 95% CI [0.73, 0.84]). In patients not on a diuretic, 151/659 (22.9%) developed aTRH, and the risk score showed moderate, but significantly lower, discriminative ability (AUC = 0.71 [0.66, 0.74]; $p < 0.001$). In the diuretic and non-diuretic cohorts, 43/265 (16.2%) and 101/265 (38.1%) of patients, respectively, had estimated risks for development of aTRH < 10%. Of these low-risk patients, 42/43 (97.7%) and 97/101 (96.0%) did not develop aTRH (negative predictive value, diuretics – 0.95 [0.93, 1.00], no diuretics – 0.96 [0.91, 1.00]).
Conclusions: We created a novel clinical score that discriminates well between those who will and will not develop aTRH, especially amongst those taking diuretics initially. Irrespective of diuretic treatment status, a low risk score had very high negative predictive value.

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095

Digital Solution Assisted Retail Pharmacists in Community Hypertension Control in China: A Real World Study of Large Samples

PrimaryAuthor.AuthorBlock:Wenhao Qu, Sinopharm Group Company Limited, Shanghai, China; Zhen Wang, Ying Chen, Yingjie Li, **Kai Liu**, CareLinker Co., Ltd., Shanghai, China

Objective: To identify the effects of a digital healthcare solution for hypertension management at retail pharmacies by pharmacists. Methods: We developed a digital healthcare solution that includes BP telemonitor, an App, and a cloud database with algorithms to assist: 1) CVD risk screening, 2) personalized meal planning, 3) personalized health coaching, and 4) medication guidance and compliance during the pharmacy consultation service. This solution has been implemented in 5,067 retail pharmacies in 141 cities in China as a decision support system to help pharmacists manage their hypertensive members. During Jan 1st 2015 to Dec 31st 2016, a total of 1,817,594 patients received in-store hypertension care and 23,024 of them were selected in this analysis following 4 inclusion criteria: 1) baseline BP was hypertensive (≥ 140/90 mm Hg), 2) the frequency of in-store hypertensive care was ≥ 1 time per month, 3) the intervention duration between the first- and last-time hypertensive care was ≥ 1 month, and 4) baseline characteristics were complete. Dynamic changes of SBP, DBP, disease staging, and population distribution over time were the major end points in this real-world, retrospective analysis. Results: SBP and DBP of the study population (n=23,024) were reduced on average by 11.2 mm Hg (baseline: 151.7 ± 14.8 mm Hg, last time: 140.5 ± 18.8 mm Hg, P< 0.001) and 6.3 mm Hg (baseline: 91.2 ± 11.9 mm Hg, last time: 84.9 ± 12.7 mm Hg, P<0.001), respectively. A total of 13,001 (56.5%) patients demonstrated improved hypertension stages by the last time BP measures with 9,625 (41.8%) of them returning to the normal range. The principal BP control effect took place after the first-time intervention, followed by gradual BP reduction over time. Simulation analysis revealed linear descending relationship of both SBP and DBP to time, and the concentration distributions were moving towards the normal BP level. The linear fitting functions were: SBP, Y = -0.103x + 140.278; DBP, Y = -0.060x + 84.799. Conclusion: Digital healthcare solution is effective in assisting retail pharmacists to deliver community hypertension care in real world.

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096

Comparing Strategies to Improve Systolic Blood Pressure Over 10 Years: A Simulation Study Using the Blood Pressure Control Model

PrimaryAuthor.AuthorBlock:**Brandon K Bellows**, Univ of Utah, Salt Lake Cty, UT; Andrew E Moran, Columbia Univ, New York, NY; Natalia Ruiz-Negrón, Univ of Utah, Salt Lake Cty, UT; Kirsten Bibbins-Domingo, Mark Pletcher, Valy Fontil, Univ of California, San Francisco, San Francisco, CA

Background: Uncontrolled hypertension increases patients' risk for cardiovascular and kidney disease. This study compared strategies to improve systolic blood pressure (SBP) among 1000 simulated patients with uncontrolled hypertension (SBP >=140 mmHg) from the National Health and Nutrition Examination Survey (NHANES).

Methods: The Blood Pressure Control Model (BPCM) is a microsimulation, health state transition model that predicts the weekly SBP of patients receiving usual care. In the BPCM, patient SBPs are estimated using office visit frequency, measured SBP accuracy and variability, probability of treatment intensification with uncontrolled SBP, effect of antihypertensive medications, and adherence. BPCM inputs are derived from national survey data, meta-analyses, and other published literature. The effects of usual care on SBP were compared to 10% and 50% increases in global strategies for SBP control (i.e., visit frequency, treatment intensification, and/or adherence) over 10 years. SBP outcomes were validated against published literature values of 44-46% prior to implementation (i.e., usual care) and 74-80% 8-10 years after implementation of aggressive hypertension management programs in large health systems.
Results: In the simulated NHANES population, the mean (SD) age was 61.1 (14.6), 52% were male, and mean baseline SBP was 153.2 (13.6) mmHg. Under usual care, the BPCM estimated a mean SBP of 140.1 (16.4) mmHg and 49% of patients achieving SBP <140 mmHg after 10 years. Compared to usual care, 50% improvements in global strategies resulted in more rapid reductions in SBP and earlier achievement of SBP control. Simultaneously improving all global strategies by 50% resulted in an estimated mean SBP of 132.4 (15.5) mmHg with 71% achieving control after 10 years.
Conclusions: Usual care and intervention BPCM predictions are consistent with hypertension control rates observed in contemporary national surveys and the observed results of recent systematic hypertension control improvement programs. These results show the BPCM may be used by health system planners to project the impact of implementing hypertension control strategies.

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097

Knockout of Matrix Metalloproteinase 9 Protects Against Hypertension-induced Renal Disease in Hypertensive Dahl S Rats

PrimaryAuthor.AuthorBlock:**Chao Zhang**, Paige N Mims, Univ of Mississippi Medical Ctr, Jackson, MS; Tongyu Zhu, Zhongshan Hosp, Fudan Univ, Shanghai, China; Fan Fan, Richard J Roman, Univ of Mississippi Medical Ctr, Jackson, MS

Matrix metalloproteinase 9 (MMP9) is a member of gelatinase family of enzymes with potentially opposing actions on tissue fibrosis (i.e. the degradation of extracellular matrix). Our previous study showed that two nonselective MMP inhibitors, XL081 and XL784, which inhibit MMP2, 9, 13 and Adam10 could delay and even reverse the development of renal fibrosis in hypertensive Dahl Salt sensitive (SS) rats. However, the specific role of MMP9 to the development of hypertensive nephropathy in MMP9 knockout (KO) rat models is unknown. In the present study, MMP9 KO rats were created on the SS genetic background using a CRISPR/Cas 9 system and the effect of KO was verified in this model. The systolic pressure, diastolic pressure and mean blood pressure (MAP) were similar in 12 weeks old MMP9 KO (n=6) and SS rats (n=9) fed a low salt diet (145 ± 3 vs. 148 ± 2mmHg; 111 ± 2 vs. 107 ± 2mmHg; 128 ± 2 vs. 126 ± 2mmHg). MAP increased to 180 ± 4 vs. 154 ± 3mmHg in SS rats versus MMP9 KO rats fed 8% high salt (HS) diet for 3 weeks. Proteinuria increased from 196 ± 18

mg/day to 718 ± 61mg/day in HS treated SS rats (n=9). It was significantly reduced in HS treated MMP9 KO rats (211 ± 30mg/day, n=6). The degree of glomerular injury (2.88 ± 0.08 vs. 3.52 ± 0.02), interstitial fibrosis (4.57 ± 0.35% vs. 10.45 ± 0.55%), vascular wall-to-lumen ratio (0.63 ± 0.04 vs. 1.22 ± 0.08) and protein cast area (6.40 ± 0.07% vs. 20.27 ± 2.65%) were all significantly reduced in MMP9 KO rats (n=6) versus the corresponding values in SS rats (n=6) fed 8% HS diet for 3 weeks. Autoregulation of renal blood flow (RBF) to elevations in perfusion was impaired in SS rats prior to the development of hypertension, for RBF rose by 20.6 ± 3.6% (n=8) when MAP was increased from 110 to 150 mmHg. Autoregulation of RBF was restored in MMP9 KO rats and only increased by 7% when pressure was increased over the same range. In contrast, there was no difference in the fall in RBF in SS versus MMP9 KO rats when pressure was reduced from 110 to 50 mmHg. These findings suggest that knockout of MMP9 in SS rats restores autoregulation of RBF and opposes the development of hypertension, proteinuria, glomerular injury and renal interstitial fibrosis.

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098

Augmenting Renal Lymphatic Vessel Density Prevents Salt-sensitive Hypertension in Mice

PrimaryAuthor.AuthorBlock:**Dakshnapriya Balasubbramanian**, Catalina A Lopez Gelston, Gabriella R Abouelkheir, Alexandra H Lopez, Kayla R Hudson, Eric R Johnson, Victoria C Garza, Reem A Daboul, Joseph M Rutkowski, Brett M Mitchell, Texas A&M Univ Health Science Ctr, College Station, TX

Salt-sensitive hypertension (SSHTN) is associated with renal immune cell infiltration and interstitial inflammation. Lymphatic vessels drain the interstitial compartment and traffic immune cells to draining lymph nodes; however little is known about the role of lymphatics and immune cell trafficking in the kidney during SSHTN. Our hypotheses were that renal lymphatic vessel density is increased in mice with SSHTN and that further augmenting renal lymphatic vessels will prevent SSHTN. SSHTN mice were made by administering L-NAME for two weeks, followed by a two week washout, and then were fed a 4% high salt diet for three weeks. Compared to control mice, mice with SSHTN (SBP: 103±3 vs. 136±2 mmHg; $p<0.05$) had markedly increased renal lymphatic vessel density. Kidneys of SSHTN mice had significantly increased gene expression of the lymphatic vessel marker *Lyve1*, the macrophage marker *Adgre1* (F4/80), the Th1 cell marker *Tbx21*, and the pro-inflammatory cytokine *Il6* while expression of the immune cell-lymphatic chemokine receptor *Ccr7* was decreased significantly. Mice solely fed a 4% salt diet for three weeks did not exhibit hypertension or increased renal lymphatic vessel density. To determine whether augmenting renal lymphatic

vessels prior to the high salt diet could prevent SSHTN, we used transgenic mice that overexpress the lymphangiogenic signal VEGF-D only in the kidney under the control of doxycycline (KidVD+ mice) and thus exhibit renal lymphangiogenesis. Doxycycline initiated one week prior to the high salt diet prevented SSHTN in KidVD+ mice while having no effect on blood pressure in KidVD- mice (SBP: 117±4 vs. 139±5 mmHg; $p<0.05$). Renal gene expression of *Tbx21* was decreased in KidVD+ mice while *Ccr7* gene expression was increased significantly. These data demonstrate that renal lymphatic vessel density is increased in SSHTN and that augmenting renal lymphatic vessel density prior to a high salt diet can prevent SSHTN by improving renal immune cell exfiltration.

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099

Smooth Muscle PPARγ Mutation Causes Salt-sensitive Hypertension

PrimaryAuthor.AuthorBlock:**Jing Wu**, Larry N Agbor, Masashi Mukohda, Anand R Nair, Pablo Nakagawa, Justin L Grobe, Curt D Sigmund, Univ of Iowa, Iowa City, IA

Abnormal increase in renal salt retention is traditionally believed to be an early pathophysiological event in the causation of salt-sensitive hypertension, whereas increase in systemic vascular resistance (SVR) is a secondary response caused by autoregulation. However, recent studies show that salt-resistant subjects vasodilate and reduce SVR during salt loading, while salt-sensitive humans fail to vasodilate and exhibit salt-induced blood pressure (BP) elevation. Therefore, we tested the hypothesis that primary vascular dysfunction predisposes to salt sensitive hypertension. We used mice with smooth muscle-specific expression of a human hypertension-causing mutation in PPARγ P467L (S-P467L). S-P467L transgenic mice and non-transgenic controls (NT) were fed regular diet (0.4% salt) or high salt diet (4% salt) for 4 weeks. S-P467L mice, but not NT controls, exhibited severe impairment in acetylcholine- and sodium nitroprusside-induced vasorelaxation (31±4.9% S-P467L salt vs. 70±9.5% regular diet, maximal relaxation at 30 μM acetylcholine). This was associated with salt-induced systolic BP elevation in S-P467L mice (142±5 mmHg salt vs 127±2 mmHg regular diet), but not in NT mice (120±2.7 mmHg salt vs 115±4.0 mmHg). These changes were not due to differences in food intake, weight gain or renal sympathetic nerve activity between the two strains. In the 3rd week of high salt diet, S-P467L mice and NT controls both had increased water intake by 3-fold compared to those on regular diet; however, S-P467L mice excreted 32% less urine and produced 36% less NO in the kidney as indicated by 24-hour urinary nitrate/nitrite. To assess renal function, mice were subjected to an acute saline challenge (10% body weight, i.p. injection). S-P467L mice exhibited a marked decline in their capacity to excrete this volume/sodium load, indicative of renal dysfunction. Of note, the impaired vasorelaxation in S-P467L occurred as early as day 3 of high salt diet, while renal dysfunction did not develop until day 10, suggesting that vascular dysfunction may serve as an initiation mechanism that reinforces salt-induced hemodynamic changes. These data supports the concept that vascular dysfunction may

predispose to renal abnormalities including increased salt sensitivity.

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Tissue-specific Deletion of Collectrin in the Proximal Tubular Epithelium Increases Arterial Pressure and Augments Salt-sensitivity

PrimaryAuthor.AuthorBlock:**Sylvia Cechova**, Univ Virginia Sch Med, Charlottesville, VA; Pei-Lun Chu, Fu Jen Catholic Univ, New Taipei City, Taiwan; Joseph C Gigliotti, Liberty Univ, Lynchburg, VA; Fan Chan, Thu H. Le, Univ Virginia Sch Med, Charlottesville, VA

Background: Collectrin (*Tmem27*) is a key regulator of blood pressure (BP) and modulator of the bioavailability of nitric oxide (NO) and superoxide. It is highly expressed in the kidney in the proximal tubule (PT), collecting duct, and throughout the vascular endothelium. We reported that collectrin plays a critical role as a chaperone for the reabsorption of all amino acids (AAs) in the PT, and for the uptake of the cationic AA L-arginine (L-Arg) in endothelial cells. Global collectrin knockout ($Tmem27^{Y/-}$) mice display baseline hypertension (HTN), augmented salt-sensitive hypertension (SSH), and decreased renal blood flow. **Objective and Methods:** To determine the PT-specific effect of collectrin on BP homeostasis and salt sensitivity, we used the Cre-*loxP* approach and PEPCK-Cre to generate a mouse line lacking collectrin specifically in the PT-- PEPCK-$Cre^{+}Tmem27^{Y/Flox}$ mice. PEPCK-$Cre^{-}Tmem27^{Y/Flox}$ mice were used as control. Radiotelemetry was used to measure BP for 2 weeks at baseline and 2 weeks on high salt diet (HSD). Renal blood flow at baseline and on HSD was measured using contrast enhanced ultrasound in the same mice. **Results:** Successful deletion of collectrin in the PT was confirmed by assessing mRNA levels using real-time RT-PCR, immunohistochemistry staining of renal tissues using anti-collectrin antibody, and quantitation of protein from kidney cortex by Western analysis. Compared to control PEPCK-$Cre^{-}Tmem27^{Y/Flox}$ mice (n=6), PEPCK-$Cre^{+}Tmem27^{Y/Flox}$ mice (n=6) displayed significantly higher systolic BP (SBP) at baseline (120.0 ± 2.5 vs 131.6 ± 2.9 mm Hg; p = 0.014) and after HSD (135.3 ± 2.6 vs 151.5 ± 5.2 mm Hg; p = 0.019). Renal blood flow was not different between groups, at baseline nor after HSD. **Conclusion:** Collectrin in the PT plays an important role in blood pressure homeostasis and response to sodium intake, independent of renal blood flow. Increasing proximal tubular collectrin activity may be a novel therapeutic strategy for the treatment of hypertension and salt-sensitivity.

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Loss of Salt Sensing Kinase, SGK1, in T Cells Abrogates Memory T Cell Formation, Hypertension, and End-organ Damage

PrimaryAuthor.AuthorBlock:**Hana A Itani**, Arvind Pandey, Allison E. Norlander, Meenakshi S. Madhur, David G. Harrison, Vanderbilt Univ Medical Ctr, Nashville, TN

Accumulating evidence indicates that NaCl can be concentrated in tissues with high salt-intake, age and in the setting of hypertension. Elevated NaCl has been shown to promote T_H17 cell formation in an SGK1-dependent fashion. We have previously shown that Memory T cells play a major role in the genesis of hypertension. These long-lived cells remain responsive to repeated hypertensive stimuli, such as salt feeding, and can be mobilized to enter the kidney where they release cytokines that promote renal dysfunction. To examine mechanisms by which T cells sense salt and contribute to salt-sensitivity, we tested the hypothesis that SGK1 in T cells is necessary for formation of memory T cells and their activation in salt-sensitive hypertension. To study the role of SKG1 in hypertension, we produced mice with T cell specific deletion of SGK1, $SGK1^{fl/fl}$ x tg^{CD4cre} mice and used $SGK1^{fl/fl}$ mice as controls. To impose repeated episodes of hypertension, we treated these mice with L-NAME (0.5mg/ml) in drinking water for two weeks, allowed a two-week normotensive interval and a then fed high salt (4% NaCl) for three weeks. L-NAME followed by high salt increased memory T cells in the kidney, aorta and bone marrow of $SGK1^{fl/fl}$ control mice but not in $SGK1^{fl/fl}$ x tg^{CD4cre} mice, as identified by the surface marker $CD44^{hi}$. To assess markers of renal injury, we measured albumin in 24-hour urine samples collected at the end of the L-NAME/high salt. L-NAME/high salt caused striking albuminuria in $SGK1^{fl/fl}$ mice and was absent in $SGK1^{fl/fl}$ x tg^{CD4cre} mice. In additional studies, we found that loss of SGK1 in T cells abrogates renal and vascular inflammation and protects against hypertensive renal and vascular injury in the L-NAME/high salt model. Thus, our data provide a potential mechanism by which SGK1 in T cells promotes their development of salt sensitivity and their mediation of renal and vascular dysfunction in hypertension.

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Decreased Ability to Excrete a Na Load and Hypertension in the ALMS1 (Alstrom Syndrome 1) Knockout Rat

PrimaryAuthor.AuthorBlock:**Keyona N King-Medina**, Ankita B. Jaykumar, Pablo A. Ortiz, Henry Ford Hosp, Hypertension and Vascular Res Div, Dept of Internal Med, Wayne State Univ Sch of Med, Detroit, MI

Few genes involved in obesity are known to be involved in hypertension. The Alstrom Syndrome 1 protein (ALMS1) is involved in obesity in humans. We found it is expressed in the thick ascending limb (TAL) where it interacts with the Na/K/2Cl cotransporter (NKCC2). ALMS1 is also expressed in other nephron segments such as the proximal tubule and collecting ducts. We hypothesized that ALMS1 deletion leads to hypertension which is likely due to a decrease ability to excrete a salt load secondary to enhanced NaCl transport by the

TAL and other nephron segments. To study the role of ALMS1 in renal function we generated ALMS1 knockout (KO) rats in a Dahl-salt sensitive genetic background via zinc-finger nuclease targeting by MCW gene targeting core. Using radio-telemetry we found the KO rats to have a higher baseline systolic blood pressure on normal Na chow compared to the Wild-type salt sensitive (WT) rats (KO:146±2 and WT:136±1 mmHg, *p=0.0012). To explore whether hypertension is related to increase renal Na reabsorption in KO rats we performed metabolic cage protocols to measure the acute excretion of a Na load (1% of body weight) in conscious rats. Our data show that it took longer for KO rats to excrete the Na load; cumulative or at individual time points (6, 9, 24 h) (cumulative Na, KO:4251±590 µmols/24h and WT:8788±994 µmols/24h, *p=0.0028). To test the role of different nephron segments in higher Na reabsorption we used a single dose of diuretics and measured urine Na and volume excretion. The NKCC2 inhibitor bumetanide (5mg/kg), induced a higher natriuretic response in KO rats (KO:485.2±37.1 µmols/8h, WT:221.8±32 µmols/8h, *p<0.05). The NCC inhibitor hydrochlorothiazide (HCTZ) 200mg/kg, caused a similar natriuretic effect in KO rats (KO:206.8±16 µmols/8h and WT:155.6±20 µmols/8h, p=0.0717). The ENaC inhibitor benzamil (10mg/kg), caused a similar natriuretic response in KO rats (KO:214.5±33 µmols/8h and WT:253.4±22 µmols/8h, p=0.3477). We conclude that ALMS1 KO have a decreased ability to excrete a salt load and this is primarily mediated by enhanced TAL-mediated Na reabsorption. Higher TAL Na absorption is likely involved in hypertension in ALMS1 KO rats. These data show that ALMS1 is important for blood pressure control and renal function.

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Cerebrovascular and Cognitive Dysfunction in DOCA-Salt Hypertension is Mediated by Perivascular Macrophages

PrimaryAuthor.AuthorBlock:**Monica M Santisteban**, Giuseppe Faraco, Gianfranco Racchumi, Josef Anrather, Costantino Iadecola, Weill Cornell Medical Coll, New York, NY

Hypertension (HTN) and high-salt diets are important risk factors for stroke and dementia. DOCA-salt is a recognized model of HTN driven by sodium retention and brain renin-angiotensin system (RAS) activation. However, it is unknown whether essential mechanisms regulating the cerebral circulation are altered in DOCA-salt mice, and, if so, whether these alterations are associated with cognitive impairment. To this end, C57BL/6 mice were implanted with 50mg DOCA pellets SQ and received 0.9% NaCl drinking water for 3 weeks. Cerebral blood flow (CBF) was measured in the somatosensory cortex by laser-Doppler flowmetry through a cranial window. DOCA-salt increased systolic blood pressure (BP; 148±3 vs 112±3 mmHg in controls; p<0.01), and attenuated the CBF increase induced by whisker stimulation (WS; 16.0±1.1 vs 22.4±0.6 %; p<0.01) or by cortical application of acetylcholine (ACh; 13.5±0.9 vs 22.8±1.1 %; p<0.01), without affecting the response to the smooth muscle relaxant adenosine. Cerebrovascular dysfunction was associated with cognitive impairment as assessed by Novel Object Recognition and Barnes Maze tasks (p<0.01). Perivascular macrophages (PVM) express AT1R and Nox2, and, as such, may be a key source of radicals mediating the cerebrovascular effects of brain RAS overactivity. To test this hypothesis, brain PVM were depleted by icv administration of

clodronate (CLO) liposomes. BP was not affected by CLO in either control or DOCA mice ($p>0.05$). PVM depletion improved novel object exploration ($p<0.01$) and time spent in the target quadrant of Barnes Maze ($p<0.05$), while also restoring the CBF responses to both WS (DOCA-CLO 19.6±0.9%; $p<0.05$) and ACh (DOCA-CLO 20.0±1.7%; $p<0.05$). Next, we tested whether reactive oxygen species (ROS) are involved in the cerebrovascular dysfunction. We observed a 45% upregulation in *gp91* mRNA in cerebral vessels from DOCA mice, which was prevented by PVM depletion ($p<0.05$). Application of the ROS scavenger MnTBAP rescued CBF responses to both WS (20.3±0.9%; $p<0.05$) and ACh (19.0±0.8%; $p<0.05$) in DOCA-salt HTN. We conclude that PVM play a previously unrecognized role in the cerebrovascular and cognitive dysfunction of DOCA-salt HTN and may represent a new therapeutic target to alleviate the neurocognitive effects of HTN.

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Therapeutic Suppression of Mtorc2 Signaling Reduces Salt-induced Hypertension and Kidney Injury in SS Rats

PrimaryAuthor.AuthorBlock:**Vikash Kumar**, Loiuse Evans, Clayton Wollner, Theresa Kurth, Allen W Cowley Jr., Medical Coll Wisconsin, Milwaukee, WI

The present study explored the protective effect of mTORC2 inhibition in salt-induced hypertension and kidney injury. We have previously reported that enhanced blood pressure salt-sensitivity with renal chronic interstitial infusion of H_2O_2 (347 nmol/kg/min) in normotensive Sprague Dawley (SD) rats. In the present study, *in vivo* experiment was performed in which H_2O_2 (347 nmol/Kg/min) was chronically infused for 3 days into renal interstitium of unilaterally nephrectomized SD rats. A significant increase of mTORC2 activity (pAKT/AKT) was observed in the renal cortex of SD rats infused with H_2O_2 compared to saline infused rats. We have recently shown that excess production of H_2O_2 in the SS rat kidneys is the hallmark of salt-induced hypertension. We hypothesized that mTORC2 in the kidney contributes to the development of salt-induced hypertension in SS rats. Rats were treated with PP242 which is an ATP-competitor inhibitor which inhibits the activities of both mTORC1 and mTORC2 whereas rapamycin specifically inhibits mTORC1. PP242 was administrated daily (i.p., 15 mg/Kg/day) for 21 days to SS rats fed a 4.0% NaCl diet. Remarkably, salt-induced hypertension was significantly reduced in SS rats which averaged 119 ± 2 mmHg in PP242 treated rats (n=7) compared to 168 ± 3 mmHg in vehicle treated rats (n=7). Albuminuria was greatly reduced with urine albumin excretion (mg/day) averaging 32.8 ± 3 in PP242 treated rats compared to 256 ± 37 in vehicle treated rats. PP242 treatment notably resulted in reduced infiltration of T lymphocytes in the kidneys of SS rats fed a 4.0% NaCl diet. $CD3^+$ cells/mm^2 averaging 157.0 ± 40.0 compared to 36.0 ± 11.0 in the renal cortex and 218.0 ± 24.0 compared to 24.0 ± 9.0 in the outer medulla in PP242 versus vehicle treated rats. These data show that mTORC2 is required for the intiation of salt-induced hypertension and therapeutic suppression of this pathway virtually abolished salt-sensitivity blood pressure and kidney injury.

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Probenecid Downregulates Kidney Pendrin and AQP-2 and Potentiates Hydrochlorothiazide-induced Diuresis

PrimaryAuthor.AuthorBlock:**Manoocher Soleimani**, Sharon Barone, Kamyar Zahedi, Jie Xu, Univ, Cincinnati, OH

Background: Concurrent inactivation of the kidney Na-Cl co-transporter NCC and the Cl^-/HCO_3^- exchanger pendrin leads to significant salt wasting, whereas single deletion of NCC does not cause any significant salt wasting, indicating that pendrin mitigates the salt excretion caused by NCC inactivation. Probenecid is a uricosuric agent that, in addition, exhibits positive ionotropic effect in the heart, downregulates/inactivates pendrin (in mammary gland cells) and blocks the ATP transporter Pannexin 1 in the proximal tubule and colleting duct. **Hypothesis:** Pretreatment with probenecid downregulates pendrin; therefore, leaving NCC as the main salt absorbing transporter in the distal nephron, and hence enhancing the hydrochlorothiazide (HCTZ) diuresis. **Results:** Male Sprague Dawley rats were treated with probenecid intraperitoneally (i.p.) at 250 or 100 mg/kg for 6 days and then received HCTZ while being maintained on probenecid for 4 more days. Urine output increased from 9.8 at baseline to 15.9 ml/24 hrs after 10 days of Probenecid at 250 mg/kg ($p<0.02$, n=5). Treatment with HCTZ alone for 4 days caused a mild diuresis, with urine output increasing to 13.8 ml/24 hrs ($p>0.05$, vs. baseline, n=5) However, rats pretreated with Probenecid for 6 days exhibited a profound diuresis when HCTZ was added for 4 additional days, with urine output increasing to 42.9 ml/day, a more than 300% increase vs. rats treated with either Probenecid or HCTZ ($p<0.003$ vs. both groups, n = 5). In the absence of pretreatment with Probenecid, the diuresis caused by concurrent Probencid plus HCTZ treatment was similar to HCTZ alone ($p>0.05$). Immunofluorescent, Northern and/or Western hybridization studies demonstrated a significant reduction in the expression of pendrin and AQP2 in the kidney cortical collecting duct/cortex of probenecid treated rats. At 100 mg/kg, Probenecid alone had no significant effect on urine output but caused a robust diuresis when HCTZ was added, with the urine output increasing from 9.93 baseline to 24.23 ($p<0.001$, n=7). **Conclusion**: Probenecid pretreatment downregulates pendrin and AQP2 and robustly enhances diuresis by HCTZ-mediated NCC inhibition in the distal nephron.

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Role of Nox4 in the Control of ENaC Activity in Dahl SS Rats During the Development of Salt-induced Hypertension and Diabetic Nephropathy

PrimaryAuthor.AuthorBlock:**Tengis S Pavlov**, Henry Ford Health System, Detroit, MI; Daria V Ilatovskaya, Gregory Blass, Oleg Palygin, Vladislav Levchenko, Allen W Cowley Jr, Alexander Staruschenko, Medical Coll of Wisconsin, Milwaukee, WI

Dahl salt sensitive (SS) rat is a well-established model for studying salt-induced hypertension and associated kidney injury. We and others have previously shown that salt-sensitive hypertension is accompanied by increased renal production of reactive oxygen species (ROS) and excessive activity of ENaC in the distal nephron. To investigate role of ROS, and specifically NADPH oxidase 4 (Nox4), a primary source of ROS in the kidney, involved in the regulation of ENaC activity during the development of SS hypertension and type 1 diabetes, we performed patch clamp analysis in the cortical collecting ducts of Dahl SS rats and SS rats lacking Nox4 ($Nox4^{-/-}$). We found that SS rats fed a 4% NaCl diet have significantly elevated ENaC activity even 3 days post diet change. ENaC activity (NP_o) was 0.57±0.08, 1.32±0.3 and 1.69±0.06 before, 3 days and 3 weeks after high salt diet, respectively. In contrast, ENaC activity was not significantly different in $SS^{Nox4-/-}$ animals after high salt diet. To study the role of Nox4 in hyperglycemic conditions, diabetes was induced in 6 weeks old male wild type or $Nox4^{-/-}$ rats with a single i.p. injection of STZ. We found that ENaC activity in the animals that were hyperglycemic for 11 weeks was elevated compared to control rats (0.71±0.10 and 1.27±0.2; $p<0.05$) and this effect was mediated via changes in channel open probability. Nox4 deficiency blunts the effect of hyperglycemia on ENaC activity (in STZ-treated animals open probability significantly increased from 0.43±0.06 to 0.86±0.07 in WT rats, but in the $Nox4^{-/-}$ group did not change: P_o was 0.51±0.06 and 0.51±0.07, respectively) that delineates the importance of Nox4-mediated ROS production for regulation of ENaC open probability. Taken together, our data indicate that ENaC activity in Dahl SS rats is elevated following a change of salt diet (3 days and 3 weeks at high salt) and after the development of type 1 diabetes. Furthermore, Nox4 plays a crucial role in these effects of high salt and hyperglycemia on ENaC activity.

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Imaging Renin Granule Exocytosis by Total Internal Reflection (TIRF) Microscopy in Mouse Juxtaglomerular Cells: Effect of cAMP and Beta Adrenergic Stimulation

PrimaryAuthor.AuthorBlock:**Mariela Mendez**, Henry Ford Hosp, Detroit, MI

Renin is stored in dense core granules in juxtaglomerular (JG) cells. Renin release is highly regulated. However, the kinetics of renin release and mode of granule exocytosis remains unexplored. We developed a new method for real-time visualization of renin granule exocytosis by TIRF microcopy. We hypothesize that renin exocytosis is due to a kiss-and-run mode of exocytosis, where fusion of the granule with the plasma membrane is transient and granule integrity is maintained. To study this, we generated a new adenoviral construct encoding full length mouse renin tagged with Carboxyl-terminus yellow fluorescent protein (Ad-Renin-YFP). First, we characterized its expression and activity in an endocrine pituitary cell line that does not express endogenous renin (Att20 cells). By Western Blot, we observed a band at the expected molecular mass of renin-YFP (70 kDa)(n=4). Ad-Renin-YFP retains its enzymatic activity in Att20 cells since angiotensin I conversion from angiotensinogen was only detected in Att20 cells transduced with renin-YFP (n=3; $p<0.01$). To monitor exocytosis of renin-YFP, we transduced primary cultures of mouse JG cells and monitored granule movement and their changes in

fluorescence intensity within 250 nm of the plasma membrane by TIRF microscopy. Under baseline conditions the average number of docked granules was 16±4 granules per cell (n=12). Translational movement (X-Y planes) of docked granules was negligible. The number of events per cell during 10 minutes was low and no full fusion was detected (1.5 ±0.5 total events per cell, n=4). After stimulation with cAMP, the number and frequency of events increased to 5.3±1.0 events per cell/10 min (n=8, $p<0.05$). Similarly the number of events increased to 4.25±0.9 when JG cells were stimulated with isoproterenol, without a decrease in the number of granules docked (n=9). While most (73.5%) of the exocytic events occurred from docked granules; only 36.5% of events were caused by recruitment of newcomer granules to the TIRF field. We conclude that in JG cells, full fusion of granules is not the main mechanism of renin exocytosis. The rapid bursts in fluorescence intensity of docked granules suggest that kiss-and-run is the main mechanism of stimulated-renin exocytosis, in a highly regulated process.

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Gamma-adducin Effects on Microvascular Function- a Common Link of Hypertension Induced Chronic Kidney Disease and Cognitive Impairments

PrimaryAuthor.AuthorBlock:**Fan Fan**, Shaoxun Wang, Paige N Mims, Chao Zhang, Richard J Roman, Univ of Mississippi Medical Ctr, Jackson, MS

Chronic kidney disease (CKD) and cognitive impairments are common complications of hypertension. Increasing evidence suggests that the cognitive impairments associated with CKD may be related to microvascular dysfunction, however, the underlying mechanisms remain to be elucidated. FHH is a genetic model of hypertension-induced nephropathy. We found that the myogenic response and autoregulation of the renal and cerebral circulation is impaired in FHH rats, and was restored in a FHH.1^{BN} congenic strain in which a small region of Chr. 1 containing 15 genes, including Add3, from BN rats was transferred into the FHH background. The present study examined whether Add3 contributes to hypertension related CKD, and is associated with the development of cognitive impairments due to microvascular dysfunction. FHH rats exhibited impaired autoregulation of RBF in comparison with FHH.1^{BN} rats. Pgc estimated from the stop flow pressure increased by 20 mmHg in FHH rats when RPP was increased from 100 to 140 mmHg versus only 4 mmHg in FHH.1^{BN}. 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.1^{BN} and FHH.Add3 rats. Glomerular injury scores were 3.31 ± 0.01, 2.54 ± 0.01 and 2.50 ± 0.03, and areas of fibrosis in renal cortex were 23.57 ± 1.04%, 8.28 ± 0.33 and 4.71 ± 0.3 in DOCA/salt induced hypertensive FHH, FHH.1^{BN} and FHH.Add3 rats, respectively. CBF rose by 99 ± 7%, 64 ± 5% and 42 ± 4% in FHH, FHH.1^{BN} and FHH.Add3 rats, respectively, when MAP was increased from 100 to 190 mmHg, demonstrating impaired autoregulation of CBF in FHH rats was partially rescued with the replacement of wildtype Add3. BBB leakage was greater in FHH rats than in FHH.1^{BN} and FHH.Add3 rats, and hypertensive FHH rats exhibited marked neurodegeneration and vascular remodeling of the neocortex and hippocampus. The hypertensive FHH rats took 2.5 times longer time to escape from an eight-

arm water maze in comparison to FHH.1^{BN} rats suggesting cognitive deficit. These results indicate that Add3 may play a role in the development of hypertension related CKD and cognitive impairments in FHH rats associated with microvascular dysfunction.

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Reduced Expression, Knockout, and Pharmacological Intervention of *Arhgef11*-RhoA Pathway Significantly Attenuates Renal Injury and Blood Pressure

PrimaryAuthor.AuthorBlock:Ashley C Johnson, Univ of Mississippi Medical Ctr, Jackson, MS; David S Pasco, Univ of Mississippi --Sch of Pharmacy, Oxford, MS; **Michael R Garrett**, Univ of Mississippi Medical Ctr, Jackson, MS

Through genetic analysis of the Dahl salt-sensitive (SS) rat, a model of hypertension and chronic kidney disease (CKD), Arhgef11, a Rho guanine nucleotide exchange factor was implicated in kidney injury. Arhgef11, via exchange of GDP for GTP, plays a role in the activation of RhoA signaling cascades through a number of cell stimuli that impact cytoskeletal structure and influence cell-cell contacts and promotes cell transformation. Previously, we demonstrated that reduced *Arhgef11* expression/protein function in an SS-*Arhgef11*^{SHR}-minimal congenic strain resulted in significantly decreased proteinuria, fibrosis, and improved renal hemodynamics compared to SS-WT, without impacting BP. More recently, an SS-Arhgef11$^{-/-}$ knockout rat model was studied. On low-salt (0.3% NaCl), SS-Arhgef11$^{-/-}$ animals demonstrated reduced proteinuria and renal injury, with no impact on BP versus SS-WT. In contrast, SS-Arhgef11$^{-/-}$ animals on an elevated-salt diet (2% NaCl) demonstrated a significant ($p<0.001$) attenuation of proteinuria (41±7.9 mg/24 hrs.), along with a substantial reduction in BP (124±2.6 mm Hg) compared to SS-WT (119±15.3 mg/24 hrs. and 151±5.9 mm Hg). This data suggests that reduced expression/loss of *Arhgef11* (on low-salt) similarly leads to renoprotection, whereas the loss of Arhgef11 (vs reduced expression) leads to blunting of salt-induced elevations in BP vs SS-WT. These animal studies, in combination with *in vitro* work, suggest that inhibition of Arhgef11-RhoA could be an effective therapeutic for CKD. Using an *in vitro* cell screen, several hundred natural product compounds were tested for ability to inhibit Arhgef11/RhoA activity and identified 3 compounds. A pilot study done using one compound (sufficient for a one-week study) was very encouraging as animals treated with XTL-019-G7 (20mg/kg/day) exhibited an ~25% reduction in proteinuria compared to vehicle (VEH) treated animals (70±8.5 versus 95±9.6 mg/24hours, $p=0.07$), demonstrating that *in vivo* testing is feasible and that the compound warrants further testing. In summary, genetic analysis of a model of CKD identified a gene/pathway involved in kidney injury that served as a target to screen natural product derived small molecules, and may ultimately lead to a new treatment for CKD.

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A Kidney Targeted Epoxyeicosatrienoic Acid Analog, EET-F01, Reduces Cisplatin-induced Nephrotoxicity

PrimaryAuthor.AuthorBlock:**John D Imig**, Md Abdul Khan, Medical Coll of Wisconsin, Milwaukee, WI; Adeniyi M Adebesin, John R Falck, Univ of Texas Southwestern Medical Ctr, Dallas, TX

Epoxyeicosatrienoic acid (EET) analogs have exceptional therapeutic potential to combat cardiovascular and kidney diseases. EET analogs combat damage in acute and chronic kidney disease models. Biological actions attributed to EET analogs such as vasodilation, anti-inflammation, anti-apoptosis, and anti-fibrosis are ideally suited to treat kidney diseases. Although EET analogs have performed well in several *in vivo* models, targeted delivery of EET analogs to the kidney can be reasonably expected to reduce the level of drug needed to achieve a therapeutic effect in the kidney and obviate possible side effects. For EET analog kidney-targeted delivery, we conjugated an EET analog to folic acid because there is a high concentration of folate receptors in renal tissue. The EET analog was conjugated to folic acid via a PEG-diamine linker. Next, we compared the kidney targeted EET analog, EET-F01, to a well-studied EET analog, EET-A. EET-A or EET-F01 was infused i.v. (10mg/kg/hr) for 6 hours via the rat jugular vein. Plasma and kidney tissue were collected and EET-A or EET-F01 measured by LC-MS-MS. EET-A plasma level was 1.6 ng/mL, but EET-A was undetectable in the kidney. On the other hand, EET-F01 was 6.5 ng/mL in plasma and 26.7 ng/mL in kidney tissue. These data demonstrate that EET-F01 targets the kidney. Experiments were conducted to compare EET-F01 and EET-A to decrease cisplatin-induced nephrotoxicity. A single injection of cisplatin (7 mg/kg ip) was administered to WKY rats treated with vehicle, EET-A (10 mg/kg ip) or EET-F01 (20 mg/kg or 2 mg/kg ip) for five days. Cisplatin increased BUN (125 ± 11 mg/dL) and NAG (12 ± 4 IU/L) compared to control (36 ± 9 mg/dL and 4 ± 1 IU/L). EET-F01 was as effective as EET-A in decreasing BUN, NAG, and renal histological injury five days following cisplatin administration. Despite it almost 2x-greater molecular weight compared with EET-A, EET-F01 was effective in lowering BUN and NAG at 20 mg/kg/d and at a 10-fold lower dose of 2 mg/kg/d. These data clearly demonstrate that EET-F01 targets the kidney and allows for a lower effective dose. In conclusion, we have developed a kidney targeted EET analog, EET-F01, that demonstrates excellent potential as a therapeutic for kidney diseases.

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Myeloid Mineralocorticoid Receptor Controls Inflammatory and Fibrotic Responses After Renal Ischemic Injury via Macrophage Interleukin-4 Receptor

PrimaryAuthor.AuthorBlock:**Jonatan Barrera-Chimal**, Insto de Investigaciones Biomédicas, UNAM, Mexico City, Mexico; Sebastian M. Lechner, Soumaya El Moghrabi, INSERM, UMRS 1138, Ctr de Recherche des Cordeliers, Paris, France; Peter Kolkhof, BAYER AG, Wuppertal, Germany; Frédéric Jaisser, INSERM, UMRS 1138, Ctr de Recherche des Cordeliers, Paris, France

Introduction: Patients who survive an episode of acute kidney injury (AKI) are at high risk of de novo chronic kidney disease (CKD) development. Pharmacological mineralocorticoid receptor (MR) antagonism is useful to prevent CKD after a single episode of ischemic AKI in the rat. **Objective:** Test the involvement of myeloid MR in the development of kidney fibrosis after an ischemic AKI episode. **Methods:** We included 18 male C57/B6 mice that were divided in: sham, renal ischemia for 22.5 min and IR plus treatment with the non-steroidal MR antagonist finerenone (10 mg/kg) at -48, -24 and -1 h before IR. MR inactivation in myeloid cells (MR^{MyKO}) was achieved by crossing mice with the MR alleles flanked by loxP sites ($MR^{f/f}$) with mice expressing the Cre recombinase under the LysM promoter activity. In $MR^{f/f}$ and MR^{MyKO} mice we induced renal IR of 22.5 min or sham surgery. The mice were followed-up during 4 weeks to test for AKI to CKD transition. In another set of mice, the macrophages were sorted from kidneys after 24 h of reperfusion and flow cytometry characterization or mRNA extraction was performed. Thyoglycolate elicited peritoneal macrophages were used for *in vitro* studies. **Results**: The progression of AKI to CKD after 4 weeks of renal ischemia in the untreated C57/B6 and $MR^{f/f}$ mice was characterized by a 50% increase in plasma creatinine, a 2-fold increase in the mRNA levels of TGF-β and fibronectin as well as by severe tubule-interstitial fibrosis. The mice that received finerenone or MR^{MyKO} mice were protected against these alterations. Increased expression of M2-anti-inflamatory markers in kidney-isolated macrophages from finerenone-treated or MR^{MyKO} mice was observed. The inflammatory population of $Ly6C^{high}$ macrophages was reduced by 50%. In peritoneal macrophages in culture, MR inhibition promoted increased IL-4 receptor expression and activation, facilitating macrophage polarization to an M2 phenotype. **Conclusion**: MR antagonism or myeloid MR deficiency facilitates macrophage polarization to a M2, anti-inflammatory phenotype after kidney IR, preventing maladaptive repair and chronic kidney fibrosis and dysfunction. MR inhibition acts through the modulation of IL-4 receptor signaling to facilitate macrophage phenotype switching.

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Beneficial or Harmful? The Role of Intensive Anti-hypertensive Treatment in the Development of Chronic Kidney Disease

PrimaryAuthor.AuthorBlock:**Ling Wang**, Donna Wang, Michigan State Univ, East Lansing, MI

Hypertension is the leading cause of end-stage renal disease, and one of the goals of anti-hypertensive treatment is to protect the kidney. However, it is unknown how low of blood pressure as the treatment target should be so that anti-hypertensive therapy would not bring harm to patients especially for those already suffer from chronic kidney disease (CKD). Thus, we used the data set from The Systolic Blood Pressure Intervention Trial (SPRINT) to study the effect of lowering systolic blood pressure on renal disease development. The SPRINT data randomly assigned patients with a systolic blood pressure (SBP) of 130 mm Hg or higher to a SBP treatment target of less than 120 mm Hg (intensive treatment, n=4678) or a treatment target of less than 140 mm Hg (standard treatment, n=4683). We examined the effect of intensive treatment on six renal outcomes: 1) CKD composite, 2)50 percent reduction in eGFR, 3) dialysis 4) albuminuria, 5) 30 percent reduction in eGFR for patients with CKD at baseline (n=2646) and 6) albuminuria for patients without CKD at baseline (n=6715). Generalized Estimating Equation is used to account the correlation of blood pressure levels over time. At the end of year 1, the mean SBP was 121.4± 0.21 mm Hg in the intensive treatment group and 136.2± 0.21 mm Hg in the standard treatment group. The patients in intensive group were found to have a higher chance of 30% reduction of eGFR (OR=3.684, 95% CI= 2.51-5.40) than in standard treatment group. There was no difference between intensive and standard treatment groups for other 5 outcomes. In addition, 1 mm Hg elevation in SBP in patients with CDK at baseline significantly increased the chance of CKD composite (OR=1.03, 95% CI=1.01-1.04), the chance of 50 percent reduction in eGFR (OR=1.02, 95% CI=1.01-1.05), and chance of 30 percent reduction in eGFR (OR=1.02, 95% CI=1.01-1.02). Thus, SBP significantly correlated with renal outcomes in CKD patients. Our data show that five renal outcomes examined using SPRINT data set are not improved by intensive management of SBP in CKD patients, rather, patients received intensive management have a higher risk of eGFR reduction by 30%, which could be detrimental. Our study indicated that intensive SBP management should not be recommended to CKD patients.

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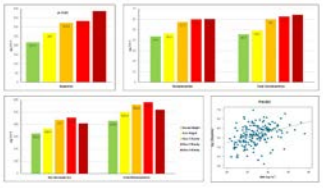
Sympathetic Activity Increases With Obesity in Hypertensive Patients

PrimaryAuthor.AuthorBlock:**Mohammed Siddiqui**, Tanja Dudenbostel, Chee Paul Lin, Suzanne Oparil, David A Calhoun, Univ of Alabama at Birmingham, Birmingham, AL

Introduction: Obesity is one of the most important risk factors for the development of hypertension; the mechanisms by which obesity raises BP are not fully understood. Obesity leads

to ↑ sympathetic autonomic nervous system (SANS) activity by several mediators such as leptin, ↓ nitric oxide, ↑ angiotensin II, ↓adiponectin, ↓ ghrelin and baroreflex dysfunction. Unknown is the role of dopamine, catecholamines and metanephrines in hypertensive individuals with obesity indexed by body mass index (BMI). **Methods:** In this prospective evaluation, 195 hypertensive patients on medications were recruited after ≥3 clinic visits at University of Alabama at Birmingham Hypertension Clinic. All patients underwent measurement of clinic BP, BMI and 24-hr urine for dopamine, total catecholamines (epinephrine and norepinephrine) and total metanephrines (metanephrine and normetanephrine). WHO obesity classification was used to categorize patients based on BMI into normal weight (BMI 18.5 to 24.9, n=19); over-weight (BMI 25 to 29.9, n=45); Class 1 obesity (BMI 30 to 34.9, n=46); Class 2 obesity (BMI 35 to 39.9, n=44) and Class 3 obesity (BMI greater than 40, n=36). **Results:** Overall, patients were 50% females, 56.8% African Americans, 58.1 ± 11.0 years old, BMI was 34.1 ± 7.1 Kg/m^2, BP was 126.5/79.9 ± 20.2/12.3 mmHg and heart rate of 73.5 ± 12.5 beats/min and total number of BP medications were 3.8 ± 1.4. 24-hr urinary dopamine, norepinephrine, total catecholamines, normetanephrine and total metanephrines increased with increasing BMI categories.



Conclusions: Increase in BMI is associated with increasing dopamine, catecholamines and metanephrines indicative of progressive SANS activation.

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Primary Hyperadrenergic Postural Tachycardia Syndrome. Evidence From Sympathetic Nerve Recordings

PrimaryAuthor.AuthorBlock:**Luis E Okamoto**, Emily M Garland, Vanderbilt Autonomic Dysfunction Ctr, Nashville, TN; Kyoko Sato, Dept of Med, Tokyo Women's Medical Univ, East Medical Ctr, Tokyo, Japan; Laura Diedrich, Middle Tennessee Res Inst, Nashville, TN; Raffaello Furlan, Dept of Biomedical Sciences – Humanitas Univ, Milano, Italy; Giris Jacob, Dept of Med & Recanati Autonomic Dysfunction Ctr, Tel Aviv Sourasky Medical Ctr, and Sackler Faculty of Med, Tel Aviv Univ, Tel Aviv, Israel; David Robertson, Italo Biaggioni, André Diedrich, Vanderbilt Autonomic Dysfunction Ctr, Nashville, TN

Postural tachycardia syndrome (POTS) is a chronic disabling condition with excessive upright tachycardia and symptoms due to enhanced sympathetic activity in otherwise healthy young individuals. Because it is not clear if sympathetic activation is a primary phenomenon, or an appropriate compensatory response to hypovolemia, deconditioning or partial neuropathy, we analyzed resting supine muscle sympathetic nerve activity (MSNA) in 38 female POTS patients (mean±SD, age 32.1±9.1, BMI 22.7±5.4). MSNA showed a wide range of burst rate (19.7±12.1 bursts/min, range 1.2 to 54, 95% CI: 15.7 to 23.6). We compared the 1st and 4th Quartiles of 28 MSNA records (MSNA_Q1: < 7.3; MSNA_Q4: > 23.4

bursts/min) to determine if resting MSNA is related to heart rate (HR) or blood pressure (BP) responses to orthostatic challenge and the Valsalva maneuver (2-way ANOVA with post-hoc tests).
Upright HR was inappropriately high in both groups (MSNA_Q1: 120±19 vs. MSNA_Q4: 110±20 bpm, NS), but the MSNA_Q4 group had higher upright diastolic BP than MSNA_Q1 (81±12 vs. 62±8 mmHg, P<0.01). Similarly, there were no differences in HR response to the Valsalva maneuver between groups, but systolic and diastolic BP were higher during late phase 2 in MSNA_Q4 (p<0.05), and this was preceded by higher MSNA spike rate (44.3±16.3 vs. 81.2 ±25 maximal spikes/secs per beat, p<0.01) during early phase 2 of Valsalva maneuver.
The greater BP responses, but comparable HR responses, to posture and Valsalva challenges provide evidence that the subset of POTS patients with higher resting MSNA have a primary sympathetic activation. These patients might benefit from sympatholytic therapy in contrast to other POTS patients with low or absent resting MSNA.

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Potent Hypertensive Actions of Angiotensin-sensitive Neurons Within the Lamina Terminalis

PrimaryAuthor.AuthorBlock:**Annette D De Kloet**, Gean Domingos-Souza, Michael D. Smeltzer, Yalun Tan, Karlena Cahill, Erin B. Bruce, Mohan K Raizada, Colin Sumners, Eric G. Krause, Univ of Florida, Gainesville, FL

It is accepted that activation of angiotensin type 1a receptors (AT1a) within the CNS elevates blood pressure by influencing sympathetic outflow and vasopressin (VP) secretion; however, the neuronal circuits mediating these effects are not completely understood. The present studies characterize the structure and function of AT1a neurons residing in the median preoptic nucleus (MnPO) and the organum vasculosum of the lamina terminalis (OVLT), thereby evaluating their potential role in blood pressure control. Using male mice that express Cre-recombinase prior to the STOP codon of the AT1a gene, initial studies combined genetic reporting with in situ hybridization to reveal that AT1a neurons in the MnPO and OVLT are largely excitatory (87±4% express vesicular glutamate transporter 2). Subsequently, AT1a-Cre mice were delivered a Cre-inducible adeno-associated virus to induce expression of channelrhodopsin-2 (ChR2) and enhanced yellow fluorescent protein (eYFP) specifically within AT1a neurons of the MnPO/OVLT (AAV-ChR2-eYFP; n = 4). Control mice were delivered

AAV-eYFP (n = 4). Analysis of eYFP immunofluorescence revealed that neurons within the MnPO/OVLT that express AT1a send projections to the paraventricular nucleus of the hypothalamus (PVN; an area involved in sympathetic outflow and VP secretion) that appear to synapse onto VP synthesizing neurons. To evaluate the functionality of this connection, we optogenetically stimulated AT1a neurons in the region while recording cardiovascular parameters in anesthetized mice. Ten-minutes of optogenetic stimulation (473 nM;15 Hz; 20 ms pulse width; 60 s ON/OFF) robustly elevated systolic blood pressure in AAV-ChR2-eYFP mice relative to AAV-eYFP controls. This effect was rapid in its onset (34 ± 9 vs. -2 ± 4 mmHg at 5 min, $p < 0.05$) but persisted for the entire 50 min of cardiovascular recording (45 ± 11 vs. -3 ± 7 mmHg at 50 min, $p < 0.05$). Intriguingly, the optogenetic stimulation also resulted in 62% increase in Fos induction in AVP neurons within the PVN relative to AAV-eYFP controls (86 ± 5 vs. 52 ± 2 %; $p<0.01$). Collectively, these results suggest that excitation of AT1a neurons in the MnPO/OVLT recruits autonomic and neuroendocrine responses that promote robust and sustained increases in blood pressure.

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Renal Nerves Mediate Renal Inflammatory Signaling Independent of Hypertension in the DOCA-salt Rat

PrimaryAuthor.AuthorBlock:**Christopher T Banek**, John W Osborn, Univ of Minnesota, Minneapolis, MN

Preclinical and clinical studies demonstrate renal denervation (RDx) may be an effective treatment for hypertension (HTN); however, the mechanisms of this effect remain unknown. We recently reported (RDx) mitigates HTN, and prevents renal inflammation in the deoxycorticosterone acetate (DOCA)-salt rat model of HTN. Although these findings suggest renal nerves directly mediate renal inflammatory signaling, this effect may also be secondary to lowering arterial pressure (AP). In this study, we aimed to elucidate the specific role of renal nerves on renal inflammatory signaling using a unilateral RDx approach to control for lowered AP. We tested the hypothesis that RDx will ameliorate the renal inflammation independent of HTN in the DOCA-salt rat. To test this hypothesis, 8 male Sprague Dawley (SD) rats were implanted with radiotelemeters to measure AP and subjected to unilateral RDx. Rats were then administered DOCA (100mg, s.c.) and 0.9% saline for 21 days. Rats were then anesthetized, and renal tissue and urine were collected from both RDx and contralateral control (CON) kidneys. Renal inflammation was assessed by assay for pro-inflammatory cytokines (IL-1β, IL-2, IL-6, GRO/KC, MCP-1) in both urine and renal tissue.

Data presented as mean ± SEM, *p<0.05. Mean AP increased from 99±3 to 135±4mmHg over DOCA-salt treatment. Renal GRO/KC content was markedly reduced in RDx kidneys compared to CON (14±5* vs. 467±96pg/mg total protein), and this was mirrored in the urinary excretion of GRO/KC in RDx vs. CON (51±11* vs. 3717±500pg/mg creatinine). Similarly, renal MCP-1 content was lower in RDx (13±2*) compared to CON (292±51). Urinary excretion of MCP-1 was also decreased by RDx (67±31* vs. 1758±721). Finally, urinary protein:creatinine was abated by RDx (13±3* vs. 19±3au). Notably, there was no effect of RDx on renal or urinary content of IL-1β, IL-2, nor IL-6. From this data, we conclude that renal nerves mediate renal inflammation associated with chemokines GRO/KC and MCP-1 release, independent of HTN. However, RDx had no effect on IL-1β, IL-2, IL-6, which suggests a chemokine specificity of renal nerve contribution to inflammatory signaling. Studies are currently underway to establish the effect of RDx on renal infiltration of specific immune cell populations.

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Barorreflex Hypofunction is Due to Increased Arterial Stiffness in Chronic Hypertension

PrimaryAuthor.AuthorBlock:**Mariano Duarte**, Jose Milei, Hosp de Clinicas Facultad de Medicina UBA, Buenos Aires, Argentina; Eleonora Savio Galimberti, Lankenau Inst for Medical Res, Philadelphia, PA; Carlos Reyes Toso, Catedra de Fisiologia Facultad de Medicina UBA, Buenos Aires, Argentina; Claudio Bellido, Hosp de Clinicas Facultad de Medicina UBA, Buenos Aires, Argentina

The autonomic nervous system (ANS) plays a central role in blood pressure (BP) homeostasis. BP changes can activate barorreflex response, which is one of the main mechanisms through which ANS rapidly regulates BP. This regulatory mechanism is compromised in chronic arterial hypertension (HT). We hypothesize that barorreflex activity is diminished in patients with high BP due to increased arterial stiffness (AS), which decreases the barorreceptors' sensitivity to mechanical deformation, and that the decreased barorreflex activity might contribute to the maintenance and progression of HT. To test this hypothesis, we studied the chronotropic response of patients (>65 years of age, n=101 patients, 75 % with HT) after Valsalva's maneuver (pressor maneuver) by measuring BP, heart rate (HR), and ECG (DII). Chronotropic response after the test was defined as the smallest R-R interval up to 5 s. post-test, normal response being considered 10-30 beats/min. of increment in HR and decreased response was defined as <10 beats/min of HR increment. We also assessed AS by measuring carotid-femoral pulse wave velocity (PWV-CF), which has a predictive value for cardiovascular events. We observed a significant increase in systolic BP in the HT group respect to control (147.2±15.7 mm Hg vs. 130.2±10.8 mm Hg). We also observed altered barorreflex function in 60 out of 76 HT patients (~79 %) but only 3 out of 25 controls (12 %). Median of increased HR was significantly decreased in the HT group respect to control response (6 beats/min. vs. 12 beats/min., P<0.001). PWV-CF significantly increased in the HT group compared to control ((10,54 m/s vs. 8,58 m/s, p<0.001). Also, changes in PWV-CF were inversely correlated to cronotropic response post-Valsalva. These results suggest that HT patients > 65 years of age are particularly prone to hypofunction of the

barorreflex mechanism. Valsalva's maneuver in combination with ECG might represent a quick, non-invasive method to assess chronotropic response and evaluate barorreflex function. Barorreflex hypofunction might contribute to the maintenance and even progression of HT in these patients. The increased AS revealed by increased PWV-CF may be the nexus between HT and barorreflex hypofunction.

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Norepinephrine (NE) Resides in Renal Perivascular Adipose Tissue: Form and Function

PrimaryAuthor.AuthorBlock:Carolina B Restini, Alex Ismail, Robert Burnett, Gregory D Fink, Michigan State Univ - MSU, East Lansing, MI; **Stephanie W Watts**, MI, East Lansing, MI

Perivascular adipose tissue (PVAT) is gaining importance as its ability to secrete and take up substances that modify vascular tone, and thus blood pressure. The kidney is inarguably important to blood pressure regulation, but little is known about renal arterial PVAT (RP) relative to its adipogenic profile or its function to modify arterial contraction as specifically conducted by a resident adrenergic system. We hypothesized that NE was present in RP, that RP had a mixture of both brown and white adipocyte gene signatures and that the NE in RP could promote renal artery contraction. Our model was the isolated renal artery +/- PVAT of the male Sprague Dawley rat, with thoracic aortic PVAT (TAP) and mesenteric PVAT (MP) as brown-like and white fat comparators, respectively. HPLC and immunohistochemistry (IHC) were used to detect NE; RT-PCR to construct an adipogenic/adrenergic profile; and myography to measure contraction. All PVATs contained NE (ng/g tissue, n=4-6): RP:524±68, TAP:740±16, MP:96±24. In RP, NE, visualized by IHC, was clearly present in adipose tissue resembling both brown and white fat. The identification of brown fat was validated by positive uncoupling protein-1 (UCP-1) staining in the RP; this made up some but not all of the RP. RT-PCR measures (2-ddCt values, n=4) supported expression of both brown-like (UCP-1: RP: 1.0±0.1, MP 3.2e-05±2.94e-05, TAP: 1.3±0.1; Cidea: RP: 1.0±0.1, MP: 0.01±0.002, TAP: 0.9±0.1) and white adipogenic genes (Tcf21: RP: 1.0±0.2, MP: 5.9±1.0, TAP: 0.3±0.1). The existence of a functional adrenergic system in the RP was supported by two findings. First, the efficacy of the indirect sympathomimetic tyramine to cause contraction was greater in isolated renal artery (n=11) +PVAT (14.4+1.8mN) vs -PVAT (7.6+1.2mN). Second, tyramine-induced contraction in +PVAT tissues was reduced by the α1-adrenoceptor antagonist prazosin (100 nM; 90+5%, n=6) and NE transporter inhibitor nisoxetine (1 uM; -logEC50 [M]- Veh: -5.0+0.4; nisoxetine = 3.7+0.2 n=4). Collectively, these data support the existence of a functional adrenergic system in a PVAT that has a profound potential to change renal arterial function. Knowledge about this fat depot may increase our ability to modify renal function in the treatment of hypertension.

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Endothelial Specific Sodium Channel Activation in Endothelium Dysfunction and Vascular Stiffness in Obese Female Mice

PrimaryAuthor.AuthorBlock:**Guanghong Jia**, Annayya R. Aroor, Michael A Hill, Sadie A Allen, Javad Habibi, Yan Yang, Yan Jia, Dongqing Chen, Camila M Manrique, Guido Lastra, Vincent G DeMarco, Adam Whaley-Connell, Luis A Martinez-Lemus, Gerald A Meininger, Univ of Missouri, Columbia, MO; Frederic Jaisser, INSERM, UMR_S 1138, Team 1, Ctr de Recherche des Cordeliers, UPMC Univ Paris 06, Univ Paris Descartes, Paris, France; James R Sowers, Univ of Missouri, Columbia, MO

Excessive activation of endothelial cell (EC) mineralocorticoid receptor (ECMR) signaling induces EC epithelial sodium channel (EnNaC) activity to promote cardiovascular stiffness. Our previous study has demonstrated that activated ECMR signaling prompts expression and translocation of EnNaC to the EC surface inducing fibrosis, inflammation, and macrophage infiltration in the vasculature of female mice fed a western diet (WD). As ECMR KO also prevented these abnormalities, we posit that ECMR/EnNaC activation was critical. Accordingly, we hypothesized that EC-specific EnNaC activation would mediate endothelium dysfunction, vascular stiffness, and impair flow-mediated vasodilation through reduction of bioavailable NO. Four week old C57BL6/J mice were fed a WD containing high fat (46%), sucrose (17.5%), and high fructose corn syrup (17.5%) with or without a low dose of amiloride (1 mg/kg/day) for 16 weeks. Female EnNaC KO and wild-type littermate females were treated with aldosterone (250 µg/kg/day) via osmotic minipumps for 3 weeks. Amiloride, an antagonist for EnNaC, significantly inhibited inward Na+ currents and EnNaC activity in the cultured endothelial cells. Amiloride treatment significantly attenuated WD-induced increases in aortic stiffness in vivo as measured by pulse wave velocity and in vitro endothelial stiffness measured by atomic force microscopy. In addition, amiloride improved flow mediated dilation in mesenteric arteries and endothelium-dependent relaxation in response to acetylcholine (10^{-9}-10^{-4} mol/L). Furthermore, amiloride prevented WD-induced increases in coronary endothelium permeability that were associated with decreased expression of claudin-5 and occluding. This also resulted in reduction of total macrophage recruitment (CD11b) and M1 polarization (CD11c). Importantly, genetic knock-out EnNaC KO also prevented aldosterone-induced endothelium stiffening and impairment of endothelium-dependent relaxation. These data indicate that EC specific EnNaC activation decreases bioavailable NO, increases vascular endothelium dysfunction, and prompts vascular stiffening in obese female mice.

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Longitudinal Associations of Childhood Hemodynamic and Metabolic Traits to Adult Aortic Stiffness Vary by Race: The Bogalusa Heart Study

PrimaryAuthor.AuthorBlock:**Justin P Zachariah**, Texas Childrens Hosp, Houston, TX; Camilo Fernandez, Rupert Barshop, Emily Harvill, Wei

Chen, Lydia Bazzano, Tulane Univ, New Orleans, LA

Background
Aortic stiffness predicts future hypertension and cardiovascular disease (CVD) events. CVD predictors like metabolic traits and arterial stiffness vary by race. We hypothesized that in longitudinal analyses, metabolic traits since childhood would be associated with adulthood aortic stiffness, and that these associations vary by race in the biracial (black vs. white) Bogalusa Heart Study cohort.
Methods
Included participants had ultrasound as young adults for aorta-femoral pulse wave velocity (afPWV) and at least three Study visits over the life course. Sampled participants (n=1081) were 24-44 years old, 31% black, 57% female, and mean follow-up was 27 years. Demographic, anthropometric, blood pressure, lipid, glucose and tobacco exposure data were extracted from each visit. Given variable visit intervals and numbers, growth mixed models were used to compute total area under the curve for risk factors on the primary outcome of interest, young adult afPWV. Childhood versus cumulative risk factors to afPWV associations were examined with multivariable adjusted linear regression. Significant race by risk factor interaction terms ($p<0.001$) led to race-specific reporting.
Results
Adult afPWV was higher in black (5.4+/-1.2 m/s) than white (5.2+/-1.1 m/s) participants ($p=0.01$). Overall, afPWV was associated with childhood and cumulative low density lipoprotein cholesterol (LDL-C, $\beta=0.13$; $p<0.001$ and $\beta=0.08$; $p<0.01$, respectively) and mean arterial pressure (MAP, $\beta=0.1$; $p=0.02$ and $\beta=0.18$; $p<0.001$) and cumulative glucose ($\beta=0.13$; $p<0.001$). For blacks and whites, higher afPWV was associated with childhood LDL-C ($\beta=0.17$; $p<0.01$ and $\beta=0.1$; $p<0.01$) and cumulative glucose ($\beta=0.14$; $p=0.02$ and $\beta=0.15$; $p<0.001$). Only in whites were childhood and cumulative MAP ($\beta=0.08$; $p=0.04$ and $\beta=0.22$; $p<0.001$) and cumulative LDL-C ($\beta=0.1$; $p<0.01$) associated.
Conclusions
Young adult aortic stiffness was associated with childhood metabolic and hemodynamic traits even after accounting for cumulative levels of these traits. The associations appear to vary by race and by child versus adult life stage. Future work may examine the mechanisms of these race-specific differences and implications for CVD event risk.

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20-HETE Antagonists Normalize Large Artery Stiffness and Systolic Blood Pressure in Metabolic Syndrome

PrimaryAuthor.AuthorBlock:**Amanda A Soler**, Ian Hunter, Gregory Joseph, Rebecca Hutcheson, Brenda Hutcheson, Jenny Yang, Frank F Zhang, Sachindra R Joshi, New York Medical Coll, Valhalla, NY; Chastity Bradford, Tuskegee Univ, Tuskegee, AL; Katherine H Gotlinger, Rachana Maniyar, New York Medical Coll, Valhalla, NY; John R Falck, Univ of Texas Southwestern Medical Ctr, Dallas, TX; Spencer Proctor, Metabolic and Cardiovascular Diseases Lab, Edmonton, AB; Michal L Schwartzman, Sachin A Gupte, Petra Rocic, New York Medical Coll, Valhalla, NY

Large artery stiffness is a causal factor in development of systolic hypertension. 20-hydroxyeicosatetraeonic acid (20-HETE), a cytochrome CYP450-derived arachidonic acid metabolite, is known to be elevated in resistance arteries in hypertensive animal models and in obesity in humans, but the role of 20-HETE in regulation of large artery remodeling in metabolic syndrome has not been investigated. Unlike normal (Sprague-Dawley (SD)) rats, large arteries (aorta, carotid and >100µM mesenteric arteries) of metabolic syndrome rats (JCR:LA-cp, JCR) express CYP4A and 4F, CYP450s which make 20-HETE in rats (2-fold increase vs. SD). Consequently, 20-HETE production is elevated in large arteries of JCR rats. We hypothesized that this elevated 20-HETE increases matrix metalloproteinase 12 (MMP12, an elastase) activation leading to increased degradation of elastin, increased large artery stiffness and increased systolic blood pressure. A 3-4 fold increase in 20-HETE production in large arteries of JCR vs. SD rats correlated with increased elastin degradation (3-6 fold) and increased arterial stiffness (~75%). 20-HETE antagonists blocked elastin degradation in JCR rats concomitant with blocking MMP12 activation. Importantly, 20-HETE antagonists and MMP12 inhibition (pharmacological and MMP12-shRNA-Lnv) significantly decreased (~60% vs. untreated JCR) large artery stiffness in JCR rats. 20-HETE antagonists also decreased systolic (182±3 mmHg JCR, 145±3 mmHg JCR+20-HETE antagonists) but not diastolic (125±4 mmHg JCR, 124±4 mmHg JCR+20-HETE antagonists) blood pressure in JCR rats. Whereas diastolic pressure was fully angiotensin II (Ang II)-dependent, systolic pressure was only partially Ang II-dependent, and large artery stiffness in JCR rats was Ang II-independent. These results suggest that 20-HETE-dependent regulation of systolic blood pressure may be a unique feature of metabolic syndrome related to high CYP4A/4F expression and resultant high 20-HETE production in large conduit arterial stiffness, which is a primary determinant of systolic blood pressure. These findings may have implications for management of systolic hypertension in patients with metabolic syndrome.

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Subtle Changes in Mechanical Properties of Carotid Artery in Overweight and Obese Children

PrimaryAuthor.AuthorBlock:**Sung-ai Kim**, Div of Cardiology, Dept of Internal Med, Hallym Sacred Heart Hosp, Hallym Univ Coll of Med, An-yang, Korea, Republic of; Young Kyun Seo, Kyung-Hee Park, Dept of Family Med, Hallym Sacred Heart Hosp, Hallym Univ Coll of Med, An-yang, Korea, Republic of

Objective: Obesity in childhood has also been associated with the development of cardiovascular abnormalities. The aim of this study was to investigate the subtle changes of mechanical properties of carotid artery in overweight and obese children. **Methods:** We prospectively recruited a cohort of overweight (n=25) and obese (n=176) children aged 7 - 17 years and non-obese children with same age and gender (controls, n=31). Overweight is defined as a body mass index (BMI) at or above the 85th percentile and below the 95th percentile and obesity as a BMI at or above the

95th percentile for children of the same age and gender. The mechanical properties of carotid artery were directly measured in a cross-sectional image of common carotid artery using a velocity-vector imaging (VVI) analysis including instantaneous vessel area and deformation parameters as fractional area change (FAC), circumferential strain, and strain rate (SR). **Results**: Compared to controls, obese children had higher blood pressures and serum glucose and worse lipid profiles than controls, whereas overweight children showed no any difference in blood pressure and laboratory findings. Among vascular properties, there were no differences in carotid intima-media thickness between the three groups. However, obese children showed larger vessel area and higher arterial stiffness as lower FAC, circumferential strain and SR than controls, whereas overweight children showed no difference in vascular properties with obese children and with controls except for circumferential SR. Circumferential SR in overweight children was significantly reduced than controls (0.47±0.19 cm/s, 0.75±0.22 cm/s, respectively, $p<0.001$) and was the only vascular parameter distinguishing overweight children from controls. In linear regression analysis, age (β=-0.13, $p=0.03$), BMI (β=- 0.37, $p<0.001$), and serum triglycerides (β=-0.14, $p=0.01$) were independent determinants predicting the circumferential SR of carotid artery in this population. **Conclusion**: A decrease in circumferential SR of carotid artery precedes the changes of other vascular properties as well as blood pressure and laboratory findings in overweight and obese children.

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RhoBTB1, a Novel PPARγ Target Gene, Rescues Hypertension and Vascular Dysfunction Caused by PPARγ Dysfunction

PrimaryAuthor.AuthorBlock:**Masashi Mukohda**, Stella-Rita C. Ibeawuchi, Chunyan Hu, Jing Wu, Ko-Ting Lu, Debbie R Davis, Deng-Fu Guo, Kamal Rahmouni, Frederick W Quelle, Curt D Sigmund, Univ of Iowa, Iowa City, IA

We reported that mice (S-DN) expressing dominant-negative peroxisome proliferator-activated receptor gamma (PPARγ) in smooth muscle cells (SMC) are hypertensive and exhibit impaired vascular relaxation due to increased RhoA/Rho kinase (ROCK) activity, and display reduced expression of a novel PPARγ target gene, RhoBTB1. We hypothesize that RhoBTB1 plays a protective role in vascular function and that the function of RhoBTB1 is disrupted in S-DN mice. To test this, we generated double transgenic mice (termed S-RhoBTB1) with tamoxifen-inducible, Cre-dependent expression of RhoBTB1 in SMC. S-RhoBTB1 mice were crossed with S-DN to produce mice (S-DN/S-RhoBTB1) in which tamoxifen-treatment (75 mg/kg, ip, 5 days) restored RhoBTB1 expression in aorta to normal. Thoracic aorta and basilar artery from S-DN showed severely impaired vasodilation to acetylcholine (ACh) and sodium nitroprusside, which was reversed by restoration of RhoBTB1 in SMC (Aorta, 46±5 vs 80±2% ACh-induced relaxation, $p<0.01$, n=6-9). Replacement of RhoBTB1 also reversed the hypertensive phenotype and aortic stiffness observed in S-DN mice within 1 week of treatment (Radiotelemetry SBP, 141±6 vs 124±3 mmHg, $p<0.01$, n=8-10; Aortic Pulse Wave Velocity, 3.8±0.2 vs 2.5±0.1 mm/ms, $p<0.01$, n=11-13). Increased phosphorylation of myosin phosphatase targeting protein was preserved in both S-DN and S-DN/S-RhoBTB1 aorta, suggesting that restoration of RhoBTB1 did not

affect increased RhoA/ROCK activity ($p<0.01$, n=6). A phosphodiesterase (PDE) 5 inhibitor, Zaprinast improved vasodilation in S-DN ($p<0.01$, n=8). Consistent with this, a cGMP analog that is resistant to PDE hydrolysis, 8-pCPT-cGMP, induced equivalent relaxation in S-DN and non-transgenic mice (n=4), while S-DN exhibited impaired relaxation induced by PDE-sensitive 8-Bromo-cGMP ($p<0.01$, n=7). PDE activity was increased in S-DN aorta and was reduced to normal levels in S-DN/S-RhoBTB1 ($p<0.01$, n=6). We conclude: a) loss of RhoBTB1 function explains the vascular dysfunction and hypertension observed in response to interference with PPARγ in smooth muscle, b) DN PPARγ in SMC causes vascular dysfunction via promoting PDE activity, and c) restoration of RhoBTB1 in SMC facilitates vasodilatation by normalizing PDE activity.

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Elevated Muscle Sympathetic Nerve Activity is Associated With Higher Aortic and Carotid Stiffness Independent of Blood Pressure in Women

PrimaryAuthor.AuthorBlock:**Seth W. Holwerda**, Rachel E. Luehrs, Nealy A. Wooldridge, Lyndsey E. DuBose, Jess G. Fiedorowicz, Gary L. Pierce, Univ of Iowa, Iowa City, IA

Central arterial stiffness, a significant contributor to the development of hypertension and cardiovascular disease with aging, is linked to elevated muscle sympathetic nerve activity (MSNA) in men. However, the extent to which MSNA is associated with central arterial stiffness in women is unknown. Given that the age-related increase in MSNA and arterial blood pressure (BP) occurs at a steeper rate among women compared to men, we tested the hypothesis that resting MSNA is more strongly correlated with central arterial stiffness in women than in men. Also, because of the parallel age-related increase in MSNA, we further hypothesized that the relation between MSNA and central arterial stiffness would not be independent of age. MSNA (microneurography), aortic stiffness (carotid-femoral pulse wave velocity, CFPWV), and carotid β-stiffness (carotid tonometry and ultrasound) were assessed in 54 healthy men (n=29; 19-72 yrs; 30 ± 1 kg/m^2; systolic BP: 128 ± 3 mmHg) and women (n=26; 26-64 yrs; 29 ± 2 kg/m^2; systolic BP: 116 ± 3 mmHg). No differences between men and women were observed for CFPWV (Men: 7.0 ± 0.3 vs. Women: 6.8 ± 0.4 mmHg, P=0.747) and carotid β-stiffness (Men: 7.6 ± 0.8 vs. Women: 7.6 ± 0.5 mmHg, P=0.975). Mean BP was lower in women compared to men (Men: 93 ± 3 vs. Women: 85 ± 2 mmHg, P=0.021) and MSNA tended to be lower in women compared to men (Men: 25 ± 3 vs. Women: 20 ± 2 bursts/min, P=0.091). After adjusting for mean BP and HR (partial correlation), CFPWV was significantly correlated with MSNA in men (R=0.44, P=0.021) and women (R=0.58, P=0.004). Interestingly, further adjustment for age abolished the association between CFPWV and MSNA in men (R=0.01, P=0.968), but not in women (R=0.43, P=0.046). A moderate relation between carotid β-stiffness and MSNA was observed in men (R=0.37,

P=0.063) and women (R=0.44, P=0.034), but was abolished after adjusting for age (Men: R=-0.001, P=0.995; Women: R=0.26, P=0.245). These preliminary data demonstrate that MSNA is positively correlated with central arterial stiffness in women and men independent of BP. Furthermore, abolishment of the relation between MSNA and CFPWV in men only when adjusting for age suggests that the association between MSNA and central arterial stiffness may be more robust in women.

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Blood Pressure in Childhood Predicts HTN in Adulthood: The International CV Cohorts Consortium

PrimaryAuthor.AuthorBlock:**Elaine M Urbina**, Cincinnati Childrens Hosp, Cincinnati, OH; Lydia Bazzano, Tulane Univ, New Orleans, LA; Trudy Burns, Univ of Iowa, Iowa City, IA; Terry Dwyer, Oxford Univ, Cambridge, United Kingdom; David Jacobs, Univ of Minnesota, Minneapolis, MN; Markus Juonala, Univ of Turku, Turku, Finland; Ronald Prineas, Wake Forest Univ, Wake Forest, NC; Olli Raitakari, Univ of Turku, Turku, Finland; Alan Sinaiko, Julia Steinberger, Univ of Minnesota, Minneapolis, MN; Alison Venn, Univ of Tasmania, Hobart, Australia; Jessica Woo, Cincinnati Childrens Hosp, Cincinnati, OH

The natural history of blood pressure (BP) tracking from childhood to adulthood is not well defined. Using data from the International Childhood CV Cohorts Consortium, consisting of 7 international longitudinal cohorts, participants (N=5042) were evaluated by self-reported adult hypertension (HTN). Correlation between mean of all measures of BP on a subject during childhood (8-11 years) or adolescence (15-18 years) and adult BP (28-31 years) was evaluated with Spearman correlation coefficients. Differences in mean BP, body mass index (BMI) and laboratory values were evaluated by t-tests. Participants at time of self-report of HTN were mean age 48.5 years (81.7% white, 17.4% black; 39% male). Prevalence of HTN (N=1562) was 31%; 3480 were normotensive (NT). Correlations between child and adolescent SBP and DBP were r=0.43, 0.42, respectively, child and young adult r=0.24, 0.23 and adolescent and young adult r=0.41, 0.27 (all $p<0.0001$). Participants self-reporting HTN were more likely to be non-white (46.8% black, 30.2% white, 29.7% other, $p<0.0001$). Males were more likely to report HTN (33% vs 28% females, $p<0.0001$). They had significantly higher SBP and BMI as children and adolescents and also significantly higher DBP and fasting glucose by adolescence and lower HDL and higher TG by young adulthood (all $p<0.01$). We conclude that adult HTN begins in childhood with higher BP and BMI which appear to be important clinical markers for progressive increases in metabolic risk factors as these individuals age through adolescence and young adulthood.

Table: CV Risk Factors Across the Lifespan of Subjects By Adult Self Report HTN Status (Mean + SD)

Parameter	Adult NT	Adult HTN	PValue
CHILDHOOD LEVELS			
No of participants	2419	983	
Age (yrs)	10.2 (0.9)	10.2 (0.9)	0.21
SBP (mmHg)	102.6 (9.8)	106.2 (11.1)	<0.0001
DBP (mmHg)	56.5 (12.0)	57.3 (13.3)	0.08
BMI (kg/m2)	17.6 (2.9)	18.8 (3.7)	<0.0001
Tchol (mg/dl)	166.6 (29.1)	165.9 (27.8)	0.60
LDL (mg/dl)	99.2 (26.9)	99.0 (26.2)	0.90
HDL (mg/dl)	59.9 (16.1)	60.1 (18.0)	0.82
TG (mg/dl)	71.8 (35.2)	73.8 (35.5)	0.13
Glucose (mg/dl)	83.6 (7.9)	84.1 (8.5)	0.43
ADOLESCENT LEVELS			
No of participants	2120	1026	
Age (yrs)	16.3 (0.7)	16.4 (0.7)	0.02
SBP (mmHg)	112.1 (10.3)	117.4 (11.9)	<0.0001
DBP (mmHg)	65.3 (10.3)	67.0 (11.8)	<0.0001
BMI (kg/m2)	21.9 (3.9)	23.2 (4.6)	<0.0001
Tchol (mg/dl)	154.3 (27.7)	156.8 (28.4)	0.06
LDL (mg/dl)	91.0 (24.7)	94.7 (26.6)	0.01
HDL (mg/dl)	53.0 (14.4)	54.1 (16.2)	0.17
TG (mg/dl)	75.1 (35.3)	76.6 (34.4)	0.34
Glucose (mg/dl)	80.2 (12.7)	83.5 (9.8)	<0.001
YOUNG ADULT LEVELS			
No of participants	1013	522	
Age (yrs)	29.6 (1.2)	29.4 (1.1)	0.01
SBP (mmHg)	110.0 (10.2)	117.6 (12.7)	<0.0001
DBP (mmHg)	67.3 (8.6)	73.5 (10.4)	<0.0001
BMI (kg/m2)	24.9 (5.2)	27.7 (6.0)	<0.0001
Tchol (mg/dl)	177.5 (34.9)	184.6 (38.3)	0.0004
LDL (mg/dl)	109.4 (31.3)	115.8 (33.5)	<0.0001
HDL (mg/dl)	50.9 (13.4)	47.9 (13.2)	<0.0001
TG (mg/dl)	94.1 (66.1)	114.0 (73.2)	<0.0001
Glucose (mg/dl)	85.0 (9.2)	85.2 (11.5)	0.85

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Disparities in Total Health Care Expenditure, and Payment Type Related to Hypertension Between Non Hispanics and Hispanics in the USA: Results From the Medical Expenditure Panel Survey (2013 - 2014)

PrimaryAuthor.AuthorBlock:**Emir Veledar**, Anshul Saxena, Emeka Osondu, Javier Valero Elizondo, Khurram Nasir, Baptist Health South Florida, Miami, FL

Background Each year in the USA, more than 75 million adults are diagnosed with hypertension (HTN), but less than 54% have this condition under control. Due to poor management, mortality due to HTN or related complications was 410,000 in 2014 and resulted in close to $50 billion spent. We sought to examine disparities in the proportion of events and related expenditure due to HTN between 54 million Hispanics, representing 17% in the USA population and non Hispanics. **Methods and Population** We used data from the Medical Expenditure Panel Survey (MEPS), the most complete source of data on the cost and use of health care and health insurance coverage for 2013 and 2014. Cost was grouped as related to ambulatory, emergency room, inpatient, home visits and medications. By source, payments were grouped as paid by family, MEDICARE, MEDICAID, private insurance, VA, Tricare and other. **Results** Overall, there were 61.2 and 61.9 million total events associated with HTN in 2013

and 2014 respectively; Hispanics accounted for 5.8 (9.5%) and 5.4 (8.7%) million events each year. On an average, HTN events involving Hispanics were costlier up to $90 - $300 more than non Hispanics ($1053 vs. $ 746 in 2013; and $890 vs. $804 in 2014). For Hispanics, payments were mainly covered by MEDICAID (42.1%) and MEDICARE (27.5%), compared to MEDICARE (39.3%) and private insurance (23.7%) for non-Hispanic population. Hispanics HTN expenditures were $6.1 billion (12.9%) in 2013 and 5.3 billion (10.3%) in 2014 and Hispanics had disproportionately fewer number of events than expected 17%, and the structure of their costs for those events was not different from non-Hispanics. In regression model, accounting for demographics and type of insurance, being Hispanic was a significant predictor of the total, ambulatory and inpatient cost, but not emergency room or medication cost. **Conclusion and discussion** Hispanics participate disproportionately less in HTN events and costs compared to their proportion in population, even when age, demographic and socioeconomic factors are accounted for. They also have on average higher and more complex events compared with non Hispanics. Almost 70% of HTN expenditure for Hispanics in 2013-2014 was covered by MEDICAID and MEDICARE indicating socioeconomic disparities.

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128

Visit-to-visit Variability of Systolic BP and Renal Outcomes in the SPRINT Trial

PrimaryAuthor.AuthorBlock:**Jesse Goldman**, Sandeep Aggarwal, Suzanne Boyle, Leslie Mcclure, Rebecca Seshasai, Meera Harhay, Drexel Univ, Philadelphia, PA

Increased visit-to-visit variability (VVV) in systolic blood pressure is a known predictor of adverse CV events & in diabetics, is associated with higher risk of incident albuminuria & chronic kidney disease. However, the impact of VVV in SBP on renal outcomes in the absence of diabetes is unknown. We investigated the association between VVV in SBP & adverse CVE & renal events among non-diabetic participants in SPRINT cohort. **Methods:** Primary exposure was quartile of within-person standard deviation of systolic blood pressure (SDBP). Primary outcomes 1) primary composite CV outcome from SPRINT study, 2) primary SPRINT renal outcome among those without CKD 3) incident albuminuria among those with & without CKD. We compared covariates by SDBP quartile, using ANOVA or chi-square tests. We fit incremental Cox hazards models to examine associations between SDBP & events. We performed sensitivity analyses for # visits in all models. **Results:** Among 9361 participants, 8589 (92%) met inclusion criteria & comprised the primary analytic sample. The mean SBP across all quartiles was similar (137 - 141 mmHg). There was no association between quartile of SDBP and the primary composite cardiovascular outcome. There was a significant association between quartile of SDBP and incident CKD among those without baseline CKD. Among 3350 participants without baseline CKD and 942 participants with CKD, higher VVV in SBP was independently associated with increased risk of incident albuminuria, regardless of baseline CKD status. This association was robust after adjustment for the number of visits. The interaction between SDBP and the primary intervention was not statistically significant in the no CKD (p=0.11) or CKD groups (p=0.93). **Discussion:** In this post-

hoc analysis of SPRINT, we found that higher VVV in SBP is associated with greater risk of incident albuminuria among non-diabetic adults with and without baseline CKD. This association remained significant after adjustment for numerous potential confounders across follow-up. Our analysis demonstrates a significant association between VVV of SBP and incident albuminuria in patients with and without baseline CKD. This novel finding in an exclusively non-diabetic, hypertensive population deserves further investigation.

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129

In Defense of Clinic Blood Pressure

PrimaryAuthor.AuthorBlock:**Srividya Kidambi**, Thomas Chelius, Irene Nunuk, Yue Ding, Brittaney Obi, Sumeet Jain, David Mattson, Allen Cowley, Purushottam Laud, Tao Wang, Jane Kotchen, Mingyu Liang, Theodore Kotchen, Medical Coll of Wisconsin, Milwaukee, WI

For genetic studies of hypertension, between 1994 and 2006, we established a cohort of 2,639 African Americans (53% female), 51% of whom were hypertensive (H). The purpose of this report is twofold: a) to evaluate the relationship of baseline hypertension status and blood pressure (BP) level with subsequent mortality in this cohort; and b) to compare the relationship of clinic and 24-hour BPs with the subsequent incidence of fatal and non-fatal cardiovascular (CV) events in a subset of the original cohort. Clinic BP was obtained in triplicate in all 2,369 subjects as part of screening procedures for a 3-day inpatient clinical study of a sub-sample (n=266). BP medications were discontinued for 1-week after the screening visit and prior to inpatient study. Detailed phenotyping along with 24-hour BPs were obtained during the 3-day study. Compared to normotensives (N) at baseline, H had higher standardized clinic BP (145 ± 20 [SD] / 94 ± 13 vs 118 ± 10/ 76 ± 8 mm Hg), were slightly older (45 ± 7 vs 42 ± 7 years; $p<0.0001$), and had higher BMI (29 ± 6 vs 27 ± 5 kg/m^2; $p<0.0001$) in the overall cohort. Mortality data were obtained from the National Death Index. Average time from baseline to mortality was 12 ± 3 years. All-cause mortality was related to clinic systolic BP ($p<0.03$) and was greater in H than N (176 vs 70 deaths; $p< 0.0002$) after adjustment for age, sex, BMI, and duration of follow-up. CV mortality was also greater in H than N (52 vs 19 deaths; $p<0.01$). During a follow-up of 14 ± 4 years of the in-patient cohort, 49 subjects had a fatal (n=11) or non-fatal (n=38) CV event. After adjustment for covariates, clinic systolic and diastolic BPs were strongly associated with CV events ($p<0.0001$). The association of clinic BPs with CV events was as robust as the associations with overall 24-hour BP, day BP, and night BP. Average difference between day and night BP was 7 ± 8/5 ± 6 mm Hg ($p< 0.0001$), but day-night BP difference was not associated with CV events ($p=0.45$). In conclusion, in middle-aged African Americans, 24-hour BPs were no more predictive of CV events than BPs measured during a single outpatient visit. In addition, both all-cause mortality and CV mortality were related to the clinic BP. These results will require confirmation with larger sample sizes.

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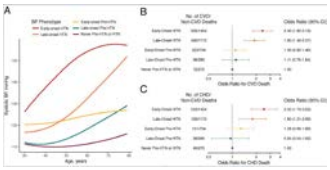
The Prognosis of Prehypertension Without Progression to Hypertension

PrimaryAuthor.AuthorBlock:**Teemu J. Niiranen**, Martin G. Larson, Framingham Heart Study, Framingham, MA; Elizabeth L. McCabe, Brigham and Women's Hosp, Boston, MA; Ramachandran S. Vasan, Framingham Heart Study, Framingham, MA; Susan Cheng, Brigham and Women's Hosp, Boston, MA

Although progression from prehypertension (preHTN) to hypertension (HTN) is preventable, the prognosis of preHTN among individuals who never progress to HTN and, in turn, the role of early- versus late-onset pre-HTN in this context remains unclear. Our sample consisted of 5593 Framingham Heart Study participants (mean age 43 years, 52% women) with mortality and serial blood pressure (BP) data available. We categorized participants into 5 BP phenotypes: never preHTN or HTN; late-onset (age ≥55 years) preHTN without ever developing HTN; early-onset (age <55 years) preHTN without ever developing HTN; late-onset HTN; and early-onset HTN. Cases were considered persons who died from cardiovascular disease (CVD) or specifically from coronary heart disease (CHD), and controls included the remaining decedents. We used multivariable-adjusted regression models to estimate case-vs-control odds ratios for the 4 preHTN/HTN categories versus those who died without ever developing preHTN or HTN. The loess-smoothed curves for mean BP values, by age, for the 5 BP phenotypes are shown in **Figure A.** Compared to individuals who maintained optimal BP throughout life, onset of preHTN earlier or later in life, without progression to HTN, did not significantly increase odds of CVD (**Figure B**) or CHD (**Figure C**) death. By contrast, late- and especially early-onset HTN were associated with considerably increased odds of CVD death and CHD death. Our study findings suggest that not all forms of preHTN are associated with the same magnitude of risk for CVD death. Further investigations are needed to determine whether interventions aimed at preventing the progression from preHTN to HTN could improve outcomes.



Disclosures:**T.J. Niiranen:** None. **M.G. Larson:** None. **E.L. McCabe:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH. **R.S. Vasan:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH. **S. Cheng:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH, AHA.

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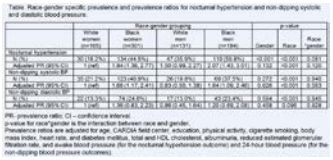
Racial Differences in Nocturnal Hypertension and Non-dipping Blood Pressure: The Coronary Artery Risk Development in Young Adults (CARDIA) Study

PrimaryAuthor.AuthorBlock:**Paul Muntner**, John N Booth III, Stephen J Thomas, Luqin Deng, Univ Alabama Birmingham, Birmingham, AL; Joseph E. Schwartz, Columbia Univ, New York, NY; Donald Llyod-Jones, Northwestern Univ

Feinberg Sch of Med, Chicago, IL; James M. Shikany, Cora E. Lewis, Univ Alabama Birmingham, Birmingham, AL; Daichi Shimbo, Columbia Univ, New York, NY

Risk factors for nocturnal hypertension are more common among blacks compared with whites. We hypothesized nocturnal hypertension and nocturnal non-dipping BP are more common among blacks compared with whites. We analyzed data for 781 participants of the population-based Coronary Artery Risk Development in Young Adults (CARDIA) study who completed ambulatory blood pressure (BP) monitoring (ABPM) in 2015-2016. Awake and sleep periods were defined using actigraphy and self-report. Nocturnal hypertension was defined as mean sleep systolic BP (SBP)/diastolic BP (DBP) ≥ 120/70 mm Hg. Non-dipping SBP and DBP, separately, were defined as a decline in mean sleep BP, relative to mean awake BP < 10%. The mean age of participants was 54.7 years, 21.1% were white women, 38.5% were black women, 16.8% were white men and 23.6% were black men. The prevalence of nocturnal hypertension was 18.2% and 44.5% among white and black women, respectively, and 35.9% and 59.8% among white and black men, respectively. After multivariable adjustment, the prevalence of nocturnal hypertension was higher among black women, white men and black men, each compared with white women (Table). The prevalence of non-dipping SBP was 21.2% and 40.9% among white and black women, respectively, and 19.8% and 37.5% among white and black men, respectively. After multivariable adjustment, non-dipping SBP was more common among black women and black men compared with white women. There were no statistically significant differences in non-dipping DBP across race-gender after multivariable adjustment. Nocturnal hypertension and non-dipping SBP are more common among blacks compared with whites even after adjustment for mean BP.



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Monocytes Activation by Salt is Associated With Cardiovascular Risk Factors

PrimaryAuthor.AuthorBlock:**Natalia Ruggeri Barbaro**, Jason D Foss, Roxana Loperena, Fernando Elijovich, Cheryl L Laffer, Wei Chen, Cristi L. Galindo, Kim R. Montaniel, Yan Guo, David G Harrison, Annet Kirabo, Vanderbilt Univ, Nashville, TN

Several studies have established a relationship between hypertension and salt intake; however the mechanisms by which salt causes hypertension are poorly understood. There is also evidence that sodium (Na+) accumulates in the interstitium with aging and hypertension in concentrations exceeding the plasma. We tested the hypothesis that increased NaCl would convert human monocytes to an inflammatory phenotype and to define

mechanisms involved. We exposed monocytes from 17 human volunteers to normal physiological NaCl (NS: 150 mM/L), elevated NaCl (HS: 190 mM/L), or an equiosmoloar concentration of mannitol. Exposure of human monocytes to high salt, but not mannitol, increased formation of immunogenic isolevuglandins (isoLG) (NS: 1688±384 vs Mann:1762±429 vs HS: 2381± 635 MFI $p<0.002$). This was associated with an increase in the dendritic cell (DC) marker CD83 (NS: 503±81 vs Mann: 530± 106 vs HS: 764 ± 136 MFI $p<0.001$). Exposure to high salt also stimulated production of IL-6 (NS: 2145±771, Mann: 1122±295 and HS: 5187±1146 pg/mL, $p=0.04$), IL-β (NS: 94±35, Mann: 62±16 and HS: 224±98 pg/mL, $p=0.01$) and TNF-α (NS:1.9±0.3, Mann: 3.42±1.4 and HS: 4.4±2.1, $p<0.0001$). In additional experiments, we found that prolonged (7 day) exposure to high salt increased surface expression of CD209, another DC marker (NS: 22±9 vs HS: 34 ± 14, $p=0.001$) and promoted conversion of the cells to a DC morphology. The propensity for monocytes to respond to NaCl was influence by the patient's risk factors. The increase in IsoLG (HS-NS) correlated with pulse pressure (mmHg, $r=0.51$, <0.04), BMI (Kg/m2, $r=0.66$, $p=0.005$), total cholesterol (mg/dL, $r=0.55$, $p<0.05$) and glucose (mg/dL, $r=0.72$, $p=0.003$). Stepwise multivariate regression revealed that BMI and pulse pressure are independent predictors of IsoLG formation in response to salt. These findings suggest that high extracellular NaCl promotes differentiation and activation of monocytes and that these pleotropic inflammatory cells exhibit a previously undefined salt sensitivity corresponding to patients' underlying risk factor profile.

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Kidney-specific Conditional Knockout of Klotho Gene Impairs Natriuresis and Causes Arterial Stiffness and Hypertension

PrimaryAuthor.AuthorBlock:**Zhongjie Sun**, Quaisar Ali, Univ Oklahoma, Oklahoma City, OK

Background and Purpose. Deletion of Klotho leads to hypertension. The purpose of this study is to investigate if renal epithelial sodium channel (ENaC) contributes to klotho deficiency-induced hypertension.
Methods and Results. We generated kidney-specific conditional klotho null ($KspKL^{-/-}$) mice and found out that renal alpha subunit of ENaC is significantly upregulated compared to wild type (WT) mice. Blood pressure measured by telemetry demonstrated systolic hypertension and elevated pulse pressure suggesting vascular dysfunction in $KspKL^{-/-}$. Pulse wave velocity, a marker of arterial stiffening was significantly increased in $KspKL^{-/-}$ mice. Amiloride, an ENaC inhibitor, completely prevented systolic hypertension and arterial stiffening in $KspKL^{-/-}$ mice. *Ex vivo* vascular relaxing responses to acetylcholine were diminished in mesenteric arteries in $KspKL^{-/-}$ group, indicating that klotho deficiency causes vascular endothelial dysfunction. Interestingly, treatment with amiloride abolished Klotho deficiency-induced vascular endothelial dysfunction. We found that urinary sodium excretion ($U_{Na}V$) was significantly impaired in $KspKL^{-/-}$ group. Na retention was also associated with a decrease in glomerular filtration rate (GFR), suggesting impaired renal function in $KspKL^{-/-}$ mice. Treatment with amiloride prevented sodium

retention and improved GFR in KspKL$^{-/-}$ mice. Total renal nitric oxide bioavailabililty and eNOS activity were significantly decreased in KspKl$^{-/-}$ mice which was ameliorated by amiloride treatment. Histological and morphometric analysis showed glomerular and tubular damage in KspKL$^{-/-}$, which was improved by amiloride treatment. Western blotting analysis demonstrated a significant decrease in the αγ subunits of ENaC in the kidney cortex following treatment with amiloride.
Conclusion. This study demonstrates for the first time that kidney-specific depletion of the klotho gene causes impairment in Na-excretion and hypertension by affecting ENaCα levels. ENaC may be a promising therapeutic target for klotho gene deficiency-induced renal and vascular dysfunction and hypertension.

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Proximal Tubule-mediated Control of Albumin Balance During the Development of Salt-sensitive Hypertension

PrimaryAuthor.AuthorBlock:**Oleg Palygin**, Vladislav Levchenko, Ashraf El-Meanawy, Alexander Staruschenko, Medical Coll of Wisconsin, Milwaukee, WI

Salt-induced hypertension and the associated renal and cardiovascular complications affect a large population and are one of the leading causes of CKD. It is widely accepted that albuminuria is a strong predictor of kidney injury and cardiovascular outcome. Despite the indisputable significance of albuminuria as one of the most important factors for predicting morbidity and mortality in patients with cardiovascular diseases, the events leading to the excessive filtration of albumin into the urine are not well understood. The goal of the current study was to determine the mechanisms contributing to albuminuria in the kidney of the Dahl salt-sensitive (SS) rat, a widely used model of salt-induced hypertension. The SS rats when fed a high salt (HS) diet develop progressive elevations in urinary albumin and mean arterial blood pressure (MAP). Intravital imaging analyses of the kidneys indicate increased loading of proximal tubules (PT) with filtered albumin at the early stage of the development of hypertension. Further progression of the disease strongly correlated with significant PT damage as indicated by the impaired albumin reabsorption, increased prevalence of granular casts, and necrosis of PT epithelial cells. To prevent PT albumin overload and further development of kidney damage PT reabsorption blocker L-Lysine (17 mg/ml) was added to the drinking water offered ad libitum, and the measurements of MAP repeated continuously on HS. Treatment of SS rats with L-lysine significantly reduced the progression of salt-induced hypertension (165±4 vs 133±2 mmHg at 14D on HS, control vs L-Lysine). L-lysine significantly attenuated the development of progressively increased albuminuria and restored serum albumin balance in hypertensive animals (1.5±0.25 vs 13.28±2.14 and 6.91±1.07 24 hrs Alb/Cre; 2.98±0.07 vs 2.46±0.08 and 2.86±0.05 g/dL of serum Alb at low salt vs 14D on HS, control, and L-Lysine treated, respectively). We conclude that salt-induced increase of albumin load at the PT results in loss of albumin reuptake, reduction of albumin serum concentrations and progression of albuminuria in SS hypertensive rats. The protection of PT against extensive albumin load can significantly reduce renal damage and development of high blood pressure.

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PVN Galphai2 Protein Over Expression Attenuates Dahl Salt Sensitive Hypertension and GNAI2 Polymorphic Variance Represents a Clinical Biomarker of the Salt Sensitivity of Blood Pressure

PrimaryAuthor.AuthorBlock:**Richard D Wainford**, Xiaoling Zhang, Boston Univ Sch Med, Boston, MA

Aim: We hypothesize that a) in the DSS rat, which in contrast to the normotensive DSR rat fails to up regulate PVN $G\alpha i_2$ proteins on a high salt intake (Wainford et al, Hypertension 2015), PVN specific $G\alpha i_2$ protein over expression will attenuate the development of salt sensitive hypertension (HTN) and, b) SNPs in the GNAI2 gene, which are associated with HTN in Japanese and Italian populations, represent a biomarker of the salt-sensitivity of BP.
Methods: Male DSS rats instrumented with a radiotelemetry probe (DSI; PA-C40) received a bilateral PVN shuttle or $G\alpha i_2$ expressing lenti-viral vector microinjection ($2x10^9$ infectious units per ml/60nl/side) and were maintained on a 7-day normal 0.4% (NS) intake for baseline BP prior to 21 day high 4% NaCl (HS) intake. On day 21 rats were sacrificed to assess PVN $G\alpha i_2$ protein expression (immunoblotting), plasma NE content (ELISA) or underwent a 5% volume expansion (VE) prior to cardiac perfusion and c-fos IHC to assess PVN neuron activation (N=4/gp/study). SNPs in the GNAI2 gene were examined in GenSalt (N=968) for associations with the salt sensitivity of BP (increase in systolic BP of 5mmHg or greater during HS intake, N=326 are salt sensitive: 172 Female, 154 male).
Results: Bilateral lenti-virus increased PVN $G\alpha i_2$ protein levels 3 fold ($p<0.05$) on both NS and HS intake, *attenuated* HTN (Day 21 HS MAP [mmHg] shuttle 160±3 vs. $G\alpha i_2$ 144±4, $P<0.05$), *abolished* Na^+ evoked global and renal sympathoexcitation (Day 21 HS plasma NE [nmol/L] shuttle 82±5 vs. $G\alpha i_2$ 50±4, $P<0.05$). PVN $G\alpha i_2$ over expression increased VE-evoked natriuresis (peak natriuresis (UNaV; [µeq/min] shuttle NS 43±3, shuttle HS 16±3, $G\alpha i_2$ HS 31±3, $P<0.05$) and PVN sympathoinhibitory parvocellular neuron activation (medial parvocellular c-Fos positive cells shuttle NS 67±8, shuttle HS 29±3, $G\alpha i_2$ vector HS 48±6, $P<0.05$). 3 GNAI2 SNPs are present in GenSalt - rs9852677 and rs2282751 did not associate with the salt sensitivity of BP. SNP rs10510755 positively correlated with the salt sensitivity of BP (MAF:6%, Z-score: 1.94, $p<0.05$) independently of sex.
Conclusion: These data suggest a critical role of PVN $G\alpha i_2$ proteins in countering the pathophysiology of salt sensitive HTN and that GNAI2 polymorphic variance represents a biomarker of the individual salt sensitivity of BP.

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Improvement of BP Control (and Simultaneous Reduction of Drug Consumption) in Treated Hypertensive Patients Compliant to a Minimal Dietary Sodium Restriction

PrimaryAuthor.AuthorBlock:**Natale Musso**, Beatrice Carloni, Maria C Chiusano, IRCCS San Martino Genova, Genova, Italy

The official guidelines for hypertension recommend a low sodium diet as a basic approach. Sodium should be restricted to 100 mEq a day. Such tight diets are seldom paralleled by an adequate compliance.
Methods: we studied the effects of a reasonable and realistic low sodium diet on the BP values in 196 pts. (157 pts. completed the study). The patients had to avoid ice creams, cheese and salt-preserved (cured) meat. They were switched from regular to salt-free bread. Otherwise they were free to follow their usual mediterranean diet. Urinary output of Na and K, weight, number of antihypertensive drugs taken, and office BP/HR were measured before diet started (Value 1 in the table) and after 9±1 weeks on diet (Value 2 in the table) (all values are mean ± SD). The BP was recorded with the patient alone, in a seated position, with automatic repeated measures, by means of Omron 907 HEM monitors. At the end of the study 88 patients showed a reduction in the urinary sodium (compliant pts.), whereas 69 did not (noncompliant pts.).

	Compliant N=88 (M/F 45/55%)			Noncompliant N=69 (M/F 40/60%)		
Variable	1	2	P value	1	2	P value
Age (yr)	62.3±13.1	--	--	64.6±11.2	--	--
Height /cm	164.3±10.4	--	--	164.3±7.9	--	--
Weight/kg	73.7±15.3	71.3±14.1	<0.001	70.2±11.8	70.1±12.3	NS
SYS BP 1	147.3±19.1	135.5±13.1	<0.01	138.4±15.3	138.3±14.8	NS
SYS BP 2	140.9±16.4	129.2±11.2	<0.01	133.2±14.8	134.6±14.9	NS
SYS BP 3	135.3±16.0	124.3±10.2	<0.01	126.5±13.9	131.4±14.3	<0.05
DIA BP 1	86.8±12.4	80.1±9.1	<0.01	82.5±11.2	83.3±13.1	NS
DIA BP 2	82.9±12.5	76.3±8.9	<0.01	79.2±10.8	81.2±12.1	NS
DIA BP 3	80.5±12.3	74.0±8.4	<0.01	77.2±10.1	79.0±11.3	NS
HR 1	74.2±10.8	73.1±10.0	NS	70.9±10.4	74.1±11.8	<0.05
HR 2	72.4±10.3	71.4±9.9	NS	71.0±10.6	73.0±11.1	NS
HR 3	72.8±16.3	70.5±10.0	<0.05	77.2±9.8	73.3±10.5	<0.05
Na^+ [U]	170.6±56.7	110.5±36.8	<0.01	141.4±49.6	166.7±57.4	<0.05
K^+ [U]	66.3±31.2	57.8±24.9	<0.05	59.3±19.8	62.7±19.4	NS
Drug N	1.91±0.94	1.39±0.93	<0001	1.80±1.14	1.80±1.06	NS

BP mmHg; HR bpm; Na and K mEq/24h; Drug (N)umber as absolute value.
Stats: ANOVA (Newman Keuls post test) & two-tailed Student's t test.

In those compliant to the low salt approach (88 out of 157 pts. i.e. 56%), a substantial improvement was attained in terms of BP control and body weight with a simultaneous significant reduction in the daily drug requirement. The responders increased from 47 to 69 (i.e. from 53% to 78%). In the noncompliant group the BP values, the responder rate (35 to 32, i.e. 50% to 46%), the weight, and the drug consumption were stable.
Conclusion: even a limited dietary salt reduction may be beneficial in terms of BP control, with a decrease of the BP levels and an increase in the responder rate, paralleled by a reduction of the drug requirement.
Take home message: even a bit of diet is better than no diet.

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Sox6 Regulates Renin Expression During Juxtaglomerular Cell Expansion

PrimaryAuthor.AuthorBlock:Jason D Foss, Liang Xiao, Kandi D Horton, Mohammad Saleem, Vanderbilt Univ Medical Ctr, Nashville, TN; Conrad P Hodgkinson, Duke Univ, Durham, NC; David G Harrison, **Jose A Gomez**, Vanderbilt Univ Medical Ctr, Nashville, TN

Introduction: Hypertension, a common condition that affects 33% of the US population, is a major risk factor for heart disease and stroke. Treatments for hypertension are limited and there is a critical need to develop new therapies. The Renin Angiotensin Aldosterone System (RAAS) plays a key role in regulating blood pressure, and renin controls its rate-limiting step. In adults, renin is produced and stored by renal Juxtaglomerular (JG) cells. During sodium restriction and other

pathophysiological stresses that require an increase in renin expression and release, the adult kidney increases the number of cells expressing renin, in a process known as JG cell expansion. JG cells formation mechanisms remain unclear. Our aim is to determine new regulators of renin and blood pressure control. ***Methods***: *In vivo:* JG cell expansion was induced by 10 days of low sodium diet (0.01% Na) and furosemide (0.1 mg/g body weight). *In vitro:* primary renal Mesenchymal Stromal Cells (MSC) were isolated from wild type mice and used until passage 5. MSC were differentiated into renin expressing cells by 7 days I&F (IBMX-100 uM and Forskolin-10 uM) treatment. Sox6 was down regulated in MSCs with lentivirus carrying vectors for Sox6 shRNA or controls scramble shRNA.
Results: *In vitro,* I&F induced renin expression in MSCs (Renin relative expression to gapdh 0.0153±0.005, n=3, P= 0.05, no renin expression in MSCs). Gene arrays comparing renal MSCs and JG cells identified Sox6 as a potential gene that controlled MSC differentiation. *In vitro* silencing of Sox6 by shRNA inhibited the differentiation of renal MSCs into renin producing cells (3.5 fold decrease compared to control shRNA, n= 4, P= 0.01). In a new transgenic mouse model, in which Sox6 is deleted specifically in renin expressing cells ($Ren1d^{Cre}/Sox6^{fl/fl}$), plasma renin concentration was not affected at baseline (0.3 vs W.T. 0.5 ug Ang I/ml/h, n=3, P>0.05). However, under conditions that promote JG cell expansion, Sox6 ablation halted the increase in the number of JG cells (8.75 fold decrease, n= 6 to 9, P= 0.001).
Conclusion: Our data indicates that Sox6 plays a novel role in renal physiology; modulating renin expression during pathological conditions. These findings suggest that Sox6 may become a new target for blood pressure control.

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Mas-related G-protein Coupled Receptor D Deficiency Leads to a Marked Dilated Cardiomyopathy in Mice

PrimaryAuthor.AuthorBlock:**Aline C Oliveira**, Marcos B Melo, Augusto A Peluso, Daisy Motta-Santos, Fernando P Neto, Rafaela F da Silva, Itamar C Jesus, Silvia Guatimosim, UFMG, Belo Horizonte, Brazil; Natasha Alenina, Michael Bader, MDC, Berlin, Germany; Maria Jose Campagnole-Santos, Robson A Santos, UFMG, Belo Horizonte, Brazil

Recently, we described a new peptide of the RAS, the heptapeptide alamandine. Alamandine [Ala^1-angiotensin-(1-7)] is formed from angiotensin-(1-7) by decarboxilation of the N-terminal Asp residue to Alanine or from the octapeptide angiotensin A (Ala^1-Ang II) by removal of the Phe c-terminal residue by ACE2. Alamandine effects were independent of Angiotensin-(1-7) receptor Mas. The Mas-related G protein receptor member D (MrgD) was identified as the alamandine receptor. Although cardioprotective effects of alamandine similar to Ang-(1-7) have been described, the significance of this peptide in the heart function is still elusive. **Objective:** To evaluate the functional role of the alamandine receptor, MrgD, in the heart using MrgD-deficient mice. **Methods:** Primary cardiomyocytes were obtained from neonatal rats and mice with up to 72 hours of life. Cells were fixed, blocked**,** permeabilized and

incubated with an anti-MrgD antibody, washed and incubated with a secondary antibody and Phalloidin. Slides were analysed using confocal microscopy. High-resolution echocardiography was performed in $MrgD^{+/+}$ and $MrgD^{-/-}$ mice (12 weeks old, n=5-6) under isoflurane anesthesia. Standard B-mode images were obtained in right and left parasternal long and short axis for morphological and functional assessment and evaluation of cardiac deformation (VevoStrain). **Results**: Immunofluorescence indicates that MrgD is present in cardiomyocytes, mainly in the perinuclear and nuclear region, with similar pattern in rats and mice. High resolution echocardiography showed left ventricular remodelling and dysfunction in MrgD-KO mice when compared to their controls. Strikingly, MrgD-deficient mice presented a pronounced dilated cardiomyopathy with a marked decrease in systolic function (EF: 34.39 ± 2.9 versus 66.49 ± 2.3 %; FS: 16.23 ± 0.6 versus 35.91 ± 1.8 %; EDV: WT = 46.47 ± 2.4 versus 81.15 ± 3.7; ESV: 15.41 ± 0.7 versus 53.21 ± 2.5). **Conclusion:** Alamandine MrgD receptor is expressed in cardiomyocytes and its deletion leads to a pronounced dilated cardiomyopathy indicating a major role for alamandine in the heart.

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Loss of Thymosin b4 Exacerbates Renal and Cardiac Damage in Angiotensin-II Hypertension

PrimaryAuthor.AuthorBlock:**Nitin Kumar**, Tang-Dong Liao, Cesar Romero, Mani Maheshwari, Ed Peterson, Oscar Carretero, Henry Ford Health System, Detroit, MI

Angiotensin-II (Ang-II)-induced hypertension is associated with tissue damage and fibrosis in the kidney and heart. Thymosin β4 (Tβ4) regulates cell morphology, inflammation and fibrosis in several organs and administration of exogenous Tβ4 is protective in diabetic nephropathy and unilateral ureteral obstruction model. However, role of endogenous Tβ4 in hypertension-induced organ damage is unknown. We *hypothesize* that, loss of Tβ4 accelerates renal and cardiac fibrosis and damage in Ang-II hypertension. To test our hypothesis, Tβ4 knockout ($Tβ4^{-/-}$) and wild-type ($Tβ4^{+/+}$) C57BL/6 mice (n=6-10) were infused continuously for six-weeks with either Ang-II (980 ng/kg/min) or vehicle via osmotic minipumps. All the results are presented in table 1. In Ang-II infusion, systolic blood-pressure were not different between both strains (Table 1). Interestingly, urinary albuminuria was significantly higher in $Tβ4^{-/-}$ mice compared to $Tβ4^{+/+}$ mice by Ang-II. High expression of Tβ4 is found in the glomeruli along with high expression of Nephrin, an important protein in the filtration barrier of the kidney. In Ang-II infusion, nephrin protein expression was greatly reduced in mice deficient of Tβ4, suggesting that loss of nephrin is one of the mechanism for elevated urinary albumin in $Tβ4^{-/-}$ mice. Additionally, renal fibrosis was higher in $Tβ4^{-/-}$ mice. We also studied cardiac damage and observed that in Ang-II infusion, cardiac hypertrophy and cardiac fibrosis were much higher in $Tβ4^{-/-}$ mice. These data indicate that loss of endogenous Tβ4 caused significant tissue damage in the kidney and heart in Ang-II hypertension, suggesting renal and cardiac protective role of this peptide.

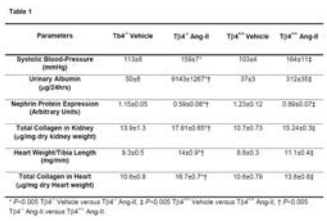

Table 1

Parameters	Tβ4$^{+/+}$ Vehicle	Tβ4$^{+/+}$ Ang-II	Tβ4$^{+/+}$ Vehicle	Tβ4$^{+/+}$ Ang-II
Systolic Blood-Pressure (mmHg)	113±8	156±7*	103±4	164±11‡
Urinary Albumin (µg/24hrs)	50±8	9143±1267*†	37±3	312±35‡
Nephrin Protein Expression (Arbitrary Units)	1.15±0.05	0.59±0.08*†	1.23±0.12	0.89±0.07‡
Total Collagen in Kidney (µg/mg dry kidney weight)	13.9±1.3	17.91±0.65*†	10.7±0.73	15.24±0.3‡
Heart Weight/Tibia Length (mg/mm)	9.3±0.5	14±0.9*†	8.8±0.3	11.1±0.4‡
Total Collagen in Heart (µg/mg dry Heart weight)	10.8±0.8	16.7±0.7*†	10.6±0.79	13.6±0.8‡

* $P<0.005$ Tβ4$^{+/+}$ Vehicle versus Tβ4$^{+/+}$ Ang-II, ‡ $P<0.005$ Tβ4$^{+/+}$ Vehicle versus Tβ4$^{+/+}$ Ang-II, † $P<0.005$ Tβ4$^{+/+}$ Ang-II versus Tβ4$^{+/+}$ Ang-II.

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Endogenous Renin-angiotensin System Activation Causes Accelerated Cerebral Vascular Dysfunction in Mice Expressing Dominant-negative Mutations in PPARγ in Endothelium

PrimaryAuthor.AuthorBlock:**Anand R Nair**, Larry N Agbor, Masashi Mukohda, Chunyan Hu, Jing Wu, Curt D Sigmund, Univ of Iowa, Iowa City, IA

Abnormal activation of the renin-angiotensin system (RAS) has been implicated in cardiovascular (CV) disease. Whereas low salt diet (LSD) may be beneficial in salt-sensitive hypertension, it has been proposed to induce CV risk due to RAS activation. Peroxisome proliferator-activated receptor gamma (PPARγ) is a ligand-activated transcription factor which regulates the actions of angiotensin II (ANG) in the vasculature and promotes anti-oxidant pathways. We hypothesize that endothelial PPARγ plays a protective role in the vasculature in response to RAS activation. Transgenic mice specifically expressing dominant-negative (DN) mutations in PPARγ in the endothelium (E-DN) were fed a LSD and endothelial function was measured. Plasma renin and ANG were significantly increased in both non-transgenic (NT) and E-DN mice fed a LSD for 6 weeks compared with normal chow (Renin - NT: 39±7 vs 20±1 ng/ml; E-DN: 34±1 vs 16±4 ng/ml; AngII - NT: 257±54 vs 47±6 pg/ml; E-DN: 294±69 vs 63±14 pg/ml $p<0.05$, n=5). At baseline, vasorelaxation to acetylcholine (ACh) was not affected in E-DN compared to NT (basilar artery: 66±12 vs 64±4%; carotid artery: 93±4 vs 91±4%, n=5). Six weeks of LSD significantly impaired ACh-mediated relaxation in basilar artery of E-DN but not in NT (42±8 vs 74±5%, $p<0.05$, n=5). Unlike basilar artery, 6 weeks of LSD was not sufficient to induce vascular dysfunction in carotid artery of E-DN (carotid artery: 86±4 vs 92±3%, n=5). The endothelial dysfunction observed in the basilar artery of E-DN was attenuated upon in vitro incubation with tempol (improved from 29±5% to 55±7%, n=6). Further, administration of the AT1 receptor blocker, Losartan (0.6g/L drinking water) for the last 2 weeks of LSD blunted the endothelial dysfunction observed in the basilar artery of E-DN (improved from 24±2% to 64±9%, n=5). We conclude that interference with PPARγ in the endothelium produces endothelial dysfunction in the cerebral circulation in response to LSD-mediated activation of the endogenous RAS and this dysfunction is mediated, at least in part, through AT1 receptor activation and ROS signaling pathways. Moreover, our data suggest that the basilar artery and perhaps cerebral circulation is particularly sensitive to inhibition of PPARγ activity and activation of the RAS.

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Adipocyte Deficiency of Angiotensin Converting Enzyme 2 Increases Systolic Blood Pressures of Obese Female Mice

PrimaryAuthor.AuthorBlock:**Robin C Shoemaker**, Wen Su, Ming Gong, Univ of Kentucky, Lexington, KY; Susan Gurley, Duke Univ, Durham, NC; Lisa Cassis, Univ of Kentucky, Lexington, KY

Background: We demonstrated that sexual dimorphism of obesity-hypertension was associated with differential activity of adipocyte angiotensin-converting enzyme 2 (ACE2) in male *versus* (*vs*) female mice. These data suggest that adipocyte ACE2 regulates blood pressure by influencing the balance of angiotensin II (AngII, a substrate of ACE2) *vs* angiotensin-(1-7) (Ang-(1-7)), which differs in male and female mice. We hypothesized that deficiency of ACE2 in adipocytes increases blood pressure of HF-fed female mice.
Methods/Results: Mice with adipocyte ACE2 deficiency were developed from breeding $Ace2^{fl/fl}$ mice to transgenic C57BL/6 mice with heterozygous transgenic expression of Cre recombinase driven by the adiponectin promoter ($Ace2^{Adipo}$). Female or male $Ace2^{fl/fl}$ and $Ace2^{Adipo}$ mice (8 weeks old) were fed a HF (60% kcal as fat) or low fat (LF; 10% kcal from fat) diet for 16 weeks, after which blood pressure was quantified by radiotelemetry. Systolic blood pressures (SBP) were not different in LF-fed female mice of either genotype (24 hr average SBP [mmHg]: $Ace2^{fl./fl}$: 121±1; $Ace2^{Adipo}$: 121±1; p>0.05). However, SBP was significantly increased in HF-fed female $Ace2^{Adipo}$ *vs* $Ace2^{fl/fl}$ mice ($Ace2^{fl/fl}$: 127±2; $Ace2^{Adipo}$: 133±3; p<0.05). As AngII is an ACE2 substrate, we quantified blood pressure responses of female mice to an acute challenge of AngII (20µg/kg body weight, sc). AngII administration increased SBPs of LF and HF-fed female mice of both genotypes. However, SBP responses to AngII were augmented in HF-fed $Ace2^{Adipo}$ female mice compared to HF-fed $Ace2^{fl/fl}$ controls (baseline *vs* AngII SBP [mmHg]: LF $Ace2^{fl/fl}$: 122±8 *vs* 180±4; LF $Ace2^{Adipo}$: 127±5 *vs* 173±7; HF $Ace2^{fl/fl}$: 121±3 *vs* 183±3; HF $Ace2^{Adipo}$: 138±7 *vs* 197±43; p<0.05). In contrast to females, HF-fed $Ace2^{Adipo}$ male mice had similar SBPs compared to HF-fed $Ace2^{fl/fl}$ males (24 hour SBP [mmHg]: $Ace2^{fl/fl}$: 127±2; $Ace2^{Adipo}$: 133±3; p>0.05).
Conclusions: Deficiency of ACE2 in adipocytes promoted the development of obesity-induced hypertension in female mice. Moreover, female adipocyte ACE2 deficient mice exhibited augmented blood pressure responses to AngII. These data support the hypothesis that adipocyte ACE2 contributes to sex differences in obesity-induced hypertension.

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Angiotensin II Type 1 Receptor Autoantibody Inhibition Improves Blood Pressure and Markers of Neurological Damage and Oxidative Stress in Brains of Placental Ischemic Rats During Pregnancy

PrimaryAuthor.AuthorBlock:**Mark W Cunningham Jr.**, Venkata Ramana Vaka, Lorena Amaral, Fan Fan, Tarek Ibrahim, Richard R Roman, Babbette LaMarca, Univ of Mississippi Medical Ctr, Jackson, MS

Preeclampsia (PE), hypertension in response to placental ischemia, is associated with angiotensin II type 1 receptor agonistic autoantibodies (AT1-AA), oxidative stress, and neurological complications, such as headaches, blurred vision, and seizures which could lead to stroke and death. We hypothesize that AT1AAs play a role in the cerebral pathology of PE. The objective of this study was to determine if administration of a specific peptide sequence to inhibit the AT1-AA from binding to the AT1 receptor, will improve blood pressure (MAP) and cerebral oxidative stress in the reduced uterine perfusion pressure (RUPP) rat model of PE. Pregnant Sprague Dawley rats were divided into 2 groups: RUPP (n=5) and RUPP+AT1-AA inhibitory peptide (7AA) (n=3). RUPP surgery was performed on gestational day (GD) 14 and the 7AA was administered (2ug/µl saline) via mini-osmotic pumps. On GD 19, MAP was determined and brains collected. Western blots were stained for Glial Fibrillary Acidic Protein (GFAP), endothelial NO synthase (eNOS), phosphorylated eNOS and NADPH oxidase activity was determined using chemilumenescence. MAP was decreased in RUPP+7AA vs. RUPP (95±2 vs. 130±6 mmHg). Brain/body weight ratio, which is indicative of edema, was reduced in RUPP+7AA (5.8±0.25 vs. 6.5±0.25 grams) vs. RUPP. NADPH oxidase activity was lower in RUPP+7AA (33275±3122 vs. 57408±10508 RLU/min/mg protein). Phosphorylated eNOS was 2 fold higher in the RUPP+7AA vs. RUPP (0.4±0.1 vs. 0.2±0.04 AU) and the phosphorylated eNOS/eNOS ratio was elevated (0.4±0.12 vs. 0.2±0.04 AU). GFAP a marker for activated astrocytes that increases during neurologic injury and serves as a compensatory mechanism for brain injury recovery was elevated in RUPP+7AA vs. RUPP (3.2±1.3 vs. 0.5±0.2 AU). Administration of AT1-AA inhibitory peptide to RUPP rats decreased blood pressure and improved markers of NO bioavailability, injury (GFAP), and cerebral swelling. In conclusion, our preliminary data suggests that AT1-AA inhibition could be a potential therapy to improve peripheral and neurological complications during PE. **Research Supported by T32HL105324 (Cunningham), RO1HD067541-06 (LaMarca), DK-104184 (Roman), 050049 (Fan), P20-GM-104357 (cores B and C-Roman; Pilot-Fan) and AHA 16GRNT31200036 (Fan).**

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Pregnancy Dependant Transcriptomic Changes in Uterine Arteries From Hypertensive and Normotensive Rat Models

PrimaryAuthor.AuthorBlock:**Hannah L Morgan**, Elisabeth Beattie, Martin W McBride, Delyth Graham, Univ of Glasgow, Glasgow, United Kingdom

Background

During normal pregnancy the uteroplacental vasculature remodels to accommodate for the increased blood flow to the placenta. In hypertensive pregnancies this remodelling is impaired. Stroke prone spontaneously hypertensive (SHRSP) rats exhibit a hypertensive pregnancy with impaired uteroplacental remodelling. This study

investigated early gene expression changes in the uterine arteries (UA) of pregnant SHRSP and normotensive Wistar Kyoto (WKY) rats.

Methods

SHRSP and WKY females were time mated and UA isolated at gestational day (GD)6 (n=3). Non-pregnant (NP) UA were isolated from virgin aged matched controls (n=3). UA RNAseq (Illumina platform) was performed. Transcript level gene changes were interpreted using Ingenuity Pathway Analysis (FDR < 0.05, FPKM>1.0). Quantitative RT-PCR was used to validate significantly differentially expressed genes.

Results

There was a greater number of differentially expressed transcripts NP v GD6 in SHRSP compared to WKY (796 vs 535). 147 pregnancy dependant gene changes were common to SHRSP and WKY; which involved cellular movement and cell growth/proliferation pathways. Further analysis revealed calcium signalling genes involved in the α-adrenergic pathway were downregulated in WKY NP v GD6, e.g. inositol-1,4,5-trisphosphate receptor (Ip3r) (*FC 2.5; p<0.01)*, but were not different in SHRSP. Inflammatory response was increased over pregnancy, specifically phospholipase A2 (Pla2ga) expression (*FC -7.2 in WKY and FC -4.3 in SHRSP; FDR<0.0001*). Gene expression differences were also observed in nitric oxide and reactive oxygen species pathways. Nox2 subunits were downregulated in SHRSP NP v GD6 e.g. p40-phox (*FC -2.2; FDR<0.001*), but did not change in WKY. qPCR confirmed significantly increased UA Ip3r gene expression in GD6 SHRSP vs GD6 WKY (*27.1CT vs 28.3CT p<0.05*) and reduced Pla2ga expression in WKY vs SHRSP NP UA (*23.3CT vs 26.4CT; p<0.001*).

Conclusion

We show that SHRSP UA have different gene expression profiles in response to pregnancy compared to WKY. These early transcript level changes occur prior to structural or functional changes, suggesting that the strains have pre-existing genetic differences that may 'prime' the UA to respond differently due to chronic hypertension in SHRSP rats.

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Reduced Placental Regulator of G-Protein Signaling-2 (RGS2) and Preeclampsia

PrimaryAuthor.AuthorBlock:**Katherine J Perschbacher**, Guorui Deng, Jeremy A Sandgren, Leslie Carillo-Saenz, Donna A Santillan, Eric J Devor, Gary L Pierce, Mark K Santillan, Rory A Fisher, Katherine N Gibson-Corley, Justin L Grobe, Univ of Iowa, Iowa City, IA

The early mechanisms and genetic risk factors driving the pathogenesis of preeclampsia (PE), a cardiovascular disorder of pregnancy, remain largely unclear. Various hormone activators of $G\alpha_q$ second-messenger signaling pathways have been implicated in PE. Regulator of G-protein Signaling 2 (RGS2) acts as an endogenous terminator of $G\alpha_q$ signaling and previous studies identified a SNP (rs4606), which results in reduced RGS2 mRNA, as a risk factor for development of PE and its sequelae. We hypothesized reduced placental expression of RGS2 may precipitate the development of PE due to disinhibited $G\alpha_q$ signaling. *In silico* reanalysis of publically available dataset GSE75010 revealed RGS2 mRNA is reduced in placentas from pre-term PE pregnancies compared to normal pre-term pregnancies (con: 8.73 ± 0.07 n=35, PE: 8.37 ± 0.055 n=49, p<0.05). Using human placental tissue samples from the University of Iowa Maternal-Fetal

Tissue Bank, we confirmed RGS2 mRNA is reduced in PE placentas (19% of control, $p<0.05$), despite a lack of correlation between the rs4606 SNP and PE. Additionally, in further reanalysis of other datasets, RGS2 mRNA is among the highest-expressed RGS member in normal human placenta, and appears to be selectively reduced in syncytio- and invasive cytotrophoblasts during PE (GSE93839, -26.3%, -23.3% of control). We next examined RGS2 expression in mouse placenta by FISH and found RGS2 mRNA colocalizes with markers of syncytiotrophoblast II (GCMA) and spongiotrophoblast (Tpbpα) layers. To test the effect of RGS2 loss during pregnancy, wildtype C57BL/6J female mice were mated with RGS2-deficient sires and developed diastolic hypertension, placental hypoxia by HIF1α ELISA (con 0.144±0.004, RGS2-KO 0.155±0.004 AU, $p<0.05$, n=5 each), and reduced placental PlGF mRNA (fold; con=1.0 n=7, RGS2-KO=0.23 n=12, $p<0.05$), compared to females mated with RGS2 littermate sires. These data support the concept that loss of RGS2 may contribute to the pathogenesis of PE rather than simply correlating with the disorder. Taken together, we have shown placental RGS2 is suppressed in PE, RGS2 is present in cytoplasm of specific layers of trophoblasts, and loss of feto-placental RGS2 is sufficient to cause placental hypoxia and maternal diastolic hypertension.

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145

Central Angiotensin-(1-7) Treatment Attenuates ERK 1/2 Expression and Oxidative Stress in the Dorsal Medulla of Betamethasone-Exposed Sheep That Associates With Improved Blood Pressure and Baroreflex Sensitivity

PrimaryAuthor.AuthorBlock:**Alexa S Hendricks**, Hossam A Shaltout, Mark C Chappell, Debra I Diz, Wake Forest Sch of Med, Winston Salem, NC

Fetal exposure to betamethasone (BMX) that is routinely administered to women threatening

premature delivery may lead to deleterious long-term effects on the central cardiovascular system. In the adult offspring of BMX-exposed sheep, we demonstrate increased mean arterial pressure (MAP) and attenuated baroreflex sensitivity (BRS). These responses are associated with dysregulation of the brain renin-angiotensin system (RAS) reflecting lower medullary expression of Angiotensin-(1-7) [Ang-(1-7)] and its beneficial actions. Moreover, mitogen activated protein kinase (MAPK), a key signaling cascade implicated in cardiovascular dysfunction and stimulation of oxidative stress is increased in the brain dorsomedial medulla (DMM). We hypothesize that loss of Ang-(1-7) tone with BMX is an underlying mechanism for the programming effects to increase MAPK and oxidative stress. Thus, we examined whether intracerebroventricular treatment with Ang-(1-7) that lowers MAP and improves BRS will impact MAPK signaling and downstream generation of reactive oxygen species (ROS). MAPK activation as detected by the ratio of phospho-ERK 1/2 to total ERK densities was significantly reduced by >80% in the Ang-(1-7)-treated BMX sheep as compared to the CSF-treated BMX controls (0.20 ± 0.07 vs 1.04 ± 0.31; p = 0.01, N=4/group). Ang-(1-7) treatment was associated with lower expression of two indices of ROS including 4-HNE (0.23 ± 0.03 vs. 0.31 ± 0.03 p = 0.03) and protein carbonyl content (9.95 ± 0.69 vs 15.94 ± 3.49; p = 0.07). Finally, regression analysis revealed that phospho-ERK 1/2 expression positively correlated with ROS (4-HNE) (r = 0.816; p = 0.01). The 4-HNE content also trended positively with MAP (r = 0.659, p = 0.08), but exhibited a negative correlation with BRS (r = -0.831; p = 0.01). We conclude that reduced central Ang-(1-7) tone may contribute to the chronic dysregulation of the MAPK and ROS signaling pathways within the DMM following BMX exposure. Moreover, Ang-(1-7) may constitute a potential therapeutic approach to improve autonomic dysfunction by attenuating both MAPK and ROS pathways. HD 047584.

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146

MicroRNA-216a Promotes M1 Macrophages Polarization Through the Activation of Telomerase

PrimaryAuthor.AuthorBlock:Shujun Yang, Jian Li, Yu Chen, Rutai Hui, **Weili Zhang**, State Key Lab of Cardiovascular Disease, Fuwai Hosp, Natl Ctr for Cardiovascular Diseases, Peking Union Medical Coll &Chinese Acad of Medical Sciences, Beijing, China

Background: Macrophages display significant phenotypic heterogeneity and plasticity. Two subtypes exist in atherosclerotic plaques: M1 pro-inflammatory subtype and M2 anti-inflammatory subtype, which can be converted to each other under certain conditions and therefore contribute to the plaque progression. But the underlying mechanisms remain unclear. Our previous work showed that m*iR-216a* mediates the activation of telomerase in macrophages differentiation via the Smad3/NF-κB pathway *in vitro*. In the present study, we aimed to examine the role of *miR-216a* in macrophages polarization and atherosclerosis *in vivo*.

Methods and results: We applied a tandem stenosis to the right carotid artery of apolipoprotein E-deficient mice model according to doctor Chen's method (Circ Res. 2013;113(3):252-65.), with local infiltration of the *miR-216a* lentivirus. At 4 weeks

postoperatively, the significant effect of *miR-216a* on plaque area was not observed; however, the results showed that *miR-216a* in the carotid atherosclerotic plaques markedly increased the numbers of M1 subtype by 1.8-fold whereas simultaneously decreased M2 subtype by 2.1-fold by applying CD16/32 and Arg1 immuno-fluorescent staining. In addition, we found that *miR-216a* significantly reduced the amounts of collagen III by Sirius-Red staining and downregulated the mRNA expression of collagen type III alpha 1 chain (COL3A1) by 70%. Furthermore, the THP-1 macrophages were used to explore the mechanism of *miR-216a* on macrophages polarization, and the results showed that *miR-216a* and telomerase reverse transcriptase (TERT) expression were only increased in M1 macrophages. Next, TERT lentivirus and siRNA were transfected, and the results showed that TERT overexpression promoted M1 polarization; on the contrary, silencing TERT inhibited M1 polarization and suppressed M2 to M1 conversion.
Conclusions: Our data indicated that *miR-216a* promotes M1 macrophages polarization through the activation of telomerase and reduces collagen III in mice carotid lesions, and ultimately accelerates the development of atherosclerosis.

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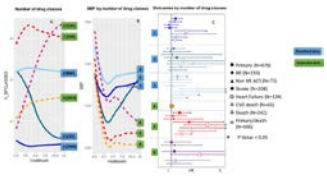
147

Detrimental Cardiovascular Effects Associated With Use of >4 Drug Classes to Achieve Blood Pressure Control in SPRINT

PrimaryAuthor.AuthorBlock:**Linsay McCallum**, Andrea Llano, Alisha Aman, John McClure, Alan Jardine, Anna F Dominiczak, Univ of Glasgow, Glasgow, United Kingdom; Phil Chowienczyk, Kings Coll, London, United Kingdom; Sandosh Padmanabhan, Univ of Glasgow, Glasgow, United Kingdom

Background
In the SPRINT trial,In SPRINT, achievement of target BP in the intensive arm required a higher number of drugs. Whilst intensive treatment of SBP was associated with an increased incidence of adverse events, it is unclear whether the use of multiple drug classes would result in adverse outcomes.
Methods
The number of drug classes prescribed at randomisation and at 1,2,3,6,9,12 months were used to identify distinct trajectory groups in the standard and intensive arm using Latent Class Mixed Modelling. Data were available in 8,449 participants. Cox-PH models, adjusted for age, sex, SBP, prevalent CVD, prevalent CKD and number of drug classes at randomisation, were used to assess the association between drug class trajectories and pre-specified outcomes from SPRINT.
Results
The SPRINT population classified into 6 groups (3 standard, 3 intensive) based on the trajectories of drug classes prescribed over the first year are shown in Panel A with corresponding SBP by drug class groups in Panel B.
Cox-PH model (reference group 4) showed group 5 (SBP 125 mmHg on >4 drug classes) had significantly higher risk of the primary outcome (HR 1.62 95% CI [1.12-2.04]; p 0.011), heart failure (HR 3.68 [1.95-6.94]; p <0.0005), CVD death (HR 4.34 [1.66-11.35]; p 0.003) and all-cause death (HR 1.75 [1.26-2.43]; p <0.0005) (Panel C).
Conclusions
In SPRINT, treatment with >4 antihypertensive

drug classes was associated with poor outcomes, specifically increased risk of death and heart failure, independent of blood pressure achieved in the first year, possibly related to the period of drug exposure. These results caution clinicians to assess need for multiple drugs and tighter blood pressure control.



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148

Association of Socioeconomic Context With Blood Pressure Response and Cardiovascular Outcomes in ALLHAT

PrimaryAuthor.AuthorBlock:**Andi Shahu**, Jeph Herrin, Sanket S Dhruva, Nihar R Desai, Harlan M Krumholz, Erica S Spatz, Yale Sch of Med, New Haven, CT

Introduction: Observational studies demonstrate that in low socioeconomic (SE) communities, where residents face social challenges and fewer opportunities for healthy lifestyle behaviors or quality care, blood pressure (BP) is higher and there are worse cardiovascular (CV) outcomes. Yet whether the effectiveness of antihypertensive therapy on BP control and CV outcomes varies by SE context in a randomized clinical trial (RCT) - where patients are treated under a standard protocol - is unknown.

Methods: We used data from the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT), which randomized 42,418 people with hypertension (HTN) to chlorthalidone, lisinopril, amlodipine or doxazosin. After excluding non-continental US sites and the doxazosin arm (terminated early), our study included 27,862 participants. We defined SE context by mapping study site ZIP codes to counties and stratifying these into income quintiles based on the national distribution of cost-of-living adjusted county median household income (2000 US Census). We compared characteristics and outcomes between participants in the lowest and highest SE quintiles using multivariable regression models. We replicated the analysis with black participants (n = 10532).
Results: Participants enrolled in low SE sites (n = 2169, 7.8%) were more likely to be female, black, Hispanic, less educated, live in the South, and have fewer CV risk factors than participants in high SE sites (n = 10458, 37.6%). Participants in low SE sites were less likely to achieve BP control (<140/90 mmHg) at 6 years (OR 0.48, 95% CI [0.37, 0.63]). They had higher all-cause mortality (HR 1.25, 95% CI [1.10, 1.41]) and heart failure hospitalization/mortality (HR 1.26, 95% CI [1.03, 1.55]), though lower angina hospitalization (HR 0.70, 95% CI [0.59, 0.83]) and coronary revascularization (HR 0.71, 95% CI [0.57, 0.89]) and no differences in stroke or MI. Results were similar among black participants.
Conclusion: Despite having access to extra resources afforded by an RCT, ALLHAT participants in low SE sites had poorer BP control, higher mortality and greater heart failure morbidity. Our findings suggest a need to address the SE context beyond the medical environment to attain equity in HTN outcomes.

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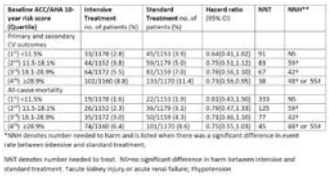
Impact of Baseline 10-year Cardiovascular Risk on Benefit and Harm of Intensive Treatment in SPRINT

PrimaryAuthor.AuthorBlock:**Robert A Phillips**, Jiaqiong Xu, Leif E Peterson, Houston Methodist, Houston, TX; Joseph A Diamond, Hofstra Northwell Sch of Med, Hempstead, NY; Adam E Schussheim, Bridgeport Hosp, Yale-New Haven Health System, Bridgeport, CT

We investigated the impact of baseline CV risk on outcomes in SPRINT. Using the ACC/AHA CVD risk algorithm, we stratified the SPRINT population into quartiles of baseline 10-year CV risk. Within each quartile, Cox proportional hazards models were used to examine the effect of intensive treatment vs. standard of care on the SPRINT CV outcomes, all-cause mortality and serious adverse events (SAEs). Number needed to treat (NNT) and number needed to harm (NNH) were calculated for each quartile. There were 9,323 participants with available baseline ACC/AHA 10-year risk scores. In each quartile of risk, the hazard ratio (HR) favored intensive treatment. For CV outcomes, NNT decreased from 91 in the 1st quartile to 38 in the 4th quartile. For all-cause mortality, NNT decreased from 333 in the 1st quartile to 45 in the 4th quartile. Although incidence of all SAEs increased with each quartile in both treatment groups (p for trend <0.0001), there was no difference in incidence of SAEs between the treatment groups in each quartile. However, SAEs classified as hypotension were more frequent in the 4th quartile for the intensive treatment group (incremental increase 1.8%, NNH = 55) and SAEs classified as acute kidney injury or acute renal failure were significantly more frequent in the 2nd, 3rd and 4th quartiles for the intensively treated group (incremental increase 1.7%, 2.4% and 2.1%; NNH 59, 42 and 48, respectively). Therefore, those with greatest baseline CVD risk got the most benefit from intensive BP treatment but were at greater risk for hypotension and renal injury. This analysis may help providers and patients make decisions regarding the intensity of BP treatment to prevent death and CV events.



Baseline ACC/AHA 10-year risk score (Quartile)	Intensive Treatment no. of patients (%)	Standard Treatment no. of patients (%)	Hazard ratio (95% CI)	NNT	NNH**
Primary and secondary CV outcomes					
(1st) <11.5%	33/1378 (2.8)	49/1153 (4.9)	0.64(0.41,1.02)	91	NS
(2nd) 11.5-18.1%	44/1352 (3.8)	59/1179 (5.0)	0.75(0.51,1.12)	83	59*
(3rd) 18.1-28.9%	64/1377 (5.5)	81/1159 (7.0)	0.78(0.56,1.10)	67	42*
(4th) ≥28.9%	102/1390 (8.8)	133/1170 (11.4)	0.73(0.56,0.95)	38	48* or 55†
All-cause mortality					
(1st) <11.5%	19/1378 (1.6)	22/1153 (1.9)	0.81(0.43,1.50)	333	NS
(2nd) 11.5-18.1%	26/1352 (2.3)	36/1179 (3.1)	0.73(0.47,1.13)	125	59*
(3rd) 18.1-28.9%	35/1372 (3.0)	50/1159 (4.3)	0.73(0.46,1.16)	77	42*
(4th) ≥28.9%	74/1390 (6.4)	101/1170 (8.6)	0.75(0.55,1.03)	45	48* or 55†

*NNH denotes number needed to harm and is listed when there was a significant difference in event rate between intensive and standard treatment.

NNT denotes number needed to treat. NS=no significant difference in harm between intensive and standard treatment. †acute kidney injury or acute renal failure; ‡hypotension

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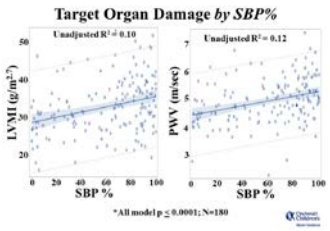
Target Organ Damage Occurs at SBP Levels Below the 95th Percentile in Adolescents: The SHIP AHOY Study

PrimaryAuthor.AuthorBlock:**Elaine M Urbina**, Cincinnati Childrens Hosp, Cincinnati, OH; Marc Lande, Univ of Rochester Medical Ctr, Rochester, NY; Kevin Meyers, Children's Hosp of Philadelphia, Philadelphia, PA; Joshua Samuels, Univ of Texas Health Sciences Ctr, Houston, TX;

Joseph Flynn, Seattle Children's Hosp, Seattle, WA

HTN leads to LV hypertrophy, increasing risk for CV events. The BP level at which LVH develops is not clear. We hypothesized that target organ damage (TOD: LVH; arterial stiffness; pulse wave velocity, PWV) occurs below the definition of HTN in youth (95th %ile of BP). We evaluated BP, anthropometrics, lab, LVM, and PWV in 180 adolescents (mean 15.8 $\pm$ 1.5 yrs, 64% white, 57% male). Subjects were classified as normal (L=104, SBP<80th %ile), mid-risk (M=38, SBP 80-<90th %ile); or high-risk (H=38, SBP >=90th %ile) by mean of 6 aneroid SBPs according age, sex and height-specific pediatric cut-points. ANOVA was used to evaluate differences in CV risk factors and TOD across groups. General linear models were used to determine if SBP %ile was an independent predictor of TOD. Logistic regression was used to determine the SBP %ile most sensitive and specific for predicting LVH. Groups did not differ by age, sex, race or BMI %ile. Mean BP increased across groups (L=109/74, M=126/82, H=135/87 mmHg, $p \leq 0.0001$). LVM, and PWV also increased (LVM L=31.5, M=34.7, H=35.3 g/m$^{2.7}$; PWV L=4.8, M=5.2, H=5.3 m/sec, $p \leq 0.01$). SBP %ile remained a significant determinant of TOD after adjusting for covariates including demographics, age, BMI, HR (LVM: SBP %ile β=0.04, R^2 0.32; PWV: SBP %ile β=0.01, R^2 0.29, all $p \leq 0.0001$). The 90th %ile for SBP resulted in the best balance between false + (14%) and false - (13%) for LVH, however even at the 80th %ile, 8% of cases of LVH were missed and 16% (29 of 180) had LVH at SBP < 95th %ile. TOD occurs at levels lower than the pediatric definition of HTN even after controlling for BMI. Guidelines should consider TOD when setting cut-points for treatment.



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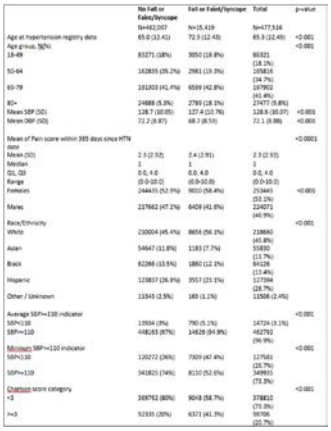
Falls and Syncope Among Treated Hypertensive Individuals With Lower Systolic Blood Pressure

PrimaryAuthor.AuthorBlock:**John J Sim**, Kaiser Permanente Los Angeles Medical Ctr, Los Angeles, CA; Hui Zhou, Kaiser Permanente Southern California, Pasadena, CA; Rong Wei, Jocelyn Tran-Nguyen, Joel Handler, Kaiser Permanente Southern California, Los Angeles, CA; Jeffrey W Brettler, Kaiser Permanente West Los Angeles Medical Ctr, Los Angeles, CA; Kristi

Reynolds, Kaiser Permanente Southern California, Los Angeles, CA

Objective: To determine whether lower treated blood pressure (BP) was associated with serious fall injuries and syncope among hypertensive patients. **Methods:** A cross sectional study was conducted within Kaiser Permanente Southern California in patients age ≥18 yrs treated for hypertension and with outpatient BP measurements btwn 7/1/2014-6/30/2015. All BP measurements were used to determine mean and minimum (single lowest BP) systolic BP (SBP) and categorized as <110 and ≥110 mmHg. Serious fall injuries and syncope were determined from emergency dept and inpatient encounters. **Results:** Among 477,516 patients (53% women, mean age 65 yrs), 14,724 (3.1%) had mean SBP<110 and 127,581 (26.7%) had minimum SBP<110. Overall, 15,419 (3.2%) experienced a fall (7,458) and/or syncope (8,763) with higher rates occurring in mean SBP<110 (5.4 vs 3.2%) and minimum SBP<110 (5.7 vs 2.3%) compared to SBP≥110. After multivariate adjustment for potential confounders, compared to patients with SBP ≥110, the odds ratios (95%CI) of falls, syncope, and falls or syncope combined were 1.17 (1.04-1.32), 1.79 (1.63-1.96), and 1.52 (1.40-1.63), respectively, for mean SBP <110; the corresponding odds ratios (95% CI) were 1.78 (1.70-1.86), 2.42 (2.31-2.52), and 2.11 (2.04 - 2.18) for minimum SBP<110 vs ≥110. Use of >2 medications, alpha blockers, older age, females sex, dementia, peripheral vascular disease, and congestive heart failure were also associated with falls and syncope. **Conclusion:** Among treated hypertensive patients, SBP <110 mmHg was associated with serious fall injuries and syncope. Low treatment related BP and variability deserve consideration as we manage our hypertension population.



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P100

Bioavailability of Arginine versus Erbumine Formulations of Perindopril in Healthy Subjects

PrimaryAuthor.AuthorBlock:**Andrew Bishop**, PNWU, Yakima, WA

Perindopril is an angiotensin converting-enzyme inhibitor with a proven track record in cardiovascular clinical trials. Traditionally formulated as the *t*-butyl amine (“erbumine”) salt, perindopril’s arginine salt has been FDA-approved in combination with amlodipine besylate. The arginine salt has the advantage of being more stable at higher temperatures or humidity.
To demonstrate bioequivalence (using the standard FDA definition of 90% confidence intervals that are within 80-125% of the values seen with the reference product), the bioavailability of the two perindopril

formulations was compared. After screening 121 healthy subjects, 30 (73% male, 38.9±9.5 years of age, 67% African American, BMI of 26.6±2.7 kg/m^2) signed informed consent, and were studied in a randomized, single-center, single-dose, open-label, 2-period, 2-way cross-over design with a 14-day washout period. After an overnight fast, perindopril was administered orally with 240 mL of water, and blood samples taken at defined time points.
After dose-normalization (the arginine salt has a molecular weight 1.43-fold greater than that of the erbumine salt), all pharmacokinetic parameters for perindopril plasma concentrations were similar between treatments. Mixed model analysis of these parameters demonstrated bioequivalence (e.g., area under the time-plasma concentration curve: 10.2 vs. 10.5 hr•ng/mL/mg, P = 0.54). Very similar results with non-significant differences were also seen for the pharmacokinetics of perindoprilat (perindopril's active metabolite): 33.3 vs. 38.3 hr•ng/mL/mg, P = 0.17. Two subjects did not complete the perindopril arginine arm of the study: one withdrew consent, and the other experienced a protocol deviation. No subjects experienced a serious adverse event or withdrew due to a treatment-emergent adverse event. Four subjects reported adverse events after perindopril arginine, and two subjects reported neck pain or presyncope after perindopril erbumine.
After dose-normalization, these two formulations of perindopril meet all traditional criteria for AB rating and interchangeability.

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P101

Degradation of Ang-(1-7) in Different Mouse Organs

PrimaryAuthor.AuthorBlock:**Andrew M Moore**, Cliona O' Mahony, Thomas Walther, Univ Coll Cork, Cork, Ireland

IntroductionAng-(1-7) has cardioprotective effects that serve to counter-regulate adverse effects of Ang II in the cardiovascular system. Angiotensin-(1-7) inhibits pressor, proliferative, and cell growth promoting effects of Ang II. Furthermore, it shows anti-fibrotic and anti-hypertrophic properties in preclinical models of myocardial infarction.
It is well accepted that Ang-(1-7) is primarily degraded to Ang-(1-5) by ACE in the cardiovascular system. However, we have preliminary results showing variations exist in Ang-(1-7) degradation pathways in various mouse organs. It is the aim of this study to quantify the metabolites in individual organs and to generate an organ-specific fingerprint of Ang-(1-7) truncation.
Methods
Mouse organs were taken from male C57BL/6J mice and homogenates/membranes were prepared and measured for protein concentration. Membrane isolations were incubated with Ang-(1-7) and analyzed for peptide identification on an LCMS system.
Results
Our results show that after 10 minutes Ang-(1-7) is degraded fastest in lung (1% [88108/6837249] of peptide remaining), followed by Kidney (8% [544423/6837249]), then liver (41% [2781701/6837249]), ventricle (83% [5699496/6837249]), and brain (86% [5868630/6837249]). Unsurprisingly, Ang-(1-5) was a major degradation product for the lung, kidney, and heart. However, we identified Ang-(1-4) as another major degradation product in lung and kidney. In contrast, the major

degradation product of Ang-(1-7) in the brain and ventricle was Ang-(2-7). While Ang-(1-7) metabolism in testes yielded a unique angiotensin metabolite not observed in other tissues, Ang-(1-7) was not at all converted in atrium membranes.

Conclusions

Ang-(1-7) degradation varies greatly in speed and metabolites between mouse organs. The necessity to update the relatively old canonical Ang-(1-7) degradation pathway is paramount for drug viability and design. By identifying the peptidases ultimately responsible for the metabolism of Ang-(1-7), its circulating concentration can be increased, thus improving the benefits of its cardiovascular protective effects.

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Liver Acquitted, Fat Indicted: Hepatic Chemerin Knockdown Does Not Reduce Blood Pressure While Whole-body Knockdown Does

PrimaryAuthor.AuthorBlock:**David J Ferland**, Bridget Seitz, Emma S Darios, Janice M Thompson, Michigan State Univ, East Lansing, MI; Steve T Yeh, Adam E Mullick, Ionis Pharmaceuticals, Carlsbad, CA; Stephanie W Watts, Michigan State Univ, East Lansing, MI

Chemerin is an inflammatory adipokine positively associated with hypertension and obesity with the majority of chemerin thought to derive from the liver and adipose tissue. The contributions of liver-derived chemerin to plasma chemerin levels and blood pressure regulation are unknown. We compared whole-body vs liver chemerin inhibition using antisense oligonucleotides (ASO) with liver-restricted activity (GalNAc) or liver and fat activity (Gen 2.5). We hypothesized that in normotensive male SD rats, circulating chemerin is predominately liver-derived and regulates blood pressure. A scrambled ASO control and phosphate-buffered saline (PBS) were used as controls and radiotelemetry was used to monitor blood pressure. Baseline mean arterial blood pressure (MAP) was recorded for one week, beginning 5 days after surgery to establish a baseline. ASOs were given weekly by subcutaneous injections for four weeks. Two days after the final injection, animals were sacrificed for tissue RT-PCR and plasma chemerin measurements using Western analysis. Gen 2.5 chemerin ASO treatments (compared to PBS control) reduced chemerin mRNA in liver, retroperitoneal fat, and mesenteric perivascular adipose tissue (mPVAT) by 99.5% ± 0.1%, 95.2% ± 0.3%, and 69% ± 2%, respectively, and plasma chemerin was reduced to undetectable levels. GalNAc chemerin ASO treatments (compared to PBS control) reduced chemerin mRNA in liver by 97.9% but had no effect on chemerin expression in retroperitoneal fat and mPVAT; plasma chemerin was reduced by 90% ± 5%. Gen 2.5 chemerin ASO treatment reduced MAP, which reached a nadir of 7 ± 2.1 mmHg 48 – 72 hours after each dose compared to the scrambled ASO controls. By contrast, MAP was unchanged in animals receiving the GalNAc chemerin ASO. Thus, although most circulating chemerin is liver-derived, plasma chemerin does not play a role in blood pressure regulation. This study suggests that while chemerin is still generally associated with increased blood pressure, circulating chemerin levels cannot directly predict this effect. In addition, local effects of chemerin from fat may explain this discrepancy and support chemerin's association with both hypertension and obesity.

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P103

Anti-hypertensive Mechanisms of Action of $G_{q/11}$ Inhibitor Ligands

PrimaryAuthor.AuthorBlock:**Matthew M. Meleka**, Shelby Dahlen, Alethia J. Edwards, Drexel Univ Coll of Med, Philadelphia, PA; Matthew Medcalf, Kevin D. Moeller, Washington Univ, Saint Louis, MO; Patrick Osei Owusu, Drexel Univ Coll of Med, Philadelphia, PA

Chronic blockade of individual G protein coupled receptors (GPCRs) has proven to be inadequate strategy for managing hypertension partly because the subcellular heterotrimeric G proteins that propagate intracellular signaling can simultaneously couple to several other vasopressor receptors. Whether blood pressure can better be controlled by directly targeting G proteins has not been thoroughly investigated due to paucity of selective, cell-permeable inhibitors. Here, we tested whether chemical inhibition of Gq/11 proteins *in vivo* and *ex vivo* using recently discovered small-molecule inhibitor ligands, YM-254890 (YM), FR-900359 (FR) and WU-07047 (WU) is sufficient to reverse hypertension in mice. Using *ex vivo* vessel reactivity assay, we found that Gq/11 inhibitors markedly reduced vasoconstriction evoked with phenylephrine (PE), vasopressin, endothelin-1, and the thromboxane analog U-46619. Blockade of PE-induced contractility by the Gq/11 inhibitors showed the following rank-order potency: $FR_{LogIC50}$ -0.008 ± 0 > $YM_{LogIC50}$ -0.49 ± 0 > $WU_{LogIC50}$ -64.95 ± 6.4. YM and WU but not FR inhibited PE-induced vasoconstriction through G protein-dependent and independent pathways by blocking L-type calcium channel-mediated Ca^{2+} influx. Acute subcutaneous injection of FR and YM (0.3 mg/kg, s.c.) in normotensive and N^{ω}-Nitro-L-arginine methyl ester (L-NAME) hypertension mice elicited marked hypotension, which was more severe (ΔSBP = -25 ± 2.7 vs. ΔSBP = -21 ± 2.2 mmHg) and long lasting ($FR_{t1/2} \cong$ 12 hr vs. $YM_{t1/2} \cong$ 4 hr) after the injection of FR relative to YM. In DOCA-salt hypertension mice, chronic injection of FR (0.3 mg/kg, s.c., daily for seven days) reversed hypertension (vehicle SBP: 149 ± 5 vs. FR SBP: 117 ± 7 mmHg) and sustained blood pressure reduction several days after terminating the injection regimen (DOCA SBP: 141 ± 2 vs. SBP 5 days post FR: 128 ± 5 mmHg). Our results together support the hypothesis that increased Gq/11 activity in blood pressure-regulating organs is involved in the pathogenesis of hypertension, and that direct systemic blockade of Gq/11 reverses hypertension. The findings provide clear evidence for targeting Gq/11 in the cardiovascular system as an effective therapy for treating hypertension.

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P104

Oxygen Drastically Increases Cardiac Output Without Changing Mean Pulmonary Arterial Pressure in a Patient With Severe, Progressive Pulmonary Hypertension

PrimaryAuthor.AuthorBlock:**Dan Zheng**, The Wright Ctr for Graduate Medical Education, Scranton, PA; Thomas Roe, Great Valley Cardiology, Scranton, PA

Background The diagnosis of PH requires a high degree of clinical suspicion and is defined as a mean pulmonary artery (mPAP) greater than or equal to 25 mmHg. Systolic pulmonary artery pressure (sPAP) can be estimated by transthoracic echocardiography (TTE) and mPAP directly measured by right heart catheterization (RHC). The hemodynamic data obtained from RHC provide diagnostic, therapeutic, and prognostic information important for the complete assessment of PH. We present a case of severe, progressive PH evidenced by serial TTE over a 6-year period. RHC confirmed severe mPAP elevation and showed a marked increase in cardiac output (CO) after giving 100% oxygen, observed with no significant change in mPAP, but decrease in heart rate (HR) and therefore increased right ventricular (RV) stroke volume (SV) in response to oxygen-induced decrease in pulmonary vascular resistance (PVR). Method A 68 year old female presented with exertional dyspnea, severe PH, but class 1 exercise capacity. Data was retrieved from serial TTE and RHC performed at rest on room air and after giving 100% oxygen. Results Serial TTE conducted over a 6-year period showed a steady progression of sPAP at a rate of 10% annually, reaching 67 mmHg at the time of the RHC, which recorded mPAP at 51 mmHg on room air (RA) and 47 mmHg with 100% oxygen. CO was at 5.84 L/min on RA and increased by 33% to 7.79 L/min with oxygen. HR was at 99 bpm on RA and at 88 with oxygen meanwhile stroke volume (SV) showed a 50% increase from 59 ml to 88 ml. Discussion/Conclusion Our patient had no significant change in mPAP facing oxygen-induced decrease in PVR. Instead, there was a marked increase in RV CO explainable only by an increase in RV SV facing decreased RV afterload, as HR decreased with oxygen. These findings are consistent with the patient's class 1 exercise capacity despite having severe PH, indicating an ability to increase CO with activity and suggesting a positive response to medications and better prognosis. Our case shows the importance of assessing hemodynamic data in the workup and management of PH. We propose using TTE assessment of CO coupled with 100% oxygen administration in addition to sPAP estimation as a noninvasive, readily available means of obtaining this critical and underutilized information.

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P105

A Pharmacist Guided Patient Driven Interdisciplinary Program to Improve Blood Pressure Control in Patients with Hypertension

PrimaryAuthor.AuthorBlock:**Charles Hopley**, Emily Andrews, Patrick Klem, Liza Wilson, Michelle Jonjak, Zhiying You, Corey Lyon, Diana Jalal, Univ of Colorado Sch of Med, Aurora, CO

Introduction Approximately 65% of patients treated for hypertension do not meet blood pressure goals. New strategies are needed to improve this number given the high impact of hypertension on CV disease. Dietary approaches and patient-driven self-titration of BP medications have been shown to be effective and safe in clinical studies but are under utilized in clinical practice. We present our experience with this ongoing project **Aims:** • Implement a pharmacist-guided, patient-driven self-monitoring and medication titration program. • Implement standardized evidence-based dietary

counseling to hypertensive patients. • **Intervention** • Patients with uncontrolled hypertension on 3 or fewer anti-hypertensive agents were contacted to participate. • BP goals were established based on individual risk factors. • Patients were referred to the clinical pharmacist who devised a personalized plan for BP medication titration. • Provide Education on dietary modification was provided to patients. The project was first introduced at the University of Colorado Hospital CKD clinic and then transitioned to the primary care setting at A.F. Williams Family Medicine Center. **Outcomes** • Appeal--both to providers and patients via pre/post survey • Adherence/effectiveness--tracked via patient EMR blood pressure entries • Adverse Events (via survey) experienced by patients will be analyzed **Results:** • At time of submission 19 patients have been enrolled and 3 patients have completed 6 month follow-up visits • Of all ongoing and completed patients adherence to protocol is 72.2% • 2 of the 3 patients completing 6 month follow-up had significant improvement in blood pressure (avg 16.26 mmHG) • Comparison of descriptive statistics gathered from pre and 6 mo program provider surveys suggest favorable acceptance of program from renal providers • All patients enrolled received dietary counseling with 3 patients receiving formal dietary consults. **Conclusion** Our model for self-titration and dietary counseling appears to be feasible, safe and well received from providers. At the time of submission this project is ongoing, however early results from patients completing the protocol appear to be promising.

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P106

Blood Pressure Regulation After Lisinopril Therapy in Persons With Diabetes

PrimaryAuthor.AuthorBlock:**Vesna Stojanov**, Clinical Ctr of Serbia, Belgrade, Serbia; Katarina Paunovic, Branko Jakovljevic, Faculty of Med, Belgrade, Serbia; Zorica Milenkovic Vicentijevic, Alkaloid, Belgrade, Serbia

Objectives: The aim of this study was to assess the regulation of blood pressure after three months of antihypertensive therapy in persons with type 2 diabetes mellitus. Design and Methods: The study sample consisted of 398 persons with arterial hypertension and type 2 diabetes, aged 63.9±9.6 years. Hypertension was treated with 10 mg lisinopril (98 patients), 20 mg lisinopril (140 patients), or with fixed combination of 20 mg lisinopril and 12.5 mg hydrochlorothiazide (160 patients). Blood pressure (BP) was measured by an oscillometric device at the beginning of the study, one month later, and after three months of therapy. A 'well-regulated' blood pressure was defined as systolic BP less than 140 mmHg, and/or diastolic BP less than 90 mmHg one month and three months after the initiation of therapy. Logistic regression was used to predict good regulation of SBP and DBP after three months of therapy in relation to age, gender, smoking habits, body mass index, and regulation of blood pressure one month after the beginning of therapy. Results: At the beginning of the study, the average SBP was 159.4±12.7 mmHg, average DBP was 96.3±8.5 mmHg. After one month, average SBP was 140.5±18.6 mmHg, average DBP was 87.8±8.1 mmHg. At that stage, SPB was well-regulated in 127 persons (31.9% of the sample), whereas DBP was well-regulated in 189 persons (47.5% of the sample). After three months of therapy, average SBP was 132.4±9.0

mmHg, and average DBP was 81.6±6.0 mmHg. At that stage, SPB was well-regulated in 286 persons (71.9% of the sample), whereas DBP was well-regulated in 339 persons (85.2% of the sample). The only significant predictor for the good regulation of SBP after three months of therapy was good regulation of SBP one month after the beginning of therapy (Odds Ratio=2.44; 95% Confidence Interval =1.44-4.13). The only significant predictor for the good regulation of DBP after three months was good regulation of DBP one month after the beginning of therapy (OR=8.08; 95% CI =3.71-17.63). These prediction models were independent of age, gender, body mass index, smoking habits, and lisinopril treatment. Conclusion: The regulation of BP after three months of antihypertensive therapy with lisinopril depends on the good regulation of BP one month after the initiation of therapy.

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P107

Hypertensive Patients Health Beliefs and Management Behaviors in a Tertiary Health Institution in Nigeria

PrimaryAuthor.AuthorBlock:**Chinwe Elizabeth Eze**, Oriyomi M Mohammed, Univ of Lagos, Yaba Lagos, Nigeria

Background/Objective: Hypertension remains a major global public health concern. Despite available effective therapies and lifestyle interventions, optimal control of blood pressure remains a very serious health challenge to healthcare professionals especially in most developing countries like Nigeria. Studies done among African Americans suggest that beliefs play important role in hypertension control or management behaviors. We therefore carried out a study to assess hypertensive patients' knowledge and beliefs about hypertension and how they affect their management behaviors.
Method: The study was centered at the outpatient cardiology clinic of Lagos University Teaching Hospital(LUTH). It was a cross sectional descriptive study with the use of interviewer-administered validated structured questionnaires. The Hypertension Knowledge-Level Scale was used to assess knowledge about hypertension. A questionnaire based on Health Belief Model was used to assess patients' beliefs and management behaviors. Ethical clearance was obtained from the Research and Ethics committee of LUTH and patients consent sought prior commencement of the study. The data obtained was analyzed using SPSS version 20.0 using descriptive and inferential statistics.
Result: A total ninety patients (52 males & 38 females) were interviewed. The level of knowledge of hypertension among the respondents was above average, 57.8%(52 of 90) had good knowledge while 42.2% (38 of 90) had poor knowledge. Majority of the respondents had poor management behaviors (65.6%, 59 of 90). This could be attributed to poor cues to action (56.7%, 51 of 90) and high positive response (70%, 63 of 90) to perceived barriers in adopting lifestyle modification and medication adherence. However, high positive response was recorded for perceived susceptibility (74.4%, 67 of 90), severity (88.9%, 80 of 90), benefits (93.3%, 84 of 90), and self-efficacy (75.6%, 68 of 90).
Conclusion: The study shows that high knowledge of hypertension as seen among the respondents did not translate into good management behavior which could be as a result of their beliefs or poor response to cues to action.

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P108

Standardised and Interdisciplinary Management of Hypertensive Urgency Improve Patients Medicalcare and Follow Up

PrimaryAuthor.AuthorBlock:**Mariano Duarte**, Javier Coyle, Analia Aquieri, Claudio Yaryour, Hosp de Clinicas Facultad de Medicina UBA, Buenos Aires, Argentina; Eleonora Savio Galimberti, Lankenau Inst for Medical Res, Philadelphia, PA

Acute severe hypertension (ASH) includes low risk to life threatening events and is defined as sustained blood pressure (BP) > 180/100 mmHg. There are 2 types of ASH: hypertensive emergency (HE), and hypertensive urgency (HU). The difference is the presence (HE) or absence (HU) of acute target organ damage (TOD). Unassisted HU can evolve to HE. More than 80 % of ASH was HU with inadequate managment. The aim of the present study was to standardize the HU management based on interdisciplinary team work and to differenciate low from high risk HU. We studied 193 patients with ASH (64±12,6 years old, 117 women and 76 men). HU evaluation consisted of physical exam, ECG, chest X-Ray, full blood and urine work, retinal exam. Patient education and patient's follow-up inmediately after acute presentation were also included. We identified 2 HU populations: High-risk HU (previous hypertensive patients or with chronic TOD), and low-risk HU (no TOD). The latter would rest for 1 h until BP <160/100 mmHg. High-risk HU typically present 4th sound, ventricular hypertrophy, creatinine>1.5 mg/dL or "arterial-venous" crosses in retinal exam, and will receive pharmacological treatment (orally labetalol 200 mg). We monitored patients for 2 h after the drug was administered, and if BP <160/100, patients were sent home, to keep rest, follow a low-sodium diet and be reevaluated 24 h later. If BP >160/100 mmHg patients will receive a 2nd dose of labetalol. The study revealed that 41 of 193 pacients (22%) were not diagnosed with high BP before, and chronic TOD was identified in 19 of them. From 152 hypertensive patients, only 31 (20%) were adequately managed and treated. The most frequent cause that triggered HU was the dietary transgression (excessive salt ingestion). In conclusion, standardized assessment and management of HU revealed that a high percentage of patients with high BP lack adequate diagnosis and/or management, and end up developing HU. Implementation of resting allowed us to achieve the goal of BP<160/100 in low-risk HU patients. High-risk HU patients were best and safe treated with orally labetalol. The new guideline also secured a medical follow-up of all HU patients, decreasing their fall-off of the medical system (<10%) and improving their long-term medical management.

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P109

Bridging the Gap: Comparison of Hypertension Management Between Internal Medicine Residency and Faculty Practices

PrimaryAuthor.AuthorBlock:**Karandeep Bumrah**, Naveed Jan, Taranjit Gill, Aishwarya Kuchikulla, Marijeta Pekez, Courtney Fay, Kshitij

Thakur, Crozer Keystone Health System, Upland, PA

Introduction: Studies have shown significant racial and geographical disparities in the management of hypertension. Not much has been studied about differences of hypertension management in a residency practice **Aim**: Our study aims to compare hypertension management between an Internal Medicine residency and faculty practice. **Methods**: We retrospectively collected data from office electronic medical record. We included all patients with diagnosis of hypertension(ICD10: I10), between the age 45-60 years, seen between August 1, 2015 and August 1, 2016. Our exclusion criteria were patients with age <45 years or > 60 years, diabetes mellitus, and pregnant patients. A total of 600 charts were reviewed. Eighty one patients were excluded. We collected the following variable; age, gender race, number of office visits in one year, total number of medications used, smoking status, BMI and rate of physician blood pressure checks. The resident group had 259 patients and faculty group had 260 patients **Results:** There was no significant difference in blood pressure control between two practices. The age and gender distribution between the two practices was similar but there was a significant difference in racial distribution. Residents saw more African American patients as compared to faculty. There were more smokers seen in resident practice. The rate of repeat physician blood pressure checks after medical assistant was similar. The two practices were in different parts of Delaware County with a significant difference in uninsured rate. The Residency practice was in a city with uninsured rate of 16.3% compared to 2.0% for the faculty practice **Conclusion**: The study showed that there is no significant difference in the hypertension management between resident and faculty practices despite significant racial, financial and insurance coverage disparities.

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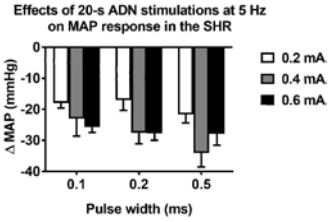
P110

Low Intensity Stimulation of Aortic Baroreceptors as a Potential Therapeutic Alternative for Hypertension Treatment

PrimaryAuthor.AuthorBlock:**Ibrahim M Salman**, Omar Z Ameer, Case Western Reserve Univ, Cleveland, OH; Arun Sridhar, Galvani Bioelectronics, Middlesex, United Kingdom; Stephen J Lewis, Yee-Hsee Hsieh, Case Western Reserve Univ, Cleveland, OH

Carotid baroreceptor stimulation has been clinically explored for antihypertensive benefits but aortic baroreceptor modulation remains untouched for clinical translation. Published studies use large current/voltage amplitudes (10V), longer pulse widths (2 ms) or ultra-high frequencies (70-100 Hz). These are energy inefficient neuromodulation methods. Our main goal was to identity optimal nerve stimulation parameters that would provide a sustained drop in mean arterial pressure (MAP) of ~30 mmHg. We stimulated aortic depressor nerve (ADN) in spontaneously hypertensive rats (SHR, *n*=4) at low ranges of frequencies, pulse amplitudes and pulse widths. Under urethane anesthesia, left ADN was stimulated for 20 seconds at varying frequencies (1, 2.5 and 5 Hz) and pulse amplitudes (0.2, 0.4 and 0.6 mA) at 0.1, 0.2 or 0.5 ms pulse width. A frequency-dependent depressor response was seen at all pulse amplitudes and widths used. Lower pulse amplitudes (0.2 mA) produced ~18 mmHg MAP drop at all pulse widths tested. Higher pulse amplitude (0.4 and 0.6 mA) produced similar

drop in MAP of ~34 mmHg. The return to baseline values was relatively prolonged with higher charge injection with 32 seconds at 0.2 mA (0.1 or 0.2 ms at 5 Hz, maximum effect size: -18 mmHg) versus 42 seconds at 0.4 or 0.6 mA (0.1, 0.2 or 0.5 ms at 5 Hz, maximum effect size: -34 mmHg). There was no added benefit of pulse amplitudes beyond 0.4 mA. We conclude that low intensity stimulation is an effective alternate way to neuromodulation of the ADN. This will enable low energy consumption for neuromodulation. Future studies will study impact of these parameters in conscious SHR rats.



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P111

Target Organ Damage in Hypertensive Patients: A Comparison Using 24h-ABPM

PrimaryAuthor.AuthorBlock:**Nelson Dinamarco**, UESC, Ilheus, Brazil; Mateus Kist Ibiapino, UESC, Ilhéus, Brazil; Bernardo Pires Sampaio, Gabriela Ticle Monteiro, Barbara Espósito Carvalho, Vanessa Brito Couto, Marileide Dinamarco, Frida Liana Plavnik, UESC, Ilheus, Brazil

Introduction: BP assessed by ABPM is better related to TOD than office measurement. Evaluate TOD patients presented at a Hypertension Lab for first screening.
Methods: 353 hypertensive (188 Female, aged 19-89) by 24h-ABPM (SpaceLabs 90307), lab. tests, LVMIU by Echo (Terason, M3000), Divided in two groups: Controlled [C] by 24h-BP (<130x80 mmHg) and Not controlled [NC] (>130/80 mmHg), albuminuria (ALB).
Results: Table shows no difference detected in lab panel, except Glu, Trygl, ALB and LVMI.
Discussion: Expected Higher, Glu, trygl, ALB levels and LVMI, in NC group, with significant statistical differences comparing C group. Expected because the high blood pressure are the trigger to TOD.
Conclusion: Screening with 24-h ABPM is a valuable tool to hypertensives patients and should be used more frequently to prevent TOD progression.

Table - Demography, lab plane, blood pressure by 24h-ABPM and clinical features of Controlled and Not Controlled hypertensive patients.

Variable	Group C (N=176)	Group NC (N=177)	P-value
Age, years mean (SD)	51.4±15.8	52.6±14.5	NS
Ethnicity, n (%)			
White	27 (15.3%)	27 (15.3%)	NS
Black	148 (84.1%)	150 (84.7%)	
Asian	1 (0.6%)	-	
24-h ABPM			
SBP (mmHg)	115.8±12.1	138.7±13.1	<0.0001
DBP (mmHg)	69.6 ±9.9	84.5±8.6	<0.0001
LVMI, mean (SD)(g/m²)	84.7 ±23.0	96.9±30.5	<0.0001
Lab panel			
Fasting plasma glucose mg/dl	93.0±25.3	102.1±42.1	<0.05
Uric acid, mg/dl	4.84±2.4	5.14±1.5	NS
Creatinine, mg/dl	0.90±1.6	0.83±0.2	NS
Total cholesterol, mg/dl	188.0±42.6	197±48.2	NS
HDL-cholesterol , mg/dl	47.2±13.5	46.4±14.9	NS
Tryglicerides, mg/dl	146.5±77.9	187.2±132.1	<0.01
Albuminuria, mg/24h	8.45±13.1	13.36±29.0	<0,0001

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P112

Bmal1 in Perivascular Adipose Tissues Regulates Blood Pressure Rhythmicity via Angiotensinogen

PrimaryAuthor.AuthorBlock:**Lin Chang**, Wenhao Xiong, Xiangjie Zhao, Yanbo Fan, Yanhong Guo, Minerva Garcia-Barria, Jifeng Zhang, Zhisheng Jiang, Yuqing Chen, Univ of Michigan, ann arbor, MI

Background:the perivascular adipose tissue (PVAT), surrounding vessel walls, constitutes a distinct functional integral layer of the vascular wall. Presence of a functional PVAT is beneficial for the regulation of vascular tone under physiological conditions while dysfunctional PVAT may promote damage of the vessel wall and development of cardiovascular diseases. However, there is little mechanistic information regarding the relationship between PVAT and blood pressure regulation. **Methods**:We investigated the vasoactivity of mouse vessel rings in response to homogenized PVAT and how Bmal1 and angiotensinogen in PVAT regulate the rhythmicity of blood pressure in mice. **Results:**Our results demonstrate that loss of Bmal1 in PVAT reduces blood pressure of mice during the rest phase. PVAT extracts significantly induce contractility of isolated blood vessel rings *in vit*ro in an endothelium independent manner, and the PVAT extracts from brown adipocyte selective Bmal1 deficient mice result in reduced vessel contractility. The capability of PVAT to stimulate vessel constriction is mediated by angiotensin II (Ang II) signaling present in PVAT evidenced by angiotensinogen being highly expressed in PVAT and the blockage of angiotensin II type 1 receptors in the isolated vessels significantly repressing the pro-contractile effect of PVAT extracts. Consistently, the angiotensinogen mRNA and Ang II levels in PVAT of Bmal1 deficient mice are significantly reduced. Deletion of angiotensinogen in PVAT results in reduced blood pressure in the rest phase as well, and vessel contractility of the PVAT extracts from angiotensinogen deficient mice is significantly reduced. Furthermore, Ang II infusion reduces Bmal1 expression in PVAT. Deletion of angiotensinogen in PVAT decreases expression and phosphorylation of casein kinase -2 alpha and -2 beta (CK2α and CD2β), in turn, enhancing Bmal1 phosphorylation. **Conclusions:**these data indicate that local Bmal1 in PVAT regulates angiotensinogen expression and secrets Ang II that acts on smooth muscle cells (SMCs) in the vessel walls to regulate vasoactivity and blood pressure.

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P113

Pairing the Mobile Device: Does the Engagement of Student Health Coaches Help Patients Use Smart-phone Enabled Devices for Blood Pressure Monitoring?

PrimaryAuthor.AuthorBlock:**Elena Vasti**, Mark Pletcher, Madelaine Faulkner, UCSF Sch of Med, San Francisco, CA

Research Question: Does pairing of health coaches with smartphone-enabled blood pressure cuffs improve hypertension control and self-monitoring in a medically underserved area (MUA)?
Introduction/Context: This pilot study aims to assess whether infusing tech-savvy youths trained as health technology coaches would (1) be a feasible approach in an MUA and (2) improve the frequency of self-monitoring, patient satisfaction, and blood pressure control of Stockton residents.
Methods: Patients with hypertension were randomly assigned to 1 of 3 intervention arms. The “Cuff Alone" (CA) group was provided a QardioArm cuff only, and was encouraged to use the cuff at their convenience. The “Student Alone" (SA) group was instructed to meet for 30 minutes once a week for 5 weeks with a student health coach who had completed a 3-day health coach training. The third group “Student Plus Cuff" (SPC) group received both a QardioArm

cuff and student coach. Student coaches and patients were surveyed about their experience in the project.
Results: Participants (n=27) were randomly assigned (9:9:9). All 15 students completed training and 6/15 students (40%) completed all 5 meetings with their assigned patient. Barriers to feasibility included transportation and patient response drop-off at the end of the study. There was a statistically significant difference in frequency of cuff use (SPC vs CA groups, 37v17, $p<0.01$). The initial blood pressures for SPC, SA and CA groups were 139/78, 142/84, and 149/85 mmHg, respectively. Final BP readings were 128/69, 150/85, and 145/85 mmHg, respectively. The SPC group was the only group that reached adequate BP control, though it was not statistically significant ($p=0.89$). 77% of the SPC group rated the project overall as excellent, compared to CA and SA (77% vs 55% vs 44%). 78% of SPC participants reported their blood pressure to be well controlled most days of the week, compared to CA (32%) and SA (22%).
Conclusions: This pilot trial demonstrated feasibility of pairing technology with that young student coaches, though challenges existed. The SPC group used their cuff more than twice the frequency of the CA group. Patients were more engaged in the SPC group, resulting in better blood pressure control by both self-reported and objective measures.

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P114

White Coat Effect is Common Among Stroke Survivors

PrimaryAuthor.AuthorBlock:**Adriana Morell**, Munachi Okpala, Sean Savitz, Anjail Z Sharrief, McGovern Medical Sch, Houston, TX

Background and Purpose: Among stroke survivors, uncontrolled hypertension is a major risk factor for recurrent stroke. Blood pressure (BP) medication titration often relies on office BP measures, which may be inaccurate due to the white coat effect (WCE). We sought to determine the prevalence of the WCE in stroke survivors and to determine whether clinical and demographic factors were associated with WCE. Methods: We followed ischemic and hemorrhagic stroke and transient ischemic attack patients with prior hypertension presenting to our stroke clinic for a BP study. Sitting BP was obtained by a medical assistant using an office automated BP machine (OABP). Patients also underwent BP measurement using BPtru, an automated machine that measures and averages five BPs with the patient alone in a room. BPtru approximate BPs obtained by the gold standard ambulatory blood pressure machine. Systolic BP (SBP) obtained by BPtru was subtracted from that obtained by OABP. WCE was defined as SBP difference ≥ 10mmHg. Uncontrolled BP was defined as SBP ≥ 135 mmHg by BPtru or ≥ 140 mmHg by OABP. We used student t-tests (continuous) and chi-squared or Fischer's exact tests (categorical) for univariate analyses. Results: Of 94 patients, mean age was 60 (SD 12), 60.6% were male, 26.6% were Non-Hispanic White, 46.8% were Black, and 23.4% were Hispanic. Systolic OABP was 13.2 mmHg (SD 19.3) higher than BPtru SBP (student t-test; $p <0.001$). WCE was present in 58.5 % of participants and BP was misclassified as uncontrolled in 21.2%. In univariate analyses, age ($p = 0.14$), sex ($p = 0.78$), race (0.07), stroke type (0.92), body mass index ($p = 0.65$), and tobacco use ($p = 0.35$) were not significantly associated with presence of WCE. The presence of normal SBP by OABP was associated with a decreased likelihood of WCE ($p = 0.006$).

Conclusions: Among hypertensive stroke patients following in a clinic, WCE was highly prevalent and one-fifth of patients were misclassified as uncontrolled. Neither race nor other previously described predictors of WCE were associated with WCE in this study. Our findings suggest that in patients with elevated office BP, findings should be confirmed with an automated machine like BPtru in order to avoid over-titration of medication or incorrect assessment of BP control.

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P115

Increased Diastolic Blood Pressure Post-mild Exercise is Associated With Early Structural and Functional Cardiovascular Abnormalities

PrimaryAuthor.AuthorBlock:**Susan Tucker**, Mahfouz El Shahawy, Cardiovascular Ctr of Sarasota, Sarasota, FL

Background: Previous research has suggested that an increase in SBP post-mild exercise is correlated with early structural and functional cardiovascular abnormalities. **Purpose:** To determine if an increase in diastolic blood pressure post-mild exercise (DBP PME) is associated with early structural and functional cardiovascular abnormalities. **Methods:** 1416 untreated, asymptomatic subjects were screened for early indicators of cardiovascular disease using the Early CVD Risk Score (ECVDRS), also known as Rasmussen Risk Score (RRS), which consists of a panel of 10 tests; large (C1) and small (C2) artery stiffness, resting BP and post mild exercise (PME), CIMT, abdominal aorta and left ventricle ultrasounds, retinal photography, microalbumuria, ECG, and pro-BNP. 267 subjects were normotensive. Of those subjects, 12 had a increase in DBP PME, 23 had no change in DBP PME, and 232 had a decline in DBP PME. Focus was placed on the three known tests recommended for early CVD assessment; C1, C2 and CIMT. **Results:** As seen in Figure 1.0, a rise in diastolic blood pressure PME is statistically evident for an increased risk of early structural and functional cardiovascular abnormalities. A significant increase in abnormalities was noted with C2 and CIMT with subjects whose DBP increased PME. **Conclusion:** Assessment of diastolic blood pressure PME is an easy, noninvasive, inexpensive test that can be performed by any health care practitioner to evaluate the risk of CVD in patients. Any increase in diastolic blood pressure PME should warrant physicians on the urgency to further investigate and treat patients to divert the progression of CVD.

Figure 1.0

	Increased DBP PME	No Change DBP PME	Decline DBP PME
Total Number of Subjects	12	23	232
Number of Males	6	11	108
Number of Females	6	12	124
Average Age	67	64	59
Average BMI	28.1	27.1	26.4
Average RRS	8	4	3
Abnormal C1	50%	34%	7%
Abnormal C2	60%	39%	17%
Abnormal CIMT	60%	40%	26%
Average Resting SBP	133	118	123
Average Resting DBP	76	71	75
Average PME SBP	150	137	147
Average PME DBP	83	71	64

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P116

Postpartum Catastrophe With Intracerebral Hemorrhage and Concurrent Cardiomyopathy

PrimaryAuthor.AuthorBlock:**Syed Rafay Ali Sabzwari**, Yan Lin, Shengchuan Dai, Florida Hosp, Orlando, FL

Introduction Postpartum Cerebral Angiopathy (PCA) or Reversible Cerebral Vasoconstriction Syndrome (RCVS) is a rare but important postpartum syndrome characterized by severe headaches with complications including seizures and intracranial hemorrhages. Peripartum cardiomyopathy (PPCM) is a known non-ischemic cardiomyopathy occurring mostly in the immediate postpartum period. This case is one of its kind to show the unique combination of PPCM and PCA. **Case Report** 32 years old Hispanic female with no past medical history presented with worsening headache since epidural anesthesia used for her uneventful spontaneous vaginal delivery six days ago. While in ED she suddenly developed left hemiplegia and an immediate CT head showed right basal ganglia intracerebral hemorrhage causing 10mm right to left midline shift. An emergent frontotemporal craniotomy and evacuation of hemorrhage was done. A cerebral angiogram the next day showed diffuse cerebral arterial vasoconstriction responding to intra-arterial verapamil suggestive of RCVS. An echocardiogram was completely normal and vasculitis workup was negative. Patient was started on nimodipine however she became hypotensive requiring pressors. A week later repeat angiography showed persistent vasospasm and a repeat echocardiogram showed dilated left and right ventricles with global impairment in systolic function and an EF of 15-20%. The subsequent day there was a change in her neurological status where she lost all brain and brainstem reflexes, pupils got fixed and dilated. CT brain showed global edema and tonsillar herniation. Apnea test was done which was consistent with the diagnosis of brain death. **Discussion** The pathophysiologic mechanism of RCVS is believed to be related to altered cerebral auto regulation in response to endothelial injury that usually resolves in 3 months. The diagnosis can be made by cerebral angiography, MRA or CTA that shows multifocal segmental arterial constrictive lesions. It is likely that a combination of diffuse persistent cerebral vasospasm accompanied by poor cardiac function that culminated in eventual brain death of this healthy, postpartum patient. Management includes calcium channel blockers, sequential cerebral angiograms and supportive care.

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P117

Hydralazine Prevents the Rupture of Experimental Cerebral Aneurysms in Hypertensive Rats

PrimaryAuthor.AuthorBlock:**Takeshi Miyamoto**, Keiko T Kitazato, Yoshiteru Tada, Kenji Shimada, Kenji Yagi, Masaaki Korai, Hidetsugu Maekawa, Tadashi Yamaguchi, Yasuhisa Kanematsu, Junichiro Satomi, Tokushima Univ Graduate Sch, Tokushima, Japan; Tomoki Hashimoto, Univ of California, San Francisco, San Francisco, CA; Shinji Nagahiro, Tokushima Univ Graduate Sch, Tokushima, Japan

Introduction. Subarachnoid hemorrhage (SAH) is a catastrophic event with high morbidity and a poor prognosis. While epidemiological studies showed that hypertension is a risk factor for aneurysmal rupture, it remains unclear whether

lowering the blood pressure (BP) prevents the rupture of aneurysms. Under the hypothesis that lowering the BP prevents the rupture of experimental cerebral aneurysms, we investigated whether hydralazine reduces the incidence and rupture of aneurysms in our rat model. **Methods**. In 10-week-old female Sprague-Dawley rats (n=34) we elicited estrogen deficiency, renal hypertension, and hemodynamic stress. Two weeks later, they were divided into 2 groups; group 1 (n=17) was treated perorally with hydralazine (100 mg/kg/day), group 2 (n=17) was the vehicle control. We recorded their death or abnormal behavior in the course of 90 days and inspected ruptured aneurysms. **Results**. At 2 weeks, both groups manifested an increase in the systolic BP (SBP) (group 1, 205 mmHg; group 2, 207 mmHg). After 30-, 60-, and 90-days, the SBP was lower in group 1 than group 2 (150 vs 210 mmHg, 163 vs 211 mmHg, and 173 vs 210, mmHg, respectively). In the course of 90 days, 9 group 2 (53%) and 8 group 1 rats (47%) developed cerebral aneurysms. The rupture rate was lower in group 1 (3/8, 38%) than group 2 rats (9/9: 100%). While hydralazine did not prevent the development of aneurysms, it prevented their rupture. qRT-PCR performed on day 35 showed the down-regulation of MCP-1 and MMP-9 in the cerebral vascular wall of group 1 rats. **Conclusion**. Our findings suggest that lowering the SBP may prevent the rupture of cerebral aneurysms via the down-regulation of MCP-1 and MMP-9 in the cerebral vascular wall.

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P118

Inhibition of Endoplasmic Reticulum Stress Prevents Intracranial Aneurysmal Rupture in a Mouse Model

PrimaryAuthor.AuthorBlock:**Daisuke Kudo**, Hajime Furukawa, Univ of California San Francisco, San Francisco, CA; Satoru Eguchi, Temple Univ, Philadelphia, PA; Tomoki Hashimoto, Univ of California San Francisco, San Francisco, CA

[Background] Aneurysmal subarachnoid hemorrhage (SAH) can cause significant mortality and morbidity. To develop a therapy for prevention of intracranial aneurysmal rupture and subsequent SAH, it is important to clarify the mechanism of intracranial aneurysmal rupture. Stimulation of the renin-angiotensin system (RAS) causes hypertension and cardiovascular remodeling. Recent evidence shows that angiotensin II enhances endoplasmic reticulum (ER) stress and inhibition of ER stress prevents angiotensin II-induced vascular remodeling but not hypertension in mice. RAS has also been implicated in intracranial aneurysms. We have previously shown that angiotensin II receptor blocker (losartan) prevented intracranial aneurysmal rupture in a mouse model without affecting systemic hypertension. To clarify the mechanism of intracranial aneurysmal rupture via RAS, we have tested our hypothesis that inhibition of ER stress prevents intracranial aneurysmal rupture in a mouse model.
[Method] We used a mouse model of

intracranial aneurysms in which spontaneous aneurysmal rupture causes neurologic symptoms. Intracranial aneurysms were induced in wild type mice by a single stereotactic injection of elastase (35mU) into the cerebrospinal fluid at right basal cistern and deoxycorticosterone (DOCA)-salt hypertension. Vehicle or 4-phenylbutyric acid (PBA, ER stress inhibitor, 100mg/kg/day) was subcutaneously injected into all mice once a day. To detect aneurysmal rupture, we performed daily neurological examinations. Symptomatic mice were euthanized immediately when they developed neurological symptoms, and all asymptomatic mice were euthanized 21 days after aneurysm induction. The incidence of aneurysms and rupture rate were compared between vehicle group and PBA group. **[Results]**The incidence of aneurysms was not significantly different between two groups (100% in vehicle, 20 of 20 vs. 87% in PBA, 20 of 23, p=0.09). However, rupture rate was significantly lower in the PBA group (60%, 12 of 20) than the vehicle group (95%, 19 of 20). (p=0.008). **[Conclusion]** Inhibition of ER stress reduced aneurysmal rupture in a mouse model of intracranial aneurysm induced by combination of elastase injection and DOCA-salt hypertension.

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P119

Hypertension Trends in Florida Stroke Patients. The Florida Puerto Rico Collaboration to Reduce Stroke Disparities

PrimaryAuthor.AuthorBlock:**Marialaura Simonetto**, Univ of Miami, Miami, FL; Nirav H. Shah, Swedish Neuroscience Inst, Seattle, WA; Kefeng Wang, Maria A. Ciliberti-Vargas, Carolina Gutierrez, Chuanhui Dong, Univ of Miami, Miami, FL; Jorge Sanchez, Oasis Pavillon, Phoenix, AZ; Dianne Foster, AHA, Marietta, GA; Jose' G. Romano, Ralp L. Sacco, Tatjana Rundek, Univ of Miami, Miami, FL

AIM: To describe hypertension prevalence (HTN) in a race diverse stroke registry and investigate associated presentation in patients admitted for acute ischemic stroke (AIS) and hemorrhagic stroke in Florida hospitals participating in the Florida Collaboration to Reduce Stroke Disparities (CReSD). **BACKGROUND:** HTN affects about 30% of U.S. adults and this prevalence doubles among stroke survivors. HTN is a major risk factor for incident stoke and recurrent stroke. Quantifying the prevalence of HTN in stroke survivors is important to guide secondary stroke prevention. **METHODS**: 121,333 stroke cases were analyzed from 69 FL hospitals participating in the AHA Get With the Guidelines-Stroke Program and FL CReSD Stroke Registry. Hypertension was defined as systolic blood pressure >140mmHg. Demographics and CV risk factors were collected at admission. We investigated the differences in HTN prevalence between race groups: white (65%), black (20%) and Hispanic (15%) as well as between age groups: 18-60, 61-80 and >80 years old. Temporal trends of HTN prevalence were also analyzed from 2010 to 2016. **RESULTS:** In our stroke population, mean age was 70±15 and 60,667 were women (50%). HTN prevalence was 65% (78,553/121,333). Patients with HTN were significantly older (mean age 72±14 vs. 67±16 in non-HTN patients) and presented significantly higher prevalence of other CV risk factors such as diabetes, hyperlipidemia and had more previous stroke/TIAs. Women had greater prevalence of HTN (66% vs. 64% in males) in all age groups. Greater HTN prevalence was in blacks (70%) compared to

white (64%) and Hispanics (58%) in all age groups. Interestingly, a significantly higher prevalence of HTN was found in AIS compared to hemorrhagic stroke but a higher SBP was observed in hemorrhagic strokes compared to AIS. Prevalence of HTN decreased of 8% from 2010 to 2016. Largest decline was observed among women (9%) and blacks (13%).
CONCLUSIONS: In our large Stroke Registry we observed higher prevalence of HTN in women, blacks and AIS. We also observe a decreasing trend over the past 7 years, especially among women and minorities (blacks and Hispanics). These findings provide an opportunity to design and implement interventions to reduce disparities in HTN and improve stroke outcome.

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P120

Down Regulation of Add3 in Astrocytes Induces Pro-inflammatory and Fibrotic Factors and May Contribute to Alzheimer'S-related Dementias

PrimaryAuthor.AuthorBlock:Paige N Mims, Sheshank Shekhar, Shaoxun Wang, Olivia Travis, **Fan Fan**, Univ of Mississippi Medical Ctr, Jackson, MS

We genetically mapped a mutation in Add3 in FHH rats that decreases Add3 expression in various tissues, including brain and cerebral vessels, and further decreases with age in comparison with FHH.1^{BN} congenic rats in which Chr. 1 from BN rats containing 15 genes, including Add3, was transferred onto FHH genetic background. More recently, we demonstrated that Add3 dysfunction contributes to cerebral vascular impairments in FHH rats, and they exhibit BBB leakage and neurodegeneration after the development of hypertension. In addition, Aβ protein expression in the brain in FHH rats is increased as early as 8 weeks of age. Reactive astrogliosis is a common finding in neurodegenerative diseases and activated astrocytes promote neurodegeneration. Moreover, astrocyte dysfunction affects Aβ clearance and Aβ accumulation is a well-defined feature of Alzheimer's disease. To further explore whether astrocytes contribute to neurodegeneration and cognitive impairments in FHH rats, we knocked down Add3 expression using Add3 Dicer-substrate RNAi (DsiRNA) in human astrocytes and found that GFAP and IL-6 expression was markedly enhanced in comparison to scramble siRNA treated cells using a q-PCR array. Moreover, the expression of TGF-β2 and TGF-β3 was elevated by 2.0 ± 2.9 and 1.8 ± 1.8 folds, respectively, in Add3 DsiRNA treated astrocytes. In addition, the actin cytoskeleton was disrupted in Add3 DsiRNA treated astrocytes. We found that FHH rats exhibit a reduction in neuronal density and neuronal size in the hippocampus following the induction of hypertension with DOCA/salt. This was

associated with loss of capillary density (22.7 ± 0.001% in FHH and 9.6 ± 0.014% in FHH.1^{BN}, n = 7 and 9, respectively) and greater expression of GFAP positive astrocytes and loss of neurons in affected areas. Hypertensive FHH rats took 2.5 times longer to escape from an eight-arm water maze as compared to control strains. These results suggest that down regulation of Add3 in astrocytes disrupts the actin cytoskeleton to induce reactive astrogliosis and release of pro-inflammatory and fibrotic factors, which promotes inflammation
and disrupts Aβ uptake, all of which may contribute to neurodegeneration and cognitive deficit associated with aging and hypertension in FHH rats.

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P121

Dipper Pattern and Albuminuria in Controlled and Uncontrolled Hypertensive Patients

PrimaryAuthor.AuthorBlock:**Nelson Dinamarco**, Mateus Kist Ibiapino, Gabriela Ticle Monteiro, Barbara Esposito Carvalho, Bernardo Pires Sampaio, Vanessa Brito Carvalho, Marileide Dinamarco, Frida Liana Plavink, UESC, Iheus, Brazil

BP assessed by ABPM is better related to TOD than office measurement. Evaluate TOD patients presented at a Hypertension Lab for first screening.
Methods: 278 hypertensive (147 Female, aged 19-89) by 24h-ABPM (SpaceLabs 90307), lab. tests, LVMI by Echo (Terason M3000); Divided in two groups: Controlled [C] by 24h-BP (<130x80 mmHg) and Not controlled [NC] (>130/80 mmHg), albuminuria (ALB) was log transformed in order to allow proper analysis. Dipping pattern 24h-ABPM: dipper (DP) (>10-20%), nondipper-absente (NDP) (<10%), reverse dipper (RDP)(> 20%).
Results: Table 1 and 2 : Demography and ALB,LVMI. No differences detected in lab panel, except Glu, Trygl, ALB and LVMI.
Discussion: Expected Higher LVMI, Glu, trygl, ALB levels, in NC group, with significant statistical differences comparing C group. Expected reverse dipping pattern would show differences when compared with dipper pattern but probably the small number of subjects didn't allow detect such differences.
Conclusion: Screening with 24-h ABPM is a valuable tool to hypertensives and dipper pattern should be achived to prevent TOD progression.

Table 1- Demography, blood pressure by 24h-ABPM and clinical features of Controlled and Not Controlled hypertensive patients.

Variable	Group C (N=169)	Group NC (N=109)	P-value
Age, years mean (SD)	51.4±15.8	50.5±13.3	NS
Ethnicity, n (%)			
White	27 (15,9%)	13 (11,9%)	NS
Black	141 (83,4%)	96 (88,1%)	
Asian	1 (0,6%)	-	
24-h ABPM			
SBP (mmHg)	116.3±11.1	143.3±13.2	<0.0001
DBP (mmHg)	70.4±6.3	88.8±7.1	<0.0001
Dipper Pattern, n (%)			
Dipper	84 (49.7)	45 (41.3)	NS
Non-dipper/absent	67 (39.6)	50 (45.9)	
Reverse dipper	17 (10.0)	14 (12.8)	
LVMI, mean (SD)	84.2 ±22.7	94.8±29.9	0.0018
Lab panel			
Fasting plasma glucose mg/dl	92.6±25.3	101.9±44.2	<0.0001
Uric acid, mg/dl	4.80±2.4	5.30±1.6	NS
Creatinine, mg/dl	0.90±1.6	0.85±0.2	NS
Total cholesterol, mg/dl	187.9±43.3	196.4±50.2	NS
HDL-cholesterol , mg/dl	47.5±13.7	45.4±14.3	NS
Tryglicerides, mg/dl	144.8±77.9	202.2±153	0,0005
Albuminuria (Ln)	1.62 (0.93)	2.04 (1.12)	<0,0001

Table 2- Albuminuria and LVMI according to 24h-ABPM blood pressure control efficacy

	Albuminuria (Ln)			LVMI (g/m²)		
	Dipper	Nondipper	Reverse dipper	Dipper	Nondipper	Reverse dipper
C	1.56 (0.87)	1.61(0.88)	1.94(1.27)	79.7±20.1	87.0±21.1	95.5±36.0**
NC	2.06 (1.15)	1.94(1.27)	2.1 (1.14)	97.0±25.6*	95.3±34.8	86±23.3

C= 24h-ABPM <130x80mmHg; NC= 24h-ABPM >130x80mmHg *p<0.0001 **p<0.05

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P122

Plasma Circulating microRNAs as Potential Biomarkers in Chronic Kidney Disease

PrimaryAuthor.AuthorBlock:**Olga Berillo**, Ku-Geng Huo, Júlio C. Fraulob-Aquino, Lady Davis Inst for Medical Res, Montreal, QC, Canada; Chantal Richer, Res Ctr, CHU Ste-Justine, Montreal, QC, Canada; Asia Rehman, Lady Davis Inst for Medical Res, Montreal, QC, Canada; Marie Briet, INSERM U1083, CNRS UMR 6214, Ctr Hospo-Univire d'Angers, Univ d'Angers, Angers, France; Pierre Boutouyrie, Univ Paris-Descartes, INSERM U970 and Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Paris, France; Mark L. Lipman, Lady Davis Inst for Medical Res, Sir Mortimer B. Davis-Jewish General Hosp, McGill Univ, Montreal, QC, Canada; Daniel Sinnett, Res Ctr, CHU Ste-Justine, Univ de Montréal, Montreal, QC, Canada; Pierre Paradis, Lady Davis Inst for Medical Res, Montreal, QC, Canada; Ernesto L. Schiffrin, Lady Davis Inst for Medical Res, Sir Mortimer B. Davis-Jewish General Hosp, McGill Univ, Montreal, QC, Canada

Background: Chronic kidney disease (CKD) is a global health burden with a worldwide prevalence of 13.4% for stage 5 and 10.6% for stage 3-5. There is an epidemiological association between hypertension (HTN) and CKD. The prevalence of high blood pressure (BP) has been reported to be over 85% in stage 3 and over 90% in stage 4-5 CKD patients. Circulating cell-free small non-coding RNAs called microRNA (miRNA) have been shown to associate with different pathologies including cancer and cardiovascular disease, and accordingly possesses potential to serve as biomarkers with clinical application. We aimed to identify differentially expressed (DE) miRNAs that may be related to CKD.**Methods and results:** Normotensive, HTN (systolic BP > 135 mmHg or diastolic BP of 85-115 mmHg with BPtru) and CKD (estimated glomerular filtration rate (eGFR) < 60mL/min/m^2) subjects (n=15-16 per group) were studied. Platelet-free plasma was isolated by a 2-step centrifugation (1000xg followed by 10,000xg) from 6 ml total blood. Plasma miRNAs were extracted using the QIAamp Circulating Nucleic Acid Kit. The quantity and quality of RNA were assessed using an Agilent 2100 Bioanalyzer. cDNA libraries were prepared using the TruSeq Small RNA Library Prep Kit, and sequenced with the HiSeq 2500 platform. FastQC was used for quality control. Sequences were mapped by STAR to the hg38 genome and annotated by miRDeep2. DE miRNAs were identified using EdgeR, which found 6 up-regulated and 3 down-regulated miRNAs uniquely associated with the HTN group, 2 up-regulated and 12 down-regulated miRNAs uniquely associated with the CKD group and 3 down-regulated miRNAs in both groups (P<0.01 & q<0.1). Two down-regulated miRNAs in the HTN group, miR-26a-5p (r=-0.33, P<0.05) and miR-151a-5p (r=-0.33, P<0.05), were correlated with SBP. One up-regulated miRNA in CKD group, let-7g-5p (r=0.31, P<0.05), was correlated with eGFR. **Conclusions and perspectives:** DE platelet-free plasma miRNAs were identified in HTN and CKD patients. Some miRNAs may have the potential to serve as biomarkers in CKD.

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P123

Obesity and Alterations in Renal Function in Adolescents Born Preterm With Very Low Birth Weight

PrimaryAuthor.AuthorBlock:**Andrew Michael South**, Patricia A. Nixon, Mark C. Chappell, Debra I. Diz, Gregory B. Russell, Elizabeth T. Jensen, Hossam A. Shaltout, Lisa K. Washburn, Wake Forest Sch of Med, Winston Salem, NC

Background:
Survival of children born preterm has improved dramatically, but prematurity and low birth weight may increase the risk of renal disease. Children born preterm have a lower glomerular filtration rate (GFR) and higher blood pressure (BP) compared to term peers, but compounding risk factors for renal disease among children born preterm remain poorly characterized. Indeed, patient factors such as obesity may influence the development of kidney disease. Thus, we hypothesize that obesity is associated with decreased renal function in adolescents born preterm with very low birth weight.
Methods:
We measured systolic and diastolic BP, serum creatinine, and urine albumin at age 14 years in 124 adolescents born preterm with very low birth weight (mean birth weight 1056 g). We calculated the GFR by the Schwartz equation and the albumin-to-creatinine ratio (ACR) on morning urine samples. We used generalized linear models to estimate the association between obesity [body mass index (BMI) ≥95th %ile for age and sex, *n*=27] and renal function, adjusting for race, sex, maternal smoking during pregnancy, and birth weight z-score.
Results:
Obesity was associated with higher systolic BP (*p*=0.03). Compared to adolescents with BMI <95th %ile, those with obesity had lower GFR (*β*: -14.68 mL/min/1.73 m^2, 95% CI -26.8 to -2.55). Adjustment for covariates attenuated this relationship (*β*: -10.05 mL/min/1.73 m^2, -21.12 to 1.03). Adolescents with obesity had a higher serum creatinine, but this did not reach statistical significance (*β*: 0.05 mg/dL, -0.01 to 0.11). There was no difference in the ACR (*β*: -0.05, -0.13 to 0.02).
Conclusions:
Obese adolescents born preterm with very low birth weight exhibit higher systolic BP and lower GFR compared to those with BMI <95th %ile, though the statistical significance of the relationship with GFR weakened after adjustment for possible confounders. Importantly, sex was a significant confounder that may influence the relationship between GFR and obesity, as female adolescents had significantly lower GFR than male adolescents. While obesity should be avoided, other factors should be considered that may contribute to worse renal function in adolescents born preterm with very low birth weight.

Disclosures: **A.M. South:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH NICHD PO1 HD047584. **P.A. Nixon:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; NIH NICHD PO1 HD047584. **M.C. Chappell:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; NIH NICHD PO1 HD047584. **D.I. Diz:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; NIH NICHD PO1 HD047584. **G.B. Russell:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; NIH NICHD PO1 HD047584. **E.T. Jensen:** None. **H.A. Shaltout:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; NIH

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P124

Role of Add3 Dysfunction in Renal Epithelial Cells in the Development of Renal Injury in Fhh Rats

PrimaryAuthor.AuthorBlock:**Bibek Poudel**, Fan Fan, Shaoxun Wang, Richard J Roman, The Univ of Mississippi Medic, Jackson, MS

The Fawn Hooded Hypertensive Rat (FHH) is a genetic model of hypertension, in which Add3 has been identified as a candidate gene for renal injury but the mechanism is unknown. The present study examined the effects of knockdown of Add3 on rat renal epithelial (NRK) cells which are the model system for podocytes and proximal tubular cells. Knockdown of the expression of Add3 using a 27-mer Dicer-substrate RNAi (DsiRNA) decreased cell viability using an MTS assay by 50% in comparison to cells treated with vehicle or scrambled DSiRNA (Add3DsiRNA, OD 0.5±0.1; untreated cells 0.9±0.1; NC-1 0.9±0.1; n=8, P<0.001). F-actin immunostaining revealed that Add3 DSiRNA markedly disrupted the cytoskeleton, and the F-actin staining intensity/cell ratio was significantly reduced (untreated 543±47, n=552 cells; NC-1 592±39, n=566 cells; Add3DsiRNA 430±58, n=385 cells, P<0.05). The maximum mitochondrial respiratory rate was reduced in Add3 DsiRNA treated cells (untreated 444±42; NC-1 414±32; Add3 DsiRNA 275±16 pmole/min/µg, P<0.01). Proton leak was higher (untreated 42±3; NC-1 40±4; Add3 DsiRNA 63±5 pmole/min/µg, P<0.002) and oxygen consumption coupled to ATP production was decreased in Add3 DsiRNA treated cells (untreated 229±12, n=9; NC-1 200±12, n=8; Add3 DsiRNA 146±11 pmole/min/µg, n=9, P<0.001). Spare respiratory capacity, an index of cell fitness, was reduced by knockdown of Add3 (untreated 154±42, n=9: NC-1 143±32, n=8; Add3DsiRNA 33±16 pmole/min/µg, n=9, P<0.001). Non-mitochondrial oxygen consumption rate (OCR/µg protein) increased in cells treated with Add3 DsiRNA (untreated 57±7; NC-1 80±11; Add3DsiRNA 116±15 pmole/min/µg, P<0.01) suggesting a possible metabolic switch to glycolytic oxygen consumption. In conclusion, knockdown of Add3 in NRK cells disrupts the actin cytoskeleton, promotes mitochondrial dysfunction and decreases cell viability. Loss of Add3 function may contribute to proteinuria in FHH rats by altering the cytoskeleton and promoting effacement and loss of podocytes and the reuptake of filtered protein in the proximal tubule.

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P125

RNA Sequencing of Left-ventricular Tissue Highlights Novel Pathways Impacted by the

Loss of miR-146b-5p in a Rat Model of Chronic Kidney Disease

PrimaryAuthor.AuthorBlock:**Mark R. Paterson**, Pengyuan Liu, Alison J. Kriegel, Medical Coll of Wisconsin, Milwaukee, WI

Chronic kidney disease (CKD) mediates pathological changes in heart and vascular tissues, yet many molecular mechanisms by which renal injury directly affects cardiovascular pathology (CVD) are unknown. miRNAs are proposed to be powerful regulators of pathophysiological signaling networks; having the potential to radically change the landscape of mRNA transcript expression and protein abundance in the context of health and disease. We have profiled the expression of miRNAs in a rat model of CKD – the 5/6 nephrectomy (5/6Nx) – with the objective to identify miRNAs with strong effects on CKD-mediated CVD. One such miRNA, miR-146b-5p (miR-146b), is highly upregulated in 5/6Nx rats compared to sham-operated controls at 7-weeks post-5/6Nx in both the kidney (5.4 fold increase; $p<0.05$) and the heart (1.6 fold increase, $p<0.05$), a time-point at which we see maximal renal and cardiac pathology. To examine the effects of miR-146b upregulation in our model of CKD, we performed RNA sequencing on cardiac tissue at 7 weeks post-5/6Nx in both wild-type (WT) Sprague-Dawley (SD) rats and a unique CRISPR-Cas9-mediated miR-146b knockout strain (miR-146b$^{-/-}$) created on the SD background. Of 3,018 genes differentially regulated by more than 1.5-fold (absolute value) in miR-146b$^{-/-}$ vs. WT rats after 5/6Nx, 135 had significantly altered expression (adjusted p-value <0.05). We filtered all differentially expressed genes through Ingenuity Pathway Analysis to identify affected signaling networks in miR-146b$^{-/-}$ vs. WT rats that correlate with observed pathological phenotypes. The expression pattern of differentially regulated genes in these networks indicates predicted activation of pathways that contribute to tissue fibrosis and mineralization, reactive oxygen species signaling, and the inflammatory and immune responses. These pathways are enriched with predicted miR-146b target genes and correlate closely with observed phenotypes in miR-146b$^{-/-}$ 5/6Nx rats; including increased renal fibrosis and mineralization of cardiovascular tissue, and the well-known role of miR-146b in negative feedback regulation of NFκB signaling. These preliminary analyses uncover unique networks of genes regulated by miR-146b that may contribute to CKD-mediated CVD.

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P126

Regulation of Short Chain Fatty Acid Receptors in the Kidney by microRNAs is Influenced by Hydrogen Sulfide Supplementation

PrimaryAuthor.AuthorBlock:**Gregory Weber**, Sathnur Pushpakumar, Utpal Sen, Univ of Louisville, Louisville, KY

Hypertension affects nearly one third of the adult US population and is a significant risk factor for chronic kidney disease (CKD). An expanding body of recent studies indicates that gut microbiome has crucial roles in regulating physiological processes through, among other mechanisms, one mode of short chain fatty acids (SCFA) and their target receptors. In addition, these SCFA receptors are potential targets of regulation by host miRNAs, however, the mechanisms through which this occurs is not clearly defined. Hydrogen sulfide (H_2S) is an important gasotransmitter involved in multiple physiological processes and is known to

alleviate adverse effects of hypertension such as reducing inflammation in the kidney. To determine the role of host microRNAs in regulating short chain fatty acid receptors in the kidney as well as the gut, C57BL/6J wild-type mice were treated with or without Ang-II (1000 $ng/kg^{-1}/min^{-1}$) and H_2S donor GYY4137 (GYY) (133 $\mu M/kg^{-1}/d^{-1}$) for 4 weeks to assess whether GYY would normalize adverse effects observed in hypertensive mice and whether this was in part due to altered gut microbiome composition. We observed several changes of SCFA receptors and predicted microRNA regulators in the kidney among the different treatments. Haptoglobin, a marker for intestinal epithelial barrier integrity, was increased in hypertensive mice (97 ng/mL) compared to control (48 ng/mL), GYY (64 ng/mL) and Ang-II+GYY (71 ng/mL). The glomerular filtration rate (GFR) was improved in mice treated with Ang-II+GYY (963 $\mu l/min^{-1}/100$ g body wt^{-1}) compared with Ang-II (480 $\mu l/min^{-1}/100$ g body wt^{-1}) indicating improved kidney function. GYY supplemented mice had increased function (1219 $\mu l/min^{-1}/100$ g body wt^{-1}) compared to controls (981 $\mu l/min^{-1}/100$ g body wt^{-1}). The *Erysipelotrichia* class of bacteria, linked with altered SCFA production, was enriched in hypertensive animals but reduced with GYY supplementation. The TM7-3 phyla of bacteria are speculated to have anti-inflammatory properties and were found enriched in GYY only mice, supporting an anti-inflammation effect of H_2S. These data point towards a role for miRNA regulation in hypertension and are beneficially influenced by H_2S in both the kidney and potentially through changes in gut microflora.

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P127

Chromogranin A Pathway: From Pathogenic Molecule to Renal Disease

PrimaryAuthor.AuthorBlock:**Sucheta M Vaingankar**, Saiful A Mir, Nilima Biswas, Nicholas Webster, Daniel T. O'Connor, Univ of California, San Diego, La Jolla, CA

Introduction Chromogranin A (CHGA) is released into circulation with catecholamine from secretory granules of chromaffin cells and post-ganglionic sympathetic neurons. Several *CHGA* gene polymorphisms contributing to traits of autonomic blood pressure control, hypertension and hypertensive nephropathy have been identified. Plasma CHGA is elevated in hypertension and renal failure. This study seeks to understand the role of CHGA in the progression of CKD/ ESRD. **Hypothesis** Kidney injury results in elevated expression of CHGA which in turn affects biochemical and physiological traits that determine progression and development of CKD/ESRD. **Methods** The remnant kidney model was used to investigate the influence of CHGA on kidney function in response to injury. Distinction in response to kidney injury in wild-type (WT) and *Chga-/-* (KO) mice was evaluated by measuring glomerular function, fibrosis and microarray analysis. Mesangial cells were treated with CHGA to delineate pathway leading to inflammation and fibrosis. **Results** Nephrectomy (Npx) exacerbated azotemia in WT mice compared to their KO counterparts. WT-Npx mice displayed far excess fibrosis as equated with KO-Npx. Gene expression profiling revealed greater mitochondrial dysfunction due to Npx in WT mice. Mesangial cells in culture treated with CHGA, triggered NO release, by a signaling

mechanism involving SR-A. CHGA treatment augmented NO production by a 2-step mechanism involving upregulation of iNOS and the arginine transporter Slc7a7. CHGA-treated vs. untreated cells exhibited differential involvement of cytokine, chemokine, complement, acute phase pro-inflammatory markers and apoptotic pathway genes. Plasma creatinine and CHGA was measured in a twin population (N=740) and an inverse correlation between GFR and CHGA was observed. Therefore, in both mice and humans an inverse correlation suggested decrease in glomerular function with increasing plasma CHGA concentration. **Conclusions** Kidney injury leads to elevated levels of CHGA which serves as an insult to the mesangial cells resulting in reduction of mitochondrial efficiency, concerted transcription of genes involved in NO production, inflammation and fibrosis, progression of CKD and development of ESRD.

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P128

Intensive Blood Pressure Management in Chronic Kidney Disease Patients: An Analysis of the SPRINT Dataset

PrimaryAuthor.AuthorBlock: Haares Mirzan, Rahul Aggarwal, Nicholas Chiu, Sang Myung Han, **Jason Park**, Benjamin Petrie, Jackson Steinkamp, Kimberly Lu, Boston Univ, Boston, MA

Introduction

Systolic Blood Pressure Intervention Trial (SPRINT) determined that among non-diabetic patients with increased CV risk, intensive management of systolic blood pressure (SBP) to a target of 120 mmHg resulted in lower rates of CV events and all-cause mortality, as opposed to the standard goal of 140 mmHg. Current management of BP in the CKD population shows conflicting evidence on target SBP. With the use of patient-level SPRINT data, our study investigates the risks and benefits of intensive BP management in patients with CKD at baseline and is the largest study of intensive BP management in CKD patients (n=2646).

Methods

The similarity between CKD patients in standard and intensive blood pressure management groups with regard to age, race, gender, estimated GFR (eGFR), and baseline SBP were assessed and no differences were found between the two groups. Differences in mortality, adverse events, and rates of achieving BP targets in intensive and standard BP management groups were examined. Cox proportional-hazards models were used for the events analysis. Multiple linear regression was used to assess the differences in achieving BP targets.

Results and Discussion

We highlight three key findings. First, the average post-management SBP was higher in CKD patients than in non-CKD patients in both standard ($p = 0.017$) and intensive ($p < .001$) groups, controlling for age, race, gender, eGFR, and baseline SBP, possibly indicating greater difficulty in controlling BP in CKD patients . Second, intensively-treated CKD patients had increased risks for intervention-related adverse events, including events that resulted in disability, hospitalization, or harm that may have required medical or surgical intervention ($p < .001$). They also experienced higher rates of AKI related adverse events ($p<.008$). Third, intensive management showed a mortality

benefit (HR: .725; 95% CI, .532 to .987), a finding that may help clarify conflicting reports in current literature.
In conclusion, we present an analysis of CKD-specific SPRINT data in order to elucidate the clinical benefits and risks of intensive BP management in the CKD population.

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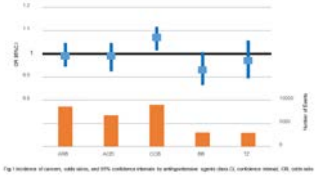
P129

Antihypertensive Drugs and Risk of Cancer: A Systematic Review and Meta-Analysis of 391, 790 Patients

PrimaryAuthor.AuthorBlock:**Nur A Che Roos**, Safaa M Alsanosi, Mohammed A Alsieni, Mayetri Gupta, Sandosh Padmanabhan, Univ of Glasgow, Glasgow, United Kingdom

Introduction: The potential risk of cancer associated with antihypertensive drugs has been disputed for decades as additional outcomes from randomized controlled trials (RCTs), observational studies, and meta-analyses showed conflicting results. **Objective**: To assess the risk of cancer in patients exposed to major antihypertensive drug classes. **Methods**: We searched bibliographic databases for RCTs published between 1950 to December 2015 studying angiotensin-receptor blockers (ARB), angiotensin-converting enzyme inhibitors (ACEi), beta-blockers (BB), calcium channel blockers (CCB), and thiazide diuretics (TZ). RCTs with at least one year duration of planned active treatment and a minimum of 100 participants per treatment arm were eligible. **Main outcome measures**: Cancer and cancer-related deaths from the RCTs. Both fixed-effect and random-effects models were conducted and results were expressed as odds ratio (OR). **Results**: We identified 91 RCTs enrolling 391, 790 participants with an average follow-up of 3.4 years. There was no evidence of excess risk for cancer with ARB, ACEi, BB, and TZ (refer Fig.1). For CCBs, there was an increased risk of cancer (OR 1.07 95%CI 1.02, 1.1) with minimal heterogeneity (I^2=13%). Subgroup analysis did not differ significantly between dihydropyridines (DHP) and non-dihiydropyridines subclasses. There was no statistically significant association between antihypertensive drug classes and risk of cancer deaths. **Conclusions**: Our results suggest that ARB, ACEi, BB, and TZ are not associated with increased risk of cancer. CCB therapy shows an increased risk of cancer. Further investigation on the risk of cancer with CCB is warranted.



Disclosures:**N.A. Che Roos:** None. **S.M. Alsanosi:** None. **M.A. Alsieni:** None. **M. Gupta:** None. **S. Padmanabhan:** None.

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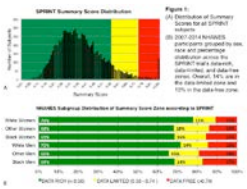
P130

A Map of SPRINT's Data-free Zone

PrimaryAuthor.AuthorBlock:**Luke Joseph Laffin**, Stephanie A Besser, Francis J Alenghat, Univ of Chicago Med, Chicago, IL

120 mmHg is an optimal SBP goal according to the SPRINT trial. However, certain inclusion and exclusion criteria cloud its broad applicability. It

is critical to understand which patients are well represented and reasonable candidates for intensive BP goals. Using only trial inclusion and exclusion criteria diminishes the fact that subjects are unevenly distributed across these criteria. A patient may fit study constraints, yet be poorly represented. Conversely, a patient may be excluded based on a parameter, and declared an inhabitant of a "data-free zone," yet in other respects resemble the trial population.
We defined and mapped the "data-rich, data-limited, and data -free zones" of SPRINT based on subjects' baseline characteristics and not on inclusion and exclusion criteria. For each participant (n=9245), a z-score was computed for 6 variables: age, SBP, glucose, non-HDL-C, creatinine, and BMI. Standardized coefficients from multivariable logistic regression, based on SPRINT's primary end-point, were used to weigh variables. Summary Scores (SS) were generated for each subject to scale with the Euclidean distance of participants from the theoretical "average patient" in six dimensional space.
A SS of 0.56 represents the 90th percentile and 0.74 represents the 97.5th. These were chosen as borders between the data-rich, data-limited, and data-free zones. SS were then calculated for 2007-14 NHANES participants with age >35, SBP≥130, and HbA1c<7. The NHANES population mapped onto SPRINT data zones shows a landscape of applicability by race and sex (Figure).
Defining data zones based on patient characteristics holds promise to refine the applicability of trial results.



Disclosures:**L.J. Laffin:** None. **S.A. Besser:** None. **F.J. Alenghat:** None.

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P131

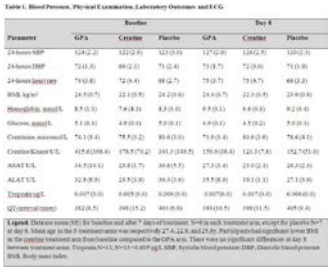
The Acute Effect of the Creatine Kinase Inhibitor Beta-GPA in Healthy Man (ABC-Trial): A Randomized Placebo Controlled First-in-human Trial

PrimaryAuthor.AuthorBlock:**Fares A Karamat Male**, Doortje L Horjus Female, Yentl C Haan Female, Gert A van Montfrans Male, Academic Medical Ctr Amsterdam, Amsterdam, Netherlands; Gajja S Salomons Femal, Dept of Clinical Chemistry, VU Univ Medical Ctr, Amsterdam, Netherlands; Joseph F Clark Male, Cincinnati Children's Hosp, Cincinnati, Ohio, USA, Cincinnati, Ohio, USA, OH; Lizzy M Brewster Female, Academic Medical Ctr Amsterdam, Amsterdam, Netherlands

Aim Increasing evidence indicates that the ATP-generating enzyme creatine kinase is involved in hypertension. Creatine kinase rapidly regenerates ATP from creatine phosphate and ADP. Recently, we showed that beta-guanidinopropionic acid (GPA), a kidney-synthesized creatine analogue and competitive inhibitor of creatine kinase, effectively and safely reduced blood pressure in spontaneously hypertensive rats. To further develop the substance as a potential blood pressure-lowering agent, we assessed the tolerability of a sub-therapeutic GPA dose in healthy men**.**
Methods In this active and placebo-controlled, triple-blind, single-center trial, we recruited 24 healthy men (18 to 50 years old, BMI 18.5 to 29.9 kg/m^2) in the Netherlands. Participants were randomized (1:1:1) to one week daily oral administration of GPA 100 mg, creatine 5 gram, or matching placebo. The primary outcome was the tolerability of GPA, in an intent-to-treat analysis.

Results Twenty four randomized participants received the allocated intervention and 23 completed the study. One participant in the placebo arm dropped out for personal reasons. GPA was well tolerated, without serious or severe adverse events. No abnormalities were reported with GPA use in clinical safety parameters, including physical examination, laboratory studies, or 12-Lead ECG (Table1). At day 8, mean plasma GPA was 213.88 (SE 0.07) in the GPA arm vs. 32.75 (0.00) nmol/L in the placebo arm, a mean difference of 181.13 (95% CI 26.53 to 335.72).

Conclusion In this first-in-human trial, low-dose GPA was safe and well-tolerated when used during 1 week in healthy men. Therefore, GPA is suitable for dose escalation trials



Disclosures:**F.A. Karamat:** None. **D.L. Horjus:** None. **Y.C. Haan:** None. **G.A. van Montfrans:** None. **G.S. Salomons:** None. **J.F. Clark:** None. **L.M. Brewster:** None.

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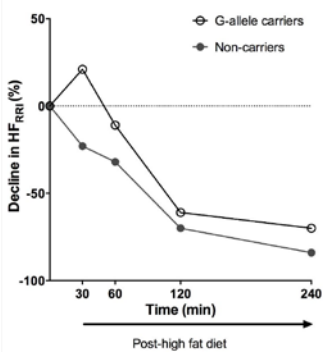
P132

Increased Cardiovascular Parasympathetic Tone in African Americans With CD36 Partial Deficiency

PrimaryAuthor.AuthorBlock:**Shahram Ejtemaei Mehr**, Vanderbilt Univ Medical Ctr, Brentwood, TN

Cardiovascular disease is the leading cause of death among African Americans (AA). Reduced parasympathetic tone as measured by high frequency heart rate variability (HF_{RRI}) predicts cardiovascular mortality. HF_{RRI} is reduced after a high fat meal through caveolar sequestration of muscarinic M2 receptors. The fatty acid translocase CD36 is a protein abundant in the myocardium and important for heart function and lipid metabolism. CD36 plasma membrane localization and function in fatty acid uptake is modulated by its interaction with caveolin. One in four AAs are G-allele carriers for CD36 SNP rs3211938 resulting in ~50% decreased CD36 expression. CD36 deficiency also reduces fat taste perception, which might lead to higher fat intake to reach taste saturation. We tested the hypothesis that obese AAs with partial CD36 deficiency have altered parasympathetic tone during fasting and after a high-fat meal. We recruited 13 G-allele carriers and 39 non-carriers. Subjects were matched by age (P=0.820), BMI (P=0.751), and blood pressure (P=0.701). There was a trend towards reduction in heart rate in carriers (P=0.07). Baseline HF_{RRI} was elevated in G carriers (557.1 [251 to 942] vs. 224 [95 to 655] ms^2, P=0.046). Eleven subjects received a high-fat meal (700 Cal/m^2 BSA, 80% fat). HF_{RRI} was measured at baseline and 30, 60, 120, 240 minutes after meal. Non-carriers (n=4) showed a time-dependent decline in the percent change in HF_{RRI} (-23, -32, -70, -84, respectively). In G-allele carriers (N=6), the decline in HF_{RRI} (21, -11, -61, -70 min) was attenuated. Conclusion: AAs with partial CD36 deficiency have enhanced fasting parasympathetic tone and a blunted response to a high fat meal.



Disclosures:**S. Ejtemaei Mehr:** None.

Funding: No

Funding Component:

P133

Angiotensin-(1-7) Does Not Acutely Lower Blood Pressure in Patients With Essential Hypertension

PrimaryAuthor.AuthorBlock:**Amy Christine Arnold**, Penn State Coll of Med, Hershey, PA; Jorge E Celedonio, Sachin Y Paranjape, Italo Biaggioni, Alfredo Gamboa, Vanderbilt Univ Medical Ctr, Nashville, TN

Angiotensin (Ang)-(1-7) is a beneficial renin-angiotensin hormone that produces vasodilation to lower blood pressure (BP) in animal models of hypertension. There are limited clinical studies, however, and it is unclear if Ang-(1-7) contributes to BP regulation in human hypertension. We propose the difficulties in showing cardiovascular effects in previous clinical studies relates to the buffering capacity of the arterial baroreflex. In this study, we tested the hypothesis that Ang-(1-7) would produce negligible effects on BP with intact baroreceptors, and that the cardiovascular effects of this hormone would be unmasked following elimination of baroreflex buffering. To test this, we examined the effects of acute intravenous Ang-(1-7) infusion (ascending doses from 0.5 to 20 ng/kg/min) on supine BP in subjects with essential hypertension under intact conditions and following acute autonomic withdrawal with the ganglionic blocker trimethaphan in a randomized, open-label, crossover study. BP was restored to baseline levels following autonomic blockade with individually titrated phenylephrine doses. Seven subjects with essential hypertension completed this study (6 male; 48±4 years of age; 29±2 body mass index). All subjects were withdrawn from antihypertensive medications for at least two weeks, and were placed on a fixed sodium diet (150 mEq/day) for three days, prior to each study day. When comparing change from baseline to maximum dose, Ang-(1-7) did not alter systemic hemodynamics under intact conditions (systolic BP: 3±4 mmHg; diastolic BP: 3±1 mmHg; heart rate (0±1 bpm). In contrast to our hypothesis, Ang-(1-7) did not elicit a BP-lowering effect under autonomic blockade (systolic BP: 10±8 mmHg, p=0.299 vs. intact; diastolic BP: 7±3 mmHg, p=0.512; heart rate: -2±1 bpm, p=0.166). Plasma Ang-(1-7) was measured in 4 subjects and showed an approximate 3-fold increase with infusions [0.8±0.7 baseline vs. 2.1±1.2 ng/mL after Ang-(1-7)]. These findings suggest that infusion of Ang-(1-7) to levels within a physiologic range does not acutely induce vasodilation to lower blood pressure in essential hypertension. We cannot exclude that Ang-(1-7) lowers BP by sympathetic inhibition, since those pathways were excluded by our study design.

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P134

Increased Gut Permeability and Dysbiosis in Patients With Pulmonary Arterial Hypertension

PrimaryAuthor.AuthorBlock:**Ruby Goel**, Seungbum Kim, Univ of Florida, Gainesville, FL; Katya Rigatto, Federal Univ of Health Sciences of Porto Alegre, Porto Alegre, Brazil; Brian Shapiro, Jordan Ray, Mayo Clinic, Jacksonville, FL; Yanfei Qi, Univ of Florida, Gainesville, FL; Marcelo B Gazzana, Marli M Knorst, Federal Univ of Rio Grande do Sul, Porto Alegre, Brazil; Elaine M Richards, Ravindra K Sharma, Carl J Pepine, Mohan K Raizada, Univ of Florida, Gainesville, FL

Introduction Pulmonary arterial hypertension (PAH) is a fatal disease and vasodilators are the mainstay for its therapy. However, they offer restricted advancement opportunities for the control and treatment of PAH. Thus, the PAH field needs a paradigm-shifting strategy for its successful management and control. **Objective** To test the hypothesis that gut microbial dysbiosis and its increased permeability are associated with PAH. **Methods** Healthy control subjects and patients with PAH were recruited from two hospitals in the Americas to ascertain the wider applicability of our hypothesis. Fecal samples of PAH patients (n=19) and control, reference subjects (n=16) were obtained at the Hospital de Clinicas de Porto Alegre, Brazil, for microbiota analysis. Plasma/serum samples were collected from PAH patients (n=22) and control, reference subjects (n=19) at the Mayo Clinic, Jacksonville, Florida, USA, for analysis of gut leakiness and inflammatory biomarkers. **Results** In PAH patients, a significant decrease in abundance, diversity and evenness of gut microbial population was observed as measured by 16S ribosomal DNA analyses of fecal samples. Analysis of fecal bacteria populations also demonstrated significant increases in gram-positive, facultative-anaerobic genera, such as *Actinomyces, Bifidobacterium Slackia,* and *Streptococcus* in PAH patients. Further, plasma zonulin and iFABP, the biomarkers for gut leakiness increased by 40.3% (p=0.0018) and 81% (p=0.0012), respectively in PAH patients. High plasma LPS, HMGB1, and TIMP1 levels are involved in increased gut inflammation and gut mucosal injury. We also observed LPS, HMGB1, and TIMP1 levels were increased by 363% (p=0.0096), 20.5% (p=0.0027), and 213% (p<0.0001), respectively in PAH patients. **Conclusions** PAH patients demonstrate profound gut microbial dysbiosis and increased permeability and inflammation. Further characterization of PAH specific microbial species holds novel management potential with the use of pre- and probiotics, designer antibiotics, appropriate fecal/bacterial transplantation, on top of background proven therapies.

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P135

Is Heart Failure With Preserved Ejection Fraction a Gut Disorder?

PrimaryAuthor.AuthorBlock:**YanFei Qi**, Ruby Goel, Avinash Singh Mandloi, Seungbum Kim, Gilberto O Lobaton, Victor P Aquino, Dana D Leach, Eileen Mary Handberg, Mohan K Raizada, Carl J Pepine, Univ of Florida, Gainesville, FL

Background and Objective: Heart failure (HF) with preserved ejection fraction (HFpEF) accounts for ~50% of HF hospitalizations, is

associated with poor quality of life and, has a mortality rate comparable with many cancers. To date, no therapies have proven effective in slowing disease progression, mainly due to limited understanding of underlying mechanisms. Mounting evidence suggests the intestine and gut microbiota play an important role in chronic inflammation in HF, and also hypertension, a history of which is present in most HFpEF patients. However, information about intestinal involvement in HFpEF is limited. Accordingly, we investigated the hypothesis that disturbed intestinal barrier function contributes to translocation of endotoxin and inflammation activation in HFpEF. **Method and Results:** Left ventricular (LV) function, intestinal permeability, circulating lipopolysaccharide (LPS, an endotoxin and strong inducer of pro-inflammatory cytokines) and high-mobility group box protein (HMGB1, a nuclear protein that triggers inflammation) were examined in a mouse model of HFpEF [4w continuous infusion of subpressor dose of angiotensin II (0.2mg/kg/d)] and 4 patients with HFpEF and 4 reference subjects. Impaired LV diastolic function (increase in LVEDP and Tau, both $p<0.05$) occurred in the mice with 3-fold increased intestinal permeability, 1.8-fold elevated LPS levels (137±7 vs control 77±6 pg/ml, $p<0.005$) and 4-fold increased HMGB1 levels (238±42 vs control 56±16 ng/ml, $p<0.05$). Stimulating human coronary artery endothelial cells with 10μg/ml LPS for 48h resulted in 3-fold elevation of HMGB1 (29±4 vs control 12±3 ng/ml, $p<0.05$) that was suppressed by butyrate (17±3 ng/ml). Patients with HFpEF had a 2-fold increase in zonulin (31±3 vs 14±2 ng/ml) and 4-fold LPS elevation (152±18 vs 32±3 pg/ml), (both $p<0.05$ vs reference subjects), confirming gut barrier dysfunction with translocation of endotoxin. **Conclusions:** HFpEF is associated with increased gut permeability that facilitates LPS translocation activating inflammation resulting in endothelial damage. Thus, the gut could be a novel target for therapeutic interventions in patients with HFpEF.

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P136

Ischemic Evaluation in Patients With Hypertensive Emergency / Urgency and Acute Systolic Heart Failure: Is Coronary Angiography Required for All?

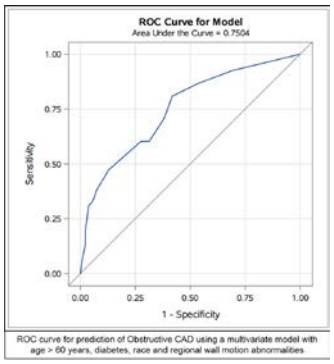
PrimaryAuthor.AuthorBlock:**Jared W Davis**, Muhannad Almubarak, Samuel K McElwee, Amrita Mukherjee, Firas Al-Solaiman, Mark F Sasse, Massoud A Leesar, Arka Chatterjee, Univ of Alabama at Birmingham, Birmingham, AL

Aim: Patients presenting with hypertensive urgency / emergency (HU/E) often have systolic heart failure(S-HF). Coronary angiography is routinely done for these patients to rule out obstructive coronary artery disease (O-CAD).

We performed a retrospective study to investigate predictors of O-CAD in this population.

Methods: Consecutive patients who underwent angiography to investigate S-HF and had hospital admissions for HU/E in the preceding 6 months were included in the study. Chart review was performed to obtain demographic, clinical and imaging / angiographic data. Statistical analysis was performed using SAS 9.4 software.

Results: 205 patients [age 58.9 ± 14.4 years; 62.4 % male; 39.5% diabetic; median EF 25% (Inter Quartile Range: 11)] were included in the study. 33.1% patients (n=68) had O-CAD. Age > 60 years (Odds Ratio: 2.3; 95% Confidence intervals: 1.3-4.3) , Diabetes (OR: 2.1; 95% CI: 1.2-3.8), history of stroke (OR: 2.7; 95% CI: 1.1-7.0) and presence of regional wall motion abnormalities (RWMA; OR: 7.4; 95% CI: 3.4-16.1) and abnormal perfusion study (OR: 7.6; 95% CI: 1.5-39.6) were significantly associated with O-CAD while African American (AA) race was a protective factor (OR: 0.4; 95% CI: 0.2-0.8). ROC curves constructed using an age cut off of 60 years along with non AA race, diabetes and RWMA yielded a good fit with a c statistic of 0.75.



ROC curve for prediction of Obstructive CAD using a multivariate model with age > 60 years, diabetes, race and regional wall motion abnormalities

Conclusions: Our results suggest that only a minority of patients with HU/E and S-HF have obstructive CAD. It may be possible to stratify patients using demographic and non-invasive tests to direct only those with high likelihood of O-CAD for coronary angiography. These results should be validated in large registry populations.

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P137

Intrauterine Growth Restriction Programs a Greater Age-related Decline in Renal Function Associated With Increased Susceptibility to Chronic Renal Injury

PrimaryAuthor.AuthorBlock:**Ashley D. Newsome**, Gwendolyn K. Davis, Norma B. Ojeda, Barbara T. Alexander, Univ of Mississippi Medical Ctr, Jackson, MS

Epidemiological studies indicate that low birth weight (**LBW**) is associated with a reduction in nephron number, hyperfiltration, hypertension, and accelerated loss of renal function. Yet, the mechanisms involved are not understood. Our laboratory uses a rodent model of LBW that results in hypertension but no change in glomerular filtration rate (**GFR**) in male intrauterine growth restricted (**IUGR**) offspring in early adulthood. The aim of this study was to test the hypothesis that IUGR programs a greater decline in GFR with aging and that it enhances susceptibility to a chronic renal insult. Male rats underwent sham (**S**) or uni-nephrectomy (**UNI-X**) with measurement of GFR two months post-surgery at 6 (young adult) and 14 months, or one month post-surgery at 19 months of age. GFR (mL/min/100 g body weight), determined by transcutaneous FITC-sinistrin clearance, was significantly decreased with age in IUGR S and IUGR UNI-X (**P<0.001

and *P<0.05 vs 6-month-old counterparts, respectively; Table). Although not significant, IUGR S had a higher mean GFR than Control S in young adulthood suggesting baseline hyperfiltration as a potential contributor to the greater age-related decline in GFR in IUGR offspring. In IUGR UNI-X at 19 months: GFR was significantly reduced (†P<0.05 vs IUGR S), urinary NGAL was increased (59872 vs 11620 ng/day in IUGR S; P<0.05), and renal TNF-α and TGF-β mRNA expression were elevated (9- and 3-fold greater than both Control UNI-X and IUGR S; P<0.05). Thus, these data indicate that IUGR programs a greater age-related decline in GFR and increased susceptibility to chronic renal injury in later life that is associated with an enhanced renal inflammatory response to injury.

	6 months old	14 months old	19 months old
Control Sham	1.10	0.99	0.78
IUGR Sham	1.36	0.96	0.71**
Control Uni-X	0.87	0.66	0.54
IUGR Uni-X	0.87	0.56	0.40**

*P<0.05 and **P<0.001 vs. 6-month-old counterpart; †P<0.05 vs. IUGR Sham

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P138

Alterations in Cardiac Structure and Function in Young Adults Born Extremely Preterm: Impact of Neonatal Bronchopulmonary Dysplasia

PrimaryAuthor.AuthorBlock:**Muhammad Oneeb Rehman Mian**, Jean-Luc Bigras, Rafael Fernandes, Mariane Bertagnolli, Li Feng Xie, Katryn Paquette, Rong Wu, Anik Cloutier, Valerie Orlando, Thuy Mai Luu, Anne Monique Nuyt, CHU Sainte-Justine, Montreal, QC, Canada

OBJECTIVE: Studies support a causal association between preterm birth and increased risk of cardiovascular diseases. Increased left and right ventricular mass and impaired systolic and diastolic function has been reported in young adults born preterm. However, the impact of extreme preterm birth and prematurity-specific complications on adult cardiac structure and function has not been evaluated. We assessed cardiac structure and function in young adults born extremely preterm (EPT) versus term, and correlated long term cardiac remodeling with neonatal bronchopulmonary dysplasia (BPD). **METHODS:** Eighty five EPT (gest. age = 27.1±1.4 weeks) were recruited along with term-born controls matched for age, sex and socioeconomic status. Birth and neonatal data (gestational age, birth weight, BPD indicated by O_2 requirements at 36 weeks postmenstrual age) was collected. Ambulatory blood pressure (Spacelabs) and echocardiographic measurements (Phillips) were taken. Comparisons were performed using ANOVA or T-test. **RESULTS:** EPT presented with increased systolic (119±9 vs 116±8 mmHg, P<0.05) and diastolic (68±5 vs 66±6 mmHg, P<0.05) blood pressures. EPT exhibited reduced septal thickness (IVS, 6.8±0.8 vs 7.1±1.1 mm, P<0.05), left ventricular internal dimension (LVID, 46±4 vs 48±5 mm, P<0.05), LV end-diastolic (98±20 vs 106±24 ml, P<0.05) and end-systolic (36±9 vs 40±11 ml, P<0.01) volumes, right ventricular internal dimension (RVID, 22±3 vs 24±4 mm, P<0.05), and LV mass (104±27 vs 115±30 g, P<0.05), but similar LV mass and volume indexes. EPT exhibited increased LV myocardial performance index (0.41±0.04 vs 0.39±0.04, P<0.01), reduced mitral lateral e' (17.6±2.8 vs

19.1±2.6 cm/s, P<0.01), mitral s' (10.7±2.3 vs 11.6±2.3 cm/s, P<0.01), tricuspid E' (15.8±2.7 vs 16.8±2.1 cm/s, P<0.05), and tricuspid S' (13.1±2.0 vs 14.0±2.0 cm/s, P<0.01) waves, and a trend in reduced mitral E wave (81±14 vs 85±15 cm/s, P=0.09). EPT with neonatal BPD exhibited greater reduction in IVS (6.5±0.8 mm, P<0.05 vs terms), LVID (45±4 mm, P<0.05), LV Mass (98±22 g, P<0.05), and RVID (20±3 mm, P<0.01). **CONCLUSIONS:** EPT exhibit cardiac structural and functional alterations compared to term-born individuals. Neonatal BPD in EPT is a key contributor to long term cardiac remodeling.

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P139

Dual Angiotensin Receptor and Neprilysin Inhibition Improves Renal Hemodynamics and Reduces Kidney Damage in Diabetic Rats

PrimaryAuthor.AuthorBlock:**Estrellita Uijl**, Marian C Clahsen-van Groningen, Erasmus MC, Rotterdam, Netherlands; Jaap A Joles, Univ Medical Ctr Utrecht, Utrecht, Netherlands; Ewout J Hoorn, Alexander H Danser, Erasmus MC, Rotterdam, Netherlands

Dual blockade with an Angiotensin Receptor/Neprilysin Inhibitor (ARNI) reduces proteinuria and glomerulosclerosis in diabetic TGR(mREN2)27 rats (i.e., rats displaying angiotensin II-dependent hypertension) more strongly than single AR blockade (ARB), despite a similar effect on blood pressure. Here we investigated whether this is due to improved renal hemodynamics and/or suppression of renal inflammation. TGR(mREN2)27 rats were made diabetic with streptozotocin for 12 weeks, and treated with placebo (n=10), valsartan (ARB; n=8) or valsartan/sacubitril (ARNI; n=8) from week 9-12. Blood pressure was measured by telemetry. Effective renal plasma flow (eRPF) and glomerular filtration rate (GFR) were assessed by quantifying para-aminohippuric acid and inulin clearances. Renal inflammation was quantified by qPCR (CD68 and CD3ε expression, representative for macrophages and T cells, respectively) and fluorescent activated cell sorting (FACS) analysis. ARNI and ARB lowered blood pressure identically, while only ARNI reduced albuminuria. Severe, chronic ischemia and globally sclerotic glomeruli occurred less frequently in kidneys of ARNI-treated animals vs. ARB-treated animals and controls. ARNI, but not ARB, increased eRPF, and a similar trend was observed for GFR. No treatment affected filtration fraction. ARNI decreased CD68 mRNA expression in both renal cortex and medulla, while ARB increased CD68 as well as CD3ε expression in renal medulla. FACS analysis revealed no differences between the treatment groups in the immune-cell fractions that had infiltrated the kidney. In conclusion, improved renal hemodynamics, combined with reduced macrophage infiltration, may underlie the stronger beneficial effects of ARNI on albuminuria and renal histology in diabetic TGR(mREN2)27 rats versus ARB.

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P140

Serum Chloride Predicts Mortality Risk in Type 2 Diabetes - Analysis of 91,159 Patients From the West of Scotland

PrimaryAuthor.AuthorBlock:**Linsay McCallum**, Univ of Glasgow, Glasgow, United Kingdom; Christopher A Sainsbury, Gregory C Jones, NHS Greater Glasgow and Clyde, Glasgow, United Kingdom; Sandosh Padmanabhan, Univ of Glasgow, Glasgow, United Kingdom

Objective

Low serum chloride (Cl^-) is associated with increased risk of death in those with heart failure, hypertension or chronic kidney disease. We sought to investigate the association of serum Cl^- with risk of cause-specific death in adults with type 2 diabetes mellitus (T2DM).

Methods

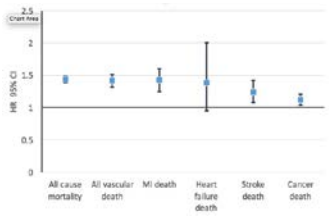
Data was available for 91,159 adults from the West of Scotland with T2DM from NHS Greater Glasgow and Clyde Safe Haven with 10 years follow up. Two groups were created: serum Cl^- <100 and Cl^- ≥100 mmol/L. Cox-PH models, adjusted for age, sex and serum sodium (Na^+), were used to assess the association between serum Cl^- and risk of death (all-cause mortality, vascular death, death from myocardial infarction (MI), death from heart failure, death from stroke, death from cancer).

Results

There were 13,459 patients with serum Cl^- <100 mmol/L; 53% were male with median age 62.5 (IQR 50.9-73.1) years and median Na+ 136 (133-138) mmol/L. There were 77,757 patients with serum Cl^- ≥100 mmol/L; 53% were male with median age 61.2 (IQR 50.2-71.4) years and median Na^+ 139 (IQR 138-141) mmol/L. Serum Cl^- <100 mmol/L was associated with an 44% increased risk of all-cause mortality (N=20,304, HR 1.44[95% CI 1.38-1.49]; p <0.0001), independent of serum Na^+ (Figure). The increased mortality risk of serum Cl^- <100 mmol/L was observed for cardiovascular mortality (N=6,323, 1.41[1.31-1.51]; p <0.0001); death from MI (N=1,986, 1.42[1.25-1.60]; p <0.0001); stroke (N=1,590, 1.24[1.08-1.42]; p 0.003); heart failure (N=200, 1.38[0.95-2.0]; p 0.09) and cancer (N=5,577, 1.12[1.04-1.21]; p 0.003).

Conclusions

Serum chloride <100 mmol/L was associated with increased risk of death in adults with type 2 diabetes mellitus.



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P141

Fetuin B, a Hepatokine, Causes Acute Endothelium-dependent Vascular Relaxation and Decrease Blood Pressure

PrimaryAuthor.AuthorBlock:**Lucas O Martins França**, Youhua Zhang, New York Inst of Technology, Old Westbury, NY; Antonio Marcus de Andrade Paes, Federal Univ of Maranhao, Sao Luis, Brazil; Maria Alicia Carrillo-Sepulveda, New York Inst of Technology, Old Westbury, NY

Non-alcoholic fatty liver disease (NAFLD) has been implicated in the pathogenesis of type 2 diabetes (T2DM) and is a direct risk factor to the development of cardiovascular diseases. Fetuin B was identified as a hepatokine secreted by steatotic hepatocytes and released in large amount in T2DM patients. While previous

reports showed that fetuin A negatively regulates endothelial function, studies related to fetuin B and vascular function remain undetermined. Thus, we tested the hypothesis that fetuin B impairs endothelial function and arterial blood pressure. Using an invasive left ventricular (LV) catheterization via the carotid artery, we found that *in vivo* acute administration of recombinant human fetuin B (0.5µg/g body weight) dropped systolic blood pressure in mice within 30 minutes (110 ± 2 vs 94 ± 2mmHg, $p<0.05$). Next, we tested whether fetuin B alters endothelial-dependent relaxation. Aortic rings were incubated acutely with fetuin B (50ng/mL) for 30 minutes prior to functional vascular studies using a wire myograph. Fetuin B potentiated acetylcholine-induced endothelial-dependent relaxation (95.2 ± 2 vs 85.0 ± 3 %, $p<0.05$, n=3). Fetuin B did not significantly affect vascular contraction induced by phenylephrine. Cultured human umbilical vein endothelial cells stimulated with Fetuin B for 12 hours displayed augmented phosphorylation of eNOS in the serine 1177 (2.5-fold of increase vs unstimulated cells, $p<0.05$). Our data showed that our hypothesis was contested since fetuin B facilitated vasorelaxation dependent of endothelium and acutely decreased blood pressure. Together the findings of this present study suggest that increased levels of fetuin B acutely play a protective endothelial vascular role. We are currently testing the long-term effects of fetuin B on the vasculature of T2DM animal models.

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P142

The CKD273 Urinary Peptidomic Biomarker is Associated with Mortality in People at Early Stages of Diabetic Nephropathy Independent of Traditional Risk Factors

PrimaryAuthor.AuthorBlock:**Gemma E Currie**, Sheon Mary, Univ of Glasgow, Glasgow, United Kingdom; Bernt J von Scholten, Morten Lindhardt, Steno Diabetes Ctr Copenhagen, Copenhagen, Denmark; Harald Mischak, Mosaiques Diagnostics GmbH, Hannover, Germany; William Mullen, Univ of Glasgow, Glasgow, United Kingdom; Peter Rossing, Steno Diabetes Ctr Copenhagen, Copenhagen, Denmark; Christian Delles, Univ of Glasgow, Glasgow, United Kingdom

Background
Mortality in type 2 diabetes (T2D) is primarily driven by cardiovascular disease. This is amplified in diabetic nephropathy (DN), even in early 'pre-clinical' stages. A urinary peptidomic classifier (CKD273) has been found to predict DN development in advance of detectable microalbuminuria. Whether it is also a determinant of mortality and cardiovascular disease in patients with established albuminuria is unknown.
Methods
We studied 155 subjects with T2D, albuminuria (geometrical mean [IQR]: 85 [34;194] mg/24hrs), controlled blood pressure (129±16/74±11 mmHg) and preserved renal function (eGFR 88±17 ml/min/1.73m^2). Blood and urine samples were collected for measurement of estimated glomerular filtration rate (eGFR), urine albumin excretion (UAE), N-terminal pro-brain natriuretic peptide (NT-proBNP; ELISA) and urinary proteomics (capillary electrophoresis coupled to mass spectrometry). Computed tomography imaging was performed to assess coronary artery calcium (CAC) score. Outcome data were

collected through national disease registries over a 6 year follow up period.

Results

CKD273 correlated with UAE (r=0.481, p=<0.001), age (r=0.238, p=0.003), CAC score (r=0.236, p=0.003), NT-proBNP (r=0.190, p=0.018) and eGFR (r=0.265, p=0.001). On multiple regression only UAE (β=0.402, p<0.001) and eGFR (β=-0.184, p=0.039) were statistically significant determinants. Twenty participants died during follow-up. CKD273 was a determinant of mortality (log rank [Mantel-Cox] p=0.004), and retained significance (p=0.050) after adjustment for age, sex, blood pressure, NT-proBNP and CAC score in a Cox regression model. Neither eGFR nor UAE were determinants of mortality in this cohort.

Conclusions

A multidimensional biomarker can provide information on outcomes associated with its primary diagnostic purpose. Here we demonstrate that the peptidomics-based classifier CKD273 is associated with mortality in albuminuric people with T2D in even when adjusted for other established cardiovascular and renal biomarkers.

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P143

Pulsatile Stress in the Hypertensive Elderly versus Young Patients

PrimaryAuthor.AuthorBlock:**Abimbola Shofu**, May Saung, G. Mustafa Awan, Bassam Omar, Christopher Malozzi, Univ of South Alabama, Mobile, AL

Background: Hypertensive patients suffer wide pulse pressure (PP) with age, an independent marker of cardiac events. Changes in the heart rate (HR) may decrease pulsatile stress (PPxHR). **Objectives:** To examine pulsatile stress in hypertensive elderly patients, as a potential compensatory mechanism to widened pulse pressure. **Methods:** Clinic charts were reviewed with hypertension as a diagnosis; 200 encounters were reviewed and divided into 2 groups; group 1 (young) were patients < 60 years of age (n=116), and group 2 (elderly) were patient ≥ 60 years of age (n=84). The difference between the groups with regards systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP), heart rate (HR), and pulsatile stress (PPxHR) were analyzed using Student's t-Test. **Results:** The mean age of patients was 68±9 years in elderly versus (VS) 49±9 years in the young. SBP was marginally higher in the elderly at 134±20 vs 128±18 mmHg in the young; P = 0.04. DBP and HR were significantly lower in the elderly vs the young at 80±13 vs 86±13 mmHg, and 67±11 vs 76±14 BPM respectively; P < 0.01. PP was significantly higher in the elderly at 55±18 vs 42±13 mmHg in the young; P < 0.01. Pulsatile stress (PPxHR), however, was not significantly different in the elderly at 3,795±1,506 vs 3,477±1,246 in the young; P = 0.11. **Discussion:** Elderly patients suffer elevated pulse pressure, an independent predictor of cardiac risk. In our cohort, the pulse pressure was significantly higher, as expected, in elderly patients. However, the heart rate in elderly patients was significantly lower, therefore causing no significant difference in the pulse pressure-heart rate product (a measure of pulsatile stress) compared with younger patients. Overall, the medical management in our cohort was guideline-based and was not significantly different among the two groups. Whether the slower heart rate

seen in elderly patients is compensatory, or merely a manifestation of intrinsic sinus node disease is unclear. Further studies to compare the response of pulsatile stress to exercise in elderly versus younger patients may help clarify this issue.

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P144

Association Between Hypertension and Thyroid Abnormalities Among Hospitalized United States Patients: Data From National Inpatient Sample

PrimaryAuthor.AuthorBlock:**Sijan Basnet**, Rashmi Dhital, Reading Health System, West Reading, PA; Biswaraj Tharu, Maharajgunj Medical Campus, Tribhuvan Univ, Kathmandu, Nepal; Sushil Ghimire, Dilli R Poudel, Reading Health System, West Reading, PA

Introduction: Disorders of thyroid, hyperthyroidism and hypothyroidism, are established risk factors for hypertension. Comparison of these risk factors in terms of association has not been done.
Methods: National Inpatient Sample database from 2009 - 2011 was utilized to explore relationship between hypertension and three categories of thyroid hormone levels (hyperthyroidism, hypothyroidism and euthyroidism). Multivariate logistic regressions were used to establish the association among all thyroid hormone level groups. We utilized STATA version 13.0 (College Station, TX) to perform the analyses.
Results: Hypertension was more common in patients with hypothyroidism and hyperthyroidism in comparison to euthyroidism (Table 1). Multivariate analysis with logistic regression (controlled for age, sex, race, smoking, obesity, dyslipidemia, diabetes, Charlson comorbidity index) demonstrated that hypertension had a greater association with hyperthyroidism (OR 1.20; CI: 1.17 - 1.23) than hypothyroidism (OR 1.09; CI: 1.08 - 1.10).
Discussion: T3 dilates peripheral arterioles and decreases systemic vascular resistance which stimulates renin secretion and increases effective arterial blood volume. Also, it increases heart rate and cardiac contractility. These mechanisms lead to increase in blood pressure. On the other hand, the mechanism with hypothyroidism is not fully recognized. It is thought to be secondary to increase in systemic vascular resistance. Through our study, we can conclude that the risk of hypertension is greater in patients with hyperthyroidism than with hypothyroidism.

Association	Odds ratio	Linearized standard error	P value	95% confidence interval
Hyperthyroidism	1.20	0.01	<0.0001	1.17 – 1.23
Hypothyroidism	1.09	0.005	<0.0001	1.08 – 1.10

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P145

Screening Rates for the Diagnostic Workup of Resistant Hypertension

PrimaryAuthor.AuthorBlock:**Gilad M Jaffe**, Stanford Hypertension Ctr, Dept of Med, Stanford Univ Sch of Med, Stanford, CA; Gomathi Krishnan, Stanford Ctr for Clinical Informatics, Stanford Univ Sch of Med, Stanford, CA; Margaret Stedman, Glenn M Chertow, Stanford Hypertension Ctr, Dept of Med, Stanford Univ Sch of Med, Stanford, CA; John T Leppert, Div of Nephrology, Dept of

Med, Stanford Univ Sch of Med; and Veterans Affairs Palo Alto Health Care System, Palo Alto, CA; Vivek Bhalla, Stanford Hypertension Ctr, Dept of Med, Stanford Univ Sch of Med, Stanford, CA

Resistant hypertension is a common clinical condition associated with higher rates of cardiovascular disease, kidney disease, and death. Among individuals with resistant hypertension, secondary causes of hypertension occur in about 20% of cases, but the rates of screening are unknown. We assessed the practice pattern of these guidelines in a major primary and tertiary care academic institution in Northern California. Using the electronic health record, we identified individuals between the years of 2008 and 2014 who were prescribed three separate classes of antihypertensive agents and had follow-up laboratory data within 24 months. We excluded individuals with known causes of secondary hypertension such as hyperaldosteronism, renal artery stenosis, fibromuscular dysplasia, adrenal disease, and end-stage renal disease. We also excluded individuals with diagnosed heart failure, who may have been prescribed selected medications for heart failure instead of hypertension. This cohort of 37,073 individuals with presumed resistant hypertension had a mean age of 58.5 years (SD 15.8), was 51.7% male, and 56.4% Caucasian, 6.3% Black, and 12.4% Asian. Among these individuals, only 520 had a serum aldosterone, and only 447 had both aldosterone and plasma renin activity levels measured. These data infer that the recommended initial screening tests for secondary causes of resistant hypertension - notably primary and secondary hyperaldosteronism - are conducted in only 1.2% of individuals. A detailed chart review of a representative sample of this cohort will be also be conducted. Thus far, these data suggest that there is significant under-screening of reversible causes of resistant hypertension. Furthermore, an electronic implementation strategy to prompt screening for secondary causes may be warranted to reduce blood pressure, optimize use of antihypertensive medications, and lower cardiovascular risk. A similar analysis will be performed in the Veterans Affairs database to evaluate screening rates in health-care delivery systems enriched with African Americans.

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P146

Uric Acid is an Independent Risk Factor for Developing Hypertension From Prehypertension: A 5-year Japanese Cohort Study

PrimaryAuthor.AuthorBlock:**Masanari Kuwabara**, Shigeko Hara, Toranomon Hosp, Tokyo, Japan; Koichiro Niwa, St. Luke's Intl Hosp, Tokyo, Japan; Minoru Ohno, Toranomon Hosp, Tokyo, Japan; Ichiro Hisatome, Tottori Univ, Tottori, Japan

Objectives: Prehypertension frequently progresses to hypertension and is associated with cardiovascular diseases, stroke, excess morbidity and mortality. However, the identical risk factors for developing hypertension from prehypertension are not clarified. This study is conducted to clarify the risks. **Methods:** We conducted a retrospective 5-year cohort study using the data from 3,584 prehypertensive Japanese adults (52.1±11.0 years, 2,081 men) in 2004 and reevaluated it 5 years later. We calculated the cumulative incidences of

hypertension over 5 years, then, we detected the risk factors and calculated odds ratios (ORs) for developing hypertension by crude analysis and after adjustments for age, sex, body mass index, smoking and drinking habits, baseline systolic and diastolic blood pressure, pulse rate, diabetes mellitus, dyslipidemia, chronic kidney disease, and serum uric acid. We also evaluated whether serum uric acid (hyperuricemia) provided an independent risk for developing hypertension. **Results:** The cumulative incidence of hypertension from prehypertension over 5 years was 25.3%, but there were no significant differences between women and men (24.4% vs 26.0%, p=0.28). The cumulative incidence of hypertension in subjects with hyperuricemia (n=726) was significantly higher than those without hyperuricemia (n=2,858) (30.7% vs 24.0%, p<0.001). After multivariable adjustments, the risk factors for developing hypertension from prehypertension were age (OR per 1 year increased: 1.023; 95% CI, 1.015-1.032), women (OR versus men: 1.595; 95% CI, 1.269-2.005), higher body mass index (OR per 1 kg/m^2 increased: 1.051; 95% CI 1.021-1.081), higher baseline systolic blood pressure (OR per 1 mmHg increased: 1.072; 95% CI, 1.055-1.089) and diastolic blood pressure (OR per 1 mmHg increased: 1.085; 95% CI, 1.065-1.106), and higher serum uric acid (OR pre 1 mg/dL increased: 1.149; 95% CI, 1.066-1.238), but not smoking and drinking habits, diabetes mellitus, dyslipidemia, and chronic kidney diseases. **Conclusions:** Increased serum uric acid is an independent risk factor for developing hypertension from prehypertension. Intervention studies are needed to clarify whether the treatments for hyperuricemia in prehypertensive subjects are useful.

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P147

Differences in the Treatment of Resistant Hypertension in African Americans and European Americans in a Clinical Setting

PrimaryAuthor.AuthorBlock:**Megan M Shuey**, Vanderbilt Univ, Nashville, TN; Nancy J Brown, Joshua C Denny, Vanderbilt Univ Medical Ctr, Nashville, TN

Using Vanderbilt University Medical Center's electronic health record (EHR), we tested the hypothesis that we could identify patients with resistant hypertension (RH) and patterns of treatment of RH in a real-world clinical setting. Patients were identified as having RH if they had blood pressure (BP) >140/90 mmHg despite concurrent treatment with three different classes of antihypertensive medications including a thiazide diuretic or amlodipine or similar dihydropyridine (DHP) calcium channel blocker (CCB) or if they were treated with four antihypertensive medications including the same classes. Secondary causes and chronic kidney disease were excluded. Among 186,015 European American (EA) and 33,576 African American (AA) hypertensive patients, 13,541 (7.3%) and 3,541 (10.5%) had RH, respectively. AA with RH were younger, heavier, more often female, had a higher incidence of type 2 diabetes, and had higher systolic and diastolic BPs than EA with RH. AA with RH were more likely than EA to be treated with vasodilators, DHP CCB, and α2-agonists. EA were more likely to be treated with angiotensin receptor blockers, renin inhibitors, and beta-blockers. Mineralocorticoid receptor (MR) antagonists were used in both EA and AA patients and use was increased in patients treated with $\geq$ four antihypertensive medications compared to

patients treated with three (2.6% vs 12.4% in EA, p<0.001; 2.8% vs 12.3% in AA, p<0.001). The number of patients treated with an MR antagonist increased to 36.6% in EA and 40.3% in AA over a mean follow-up period of 7.4 and 8.7 years, respectively.
Our results demonstrate that clinicians use different classes of medication to treat RH in AA and EA.

Table: Characteristics and medication use in European American and African American patients with RH in the Vanderbilt University Medical Center EHR

Variable	European American (n=13,541)	African American (n=3,541)	p-value
SBP, mmHg	145.4 (145.1-145.6)	148.8 (148.2-149.4)	<0.001
DBP, mmHg	78.0 (77.8-78.2)	84.7 (84.3-85.2)	<0.001
Age, years	64.19 (64.0-64.4)	56.0 (55.4-56.5)	<0.001
BMI, kg/m2	32.3 (32.1-32.6)	34.8 (34.4-35.3)	<0.001
Female, n (%)	3,495 (50.4%)	2,092 (59.1%)	<0.001
T2DM, n (%)	2,694 (19.9%)	954 (26.9%)	<0.001
Sleep Apnea, n (%)	868 (6.4%)	252 (7.1%)	0.14
Thiazide/CCD, n (%)	13541 (100%)	3,541 (100%)	1.0
ACE inhibitor, n (%)	6,999 (51.7%)	1,916 (54.1%)	0.01
ARB, n (%)	5,178 (38.2%)	1,161 (32.8%)	<0.001
BB, n (%)	8,697 (64.2%)	1,996 (56.4%)	<0.001
Alpha 2 agonist, n (%)	1,921 (14.2%)	736 (20.8%)	<0.001
CCB, n (%)	9272 (68.5%)	2,625 (74.1%)	<0.001
Amlodipine, n (%)	6,759 (49.9%)	1,749 (49.4%)	0.58
DHP CCB, n (%)	1,508 (11.1%)	585 (16.5%)	<0.001
Non-DHP CCB, n (%)	1,005 (7.4%)	291 (8.2%)	0.12
Thiazide Diuretic, n (%)	8,812 (65.1%)	2,319 (65.5%)	0.65
Aldosterone antagonist, n (%)	854 (6.3%)	240 (6.8%)	0.32
Non-thiazide Diuretic, n (%)	4,271 (31.5%)	1,190 (33.6%)	0.02
Vasodilator, n (%)	903 (6.7%)	422 (11.9%)	<0.001
Minoxidil, n (%)	161 (1.2%)	101 (2.9%)	<0.001
Hydralazine, n (%)	742 (5.5%)	321 (9.1%)	<0.001
Alpha antagonist, n (%)	626 (4.6%)	159 (3.9%)	0.08
Renin inhibitor, n (%)	275 (2.0%)	43 (1.2%)	0.001
Misc antihtn, n (%)	3 (0.0%)	0 (0.0%)	1.0

Data are presented as mean (95% Confidence Interval) unless otherwise indicated.
Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; T2DM, type II diabetes mellitus

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P148

Effect of Apparent Treatment Resistant Hypertension on Cardiovascular Disease Events and Modification by Sex

PrimaryAuthor.AuthorBlock:**Julianne Nelson**, Longjian Liu, Drexel Univ, Philadelphia, PA

Apparent treatment resistant hypertension (ATRH) is an important health concern in the U.S. affecting approximately 9% of all hypertensive adults and growing. However, long-term prognosis of those with ATRH remains to be fully investigated, especially with regard to the presence of differences by sex. Using data from the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT) we examined the relationship between ATRH and CVD and effect modification by sex. Subjects in ALLHAT were 55 years or older (N male = 13,597 and N female = 11,919) and were randomized to one of four antihypertensive medications for the purposes of the trial. We used Cox Proportional Hazard models and tested for interaction of sex through use of a multiplicative interaction term, stratified analyses, the Aalen Additive Hazards model, and joint effects. Missing blood pressure readings were imputed using multiple imputation as a sensitivity analysis. Due to the design of ALLHAT, ATRH was assessed at the year 2 follow-up visit and subjects were then followed for an additional 6 years (average follow-up 4.7 years) during this time there were 5,030 CVD events. Overall (N=25,516), ATRH was associated with an approximate 30% increased risk of CVD (HR 1.30, 95% CI 1.19 – 1.42) compared to those without ATRH. This risk was greater in women than in men ($p_{interaction}$ <.0001). In women, ATRH was associated with an approximate 62% increase in risk of CVD (HR 1.62, 95% CI 1.41 – 1.86) while in men, ATRH was associated with an approximate 13% increase in risk of CVD (HR 1.13, 95% CI 1.01 – 1.27). Sex was also a modifier of the relationship on the additive scale (p-value 0.003). ATRH is associated with an increased risk of developing CVD and women with ATRH were at a greater risk of developing CVD compared to men. These findings provide important insights into the complex relationship between ATRH and cardiovascular health, and suggest that women, in particular, may be a high-risk subgroup. Thus future studies should examine these differences to aid in development of targeted treatment and

interventions which would have a significant public health benefit.

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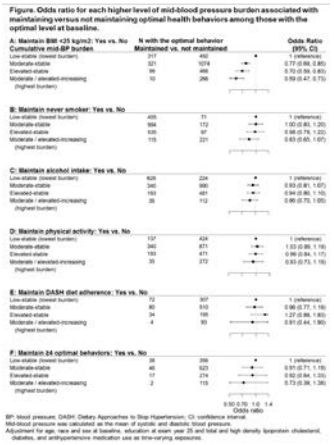
P149

Maintenance of Optimal Health Behaviors Over 25 Years and Cumulative Blood Pressure Burden: Prospective Data From the Coronary Artery Risk Development in Young Adults Study

PrimaryAuthor.AuthorBlock:**John N Booth**, Univ of Alabama at Birmingham, Birmingham, AL; Norrina B Allen, Northwestern Univ, Birmingham, AL; April P Carson, David Calhoun, Univ of Alabama at Birmingham, Birmingham, AL; Daichi Shimbo, Columbia Univ, New York, NY; James M Shikany, Cora E Lewis, David T Redden, Paul Muntner, Univ of Alabama at Birmingham, Birmingham, AL

Introduction: Healthy behaviors lower blood pressure (BP) in short-term randomized trials.
Hypothesis: Maintaining healthy behaviors for 25 years will be associated with low cumulative BP burden.
Methods: In 1985-86, the Coronary Artery Risk Development in Young Adults (CARDIA) study enrolled 5115 adults 18-30 years old. BP and health behaviors were measured at 8 exams over 25 years. Optimal health behaviors were body mass index (BMI) < 25 kg/m^2, never smoking, moderate / vigorous physical activity ≥ 150 minutes weekly, no / moderate alcohol intake (women / men: 0-7 / 0-14 drinks weekly) and Dietary Approaches to Stop Hypertension (DASH) adherence score ≥ 15. Also, maintaining ≥ 4 optimal behaviors was assessed. Using Proc Traj in SAS, 4630 adults who had ≥ 1 optimal behavior at baseline and attended ≥ 3 exams were grouped by change in mid-BP with age. Odds ratios (OR) were calculated for each higher level of BP burden associated with maintaining versus not maintaining optimal behaviors at all exams.
Results: Those who did versus did not maintain optimal BMI were less likely to have moderate-stable, elevated-stable or moderate / elevated-increasing versus low-stable BP burden (**Figure, Panel A**). There was a non-statistically significant lower OR for the highest versus low-stable BP burden in adults who maintained optimal smoking and alcohol intake (**Figure, Panels B-C**). Optimal physical activity and DASH diet were not associated with mid-BP burden (**Figure, Panels D-E**). The OR (95% CI) for moderate / elevated-increasing versus low-stable BP burden in those maintaining ≥ 4 optimal behaviors was 0.73 (0.39 - 1.38).
Conclusion: Maintaining optimal BMI into middle age preserves low BP burden.



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P150

Sex Difference of Hypertension in Spontaneously Hypertensive Rats: Role of Mitochondrial Oxidative Stress

PrimaryAuthor.AuthorBlock:**Rodrigo Oscar Maranon**, Carolina Dalmasso, Chetal N. Patil, Jane F. Reckelhoff, UMMC, Jackson, MS

Men have higher blood pressure (BP) than premenopausal women. Pressor response to oxidative stress may be a major contributor to the sex difference in BP control. Mitochondrial oxidative stress is associated with hypertension; however, whether mitochondrial oxidative stress plays a role in the sex difference in BP is unknown. In the present study, we tested the hypothesis that mitochondrial oxidative stress contributes to the sex difference in BP regulation in spontaneously hypertensive rats (SHR). Young intact (iYMSHR) and castrated males (cYMSHR), and females SHR (YFSHR) (3 mos of age) were implanted with radiotelemeters, and after a 4 day baseline BP, were treated with mitoTempo (0.75 mg/kg/d, sc minipumps), a specific scavenger of mitochondrial superoxide, for 7 days. Following 10 days washout of mito-tempo, rats were treated with Tempol (30 mg/kg/day, po drinking water) for 7 days. iYMSHR have higher blood pressure (by telemetry) than cYMSHR and YFSHR (148±1 mmHg, n=5, vs 132±1 mmHg, n=5, and 139±1 mmHg, n=5; $p<0.01$, respectively). MitoTempo reduced BP by 6% in iYMSHR (147±1 vs 139±1, n=5; $p<0.05$) compared to females (3%: 139±1 vs 136±1; n=5; p: NS) and castrated males (4.5%: 132±1 vs 126±1, n=5; $p<0.05$). After 10 days washout, tempol reduced BP only in iYMSHR (144±1 vs 130±1 mmHg, n=5; $p<0.05$). Our results suggest

that mitochondrial oxidative stress may contribute to BP regulation in male SHR, but has no effect in females. The data also suggest that the presence of testosterone is necessary for the pressor response to oxidative stress in males since Tempol had no effect on BP in castrated males. Further studies examining the effect of steroid hormones and mitochondria in BP regulation are necessary to elucidate the importance of mitochondrial oxidative stress on sex difference of hypertension.

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P151

Salt Sensitivity in Male and Female C57BL/6J Mice: Role of Renal Angiotensin and Dopamine Receptors and Sodium Transporters

PrimaryAuthor.AuthorBlock:**Xiaobo Ma**, George Washington Univ, Washington, DC; Donghai Zhou, Coll of Veterinary Med, Huazhong Agricultural Univ, Wuhan, Hubei, China, Huazhong, China; Zhenyu Diao, Dept of Obstetrics & Gynecology, Nanjing Drum Tower Hosp, Nanjing Univ, Nanjing, China; Laureano D. Asico, George Washington Univ, Washington, DC; Crisanto S. Escano, Div of Nephrology, Dept of Med, Univ of Maryland Sch of Med, Baltimore, MD; Santiago Cuevas, Ines Armando, Pedro A. Jose, Xiaoyan Wang, George Washington Univ, Washington, DC

To test if there is a sex difference in the salt sensitivity of C57Bl/6J mice, we studied blood pressure (BP), renal dopamine receptors, and sodium transporters in response to high salt diet (4% NaCl, 1 wk) and candesartan. Similar to males, the night-time systolic BP (SBP, telemetry, n=4) in females started to increase on day 1, peaked on day 2 (130±1 vs 117±1, mm Hg) and remained at high levels (126±1); the daytime SBP started to increase on day 2 that became significant on day 6 (124±1 vs 111±1). The high salt-diet induced-increase in SBP was prevented by candesartan (1 mg/kg, 1 wk, subcutaneously via osmotic mini-pump) (82±3 vs. 117±2 in males; 86±2 vs. 121±1 in females, under anesthesia, n=5/group). There were no sex differences in the SBP response to diet and candesartan, food and water intakes, urinary excretions of water and electrolytes, and serum concentrations of creatinine and electrolytes on high and normal salt diet. In females fed a high salt diet, candesartan increased renal D_1R (151±12, immunoblotting, % of control, n=5/group) and D_5R (156±5) but not D_2R, D_3R, or D_4R protein expression, increased renal mRNA expression of D_1R(160±17) but not D_5R (real-time PCR), and decreased the renal protein expressions of sodium-hydrogen exchanger isoform 3 (36±6), sodium-potassium-2 chloride cotransporter (8±2), sodium-chloride cotransporter (30±3), and α (55±4), β (60±8) and γ (9±1) epithelial sodium channel, but not type 2 sodium phosphate cotransporter and $\alpha 1Na^+K^+$ATPase. Those changes were also seen in males except that renal D_1R protein expression was not increased. Under normal salt intake, $AT_{1A}R$ KO females (C57Bl/6J background) had increased renal protein expressions of D_1R (152±8), D_5R (131±6), and D_4R (114±4), but not D_2R and D_3R; renal protein expressions of sodium-hydrogen exchanger isoform 3 (47±8), sodium-potassium-2 chloride cotransporter (52±11), and α (42±9) and γ (46±7) epithelial sodium channel were decreased. We conclude that the increase in BP caused by high salt diet and the effect of AT_1R blockade on BP, renal dopamine receptors and sodium transporters are similar in male and female C57Bl/6J mice . The consequences of the

sex-related differential regulation of D_1R and D_5R, in pathophysiology, other than BP, remain to be determined.

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P152

Female Mice Are Not Protected From High Fat Diet-induced Metabolic Dysfunction

PrimaryAuthor.AuthorBlock:**Taylor Friemel**, Justin Loloi, Sarah S Bingaman, Amy Christine Arnold, Penn State Coll of Med, Hershey, PA

Female clinical populations are generally protected from obesity-related blood pressure and metabolic complications such as hypertension and type II diabetes, despite increased adiposity compared to males. Similar cardiovascular protection has been observed in obese female mice produced by high fat diet (HFD) feeding, a well-established animal model of obesity. The impact of HFD on glucose homeostasis and insulin action in relation to gender differences, however, remains unclear. In this study, we tested the hypothesis that female mice would be protected from HFD-induced glucose intolerance and insulin resistance. To test this, five-week old male and female C57BL/6J mice were placed on control chow diet or 60% HFD for 12 weeks (n=11 chow male; n=6 chow female; n=15 HFD male; n=5 HFD female). Body composition was measured and glucose tolerance testing (GTT; 2g/kg of 50% dextrose) and insulin tolerance testing (ITT; 0.75 units/kg) were performed during the last week of diet. Females had lower body mass compared with males over the 12-week study period. Both genders increased body mass to a similar extent in response to chow diet (24.6±1.7 male vs. 26.5±2.3% female; p=0.517), and HFD (85.0±2.1 males vs. 79.8±6.7% females; p=0.332). HFD females had similar adiposity compared with males (22.9±0.7 males vs. 22.5±1.6% females; p=0.780). HFD reduced insulin sensitivity in male mice [area under the curve (AUC) for blood glucose levels during ITT: -5903±662 chow vs. -1748±1024 HFD; p=0.005] and female mice (-6599±966 chow vs. -2904±1507 HFD; p=0.041), with no differences between genders (p=0.567). HFD also resulted in glucose intolerance in male (AUC for blood glucose levels during GTT: 40262±1769 chow vs. 52800±2502 HFD; p=0.001) and female (36951±2270 chow vs. 55507±3582 HFD; p=0.001) mice, with no gender differences (p=0.582). These data suggest that in contrast to our hypothesis, HFD female mice develop obesity-related insulin resistance and glucose intolerance to a similar extent as males. These findings have important implications for use of this mouse model to study impact of gender on metabolic function in obesity.

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P153

Mineralocorticoid Receptor Inhibition Abolishes Sex-specific, Renin Angiotensin Aldosterone System-associated Salt Sensitivity in Female Mice

PrimaryAuthor.AuthorBlock:**Jessica L Faulkner**, Simone Kennard, Daisy Harwood, Galina Antonova, Jane Morwitzer, Eric J Belin de Chantemele, Augusta Univ, Augusta, GA

Up to half of essential hypertension cases in women are associated with salt sensitive blood pressure (BP) increases, however, the sex-specific mechanisms of salt sensitivity in women are unknown. Our lab has shown that female mice are more sensitive to the hypertensive effects of aldosterone than male mice but it is unknown if aldosterone plays a role in salt sensitivity in female mice. We hypothesized that increasing dietary sodium via a high salt diet would increase blood pressure in female mice which would be abrogated by mineralocorticoid receptor (MR) antagonism. To determine salt sensitivity male and female Balb/C mice were implanted with radiotelemeters for continuous recording of BP. BP was recorded during baseline (7 days) and throughout the administration of a high salt (4% NaCl, HS) diet for 7 days with or without concurrent eplerenone supplementation (daily 200 mg/kg/day). Plasma and kidneys were then harvested. Systolic (SBP) and diastolic (DBP) BP were increased in female mice, but not in males, on HS (7.8±3.3 SBP and 7.8±4.0 DBP Δ change in mmHg in female ($P<0.05$) vs -3.7±3.1 SBP and 3.1±2.1 DBP in male, respectively, n=7-8). Plasma aldosterone levels were decreased in HS male mice compared to control (224±57 vs 151±19 pg/ml, n=3-5), however, increased modestly in HS females (254±56 vs 394±158 pg/ml, n=3-6). Preliminary data indicated that MR antagonism with eplerenone ablated increases in SBP and DBP in HS female mice, while having no effect on blood pressure in HS males (-22.8 SBP and -23.9 DBP Δ change in mmHg in female vs 1.0 SBP and -1.5 DBP in male, n=2). In addition, and in association with an absence of aldosterone suppression with HS, renal mRNA expression of renin (1.4±0.1-fold, $P<0.05$, n=5), angiotensinogen (4.4±0.2-fold, $P<0.05$, n=5), AT1 receptor (52.9±0.9-fold, $P<0.05$, n=5), MR (1.6±0.2-fold, $P<0.05$, n=5) and α-ENAC (1.3±0.0-fold, $P<0.05$, n=5) were increased in HS female mice compared to control females. These data indicate that BP increases in HS female mice are associated with unexpected increases in plasma aldosterone as well as mRNA expression of proteins associated with renin angiotensin aldosterone system activation, which may be novel mechanisms via which females have increased risk for salt sensitive hypertension.

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P155

Renal Responses to Experimental Angiotensin II Induced Hypertension (Ang-HTN) in Female Rats

PrimaryAuthor.AuthorBlock:**Luciana C Veiras**, Jessica Prescott, Donna Ralph, Alicia McDonough, Keck Sch of Med USC, Los Angeles, CA

Compared to males, female Sprague Dawley rats (SDR) at baseline exhibit lower abundance of Na^+/H^+ exchanger (NHE3), Na^+-Pi transporter (NaPi-IIa), paracellular claudin-2 (Cld2), and higher NHE3p (an inactivation marker) as well as lower fractional reabsorption along the proximal tubule (PT); along the distal tubule-collecting duct (DT-CD) females exhibit greater Na-Cl cotransporter (NCC) abundance and activating phosphorylation (NCCp), as well as greater epithelial Na^+ channel (ENaC) subunits'

abundance and activating cleavage. In male SDR, AngII-HTN (400 ng/kg/min, 14 days) increases DT-CD NCC, NCCp and ENaC abundance and activating cleavage, while PT NHE3 and NaPi-IIa are decreased or unchanged, accompanied by a natriuresis and diuresis. This study aimed to determine the effect of AngII-HTN in female SDR using the same protocol applied to males. AngII-HTN: increased BP (tail cuff) from 111 ± 3 to 193 ± 8 mmHg (equivalent to males), increased UV 1.7-fold (vs. 3 fold in males) and decreased UOsm 40% (equivalent to males). AngII-HTN increased: cortical thick ascending limb Na^+-K^+- $2Cl^-$ cotransporter (TAL NKCC2) and NKCC2p in females by 2.2 and 1.8-fold ($p<0.002$), did not change NCC or NCCp abundance, increased ENaC subunits: α by 1.7-fold, cleaved α by 2.4-fold, β by 1.4-fold, cleaved γ by 1.3-fold (all $p< 0.01$). In the PT of females, AngII-HTN increased NHE3p by 1.4-fold, NaPi2 by 1.2-fold and Cld2 by 2.2-fold (all $p<0.005$). In conclusion, despite similar hypertensive and diuretic responses to AngII-HTN in females as males, a distinct pattern of sodium transporters' regulation along the nephron during AngII-HTN is observed in females: 1) abundance of TAL NKCC2, NKCC2p, not increased in males, is increased in females while DCT NCC and NCCp, stimulated in males and higher at baseline in females, is not increased in females, 2) CD ENaCs are similarly stimulated in both sexes, and 3) PT NHE3p, higher at baseline in females, is further increased by AngII consistent with NHE3 inactivation that could contribute to pressure diuresis.
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P156

Estrogen Regulation of Endothelin-B Receptor Mediated Vasodilation

PrimaryAuthor.AuthorBlock:**Katherine Haigh**, Ronald F. Feinberg, Univ of Delaware, Newark, DE; Hugh S. Taylor, Yale Univ Sch of Med, New Haven, CT; Megan M. Wenner, Univ of Delaware, Newark, DE

Our laboratory has recently demonstrated that a loss of endothelin-B (ETB) receptor mediated dilation contributes to impaired vasodilatory function in postmenopausal women. It is unclear if these changes are due to aging, or alterations in ovarian hormones that occur after menopause. The purpose of this study was to test the hypothesis that in a low estradiol state, there is a loss of ETB mediated dilation, and that estradiol administration reverses these responses and mediates dilation. **Methods:** We tested 8 young women (YW: 24±2 years, 23±1 kg/m^2, mean arterial BP 84±2mHg) and 6 postmenopausal women (PMW: 56±1 years, 24±1 kg/m^2, mean arterial BP 94±2mHg). In YW, we suppressed endogenous ovarian hormone production with daily gonadotropin-releasing hormone antagonist (GnRHant; Ganirelix) administration for 10 days, adding estradiol (E2, 0.1 mg/day, Vivelle dot patch) on days 4-10. PMW were tested at baseline and after 1-week E2 administration (0.1 mg/day, Vivelle dot patch). We measured nitric-oxide mediated vasodilation in the cutaneous circulation during local heating (42°C) via laser Doppler flowmetry, followed by microdialysis perfusions of sodium nitroprusside (28mM) with local heating to 43°C to elicit maximal dilation. Cutaneous vascular conductance (CVC) was calculated as cutaneous blood flow/mean arterial blood pressure, and expressed as a percent of maximal dilation.

Results: ETB receptor blockade increased vasodilation in YW during hormone suppression with GnRHant (control: 88±3 vs. BQ-788: 94±2 CVC %max, *P*<0.05). However, ETB receptor blockaded tended to reduce vaodilation during E2 administration (control: 88±3 vs. BQ-788: 82±2 CVC %max, *P*=0.12). In PMW, ETB receptor blockade had no significant effect on vasodilatory responses (control: 90±4 vs. BQ-788: 95±2 CVC %max, *P*=0.20). Similarly, ETB receptor blockade did not alter vasodilation after E2 administration (control: 88±7 vs. BQ-788: 88±4 CVC %max). **Conclusions:** These preliminary data suggest that suppression of endogenous ovarian hormone production alters ETB receptor responses in young women, which is partially mediated by E2. Additional data are needed to determine ETB receptor sensitivity to E2 after menopause.

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P157

Genetic Stratification of Cardiovascular Risk in Patients With Arterial Hypertension

PrimaryAuthor.AuthorBlock:**Vadym Nicolajevich Bondar V**, Kateryna Chernyshova, Helen Kobeleva, Viktoria Tbileli, Yegor Maznichenko, Odessa Natl Medical Univ, Odessa, Ukraine

The purpose of the study was to form a model of genetic stratification of cardiovascular risk (CVR) based on the evaluation of polymorphisms of candidate genes and total gene modification index (GMI), to compare this model with existing clinical stratification scores of CVR and to evaluate the possibility of its use with prognostic purpose in patients with arterial hypertension (AH) in Ukrainian population. Materials and methods. 240 patients with AH were examined and stratificated depend from prognostic CVR according to ESC 2013. The patients were divided into 4 groups: with low, moderate, high or very high CVR. Then we performed the analysis of polymorphisms of the following candidate genes by PCR: ADD1:1378, AGT:704, AGT: 521, AGTR1:1166, AGTR2: 1675,CYP11B2:-344, GNB3:825, NOS3:-786, NOS3:894. We formed GMI, in which the proportion of "pathological" homozygous polymorphism of one gene was 1.5 points, the heterozygous polymorphism - 1 point, "normal" genotype - 0 points. Then points were summed up and divided by maximum possible amount and GMI was formed as proportion of the "pathological" genotypes, expressed as a percentage. We offered the genetic stratification, in which GMI from 0 to 20% was considered as low genetic risk, from 21 to 40 % - moderate risk, from 41 to 70% - high risk, from 71 to 100% - very high risk. The statistical analysis of correlations was made between the traditional and genetic stratification scores. Results. As a result of traditional stratification of risk factors in group with low CVR were 24 patients, while in 75% of patients was similar low genetic risk (GR) according to the GMI (r=0,72, p<0,01). In group with moderate CVR were 96 patients, of which 75% had moderate GR (r=0,71, p<0,01). In group with high CVR were 72 patients, 72% of them had high GR (r=0,71, p<0,01). In group with very high CVR were 48 patients, 67% of them had same very high GR (r=0,70, p<0,05). Thus, proposed genetic stratification risk score based on

evaluation of proportion of "pathological" genotypes of candidate genes, has a significantly high correlation with traditional stratification score of CVR and can be used with prognostic purpose in patients with AH. Genetic stratification risk score can have obvious benefits for the purposes of primary prevention of AH.

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MicroRNA Profiling in Peripheral Blood Mononuclear Cells From Hypertensive Patients With or Without Chronic Kidney Disease

PrimaryAuthor.AuthorBlock:**Ku-Geng Huo**, Julio C Fraulob-Aquino, Lady Davis Inst, Montreal, QC, Canada; Chantal Richer, Div of Hematology-Oncology, Res Ctr, CHU Ste-Justine, Montreal, QC, Canada; Asia Rehman, Lady Davis Inst, Montreal, QC, Canada; Marie Briet, Ctr Hospo-Univire d'Angers, PARIS, France; Pierre Boutouyrie, Univ Paris Descartes, PARIS, France; Mark Lipman, Lady Davis Inst, Montreal, QC, Canada; Daniel Sinnett, Div of Hematology-Oncology, Res Ctr, CHU Ste-Justine, Montreal, QC, Canada; Pierre Paradis, Ernesto L. Schiffrin, Lady Davis Inst, Montreal, QC, Canada

Background: Hypertension (HTN) and chronic kidney disease (CKD) are global health disorders that are epidemiologically associated. The immune system has been shown to play a role in HTN and CKD in animal models. Activation of T cells has been observed in peripheral blood mononuclear cells (PBMCs) of patients with HTN. MicroRNAs (miRNAs) are crucial post-transcriptional regulators of immune cells. Whether miRNAs play a role in the activation of immune cells in HTN and CKD in humans remains unknown. We aimed to address this question by identifying differentially expressed (DE) miRNAs and their mRNA targets in PBMCs of HTN and CKD patients. **Methods and results:** Normotensive, HTN (systolic blood pressure (BP) >135 mm Hg or diastolic BP of 85-115 mm Hg with BpTRU) and CKD subjects (estimated glomerular filtration rate <60mL/min/m^2) (n=15-16) were studied. PBMCs were isolated from 30 ml of whole blood and used for total RNA extraction with the mirVana miRNA isolation kit. cDNA libraries were prepared using the TruSeq small RNA prep kit and the TruSeq stranded total RNA prep kit, and were sequenced by Illumina HiSeq 2500. DE miRNAs (P<0.05) were identified using EdgeR, which found 41 up- and 38 down-regulated miRNAs, as well as 101 up- and 316 down-regulated mRNAs uniquely associated with the hypertensive group, while 11 up- and 18 down-regulated miRNAs, as well as 153 up- and 73 down-regulated mRNAs were found uniquely associated with the CKD group. Meanwhile, 4 up- and 8 down-regulated miRNAs, as well as 13 up- and 19 down-regulated mRNAs were found in both groups. Target Scan was used to predict DE miRNA targets in the DE mRNAs. Enrichment analysis showed that the HTN-associated DE miRNA-targeting DE genes were highly enriched in gene ontology (GO) terms involved in cytosolic transport, protein kinase B (PKB) signalling and RNA 3’ processing (q<0.001), while the CKD-associated DE miRNA-targeting DE genes were highly enriched in GO terms involved in immune response, ribosomal process and metal ion homeostasis (q<0.001). **Conclusions:** DE miRNAs were identified in PBMCs of HTN and CKD patients. Enrichment analysis in DE miRNA-targeting DE mRNAs revealed GO terms that could be linked to different degrees of immune cell activation in HTN and CKD.

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MicroRNA Profiling in Small Resistance Arteries of Hypertensive Patients With or Without Chronic Kidney Disease

PrimaryAuthor.AuthorBlock:**Ku-Geng Huo**, Julio C Fraulob-Aquino, Lady Davis Inst, Montreal, QC, Canada; Chantal Richer, Div of Hematology-Oncology, Res Ctr, CHU Ste-Justine, Montreal, QC, Canada; Asia Rehman, Lady Davis Inst, Montreal, QC, Canada; Marie Briet, Ctr Hospo-Univire d'Angers, PARIS, France; Pierre Boutouyrie, Univ Paris Descartes, PARIS, France; Mark Lipman, Lady Davis Inst, Montreal, QC, Canada; Daniel Sinnett, Div of Hematology-Oncology, Res Ctr, CHU Ste-Justine, Montreal, QC, Canada; Pierre Paradis, Ernesto L. Schiffrin, Lady Davis Inst, Montreal, QC, Canada

Background: Hypertension (HTN) and chronic kidney disease (CKD) are global health disorders that are epidemiologically associated. Vascular injury is an early manifestation in HTN and contributes to CKD. It is characterized by endothelial dysfunction and vascular remodeling that are accompanied by gene expression changes. MicroRNAs (miRs) are important non-coding RNA regulators of gene expression. Dysregulation of miRs has been shown in HTN and CKD, but their implication in vascular injury remains unclear. We aimed to identify differentially expressed (DE) miRs in small arteries of HTN and CKD human subjects to get further insight into pathophysiological molecular mechanisms in these conditions.
Methods and results: Normotensive, HTN (systolic blood pressure (BP) > 135 mm Hg or diastolic BP of 85-115 mm Hg with BpTRU) and CKD subjects (estimated glomerular filtration rate <60mL/min/m^2) (n=15-16) were studied. Small arteries were dissected from a subcutaneous gluteal biopsy under RNAse free condition and used for total RNA extraction with the mirVana miR isolation kit. cDNA libraries were prepared using the TruSeq small RNA prep kit and the TruSeq stranded total RNA prep kit, and sequenced by Illumina HiSeq 2500. DE miRs and DE mRNAs (P<0.05) were identified using EdgeR, which found 3 up- and 6 down-regulated miRs, as well as 134 up- and 149 down-regulated mRNAs uniquely associated with HTN, 42 up- and 39 down-regulated miRs, as well as 743 up- and 348 down-regulated mRNAs uniquely associated with CKD, while 2 up-regulated miRs and 101 up- and 75 down-regulated mRNAs were found in both groups. Target Scan was used to predict DE miR targets in the DE mRNAs. Enrichment analysis showed that the HTN-associated DE miR-targeting DE mRNAs were highly enriched in gene ontology (GO) terms involved in peptidase activity, mitochondrial activity and immune response (q<0.01), while the CKD-associated DE miR-targeting DE genes were highly enriched in GO terms involved in tube formation, fibroblast proliferation and EGF response (q<0.001).
Conclusions: DE miRs were identified in small arteries of HTN and CKD patients. Enrichment analysis in DE miR-targeting DE mRNAs revealed

GO terms that could be linked to different degrees of vascular changes in HTN and CKD.

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P160

A Genome-spanning Map of Chromatin Structure Near the Renin Proximal Promoter

PrimaryAuthor.AuthorBlock:**Timothy Stodola**, Pengyuan Liu, Yong Liu, Andrew Vallejos, Aron Geurts, Andrew Greene, Mingyu Liang, Medical Coll of Wisconsin, Milwaukee, WI

Chromatin conformation capture technologies (3C, 4C-seq) allow mapping of the three dimensional spatial structure of the genome. Physical proximity mapping, which captures these 3 dimensional interactions, fills an important gap in our understanding of enhancer-gene relationship by linking possible regulatory regions to specific genes that may be distant from the enhancer by thousands or millions of base pairs or even located on different chromosomes. The goal of the current study was to expand our understanding of renin regulation by identifying regions containing potential regulatory elements of renin more than a few kilobases from the renin proximal promoter, using 4C-seq technology, in isolated SS/JrHrdMcwi cardiac microvascular endothelial cells. Three replicates of ten million cells were fixed, digested with EcoRI, ligated, and then decrosslinked. Secondary digestion with NlaIII followed by a second ligation captured an unknown sequence surrounded by the sequence of the renin promoter. A sequencing library was prepared using primers specific to the renin promoter to identify the captured sequences. Sequence reads were mapped to rat genome (Rn 5) divided into fragments cut by EcoRI. Reads were verified to contain the renin proximal promoter sequence and a uniquely mapped captured sequence. The read counts were binarized and Z-scores were calculated based on a windowed average reads relative to background, and Z-scores corresponding to FDR < 0.01 were considered significant. We found 62 loci spanning the genome in contact with the renin proximal promoter. Clusters of interactions were found on Chr13 at 39.6-40.5Mpb and at 53.9-56.3Mbp where the Renin gene is located. Additional contacting loci were found on all chromosomes except X. Quantitative PCR in newly isolated endothelial cells processed as 3C samples (fixed, digested, ligated, decrosslinked) were used to test 9 of the interactions, of which 8 were validated. These loci are enriched with genes whose expression is correlated with renin (53 of 268 genes, $p = 7x10^{-10}$) in humans, rats and mice. The present study generated a genome-wide map of segments physically interacting with the renin proximal promoter producing the first such map for a gene that is essential for cardiovascular physiology.

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Inhibition of Class I HDAC Enhances the Expression of *Npr1* and Repairs Renal Pathology in Gene-targeted Haplotype Mice

PrimaryAuthor.AuthorBlock:**Prerna Kumar**, Venkateshwara R. Gogulamudi, Christian Nguyen, Ramachandran Samivel, Kailash N. Pandey, Tulane Univ, New Orleans, LA

The objective of the present study was to determine the effect of class I histone deacetylase (HDAC) inhibitor, mocetinostat (MGCD0103) on the expression of *Npr1* (coding for natriuretic peptide receptor-A; NPRA) and renal pathology in *Npr1* gene-disrupted haplotype (50% of normal) mice. Adult male *Npr1* haplotype (1-copy; $Npr1^{+/-}$), wild-type (2-copy; $Npr1^{+/+}$), and gene-duplicated (3-copy; $Npr1^{++/+}$) mice were injected intraperitonealy with MGCD0103 (2 mg/kg) at alternate days for 2-weeks. After MGCD0103 treatment, the renal *Npr1* mRNA was increased in $Npr1^{+/-}$ mice by 7.3-fold ($p < 0.01$), $Npr1^{+/+}$ mice by 4.4-fold ($p < 0.05$) and $Npr1^{++/+}$ mice by 3.6-fold ($p < 0.05$) compared with vehicle-treated controls. The MGCD0103 also enhanced renal cGMP levels (pmol/mg protein) in $Npr1^{+/-}$ (57 ± 7 vs. control, 12 ± 1; $p < 0.01$), $Npr1^{+/+}$ (125 ± 9 vs. control, 66 ± 5; $p < 0.01$), and $Npr1^{++/+}$ (202 ± 7 vs. control, 127 ± 15; $p < 0.05$) mice. An increased HDAC activity (ng/min/mg protein) was observed in $Npr1^{+/-}$ mice (24.4 ± 2.8; $p < 0.05$); however, $Npr1^{++/+}$ mice showed lower HDAC activity (6.4 ± 0.7; $p < 0.05$); compared with $Npr1^{+/+}$ mice (15.9 ± 1.3). Treatment with MGCD0103 significantly attenuated HDAC activity by almost 50% (15.3 ± 1.2; $p < 0.01$) in *Npr1* haplotype mice. A significant decrease in systolic blood pressure was observed in MGCD0103-treated $Npr1^{+/-}$ mice (106 ± 0.6 vs. control, 131 ± 1.9, $p < 0.001$). Significantly lower creatinine clearance (µl/min) was observed in $Npr1^{+/-}$ (51 ± 11; $p < 0.05$) vs. $Npr1^{+/+}$ mice (130 ± 14.0; $p < 0.05$) and treatment with MGCD0103 increased creatinine clearance in $Npr1^{+/-}$ mice (105 ± 13; $p < 0.05$) vs. controls. Higher urinary albumin to creatinine ratio was detected in $Npr1^{+/-}$ mice (0.7 ± 0.05) vs. $Npr1^{+/+}$ animals (0.5 ± 0.05; $p < 0.05$) and a complete reversal was observed in drug-treated $Npr1^{+/-}$ mice (0.4 ± 0.04). The present results provide direct evidence that class I HDAC inhibition upregulates NPRA expression *in vivo* and repairs the renal pathology in $Npr1^{+/-}$ haplotype mice. The present findings will have important implications in the treatment and prevention of hypertension and renal pathophysiological conditions.

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P162

***In vivo* miR-431 Inhibition Protects Against Vascular Damage and Hypertension**

PrimaryAuthor.AuthorBlock:**Ku-Geng Huo**, Julio C. Fraulob-Aquino, Tlili Barhoumi, Lady Davis Inst, Montreal, QC, Canada; Chantal Richer, Div of Hematology-Oncology, Res Ctr, CHU Ste-Justine, Montreal, QC, Canada; Suellen C. Coelho, Sofiane Ouerd, Antoine Caillon, Lady Davis Inst, Montreal, QC, Canada; Mathieu Lajoie, Daniel Sinnett, Div of Hematology-Oncology, Res Ctr, CHU Ste-Justine, Montreal, QC, Canada; Pierre Paradis, Ernesto L. Schiffrin, Lady Davis Inst, Montreal, QC, Canada

Background: Vascular injury is an early manifestation of hypertension. microRNAs (miRNAs) play an important role in

cardiovascular disease, but their implication in vascular injury remains unclear. Using small and total RNA sequencing, we identified in murine mesenteric arteries (MAs) a conserved angiotensin (Ang) II-upregulated Dlk1-Dio3 miRNA miR-431 that correlated with blood pressure (BP), and an Ang II-downregulated BP-correlated conserved putative miR-431 target, the transcriptional factor ETS homologous factor (*Ehf*). miR-431 might be involved in vascular remodeling as *Ehf* regulates expression of extracellular matrix genes including alpha-1 type I collagen (*Col1a1*) and of other Dlk1-Dio3 miRNAs. In this study, we proposed to validate the miR-431-*Ehf*-*Col1a1* interaction *in vitro* and *in vivo*, and determine whether miR-431 inhibition antagonizes angiotensin (Ang) II-induced hypertension and vascular injury. **Methods and results:** Transfection of miR-431 mimics into human aortic smooth muscle cells decreased *Ehf* expression (0.13±0.05 fold, *P*<0.001) and increased *Col1a1* (1.7±0.5 fold, *P*<0.01), whereas miR-431 inhibitors increased *Ehf* (1.5±0.2 fold, *P*<0.001) and decreased *Col1a1* (0.89±0.11 fold, *P*<0.05). *Ehf* siRNA transfection increased 1.2±0.1 fold *Col1a1* (*P*<0.01). Co-transfection of miR-431 mimics with luciferase reporter vectors that contain the wild-type but not mutated miR-431 human *EHF* 3' UTR binding site decreased 0.51±0.01 fold (*P*<0.05) luciferase expression compared to scrambled mimics. miR-431 inhibitor IV injection in mice at day 0 and 7 of Ang II infusion decreased miR-431 (0.16±0.05 fold, *P*<0.01), *Col1a1* (0.58±0.11 fold, *P*<0.05), increased *Ehf* (2.9±0.8 fold, *P*<0.05) in MAs, delayed BP elevation (*P*<0.01), improved endothelium-dependent relaxation (33±8 vs 64±7%, *P*<0.05) and reduced vascular stiffness (strain at 140mmHg: 0.68±0.02 vs 0.58±0.02 ΔD/D, *P*<0.01) compared to scrambled mimics-injected Ang II-infused mice. **Conclusion and perspectives:** miR-431 and its target *Ehf* may act as master regulators in the pathophysiology of vascular damage in hypertension. miR-431 inhibition has the potential to serve as a novel therapeutic approach for treatment of vascular damage and hypertension.

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P167

Aging Exacerbates Increased MAP and Proteinuria in Male Rat Offspring of Hyperandrogenemic Mothers

PrimaryAuthor.AuthorBlock:**Carolina Dalmasso**, Rodrigo O. Maranon, Chetan N. Patil, Jane F. Reckelhoff, UMMC, Jackson, MS

Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting 6-10 % of women, and is associated with hyperandrogenemia, oligo/anovulation and polycystic ovaries. Many women with PCOS have difficulty getting pregnant and, when they do, the infants are predisposed to preterm birth, metabolic disorders during childhood, and potential risk for the daughters to develop PCOS. The mechanisms by which the offspring of women with PCOS develop negative health outcomes are unknown. Our lab has already characterized

the phenotype of young PCOS offspring showing an increased MAP and proteinuria in the males. In this study we tested the hypothesis that aging will exacerbate the negative health outcomes observed in the offspring of PCOS dams. Twelve months old female (F) and male (M) offspring of control (C) and PCOS (P) dams (SD; DHT 7.5mg/90d) were divided into 4 groups: F-C, F-P, M-C and M-P. At 12 months old, there is a significant decrease in body weight in F-P and M-P, compared to F-C and M-C, respectively (F-P: 284±4 vs. F-C: 301±5; M-P: 533±8 vs. M-C: 575±8 mg/24h, $p<0.001$) and the body weight remains lower until 18 months of age. Body composition analysis showed that there is a decrease in lean mass in F-P and M-P, compared to F-C and M-C, respectively (F-P: 284±4 vs. F-C: 301±5; M-P: 533±8 vs. M-C: 575±8 mg/24h, $p<0.01$). Proteinuria at 12 months, is higher in F-P and M-P, compared to F-C and M-C, respectively (F-P: 18±2 vs. F-C: 9±1; M-P: 169±8 vs. M-C: 108±6 mg/24h, $p<0.001$). This increased proteinuria is still observed at 15 months (F-P: 33±5 vs. F-C: 5±1; M-P: 143±7 vs. M-C: 103±4 mg/24h, $p<0.001$). Baseline mean arterial pressure (MAP, telemetry, 17 months old) was similar F-P vs. F-C (F-C: 109±2 mmHg vs. F-P: 107±1 mmHg; p=NS). However, in males, MAP was significantly higher in M-P than M-C (M-C: 114±1 mmHg vs. M-P: 127±1 mmHg; $p<0.05$). These results showed that aging exacerbates the increased MAP and proteinuria in males. Female offspring do not develop a PCOS phenotype with aging. Future studies will be necessary to determine the exact mechanisms responsible for the increase in blood pressure and renal injury.

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P168

Prevalence of Spontaneous Hypertension in African Green Monkeys is Age-related

PrimaryAuthor.AuthorBlock:**Megan K Rhoads**, Jeffrey Osborn, Univ of Kentucky, Lexington, KY

Chlorocebus aethiops sabaeus, the African Green Monkey (AGM), is a novel translational model of hypertension and cardiovascular disease. The AGM is a diurnal, primarily bipedal nonhuman primate, in which ~ 1/3 of the population exhibits spontaneous hypertension (systolic blood pressure > 140mmHg). Since age and sex are related to the development of human hypertension, we hypothesized that age and sex may be crucial to the development of hypertension in this primate species. Hypertension prevalence was compared in animals with known ages or highly confident age estimates. Age estimates were performed by staff with over 50 years combined experience working with AGMs. Behavior, weight, incisor length, fur coarseness, tail stiffness, and troop hierarchy were used to reasonably estimate an animal's age. Animals were binned into 1 of 4 age categories: juvenile, 0-4 years; young adult, 5-10 years; adult, 11-15 years; or mature adult, ≥ 16 years. Overall, hypertensive (HT) AGMs comprised 31.3±22.8 % (mean± 95%CI) of the juvenile group, n=16; 31.9±6.6% of the young adult group, n=244; 48.3±12.6% of the adult group, n=60; and 61±18.1% of the mature adult group, n=28. The prevalence of HT is greater in the adult and mature adult groups ($Z = -2.3$, $p<0.05$; $Z = -2.9$, $p<0.05$, respectively) compared to the young adult age group. No significant differences in prevalence were found between sexes within the same age ($p>0.05$). In males, HT animals

comprised: 30±28.4% of the juvenile group, n=10; 28.9±8.1% of the young adult group, n=121; 50±13.6% of the adult group, n=52; and 61.5±18.7% of the mature adult group, n=26. In females, HT animals comprised: 33.3±38.1% of the juvenile group, n=6; 36.9±11.1% of the young adult group, n=73; 37.5±33.5% of the adult group, n=8; and 50±50% of the mature adult group, n=2. In males, the prevalence of HT is increased in the adult ($Z = -2.6$, $p<0.05$) and the mature adult age groups ($Z = -3.1$, $p<0.05$) compared to the young adult group. No differences were found in female age groups. Similar to humans, age is a strong contributor to the development of spontaneous hypertension in the AGM. Sex may contribute to the development of AGM spontaneous hypertension and future studies will assess the impact of age, sex hormones, and hypertension in female AGMs.

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P169

Difference of Risk Factors for Controlled Blood Pressure in Three Types of Heart Failure

PrimaryAuthor.AuthorBlock:**Hong Seok Lee**, New York Medical Coll, Univ of California Riverside, New York, NY; Nunez Belen, New York Medical Coll, New York, Riverside, NY; Hans Cativo, Amrut Savadkar, Visco Ferdinand, Savi Mushiyev, Gerald Pekler, New York Medical Coll, New York, NY

Background: Hypertension is the most important modifiable risk factor for worsening heart failure (HF) because hypertension increases cardiac work, which results in worsening left ventricular hypertrophy and development of coronary artery disease. We will determine risk fctors of BP control in different types of heart failure according to JNC 8 guideline. **Method:** Based on ACC/AHA guidelines, heart failure is classified as a reduced ejection fraction(HFrEF, EF <40), preserved ejection fraction (HFpEF, EF>50) and heart failure with an improved ejection fraction(HFpEF(i),EF≥40). 732 patients enrolled in our heart failure program were analyzed retrospectively. And 672 patients who had been followed from Jan 1st ,2013 to June 30st 2015 were included. Multiple logistic regression analysis was performed to determine the relationship between hypertension and heart failure after adjusting for potential confounders. **Results:** Patients with three types of heart failure had different BP control rate. It was 67.5% (308/456) ,76.5%(104/136), 77.5%(62/80) in HFrEF, HFrEF, and HFpEF(i) based on JNC 8 guideline, respectively. Mean systolic BP was 127.1±17 mmHg in HFrEF, 129.0±21 mmHg in HFpEF and 124.4±18 mmHg in HFpEF(i). Obesity [Odds ratio (OR): 0.12,95% Confidence Interval(CI): 0.048-0.284] , ACE inhibitor or ARB [OR: 2.66, CI: 1.50-3.42] and lasix [OR: 1.90,CI: 1.07-3.40] and aspirin [OR 0.53, CI: 0.37-0.96] were noted to be related to controlled BP in HFrEF. Aspirin [OR 0.17, CI: 0.05-0.60] was significantly associated with controlled BP in HFpEF. And beta-blocker [OR: 0.07, CI: 0.01-0.62] and anti-lipid medication [OR: 4.76, CI: 1.73-5.89] were associated with BP control in HFpEF(i). **Conclusion:** In each type of heart failure, there was difference of risk factors related to BP control. Different medications were associated with control of BP

in different types of heart failure. Patients may need to modify risk factors including types of medication to control BP according to types of heart failure. It might be leading to better heart failure management.

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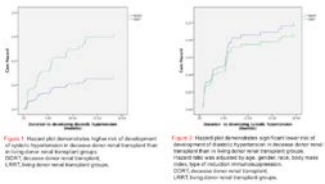
P170

Hypertensive Pattern After Kidney Transplantation

PrimaryAuthor.AuthorBlock:**Ekamol Tantisattamo**, Multi-Organ Transplant Ctr, Div of Nephrology, Dept of Internal Med, Oakland Univ William Beaumont Sch of Med, William Beaumont Hosp, Royal Oak, MI; Possawat Vutthikraivit, Phramongkutklao Coll of Med, Mahidol Univ, Bangkok, Thailand; Haritha Mopuru, Multi-Organ Transplant Ctr, William Beaumont Hosp, Royal Oak, MI

Background: The patterns and factors contributing to post-transplant hypertension are unknown. ***Methods:*** Seventy kidney transplant recipients enrolled during a 10-month period were divided into deceased donor renal transplant (DDRT) or living donor renal transplant (LDRT) groups. Risk of development for HTN (SBP ≥140 or DBP ≥90 mmHg) between these 2 groups were compared. ***Results:*** There was 50 patients (71.4%) in a DDRT group. Mean age±SEM of the DDRT and LRRT were 52.8±1.7 and 52.3±2.7 years, respectively (p=0.872). Mean pre-transplant SBP and DBP were not significantly different between both groups. DDRT group was at greater risk to develop post-transplant systolic HTN than the LRRT group but not statistically significant (HR 1.145, CI 0.616 - 2.130, p=0.668; Figure 1). However, the risk to develop diastolic HTN was significantly lower in DDRT than LRRT groups (HR 0.506, CI 0.265 - 0.965, p=0.039). The risk of developing post-transplant diastolic HTN were not modified by age (<65 and ≥65 years old; HR of interaction 0.246, CI 0.025 - 0.450, p=0.232). After controlling for several factors including age, the risk for developing diastolic HTN was 0.422 times lower in DRRT than that of LRRT group (CI 0.187 - 0.956, p=0.039; Figure 2). ***Conclusion:*** DDRT recipients are at higher risk of developing systolic HTN but lower risk for diastolic HTN in DDRT recipients. This pattern of post-transplant HTN is similar to non-transplant elderly patients, whose pulse pressures are widened. Since there was no difference in mean age between DDRT and LRRT groups, underlying process apart from age may affect post-transplant HTN in DDRT and subsequently poorer renal allograft survival in DDRT than that of LRRT.



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P171

Smaller Body Mass Index is Associated With Mild Cognitive Impairment in Elderly Hypertensive Patients

PrimaryAuthor.AuthorBlock:**Yoshihiro Saito**, Joji Ishikawa, Ayumi Toba, Ayumi Suzuki, Yoshiaki Tamura, Atsushi Araki, Kazumasa Harada, Tokyo Metropolitan Geriatric HP, Tokyo, Japan

Backgrounds: In elderly hypertensive patients, it might be important to pay attention to cognitive function when achieving aggressive blood pressure goal; therefore we evaluated factors associated with cognitive impairment in elderly hypertensive patients. **Methods:** We performed Mini Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA) in 209 elderly hypertensive patients who were evaluated frailty (Aged $\geq$ 65 years, Male 38.3 %). Dementia was diagnosed as MMSE scores $\leq$23 and/or anti-dementia drug use; mild cognitive impairment (MCI), MoCA<24 points and MMSE$\geq$24 points. **Results**: Mean age was 78.6±6.2 years. There were 84.2 % of patients who were taking antihypertensive drug. Clinic blood pressure was 133.2±17.6 / 74.3±11.9 mmHg and body mass index (BMI) was 23.7±7.6 kg/m^2. MMSE score was 27.0±3.8 and MoCA was 21.1±4.9 points. There were 53.0 % of patients with MCI; 12.9 % with dementia. Patients with MCI and dementia had lower BMI than those without (MCI group, BMI=23.2±3.5 kg/m^2; dementia group, 23.4±2.7 kg/m^2; no MCI or dementia group, 24.8±3.9 kg/m^2; P=0.012). The risk of MCI increased by 13.2 % per smaller BMI of 1 kg/m^2 (P=0.006); that of dementia increased by 18.6 % per smaller BMI of 1 kg/m^2 (P=0.055) after adjustment for age, gender, antihypertensive drugs use, diabetes, dyslipidemia, smoking habit, alcohol drinking, history of stroke, and systolic and diastolic blood pressure. Patients with the lowest quartile of BMI (< 21.34 kg/m2) had 3.82 (95%CI 1.42-10.27) times greater risk of MCI and had 7.59 (95%CI 1.22-47.27) times greater risk of dementia than those with highest quartile of BMI (> 25.69 kg/m2). **Conclusion:** In elderly hypertensive outpatients who were suspected of frailty, smaller BMI was associated with presence of MCI and dementia, suggested that we needed to decide optimal blood pressure level carefully in lean hypertensive patients.

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P172

Methylation of Mitochondrial Genes and Mitochondrial Damage in Aged Kidney of Hypertensive Mice

PrimaryAuthor.AuthorBlock:**Lu Ren**, Utpal Sen, Sathnur Pushpakumar, Univ of Louisville, Louisville, KY

Hypertensive nephropathy is a major global health issue. Accumulating evidence from recent studies suggest that mitochondrial injury may contribute to the pathogenesis of renal damage in hypertension leading to glomerular and tubular necrosis. It is well-known that aging is associated with declining mitochondrial function in several organ systems. However, the role of aging and its correlation with mitochondrial injury and kidney damage in hypertension remains unclear. The purpose of the present study was to determine whether methylation of mitochondrial genes contributes to mitochondrial dysfunction in the aged kidney in Ang-II induced hypertension. Wild type (WT, C57BL/6J) mice aged 12-14 wk and 75-78 wk were used in this study and treated without or with Ang-II (1000 ng/kg/min) for 4 weeks. In response to Ang-II, aged mice exhibited higher blood pressure (145.21±5.32 vs. 127.32±3.47 mmHg) and reduced glomerular filtration rate compared to young mice (362.7±8.3 vs.

825±10.2 µL/min/100 g b.w.). Ang-II upregulated DNA methyltransferases and increased Dnmt activity in aged kidney and bisulfite conversion and methylation specific PCR revealed increased methylation of mitochondrial superoxide dismutases 1 and 2 and Sirtuin-1 genes which correlated with lower protein levels suggesting impaired anti-oxidant defense. Aged mice exhibited reduced mitochondrial biogenesis as suggested by downregulation of peroxisome proliferator-activated receptor (PPAR)-α and -γ, and PPAR-γ-co-activator (PGC)-1α. Furthermore, reduction of optic atrophy 1 and mitofusin-2 indicated increased mitochondrial fragmentation and downregulation of ATP synthase was associated with impaired ATP generation in aged mice receiving Ang-II compared to young mice. Our results suggest that increased DNA methylation of mitochondrial antioxidant genes leads to oxidative stress induced mitochondrial damage in aged kidney during hypertension.

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P173

Target Blood Pressure in Elderly Hypertensive Patients: A Korean National Health Insurance Service Health Examinee Cohort Study

PrimaryAuthor.AuthorBlock:**Jiwon Seo**, Sungha Park, Chan Joo Lee, Yonsei Univ Coll of Med, Seoul, Korea, Republic of; Jinseub Hwang, Daegu Univ, Daegu, Korea, Republic of

Background: We aimed to investigate the benefits of strict blood pressure control and determine the optimal blood pressure in elderly hypertensive subjects.

Methods: This study used the National Health Insurance Service Health examinee Sample Cohort. We selected subjects who were over the age of 60 years and who had received a newly diagnosis of hypertension (Korean Classification of Disease [KCD] codes I10–I13) from 2003 to 2006. Subjects who had previous major cardiovascular (CV) events, such as heart failure, ischemic heart disease, stroke, or end stage renal disease (ESRD) were excluded. The remaining 25,858 individuals were divided into three groups; 1) mean systolic blood pressure (SBP) < 130mmHg (N = 7,494), 2) mean SBP 130 to 140 mmHg (N = 9,272), 3) mean SBP above 140mmHg (N = 9,092). The primary outcome of the study was to compare all-cause mortality and CV mortality in each group.

Results: Compared to mean SBP ≥140 mmHg group, mean SBP 130 to 140 mmHg group had significantly lower rate of all-cause mortality (HR=0.63, 95% CI= 0.58-0.69, p<0.001) and CV mortality (HR=0.58, 95% CI= 0.48-0.69, p<0.001). The diastolic blood pressure (DBP) between 80 to 90 mmHg group also had lower risk of all-cause death (HR=0.51, 95% CI= 0.47-0.56, p<0.001) and CV death (HR=0.44, 95% CI= 0.37-0.53, p<0.001) in comparison with DBP ≥90 mmHg group. However, the SBP below 130 mmHg subjects and the DBP below 80 mmHg subjects did not have significant lower rate of all-cause and CV mortality compared to SBP 130 to 140 mmHg and DBP 80 to 90mmHg subjects, respectively. In subgroup analysis, mean SBP <120 mmHg subjects were associated with higher risk of all-cause death (HR=1.47, 95% CI= 1.27-1.72, p<0.001) and CV death (HR=1.57, 95% CI= 1.15-2.14, p=0.004) than SBP 130 to 140 mmHg subjects.

Conclusion: Mean SBP between 130 to 140 mmHg and mean DBP below 90 mmHg for elderly hypertensives without previous major CV disease was beneficial in reducing all-cause and CV mortality. However, mean SBP below 120mmHg was associated with a raised risk of all-cause mortality and CV mortality.

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P174

Free Triiodothyronine Level Correlates with Statin Responsiveness in Acute Myocardial Infarction

PrimaryAuthor.AuthorBlock:**Kuo Zhang**, Wenyao Wang, Fuwai Hosp, Beijing, China; A. Martin Gerdes, New York Inst of Technology, Old Westbury, NY; Yi-Da Tang, Fuwai Hosp, Beijing, China

Background: Although thyroid hormone (TH) has important effects on lipid metabolism, the relationship between TH and statin responsiveness has never been investigated. We hypothesized that TH plays an important role in statin responsiveness in AMI patients.
Methods: Consecutive 1091 hospitalized AMI patients in Fuwai hospital were enrolled. The study population was divided into three groups based on the intensity of statin treatment: low (n=221), moderate (n=712) and high (n=158). Lipid levels were measured after statin therapy lasting for 10-14 days. We explored the association between TH, lipid levels and achievement of low density lipoprotein cholesterol (LDL-C) lowering goals in patients with acute myocardial infarction (AMI) on statin therapy.
Results: By general linear analysis, a significant linear trend between FT3 and LDL-C level (linear coefficient=-0.082, P =0.001) and FT3 and total cholesterol (TC) level (linear coefficient=-0.105, P =0.031) was observed in the moderate-intensity statin group. A more apparent linear trend was detected in the high-intensity statin group (for LDL-C: linear coefficient=-0.113, P =0.005; for TC: linear coefficient=-0.172, P=0.029, respectively). However, no significant correlation was observed in the low-intensity statin group. Compared with the low-T3 group (defined as FT3<1.79 pg/ml), the OR (95% CI) for attaining a LDL-C<3.0mmol/L was found to be 2.217 (1.001-4.839) in the higher FT3 group (>2.95 pg/ml).The OR for attaining the more intensive goal (LDL-C<1.8mmol/L) (95% CI) was 2.836 (1.014-5.182).
Conclusions: Variation in FT3 levels is related to the lipid-lowering responsiveness of statins in AMI patients.

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P175

Chronic Exercise Attenuates Blood Pressure Elevation and Albuminuria With Improvement of Renal Lipid Metabolism in High Fructose-fed Rats

PrimaryAuthor.AuthorBlock:**Gaizun Hu**, Graduate Sch of Med, Tohoku Univ, sendai, Japan; Osamu Ito, Tohoku Medical and Pharmaceutical Univ, sendai, Japan; Lusi Xu, Graduate Sch of Med, Tohoku Univ, sendai, Japan

High fructose diet (HFr) can lead to metabolic disorder, hypertension, and renal disease. Although chronic exercise (Ex) provides various beneficial effects on hypertension and kidney disease, the precise mechanism is not fully clarified. Thus, present study examined the effects of Ex on the blood pressure, renal function and renal lipid metabolism in rats fed with HFr. Sprague-Dawley rats were allocated to 3 groups. The HFr and Ex groups were fed

with HFr (60%, w/w), the control group was fed with the diet in which fructose was replaced by starch. The Ex group underwent treadmill exercise at aerobic intensity. After 12 weeks, renal triglyceride (TG) content were measured, and expression of enzymes and regulators of fatty acid metabolism were analyzed by Western blot. HFr increased systolic blood pressure (SBP) and albuminuria and Ex decreased the HFr-increased SBP and albuminuria (85±4 vs. 122±9 vs. 91±4 mmHg, P<0.01, 326±67 vs. 534±79 vs. 176±54 mg/day, P<0.01). HFr increased plasma TG and uric acid (UA) and Ex decreased the HFr-increased TG and UA (124±20 vs. 474±35 vs. 238±23 mg/dL, P<0.01, 1.15±0.10 vs. 2.14±0.10 vs. 1.50±0.13 mg/dL, P<0.01), whereas HFr or Ex did not affect plasma creatinine. HFr increased renal TG content and Ex decreased the HFr-increased TG content (12.2±0.5 vs. 14.1±0.5 vs. 10.3±1.1 mg/100mg tissue, P<0.01). Among enzymes of fatty acid synthesis, HFr increased the renal expression of fatty acid synthase (FAS), and Ex decrease the expression of FAS (P<0.01). Among enzymes and regulators of fatty acid oxidation, HFr decreased the renal expression of PPARα, carnitine palmitoyltransferase I (CPTI), medium-chain acyl-CoA dehydrogenase (MCAD) (P<0.05), and Ex increased the expression of PPARα, CPTI, MCAD, and acyl-coenzyme A oxidase (ACOX). These results indicated that Ex attenuates blood pressure elevation, albuminuria with an improvement of renal lipid metabolism in the HFr-fed rats. These effects of Ex may relate to an improvement of the renal lipid metabolism.

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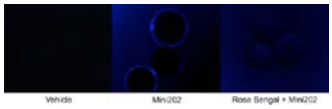
P176

A New Function for Adipocytes: Norepinephrine Storage

PrimaryAuthor.AuthorBlock:**Maleeha Ahmad**, Michigan State Univ, East Lansing, MI; Nadia Ayala-Lopez, Yale Univ, New Haven, CT; Emma Darios, Stephanie W. Watts, Michigan State Univ, East Lansing, MI

Activation of the sympathetic nervous system, and the subsequent release of the neurotransmitter norepinephrine (NE), contracts blood vessels and increases blood pressure, a mechanism altered in obesity-related hypertension. Perivascular adipose tissue (PVAT) is a functional reservoir for catecholamines, including NE, and PVAT adipocytes have plasma membrane transporters that can transport NE, pointing to similarities with the neuronal handling of NE. Thus, we hypothesize that PVAT adipocytes store NE, and use the vesicular monoamine transporter (VMAT) to do so, similar to sympathetic neurons. High-pressure liquid chromatography showed NE present in normal male Sprague Dawley rat superior mesenteric artery (SPVAT; 503.60 ± 34.70 ng mg tissue^{-1}) and mesenteric (MPVAT; 148.20 ± 15.00 ng mg tissue^{-1}) PVATs (N=6). Using immunofluorescence, we tested for VMAT1 and VMAT2 in these PVATs (N=6). Both transporters were present surrounding the lipid of the adipocytes. We developed a novel protocol imaging live primary rat MPVAT adipocytes to capture real-time uptake of Mini202, a fluorescent VMAT probe functioning as a false neurotransmitter (N=4). Cells stained with 500 µM Mini202 (Corrected Total Cell Fluorescence x 10^6 = 9.29 ± 0.88). Addition of 100 µM Rose Bengal, a potent VMAT inhibitor, eliminated Mini202 signal (CTCF x 10^6= -1.72 ± 0.30. Negative because Mini202 background brighter than abolished signal). These data support a

new function of adipocytes in storing catecholamines. Understanding NE uptake and storage in PVAT is crucial for research on obesity-related hypertension and is important considering the growing prevalence of obesity and cardiovascular disease.



Disclosures:**M. Ahmad:** None. **N. Ayala-Lopez:** None. **E. Darios:** None. **S.W. Watts:** None.

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P177

Intermittent Hypoxia Attenuates Corticotropin-Releasing Hormone Receptor 2 (CRHR2) mRNA and CRHR2-Mediated Calcium Influx in Neurons in the Nucleus of the Solitary Tract

PrimaryAuthor.AuthorBlock:**Lei Wang**, Steve Mifflin, UNT Health Science Ctr, Fort Worth, TX

Studies have suggested that corticotropin-releasing hormone (CRH) originating within the hypothalamic paraventricular nucleus (PVN) acts in the nucleus of the solitary tract (NTS) to regulate sympathetic and cardiovascular functions, but the anatomical distributions and cellular actions of CRH receptors (CRHRs) in the NTS are not fully understood. We conducted *in situ* hybridization for CRH type 1 and type 2 receptors (CRHR1 and CRHR2) in Crl:CD(SD) rats and present mRNA levels as the total pixel gray values of positive staining calculated by ImageJ. The mRNA level of CRHR2 was significantly higher than CRHR1 ($1.97 \pm 0.1 \times 10^7$, n=4 vs. $4.56 \pm 0.92 \times 10^6$, n=4; $p < 0.0001$) in the caudal NTS (13.68 mm - 14.76 mm caudal to bregma). We then investigated how the NTS CRHRs and CRHRs signaling pathways were influenced by 7 days exposure to intermittent hypoxia (IH), a model of the arterial hypoxemias that occur during sleep apnea. IH significantly decreased CRHR2 mRNA level in caudal NTS by 34 ± 14% ($1.97 \pm 0.1 \times 10^7$, n=4 vs. $1.3 \pm 0.22 \times 10^7$, n=3; $P < 0.05$) but did not affect CRHR1 mRNA level. To investigate the cellular actions of CRHRs in the NTS, we performed calcium imaging on NTS slice preparations using Fura-2-acetoxymethyl ester and present intracellular calcium levels as the ratio of fluorescent intensity excited by 340nm vs. 380nm. CRH induced a transient increase of intracellular calcium level (n=19) that was abolished by the voltage-dependent calcium channel blocker nifedipine. Calcium influx was attenuated by the CRHR2 antagonist K41498 (0.06 ± 0.006, n=19 vs. 0.041 ± 0.004, n=11; $P < 0.01$) but not by CRHR1 antagonist NBI-35965 (n=5). The CRHR2 agonist Urocortin-II induced calcium influx (0.05 ± 0.005, n=9) but not the CRHR1 agonist Stressin-I. IH also attenuated the CRH-induced calcium influx in the NTS (0.06 ± 0.006, n=19 vs. 0.04 ± 0.004, n=19; $P < 0.01$). Collectively, these results demonstrate that CRHR2 mediates calcium influx in NTS neurons and IH diminishes this effect by down-regulating CRHR2.

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P178

The Role of Lateral Hypothalamic Leptin Signaling in Metabolic and Cardiovascular Regulation

PrimaryAuthor.AuthorBlock:**Huxing Cui**, Eva Rodriguez Cruz, Univ of Iowa, Iowa City, IA

While neurons in the lateral hypothalamic area (LHA) are clearly involved in feeding,

metabolism, and cardiovascular regulation, neurochemically and neuroanatomically heterogeneous nature of LHA neurons has been a challenge in understanding their function in physiological regulation. We have recently identified a unique subset of LHA GABAergic neurons, which are distinct from well-known orexin and MCH neurons, that co-express two metabolically important leptin receptor (LepR) and melanocortin-4 receptor (MC4R), suggesting that these neurons might be the important targets of leptin and melanocortin for metabolic and cardiovascular regulation. Here we show that LHA LepR-positive neurons innervate broadly to intra- and extra-hypothalamic brain regions important for feeding, sympathetic nervous activity, and cardiovascular function, including but not limited to arcuate nucleus, paraventricular nucleus of hypothalamus, parabrachial nucleus, and nucleus of the solitary tract. Stereotaxic microinfusion of leptin into the LHA increases renal sympathetic nerve activity (RSNA) (% changes from baseline at 4th hour: vehicle -25.03 ± 7.09 % vs leptin 100.23 ± 26.94 %, $p<0.001$); and specific deletion of LepR from LHA significantly increase body weight when fed high-fat diet (44.5 ± 1.9g vs 52.5 ± 2.5g, $p<0.01$); and selective chemogenetic activation of LHA LepR+ neurons decrease feeding and increase physical activity. Our findings identify the LHA as a novel brain site for leptin to integrate feeding, metabolism and sympathetic regulation. Further investigation of the role of LHA leptin signaling in blood pressure regulation is underway.

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P179

Pulmonary Spinal Sympathetic Afferent Activation in Post-myocardial Infarction Rats

PrimaryAuthor.AuthorBlock:**Julia Shanks**, Zhiqiu Xia, George J Rozanski, Harold D Schultz, Steven J Lisco, Irving H Zucker, Han-Jun Wang, UNMC, Omaha, NE

Recently, we reported activation of pulmonary spinal afferents by topical application of bradykinin (BK) to lung surfaces evokes a potent sympatho-excitatory reflex, including increased blood pressure (BP), heart rate (HR) and renal sympathetic nerve activity (RSNA) in urethane-α-chloralose anesthetized vagotomized rats. This finding suggests a new previously undocumented pulmonary sympathetic afferent reflex (PSAR). Preliminary data was reported in abstract form at the Association of University Anesthesiologists 64th Annual meeting. Expanding this work, we examined the activation of the PSAR at different time points post myocardial infarction (1 month vs. 5 months post MI) and at different degrees of infarct size (15%-35% (moderate) vs. above 35% (large) of left ventricle). Following bilateral vagotomy, we applied filter paper (3 × 3 mm) saturated with BK (10 μg/ml) to the ventral lung surface to stimulate the PSAR in both urethane-α-chloralose anesthetized sham-operated and MI rats. Application of BK to the lungs resulted in increased BP, HR and RSNA in all sham and MI rats. Compared to sham rats, the BK-induced sympatho-excitatory response was significantly increased in 1-month moderate and 1-month large MI rats as well as in 5-month moderate MI rats. The BK-induced sympatho-excitatory response was significantly blunted in 5-month large MI rats (Table 1). These data suggest that the activation of pulmonary spinal afferents in

the post-MI state may be an independent risk factor for sympatho-excitation. Further studies are needed to understand the differential effects of pulmonary spinal afferent activation at the transition from the post MI state to heart failure.

Table 1. Topic application of BK to the lung causes a sympatho-excitatory response in sham and MI rats

	1 month post MI			5 months post MI		
	Sham n=5	moderate MI n=5	Large MI n=6	Sham n=5	moderate MI n=5	Large MI n=5
ΔMAP (mmHg)	18.8±2.4	32.3±1.4*	30.0±1.5*	22.0±1.4	34.4±1.7*	10.1±1.1*
ΔHR (bpm)	21.2±3.1	63.0±10.3*	54.2±6.7*	23.0±2.7	65.0±10.6*	8.8±3.5*
RSNA (%baseline)	90.0±9.4	166.7±28.2*	191.9±18.2*	90.6±10.2	170.8±23.0*	60.7±4.8*

Mean±SE. *P<0.05 vs. sham.

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P180

Blood Pressure Lowering During Chronic Baroreflex Activation: Don't Forget the Heart

PrimaryAuthor.AuthorBlock:**John S. Clemmer**, Thomas E. Lohmeier, Univ of Mississippi Medical Ctr, Jackson, MS; Radu Iliescu, Gr. T. Popa Univ of Med and Pharmacy, Iasi, Romania; W. Andrew Pruett, Robert L. Hester, Univ of Mississippi Medical Ctr, Jackson, MS

Electrical stimulation of the baroreflex chronically suppresses sympathetic activity and arterial pressure (AP) and is currently being evaluated for the treatment of resistant hypertension. The antihypertensive effects of baroreflex activation (BA) are often attributed to renal sympathoinhibition. However, BA also decreases heart rate (HR) and, surprisingly, robust blood pressure lowering occurs even after renal denervation. Accordingly, responses to BA were simulated under normal conditions and after blockade of changes in renal sympathetic nerve activity (fixed RSNA) and autonomic activity to the heart (fixed HR) using an established mathematical model of human physiology (HumMod). Three week responses to BA closely mimicked our actual experimental observations in dogs including a 40% decrease in plasma [norepinephrine] (and RSNA in the simulation). With RSNA and HR fixed at control levels, mean AP (MAP), HR, right atrial pressure (RAP) and plasma atrial natriuretic peptide (ANP) responses to BA were:

Condition	MAP (mmHg)	HR (BPM)	RAP (mmHg)	[ANP] (pMol/L)
Control	95	72	1.3	20
BA	75	52	2.3	47
BA+fixed RSNA	75	52	3.0	68
BA+fixed HR	85	72	0.9	15
BA+fixed RSNA+HR	90	72	1.3	22

Changes in autonomic activity to the heart during BA lead to bradycardia, increases in atrial pressure and ANP secretion. Increased ANP and suppression of RSNA enhance renal excretory function and normally account for most of the hypotensive response to BA. However, when suppression of RSNA is not possible, blood pressure lowering in response to BA is not appreciably impaired due to inordinate fluid accumulation and further increases in atrial pressure and ANP secretion. These simulations provide a mechanistic understanding of experimental and clinical observations showing that BA effectively lowers blood pressure in subjects with previous renal denervation.

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P181

Contrasting Effects of High Fat Diet and DOCA-salt on Primary Neuronal Cilia

PrimaryAuthor.AuthorBlock:**Jingwei Jiang**, Kamal Rahmouni, Univ of Iowa, Iowa City, IA

Virtually, every mammalian cell is equipped with an antenna like primary cilium, a cell surface protrusion that is thought to act as a sensory organelle. Many of the rare genetic disorders that cause shorter, absent or disrupted cilia are associated with obesity and cardiovascular dysfunction in humans and rodents, which suggest that cilia length contribute to energy balance and cardiovascular homeostasis. Here, we examined the length of the primary neuronal cilia in the brain nuclei that contribute to metabolic and cardiovascular regulation in high fat diet-induced obese (DIO) mice and DOCA-salt mice. Cilia length was examined by adenylate cyclase 3 (AC3) immunostaining, followed by confocal 3D reconstruction, and quantification by IMARIS imaging analysis software. Analysis of the cilia length and distribution showed reduced frequency of cilia that are over 10 µm in the brain of DIO mice compared to control mice fed normal diet fed mice (17.02±1.36% vs 23.78±1.15%, p=0.032). Interestingly, the most pronounced difference in cilia length was observed in the dorsomedial hypothalamus with the DIO mice displaying significantly shorter cilia (6.90±0.06 µm) relative to controls (7.32±0.14µm in controls, n=5/group $p<0.05$). Conversely, we found that average neuronal cilia length was elongated in 3-week DOCA-salt treated mice compared to sham group. The number of primary neuronal cilia that are over 10 µm was significantly increased in DOCA-salt mice by 8% (p=0.0114). On the other hand, the number of cilia that are 4-5 µm in length was significantly decreased in DOCA-salt mice compared to sham controls (11.73±1.70% vs 18.73±2.02%, p=0.0385). The supraoptic nucleus was the only nucleus that displayed difference in the length of cilia that are 5-10 µm in length (7.46±0.24 µm vs 6.76±0.15µm, n=5/group, p=0.0509). Our data demonstrate plasticity of neuronal cilia in response to high fat diet and DOCA-salt treatment in defined brain regions. Our results raise the possibility that primary neuronal cilia may function as part of environmental surveillance system in the brain that control energy homeostasis and cardiovascular function. Further analysis of the role of primary neuronal cilia in cardiovascular regulation is underway.

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P182

Chronic Central Melanocortin 4 Receptor Blockade Does Not Prevent Cardiac Dysfunction After Myocardial Infarction in Rats

PrimaryAuthor.AuthorBlock:**Fabio Gava**, UMMC, Jackson, MS; Alexandre A da Silva, Organizacao Educacional Barao de Maua, Ribeirao Preto, Brazil; Jussara M do Carmo, UMMC, Jackson, MS

Progression of myocardial infarction (MI) to heart failure may be exacerbated by excessive activation of the sympathetic nervous system (SNS). We previously showed that the central nervous system (CNS) melanocortin system plays a key role in regulating SNS activity and blood pressure (BP) in several different conditions, including hypertension and obesity. Therefore, we tested whether melanocortin-4 receptor (MC4R) blockade protects against cardiac dysfunction associated with MI in rats. Male Sprague-Dawley rats at 12 weeks of age

were implanted with BP telemetry transmitters and an intracerebroventricular (ICV) cannula was inserted into the lateral ventricle. After 10 days of recovery, food intake, mean arterial pressure (MAP) and heart rate (HR) were measured 24-hrs/day by telemetry and cardiac function was assessed by echocardiography (VEVO 2100$^{@}$). After stable baseline measurements for 4 days, the left coronary artery was permanently ligated and vehicle (n=5) or the MC4R antagonist (SHU-9119, 1 nmol/h, n=7) was infused ICV via osmotic minipump for 28 consecutive days. Chronic MC4R antagonism significantly increased food intake and body weight (25±1 to 37±4 g/day and 365±4 to 497±10 g) compared to vehicle treatment (24±1 to 20±1 g/day and 372±6 to 417±5 g). Chronic SHU-9119 infusion did not alter MAP (108±1 to 105±2 mmHg) whereas MAP was significantly reduced in vehicle-treated rats with MI (104±1 to 97±2 mmHg). However, HR decreased more in SHU-9119-treated rats (~-70 bpm) than in vehicle group (~-42 bpm). Compared to vehicle, SHU-9119 infusion for 4 weeks did not prevent cardiac dysfunction caused by MI as evidenced by low cardiac radial strain (40±1 to 18±3 vs. 44±2 to 23±5 %), cardiac output (106±7 to 68±4 vs. 114±5 to 86±8 ml/min) and ejection fraction (71±2 to 33±2 vs. 71±2 to 38±4 %). These results suggest that chronic central MC4R inhibition does not attenuate the progression to heart failure after MI in rats. (NHLBI-PO1HL51971, NIGMS- P20GM104357)

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P183

Influence of Insufficient Sleep on Endothelial Fibrinolytic Capacity in Adults With Elevated Blood Pressure

PrimaryAuthor.AuthorBlock:**Anthony R Bain**, Caitlin A Dow, Kyle J Diehl, Tyler D Bammert, Jared J Greiner, Univ of Colorado, Boulder, Boulder, CO; Brian L Stauffer, Univ of Colorado Anschutz Medical Ctr, Denver, CO; Christopher A DeSouza, Univ of Colorado, Boulder, Boulder, CO

The capacity of the endothelium to release tissue-type plasminogen activator (t-PA) is impaired in adults with elevated BP, leading to an increased risk of thrombotic events. Insufficient sleep is independently associated with elevated BP and impaired t-PA release. However, the compounded influence of insufficient sleep on t-PA release in adults with elevated BP is unknown. We tested the hypothesis that impairments in the capacity of the endothelium to release t-PA in adults with elevated BP is worse in those who sleep <7 h/night (short sleep duration) compared with those who sleep 7 to 9 h/night (normal sleep duration). We studied 38 sedentary, middle-aged adults: 10 with normal BP and normal nightly sleep duration (6M/4F; age: 55±2 yr; BP: 114/94±2/3 mmHg, sleep duration: 7.4±0.2 h); 14 with elevated BP and normal nightly sleep duration (8M/6F; 60±2 yr; 141/87±2/2 mmHg; 7.8±0.1 h); and 14 with elevated BP and short nightly sleep duration (10M/4F; 57±2 yr; 139/85±2/2 mmHg; 6.1±0.2 h). All subjects were free of overt metabolic and coronary disease. Net endothelial release of t-PA was determined, in vivo, in response to intra-brachial infusions of bradykinin (BK: 125-500 ng/min) and sodium nitroprusside (SNP: 2.0-8.0 µg/min). In the normal sleep groups, as expected, endothelial t-PA release in response to BK was significantly blunted (~30%) in the

adults with elevated BP (from -1.2±0.8 to 50.2±4.8 ng/100mL tissue/min) compared with normal BP (from 0.9±3.4 to 73.0±8.0 ng/100mL tissue/min); and total t-PA release (area under the BK curve) was ~25% lower ($p<0.05$) in the adults with elevated (307±33 ng/100mL tissue) vs. normal (396±27 ng/100mL tissue) BP. Importantly, net endothelial release rate (from -1.5±1.0 to 40.6±4.3 ng/100mL tissue/min) and total amount of t-PA released (222±28 ng/100mL tissue) in response to BK were markedly lower (~25% and 30%, respectively, $P<0.05$) in the elevated BP and short sleep duration group compared with the elevated BP and normal sleep duration group. In the elevated BP population, sleep duration was positively correlated with total t-PA release ($r=0.46$, $P<0.05$). There was no effect of SNP on t-PA release in any group. In summary, insufficient sleep is associated with exacerbated impairments in t-PA release in adults with elevated BP.

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P184

Muscarinic Receptor Modulators' Effect on Cilia Structure and Nitric Oxide Release

PrimaryAuthor.AuthorBlock:**Wissam A AbouAlaiwi**, Hayley Gibson, Univ of Toledo, Toledo, OH

Primary endothelial cilia are microtubule-based organelles that act as mechano-sensors to help detect, and respond to changes in the extracellular environment. One of these responses includes its ability to detect fluid shear stress and translating that signal into a biochemical process to synthesize nitric oxide, an endogenous vasodilator. Abnormal cilia structure and function has been known to cause a myriad of human disorders, including polycystic kidney disease and hypertension. It is also well documented that muscarinic acetylcholine receptors subtype M1 also plays a key role in producing a vasodilation response in the vasculature. However, nothing is known about the relationship between primary endothelial cilia and the M1 receptor. To further explore the relationship between AChM1R and the mechanosensory function of primary cilia, the effects of muscarinic modulators on cilia length and function in wild-type, and mechano-insensitive cilia mutant endothelial cells, $Pkd1^{-/-}$ (dysfunctional cilia) and $Tg737^{orpk/orpk}$ (cilia-less) were examined. We show that AChM1R localizes to primary endothelial cilia. AChM1R activation lead to the significant increase cilia length in endothelial cells treated with CDD102A, an AChM1R agonist (1.21 ± 0.07 vs. 2.33 ± 0.04 for wild-type, 1.02 ± 0.01 vs. 1.44 ± 0.01 for $Pkd1^{-/-}$, and 0.024 ± 0.005 vs. 0.3 ± 0.004 for $Tg737^{orpk/orpk}$) compared to non-treated cells. Treating endothelial cells with pirenzepine, an AChM1R antagonist, led to a significant decrease of cilia length (1.34 ± 0.02 vs. 1.15 ± 0.01 in wild-type, and 1.16 ± 0.01 vs. 0.71 ± 0.01 in $Pkd1^{-/-}$) compared to non-treated cells. Treatment with CDD102A also significantly upregulated the expression of AChM1R, and phosphorylated eNOS. Our data clearly suggested that modulating cilia sensory function could have a positive influence on nitric oxide generation and blood pressure regulation.

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P185

Prognostic Value of Endothelial Markers in Hypertensive Nondiabetic Patients

PrimaryAuthor.AuthorBlock:**Natalia Murashko**, Anna Bragina, Galina Bragina, Valery Podzolkov, I.M. Sechenov First Moscow State Medical Univ, Moscow, Russian Federation

Aim: to study the prognostic value of the serum levels of stable nitric oxide metabolites (NOx), endothelin-1 (E1), homocystein (HC), von Willebrand factor (Wf), tissue plasminogen activator (tPA), in hypertensive nondiabetic patients without cardiovascular disease.
Methods: 124 patients with hypertension (HTN), without diabetes were enrolled, the mean age was 51.4±6.5y, the mean duration of HTN was 8.5±7.6 y, 36 % were males, 35% were smokers,
54% were obese. The cardiovascular risk was estimated according to the SCORE (Systematic Coronary Risk Evaluation) system. Plasma NOx levels were determined by spectrophotometry, E1, HC, Wf, tPA levels by enzyme immunoassay. The follow-up period was 8±1.1y. Statistical analysis was done using the SPSS 11.1 software.
Results: On initial investigation all patients were stratified according to the SCORE risk levels. The increase of SCORE risk levels were associated with the elevation of serum NOx, E1, HC, and Wf concentrations. The mean plasma NOx concentration increased along with the SCORE risk level: it was 36.4±12.7 in low risk, 41.1±15.7 in moderate risk, 44.9±19.8 in high risk, and 50.4±19.1 umol/L in very high risk categories (<0.05 between the low and high risk groups). The tPA levels did not differed in relation to the SCORE risk estimates. The SCORE risk level correlated with serum concentrations of NOx (r=0.74, p=0.0001), HC (r=0.54, p=0.0001), and Wf (r=0.43, p=0.001).
Conclusions: The stable nitric oxide metabolites, homocystein, and von Willebrand factor are the markers of oxidative/nitrosative stress severity. Our results are indicative that this substances may be of prognostic value in hypertensive nondiabetic patients.

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P186

Selective ET_A Receptor Antagonism versus Dual ET_A/ET_B Receptor Blockade for Preventing Angiogenesis Inhibitor-induced Hypertension

PrimaryAuthor.AuthorBlock:**Katrina M Mirabito Colafella**, Richard van Veghel, Renè de Vries, Jorie Versmissen, Anton H van den Meiracker, A. H. Jan Danser, Erasmus MC, Rotterdam, Netherlands

Angiogenesis inhibitors are a mainstay treatment for cancer. While effective in preventing tumor growth, angiogenesis inhibitors cause off-target effects including cardiovascular toxicity and renal injury, most likely via endothelin-1 (ET-1) upregulation. ET-1 via stimulation of the ET_A receptor causes pro-hypertensive effects whereas stimulation of the ET_B receptor can elicit both pro- or anti-hypertensive effects. In the present study, we hypothesized that selective ET_A receptor blockade versus dual ET_A/ET_B blockade provides better cardiovascular protection from angiogenesis inhibitor-induced hypertension. Male WKY rats were treated with vehicle, sunitinib (VEGF inhibitor; 14 mg/kg/day) alone or in combination with macitentan ($ET_{A/B}$

receptor antagonist; 30 mg/kg/day) or sitaxentan (ET_A receptor antagonist; 30 or 100 mg/kg/day) for 8 days. Mean arterial pressure (MAP) was measured via radiotelemetry at baseline and days 1-6 of treatment. Vasoreactivity to acetylcholine (ACh) and ET-1 was assessed in iliac vessels. Compared to vehicle treatment, sunitinib treatment caused a rapid and sustained increase in MAP (1±1 versus 23±2 mmHg on day 6 of treatment, respectively, P<0.001). Co-treatment with macitentan blunted the pressor response to sunitinib, such that on day 6 of treatment the increase in MAP was 7±4 mmHg (P<0.01 versus sunitinib-treated). Similar results were observed for co-treatment with 30 mg/kg/day of sitaxentan. Conversely, co-treatment with 100 mg/kg/day sitaxentan, which has been shown to induce a depressor response in normotensive Wistar rats, completely abolished sunitinib-induced hypertension, with MAP actually decreasing before returning to near baseline level (-1±1 mmHg on day 6 of treatment, P<0.001 versus sunitinib-treated). Compared to vehicle treatment, ET_B receptor stimulation yielded a constrictor response after 8 days of sunitinib treatment, while the response to ACh was unaltered. Both macitentan and sitaxentan reversed the constrictor ET_B receptor response. Our results support a key role for the ET-1 system in the development of sunitinib-induced hypertension and suggest that selective ET_A receptor blockade is sufficient to block this effect.

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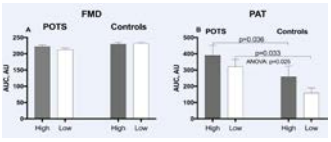
P187

Nitric Oxide Function in Postural Tachycardia Syndrome During High and Low Sodium Diets

PrimaryAuthor.AuthorBlock:**Jorge E Celedonio**, Victor C Nwazue, Emily M Garland, Cyndya A Shibao, Luis E Okamoto, Italo Biaggioni, Satish R Raj, Alfredo Gamboa, Vanderbilt Univ Medical Ctr, Nashville, TN

Background - Postural tachycardia syndrome (POTS) is characterized by an increase in sympathetic activity, with an exaggerated rise in heart rate upon standing and symptoms of cerebral hypoperfusion. Endothelial/Nitric Oxide (NO) dysfunction might be involved in POTS pathophysiology. As part of the non-pharmacologic treatment of POTS, a high sodium diet is often recommended to increase plasma volume. We assessed endothelial/NO function in conduit vessels (using flow-mediated dilation, FMD) and resistance vessels (using finger pulse arterial tonometry, PAT) in POTS patients during high and low salt diets. **Methods** - In 14 female POTS patients (34±9 years, BMI 23±3 kg/m^2) and 13 matched healthy control subjects (29±4 years, BMI 24±3 kg/m^2), we evaluated the time course responses to FMD and PAT. Subjects were randomly assigned to either high salt diet (300mEq/day) or low salt diet (10mEq/day) for 6 days and crossed over to the other arm after 1 month. The areas under the curve for brachial artery diameter by FMD and finger artery dilation by PAT were compared between interventions and groups. **Results** - No differences in NO function in a conduit artery were seen. In contrast, in resistance vessels, high salt diet increased vasodilation in both POTS and healthy subjects (figure). In addition, POTS patients have greater vasodilation than healthy subjects during both low and high salt diets (p=0.036 and 0.033 for

high and salt diets respectively).
Conclusions - POTS patients may have an exaggerated NO response to reactive hyperemia in resistance vessels, but not in conductance vessels. This excessive vasodilation could contribute to POTS symptoms on standing.



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P188

Nephron-wide Deletion of Nos3 Impairs Salt Excretion and Causes Hypertension

PrimaryAuthor.AuthorBlock:**Yang Gao**, Deborah Stuart, Univ of Utah Health Sciences Ctr, Salt Lake City, UT; Takamune Takahishi, Div of Nephrology, Vanderbilt Univ, Nashville, TN; Donald E. Kohan, Univ of Utah Health Sciences Ctr, Salt Lake City, UT

In vitro studies have suggested that nephron nitric oxide synthase 3 (NOS3) plays a role in the regulation of urinary Na^+ excretion (UNaV) and potentially contributes to blood pressure (BP) control. To assess whether NOS3 is indeed involved in the physiological regulation of UNaV and BP, mice with doxycycline-inducible nephron-wide deletion of NOS3 were generated. These mice were homozygous for loxP-flanked exons 9-12 of the NOS3 gene (contains the calmodulin binding site) and hemizygous for Pax8-rtTA and LC-1 transgenes (Pax8 promoter-rtTA confers nephron-specific targeting and LC-1 transgene contains doxycycline/rtTA-inducible Cre recombinase). Mice were treated with either vehicle (controls) or doxycycline (knockouts, KO) at 1 month of age for 12 days and studied at ~3 months of age. Nephron-specific NOS3 KO mice had renal-specific NOS3 gene recombination and reduced mRNA levels in microdissected nephron segments. Renal vascular NOS3 mRNA levels were markedly increased in KO mice, presumably as a compensatory response to nephron NOS3 KO. KO mice had modestly increased mean arterial pressure (100 ± 2 mmHg in controls and 103 ± 1 mmHg in KO, N=6, $p<0.05$) on a normal (0.3% Na^+) salt diet that increased further during high (3.2% Na^+) salt intake (103 ± 2 mmHg in controls and 118 ± 3 mmHg in KO, N=6, $p<0.05$). KO mice had reduced daily UNaV and greater cumulative Na^+ retention compared to controls over the 7 days of high salt feeding. PRC tended to be lower in KO mice on a high salt diet compared to controls (85.8 ± 13.5 ng/ml in controls and 60.1 ± 10.3 ng/ml in KO, N=12, $p<0.07$). Control mice excreted most of an acute I.P. salt load by 4 hr post-load, while KO mice excreted the acute salt load between 5-7 hr after loading. Furosemide, but not hydrochlorothiazide, abolished the differences in UNaV between control and control mice following an acute salt load. No sex-dependent differences in UNaV or BP were observed. In summary, these findings indicate that nephron NOS3 is involved in acute and chronic control of renal Na^+ excretion and BP during high salt intake. Taken together with previous studies showing that collecting duct-specific KO of NOS3 does not alter UNaV or BP, our data suggest that thick ascending limb is at least partly involved – further mechanistic studies are ongoing.

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P189

Endothelial Cullin3 Mutation Causes Vascular Dysfunction, Arterial Stiffening, and Hypertension

PrimaryAuthor.AuthorBlock:**Jing Wu**, Larry N Agbor, Shi Fang, Chunyan Hu, Ko-Ting Lu, Xuebo Liu, Masashi Mukohda, Anand R Nair, Curt D Sigmund, Univ of Iowa, Iowa City, IA

Mutations in CULLIN3 gene (causing in-frame deletion of exon 9) cause hypertension in humans. The hypertension phenotype is unlikely to be driven by renal tubular mechanisms, as kidney-specific deletion of Cullin3 (Cul3) in mice results in hypotension, not hypertension. We have recently shown that smooth muscle expression of Cul3Δ9 causes vascular dysfunction and elevation of blood pressure (BP) via augmented RhoA/Rho-kinase signaling, strongly supporting a vascular role of Cul3 in BP regulation. To test the importance of endothelial Cul3 *in vivo*, we bred the conditionally activatable Cul3α9 mice with Tek-CRE^{ERT2} mice specifically expressing tamoxifen-inducible Cre-recombinase in the endothelium. The resultant mice (E-Cul3α9) exhibited impaired endothelial-dependent relaxation in the basilar artery (maximal relaxation in response to 30 μM acetylcholine, 45% vs 85% in control mice) and carotid artery. No difference in smooth muscle function was observed. Moreover, E-Cul3α9 mice exhibited nocturnal hypertension as determined by radiotelemetry (night time peak BP, 141±3 mmHg vs 122±3 mmHg). However no difference was seen in daytime pressure. E-Cul3α9 mice also exhibited arterial stiffening as indicated by elevated pulse wave velocity (3.7±0.3 m/s vs 2.7±0.1 m/s). To determine the molecular mechanism of endothelial dysfunction, primary aortic endothelial cells were isolated from mice carrying the inducible Cul3α9 construct and Cul3α9 expression was robustly induced by adenovirus carrying Cre recombinase gene *in vitro*. Cul3α9 acted in a dominant negative manner by interfering with expression and function of wild type Cul3, leading to impaired turnover of a Cul3 substrate protein phosphatase 2A, marked reduction in phosphorylated eNOS, and decreased nitric oxide production. Treatment with a selective PP2A inhibitor Okadaic Acid (1 nM) rescued Cul3α9-induced impairment of eNOS activity. These data define a novel pathway involving Cullin-3/PP2A/phospho-eNOS in the endothelium. Selective endothelial expression of Cul3α9 partially phenocopies the hypertension observed in Cul3α9 patients, suggesting that mutations in Cullin-3 cause human hypertension in part through a vascular mechanism characterized by endothelial dysfunction.

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P190

Long-term Intake of Muscadine Grape Extract Markedly Improves Exercise Capacity in Older Female (mRen2)27 Hypertensive Rats

PrimaryAuthor.AuthorBlock:**A'ja V. Duncan**, Wayne Graham, Liliya Yamaleyeva, Patricia E Gallagher, E. Ann A Tallant, Mark C Chappell,

Debra I Diz, Wake Forest Sch of Med, Winston-Salem, NC

Muscadine grapes (*Vitis rotundifolia*) contain significant levels of polyphenols and their antioxidant capacity may have cardiovascular benefit. We determined the cardiovascular effects of a water soluble muscadine grape extract (MGE) from Piedmont Research & Development Corporation (PRDC, Winston-Salem, NC) that we confirmed has a high content of epicatechin, gallic acid, ellagic acid and procyanidin B2. We hypothesize that this soluble MGE with a potentially high phenolic bioavailability exhibits cardioprotective actions. The effect of long-term MGE intake on blood pressure, cardiac function and exercise tolerance was established in female and male hemizygous (mRen2)27 [mRen2] transgenic rats, an Ang II-AT_1R-dependent model of hypertension. The mRen2 were administered MGE (8 mg of total phenolics/mL) in the drinking water for 26 weeks (7 female; 7 male); control rats (9 female; 11 male) were given water only. At 40 weeks of age, exercise tolerance was assessed by treadmill (10.2 m x min^{-1}, 5% inclination). The MGE-treated female mRen2 exhibited a >300% improvement in workload (*W: g X m*) as compared to the untreated group [14551 ± 1313 vs. 4498 ± 481; p<0.01]. MGE intake also extended the time to fatigue (TTF) by 300% in females (4943 ± 443 vs.1615 ± 166 sec; p<0.01). In contrast, chronic intake of MGE had no effect on either exercise workload or TTF in the male mRen2. MGE intake did not alter systolic blood pressure in females (162 ± 3 vs. 166 ± 6 mm Hg, p>0.05) and males (165 ± 5 vs. 160 ± 3 mm Hg) nor alter body weight in either group [females: 334 ± 7 g vs. 339 ± 8 g; males: 613 ± 12 g vs. 590 ± 6 g). Echocardiographic analyses revealed that although the female mRen2 exhibited a higher ejection fraction [73.4±5.1 vs. 42.3±4.5%, n=4-5, p<0.05] and a higher fractional shortening [44.2±4.2 vs. 22.1±2.7%, n=4-5, p<0.05] as compared to males, MGE treatment did not improve these indices in either group. We conclude that the marked improvement in exercise capacity in older adult hypertensive females by chronic MGE intake is not accompanied by augmented cardiac performance, perhaps reflecting the lack of an effect on blood pressure. Thus, it is possible that MGE may directly impact the skeletal muscle to improve exercise in the hypertensive mRen2 females, but not the males.

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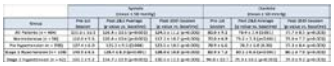
P191

Improved Systolic and Diastolic Blood Pressure After Enhanced External Counterpulsation Therapy Combined With a Lifestyle Modification Program

PrimaryAuthor.AuthorBlock:**Murtaza Bharmal**, Michael Share, Cedars Sinai Medical Ctr, Los Angeles, CA; Sara Soulati, Dean Chiaro, Sara Soulati Health for Life Program, Los Angeles, CA; Ronald Weaver, GlobalCardioCare, Los Angeles, CA; Ronald Victor, Timothy Henry, Cedars Sinai Medical Ctr, Los Angeles, CA

Background: Enhanced external counterpulsation (EECP) is a noninvasive, FDA approved therapy for refractory angina. Because EECP increases nitric oxide release, it may have an ancillary benefit on blood pressure (BP). **Methods:** We measured changes in BP at baseline over 35 sessions of EECP, which included lifestyle coaching. Supine blood pressure was measured before and after every EECP session by trained healthcare technicians

with calibrated manual sphygmomanometers. **Results:** 404 patients (age 67.2 ± 10.0, 50% male - 200/404, 77% African American - 311/404) completed the program. Hypertensive subjects at baseline (37% - 150/404) showed an improvement in both systolic and diastolic BP (Table). Notably, there was a marked reduction in BP after the first EECP session, with a further, more modest reduction by the end of the 35 sessions. Weight decreased by a mean 3.0 lbs ($p<0.001$) and there was no significant difference in the number of patients receiving antihypertensive medications before or after completing EECP (46% - 184/404 vs. 47% - 189/404, p=0.62). **Conclusions:** EECP therapy leads to a reduction in both systolic and diastolic blood pressure, which is most prominent in patients with baseline hypertension. These results maintain throughout the 35 sessions. These results reflect observational data from one highly experienced clinic in which EECP is routinely combined with daily coaching on vegan plant-based lifestyle modifications and exercise. Further studies are indicated to investigate this observed phenomenon to both confirm these findings as well as assess for the length and durability of the effect.



Disclosures:**M. Bharmal:** None. **M. Share:** None. **S. Soulati:** 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; CEO of GlobalCardioCare. **D. Chiaro:** 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; Chief Clinical Officer. **R. Weaver:** None. **R. Victor:** None. **T. Henry:** None.

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P192

Psychosocial Correlates of Adherence to Non-pharmacological Therapy: An Examination of Baseline Results From the *SisterTalk Adhere* Intervention for Black Women

PrimaryAuthor.AuthorBlock:**Augustine W Kang**, Patricia M Risica, Brown Univ, Providence, RI

Significance: Non-pharmacological treatment for blood pressure, such as dietary and Physical Activity (PA) adherence, is a key determinant of effective blood pressure management. Psychosocial variables such as self-efficacy, outcome expectations and motivation to engage in health behavior have been reported to be predictors of non-pharmacological adherence. However, few studies have studied these constructs among minority and at-risk populations such as hypertensive Black American women. Identifying psychosocial correlates of adherence is key to the development of efficacious interventions to improve adherence and overall health outcomes.
Methods: We examined baseline measures (n = 323) of a sample of self-reported hypertensive Black women from *SisterTalk Adhere*, a randomized controlled trial to promote weight loss for hypertension control. Independent variables include (1) self-efficacy, (2) outcome expectations and (3) motivation for health behavior (further divided into PA and diet domain specific) Dependent variables are dietary and PA non-pharmacological adherence (operationalized as “none/some/most/all of the time” for diet and PA adherence in the past 4 weeks).
Results: The mean age of the sample was 52.17±10.32. Regression analyses revealed that

age was significantly associated with dietary adherence (p = .037). Dietary self-efficacy and positive outcome expectations from healthy eating were significantly associated with dietary adherence (both p < .001). Motivation for healthy eating was also significantly associated with dietary adherence (p = .043). Self-efficacy for PA and positive outcome expectations from PA were significantly associated with PA adherence (p = .006 and p < .001 respectively). Motivation for PA was not found to be associated with PA adherence.
Conclusion: Results suggest that self-efficacy, outcome expectations and motivation for health behavior may be important targets to improve engagement in health behavior and non-pharmacological adherence among hypertensive Black women.

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P193

Anti-fibrotic and Anti-oxidant Actions of a Muscadine Grape Extract Supplement on Hypertension-induced Cardiac Damage

PrimaryAuthor.AuthorBlock:**Pooja D Patil**, Brian M Westwood, Patricia E Gallagher, E. Ann Tallant, Wake Forest Sch of Med, Winston-Salem, NC

Approximately 75 million U.S. adults suffer from hypertension, an independent risk factor for pathological cardiac hypertrophy. The current study examines the effects of a muscadine grape extract formulation (MGE; Piedmont Research & Development Corp.) with anti-oxidant, anti-inflammatory, and anti-proliferative properties on hypertension-induced cardiac damage in Sprague-Dawley rats receiving a 4-week Ang II infusion (24 µg/kg/h). We previously showed that MGE in the drinking water had no effect on blood pressure or systolic function in normotensive or hypertensive rats; however, MGE ameliorated the Ang II-induced decrease in diastolic function and increase in cardiomyocyte cross-sectional area. In the current study, MGE supplementation of Ang II-treated rats decreased interstitial cardiac fibrosis, reducing collagen III staining in the myocardium (0.9 ± 0.2% control, 6.8 ± 1.0% Ang II, and 2.8 ± 0.4% Ang II/MGE; p<0.01). MGE alone had no effect on any parameters assessed. The positive correlation between collagen and diastolic function with Ang II compared to control (p<0.01) was significantly abrogated by co-administration of MGE. TGFβ is a critical cytokine that stimulates cardiac fibrosis. The Ang II-mediated increase in cardiac TGFβ mRNA was attenuated by MGE (relative gene expression: 1.0 ± 0.1 control, 2.0 ± 0.4 Ang II, 1.1 ± 0.2 Ang II/MGE; p<0.05) as was downstream pSmad2 protein in cardiomyocytes (% nuclei: 26.1 ± 6 control, 53.8 ± 9.6 Ang II, 27.6 ± 5.5 Ang II/MGE; p<0.05) and cardiac fibroblasts (2.3 ± 1.1 control, 11.7 ± 4.9 Ang II, 2.2 ± 0.5 Ang II/MGE; p<0.05). NADPH oxidase is a primary contributor to cardiac oxidative stress and excess ROS production can lead to activation of pro-fibrotic pathways. MGE significantly reduced the Ang II-induced increase in p22phox mRNA (relative gene expression: 1.0 ± 0.1 control, 1.9 ± 0.1 Ang II, 1.0 ± 0.1 Ang II/MGE; p<0.0001) and protein (relative density: 1.0 ± 0.2 control, 5.4 ± 1.6 Ang II, 2.9 ± 1.0 Ang II/MGE; p<0.05), a subunit required for NADPH oxidase activity. These results indicate that MGE ameliorates cardiac fibrosis by targeting NADPH oxidase and the TGFβ pro-fibrotic pathway, suggesting that MGE supplementation may be an effective, natural therapeutic for hypertension-induced cardiac damage.

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P194

High Intensity Interval Training Protects the Heart

PrimaryAuthor.AuthorBlock:**Nevena Jeremic**, Nicholas Todd Theilen, Gregory J Weber, Univ of Louisville, Louisville, KY; Vladimir Zivkovic, Ivan Srejovic, Vladimir Jakovljevic, Univ of Kragujevac, Kragujevac, Serbia; Suresh Chand Tyagi, Univ of Louisville, Louisville, KY

BACKGROUND Human studies have shown high intensity interval training (HIIT) can have beneficial effects on the heart. The main goal of this study is to show 15 minutes of exercise with high intensity can has the same protective effects on the cardiovascular system as endurance training (ET) in healthy and diseased animals. **METHODS** For this study we used mouse (C57BL/6J) and rat (Wistar albino) models. We had three group of mice and rats: sedentary-control (CTRL), ET, and HIIT groups. First, 10-12 week old mice were treated with either ET or HIIT for 4 weeks. We performed heart ultrasonography, cardiomyocyte contractility, blood flow of the abdominal aorta and Western blot analysis for SOD-1, SOD-2, and citrate synthase to test cardiovascular differences between groups. Further, we used DHE staining, ATP production assay and O_2^- consumption kits. Second, 10-12 week old rats were treated with either ET of HIIT prior to ischemia/reperfusion injury on a Langendorff apparatus. Cardiodynamic data on dp/dt max, dp/dt min, SLVP, DLVP, heart rate, coronary flow and blood pressure were collected to observe differences between groups. **RESULTS** Our data reviled an increase in blood flow of mice abdominal aorta in both exercise groups compared to control but not between exercise groups itself (BF CTRL: 2218.7±10.0; BF ET: 2481.9±10.5; BF HIIT:2450.6±12.1). Ejection fraction was increased in HIIT compared to other groups (EF CTRL: 76.58±1.8; EF ET: 74.5±2.0 and EF HIIT: 84.83±2.4); Fractional shortening was increased in HIIT group compared to other groups (FS CTRL: 44.49±0.9; FS ET: 42.79±1.08; FS HIIT: 52.51±1.9). LIVDs was decreased in HIIT group compared to endurance, while in endurance was increased compare to control group (LIVDs CTRL: 1.11±0.3; LIVDs ET: 2.33±0.2; LIVDs HIIT: 1.4±0.2). DHE staining for ROS revealed no differences among groups. **CONCLUSION** These findings indicate that HIIT have specific protective effects on the cardiovascular system compared to endurance training, even with possible higher heart contractility. People often cite the time as an excuse for lack of physical activity, however time is no longer an excuse.

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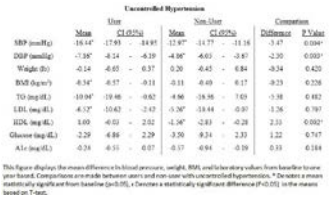
P195

Digital Health Tool Use and Outcomes in People With Uncontrolled Hypertension

PrimaryAuthor.AuthorBlock:**Conor Senecal**, Robert J Widmer, Amir Lerman, Mayo Clinic, Rochester, MN

Background: Digital health applications are increasingly ubiquitous, however the effectiveness remains mixed. Using a large

working age cohort we sought to evaluate use of a digital health intervention (DHI) in people with uncontrolled hypertension and their changes in blood pressure control. Methods: We analyzed usage of DHI and change in blood pressure control and other CVD risk factors over one year in 9,724 participants of a work health program across 81 organizations in 42 states. Patients were selected from a larger cohort based on a previous ICD-9 diagnosis of hypertension. Patients with uncontrolled hypertension, systolic blood pressure (SBP) greater than 140 mmHg, at the initial visit were included. Application users, defined as at least one login, were compared to non-users. Changes in blood pressure, weight, and lipids were compared at one year. Results: Users and non-users were similar in age, weight, blood pressure and lipids at baseline, though users were more likely to be female. At one year users had 16.44 mmHg reduction in SBP and 7.16 mmHg reduction in diastolic blood pressure (DBP), both statistically significantly more than non-users (Table 1). Both users and non-users had an improvement in LDL from baseline, while non-users also had an improvement in BMI and TG from baseline. Discussion: These results show that the use of a DHI is associated with improved systolic and diastolic blood pressure control compared to a workplace health program alone.



Uncontrolled Hypertension

	User			Non-User			Comparison	
	Mean	CI (95%)		Mean	CI (95%)		Difference	P Value
SBP (mmHg)	-16.44*	-17.93	-14.95	-12.97*	-14.77	-11.16	-3.47	0.004*
DBP (mmHg)	-7.16*	-8.14	-6.19	-4.96*	-6.03	-3.87	-2.20	0.003*
Weight (lb)	-0.14	-0.65	0.37	0.20	-0.45	0.84	-0.34	0.420
BMI (kg/m²)	-0.34*	-0.57	-0.11	-0.11	-0.40	0.17	-0.23	0.226
TG (mg/dL)	-10.94*	-19.48	-0.62	-4.96	-16.96	7.03	-5.98	0.402
LDL (mg/dL)	-4.12*	-10.62	2.43	-1.26*	-10.44	-0.87	-2.86	0.797
HDL (mg/dL)	1.00	-0.01	2.02	-1.56*	-2.83	-0.28	2.33	0.002*
Glucose (mg/dL)	-2.29	-4.86	2.29	-3.50	-9.24	2.33	1.22	0.767
A1c (mg/dL)	-0.24	-0.51	0.07	-0.37	-0.94	-0.19	0.33	0.184

This figure displays the mean difference in blood pressure, weight, BMI, and laboratory values from baseline to one year based. Comparisons are made between users and non-user with uncontrolled hypertension. * Denotes a mean statistically significant from baseline (p<0.05). ± Denotes a statistically significant difference (P<0.05) in the means based on T-test.

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P196

Effect of Combined Oral Intake of Ginger Extract and Rice Vinegar on Blood Pressure with 2-kidney,1-clip Renovascular Hypertensive Rats

PrimaryAuthor.AuthorBlock:Natsumi Saito, Yukiko Segawa, Saki Maruyama, Ayuna Yamaoka, Kobe Women's Univ, Kobe, Japan; Hiroko Hashimoto, Osaka Seikei Junior Coll, Osaka, Japan; Tomoko Osera, **Nobutaka Kurihara**, Kobe Women's Univ, Kobe, Japan

Objective: Ginger is widely used as traditional Asian herbal medicine. Ginger has the same pungent ingredient as chili and vanillyl. We showed that administration of capsaicin to renovascular hypertension (RH) model rats increased endothelial nitric oxide (NO) synthase (eNOS) mRNA expression and NO production, and suppressed blood pressure (BP). Traditionally in Japan, ginger is pickled and eaten. Ginger and vinegar each are supposed to have an effect of suppressing an increase in BP in RH rats. The aim of this study was to investigate the effect. Method: Male Sprague-Dawley rats (6wks) were treated with sham operation (SHAM) as controls or clipping the left renal artery (2K1C) as RH model. After surgery, the rats started receiving a control diet (C) or a diet with 0.08% (w/w) of Ginger Extract (GE) for 6 weeks, and a tap water (W) or a water with 4.5% (v/v) rice vinegar (V). The systolic BP (SBP) was measured by a tail-cuff method every week. At the end of the protocol, the mean arterial BP (MAP) was measured under anesthesia. Then, the aortas were removed for extracting mRNA. mRNA for angiotensin type 2 receptor (AT_2) and eNOS was evaluated by real-time RT-PCR. Results: Through the experiment period, SBP was significantly effects in time, model (SHAM vs 2K1C), diet (C vs GE) , time×animal (P <0.001, each) and water (W vs V) (P <0.05). At the end of the protocol,

2K1C-C+W was higher in SBP than SHAM-C+W (176 ± 6 vs 138 ± 1 mmHg, *P* <0.05). 2K1C-GE+W showed lower SBP (150 ± 2 mmHg) than -C+W (*P* <0.05). SBP was not significantly different in 2K1C-GE+V (149 ± 4mmHg) from in -GE+W. The observations in MAP were similar to those in SBP. AT_2R mRNA expression showed significant effects in model (*P* <0.05) : the mRNA in 2K1C-C+W (0.9 ± 0.2) was significantly greater than in SHAM-C+W (0.4 ± 0.1) (*P* <0.05). There were no significant differences among the 2K1Cs: -C+W, -C+V (0.9 ± 0.1), -GE+W (0.8 ± 0.1) and -GE+V (0.9 ± 0.2). eNOS mRNA expression showed significant effects only in diet (CTL vs GE, *P* <0.05), but not in water and any interactions. Conclusion: Continuous ingestion of GE and V may suppress BP increase in 2K1C, respectively. Simultaneous ingestion of GE and V showed no enhanced effects compared to GE or V solo ingestion in 2K1C. The roles of eNOS and AT_2R in the mechanism did not become clear in this study.

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P197

Vitamin D, Paratohormone and Cardiovascular Risk: The Good, the Bad and the Ugly

PrimaryAuthor.AuthorBlock:**Rocco Giannotti**, Valeria Pascale, Rosa Finelli, Valeria Visco, Ida Matula, Univ of Salerno, Salerno, Italy; Nicola Ragosa, ASL Salerno, Vallo della Lucania, Italy; Angelo massari, Univ of Salerno, Salerno, Italy; Maria G Di Sevo, ASL Salerno, Polla, Italy; Michele Ciccarelli, Guido Iaccarino, Univ of Salerno, Salerno, Italy

25OH Vitamin D (VitD) insufficiency and increased cardiovascular risk (CVR) association is still debated. The VitD dependendent paratohormone (PTH), is considered as the possible actuator of VitD effects on CVR. In an overall population observational study, we assessed the role of PTH in predicting CVR. We recruited 412 persons during the World Hypertension Day, in the area of Salerno, Southern Italy. Through means of dedicated questionnaires, blood pressure measurements and a blood draught, we collected dietary habits, anamnestic, clinical and metabolic data. CVR was calculated according to the Framingham CVR charts. The overall population mean age±SD was 49.9±20 yrs, and female sex was slightly prevalent (53.2%). VitD deficiency (<20 uMol/ml) was most frequent (65.4%). In this population VitD and CVR did not correlate. VitD and PTH inversely correlated (r=-0.236, p<0.001) as expected. PTH was in direct linear regression with CVR (F=14,982, p<0.0001). Elevated PTH (>=56 mg/dl) levels identify a population with higher CVR (11.9±6.2 vs 9.2±7.1, p<0.01) and larger prevalence of cardiovascular events (10,2 vs 4.1, p<0,04). Since PTH increases with age and kidney function, we selected a 41-60 years old population (N=150). Also in this homogenous group, people with elevated PTH present a larger cardiovascular risk (8.4±10.5 vs 4.1±4.8, p<0,01) due to higher systolic blood pressure (137.2±20.4 vs 130.1±16.0, p<0.05). In conclusion, VitD deficiency causes PTH elevation that in the overall population increases the CVR. Our data let hypothesize that VitD supplementation may represent a major presidium to reduce PTH level and consequently CVR.

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P198

Combination of Undaria Pinnatifida Sporophyll and Vinegar Remarkably Decreases Blood Pressure in 2-kidney, 1-clip Renovascular Hypertensive Rats

PrimaryAuthor.AuthorBlock:Saki Maruyama, Yukiko Segawa, Natsumi Saito, Ayuna Yamaoka, Kobe Women's Univ, Kobe, Japan; Hiroko Hashimoto, Osaka Seikei Junior Coll, Osaka, Japan; Tomoko Osera, **Nobutaka Kurihara**, Kobe Women's Univ, Kobe, Japan

Objective: One of foods necessary to Japanese cuisine "Washoku" is algae, including Saccharina japonica (SJ) and Undaria pinnatifida (UP). The intake of SJ was observed to decrease blood pressure (BP) in spontaneously hypertensive rats in some studies, and in 2-kidney, 1-clip hypertensive (2K1C) rats in our studies. Furthermore, we observed the interactive effects of SJ and vinegar on BP in 2K1C rats (Kitamura S et al; Hypertension 2013, Maruyama S et al; Hypertension 2016). In the present study, we investigated the interactive effects of UP sporophyll (UPS) and vinegar, as well as the effect of UPS, on BP in 2K1C rats. We also evaluated angiotensin II type 1 receptor (AT1R) mRNA expression, in the mechanism. 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 with 5.0% (w/w) UPS, and a tap water or a water containing 5.0% (v/v) rice vinegar (V) for 6 weeks. Systolic BP (SBP) was measured by a tail-cuff method every week. At the end of the protocol, mean arterial pressure (MAP) was measured in each rat under anesthesia. Then, the aortas were removed for extracting mRNA. AT1R mRNA expression was evaluated using real-time RT-PCR. Results: Through the experiment period, SBP was significantly higher in 2K1C-C than in SHAM-C ($P < 0.001$). Neither 2K1C-V nor -UPS showed significant differences in SBP from 2K1C-C. However, 2K1C-UPS+V provided a significant reduction in SBP compared with 2K1C-C, -V, and -UPS ($P < 0.01$). At the end of the protocol, 2K1C-C was higher in SBP than SHAM-C (166 ± 3 vs 137 ± 3 mmHg, $P < 0.001$). 2K1C-UPS+V (155 ± 4 mmHg) was tended to be lower than 2K1C-C, -V (162 ± 8 mmHg), and -UPS (164 ± 3 mmHg). Thus, BP was reduced only in 2K1C-UPS+V in 2K1C animals. The observations in MAP were similar to in the SBP. No significant differences in AT1R mRNA expression were observed among SHAM-C (1.3 ± 0.2), 2K1C-C (1.2 ± 0.3), -V (1.0 ± 0.2), -UPS (1.3 ± 0.2) and -UPS+V (1.0 ± 0.2). Conclusion: UPS and vinegar may decrease BP collaboratively in 2K1C rats. AT1R mRNA may not play an important role in the mechanism.

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P199

Higher Circulating Triglycerides are Independently Associated With Greater Short-term Blood Pressure Variability in Middle-aged/older Adults With Obesity

PrimaryAuthor.AuthorBlock:**Rachel E. Luehrs**, Graziela Z. Kalil, Seth W. Holwerda, Nealy A. Wooldridge, Jess G. Fiedorowicz, Lyndsey E. DuBose, Melissa M. Rader, Gary L. Pierce, The Univ of Iowa, Iowa City, IA

Elevated short-term (24 hour) blood pressure variability (BPV) is associated with subclinical target organ damage and cardiovascular disease (CVD) among middle-aged/older (MA/O) adults with hypertension and obesity. Circulating total cholesterol (TC), low-density cholesterol (LDL-C) and triglycerides (TGs) increase with human obesity and are independent risk factors for CVD. In addition, BPV is increased in mouse models of hyperlipidemia and is normalized with statins. However, whether higher circulating lipoproteins independently contribute to greater short-term BPV among adults with obesity remains unclear. We hypothesized that higher LDL-C, TGs and lower high-density lipoprotein (HDL-C) would be associated with greater short-term BPV among individuals with obesity. Fasting plasma lipids and 24 hour ambulatory BP monitoring were assessed in fifty-six MA/O adults with obesity defined as body mass index (BMI) ≥ 30 kg/m^2 (56% F; age 54±7 yrs; BMI, 38.2±5.6 kg/m^2) and at least one other CVD risk factor. There was a significant relation between 24 hour systolic BPV and TC (r=0.30, P=0.03), TGs (r=0.34, P=0.01) and LDL-C (r=0.25, P=0.059), but not HDL-C (r=-0.07, P=0.61). Interestingly, these findings remained significant after adjusting for age, sex, BMI and 24 hour systolic BP (TC: r=0.34, P=0.01; TGs: r=0.39, P<0.01; LDL-C: r=0.31, P=0.03) but HDL-C remained non-significant (r=-0.16, P=0.27). In contrast, other cardiometabolic risk factors such as fasting glucose, insulin, c-reactive protein concentrations, carotid-femoral pulse wave velocity and HOMA-IR were not associated with 24 hour systolic BPV. In a multiple linear regression model that included age, sex, BMI, 24 hour systolic BP, TGs and LDL-C, only fasting TGs (β=0.02 ± 0.01, P=0.02) were a significant correlate of 24 hour systolic BPV (Model R^2 =0.24, P=0.03). Results were the same if TC was substituted for LDL-C in the model. In conclusion, higher plasma TC, LDL-C and TGs are associated with greater 24 hour BPV among MA/O adults with obesity with only TGs being independently associated with BPV. These data suggest that greater variability in BP among MA/O adults with obesity is mediated in part through circulating TGs suggesting that TGs may be a therapeutic target to modify short-term BPV.

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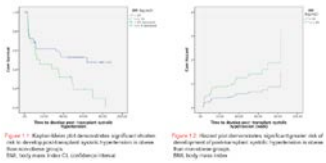
P200

Post-transplant Weight Control and Hypertension After Kidney Transplantation

PrimaryAuthor.AuthorBlock:**Ekamol Tantisattamo**, Oakland Univ William Beaumont Sch of Med, Royal Oak, MI; Possawat Vutthikraivit, Phramongkutklao Coll of Med, Mahidol Univ, Bangkok, Thailand; Haritha Mopuru, Multi-Organ Transplant Ctr, William Beaumont Hosp, Royal Oak, MI

Background: Pre-transplant obesity is associated with post-transplant hypertension

(HTN). The contribution of weight change after kidney transplantation and BP is unclear.
Methods: A retrospective cohort study during a 24-month follow-up was conducted in 70 kidney transplantation recipients whose BMI either <30 and ≥30 kg/m^2. The association between pre-transplant BMI as well as change of post-transplant BMI in each group and post-transplant HTN (SBP ≥140 mmHg), was determined.
Results: Of 70 patients (48 (69%) non-obese and 22 (31%) obese), mean age±SEM was 52.66±1.43 years. Mean pre-transplant SBP in non-obese group was not significantly different from SBP in obese group (141.6±21.5 VS. 139.59±22.41; p=0.726, CI -9.2036 to 13.1468). Post-transplant HTN occurred earlier in obese than non-obese groups (mean time to develop post-transplant HTN was 29.4±6.1 VS. 50.6±5.7 weeks; Log Rank 6.068, P=0.014; Figure 1.1). During 24-month follow-up period, followed-up mean BMI appears to increase compared with pre-transplant BMI and only non-obese group had a significant increase in BMI; whereas, BMI decreased in obese group. After controlling for age, gender, type of kidney transplantation, and type of induction immunosuppression, the hazard of post-transplant HTN is 2.152 times greater in obese group than that of non-obese groups (p=0.024, CI 1.108 to 4.178; Figure 1.2).
Conclusion: Although post-transplant weight gain occurred only in non-obese patients, the risk to develop post-transplant HTN in obese patients appears greater than in non-obese patient transplant. Therefore, pre-transplant weight loss remains important to minimize the risk of post-transplant HTN.



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P201

Prevalence of Metabolic Syndrome in Hypertensive Patients

PrimaryAuthor.AuthorBlock:**Nirankar Singh Neki**, Jaswinder Singh Student, Govt Medical Coll, Amritsar Punjab, India

Introduction and Objectives:
Metabolic syndrome (MS) is strongly predictive of developing diabetes mellitus, stroke and cardiovascular diseases. This study was done to find out the prevalence of metabolic syndrome among newly diagnosed hypertensive patients in a tertiary care hospital of northern India, Guru Nanak Dev hospital, attached to Government Medical College, Amritsar, India.
Methods
It is a hospital based cross sectional study involving one hundred newly diagnosed subjects with hypertension above the age 20 years. High blood pressure was defined according to Joint National committe-7 (JNC-7) guidelines, taking systolic BP> 140 and diastolic BP>90 as hypertension. Exclusion criteria included secondary hypertension, secondary cause of obesity, pregnancy, acute illness, or on steroids or any other medications known to elevate plasma glucose. The diagnosis of MS was defined as per Modified Cholesterol Education Program- Adult treatment Panel -III (MCEP-ATP III) criteria.
Statistical analysis was done using student's t-test and chi- square test, and p value of less than 0.05 was considered significant.
Results
Table1

Hypertensive MS patients Vs hypertensive patients without MS

Table 1

Discussion

The increased prevalence of MS in our study shows the impact of life style changes, low socioeconomic status etc. High prevalence of MS in females is possibly because of sedentary habits and staying at home. In our study, high body mass index and central obesity and hypertriglyceridemia were shown to be strongly linked to MS.

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P202

The Metabolic Syndrome and Shift Work Among firefighters in South Korea

PrimaryAuthor.AuthorBlock:**Jee eun Choi**, Mun-Joo Bae, Sungha Park, Yonsei Univ, Seoul, Korea, Republic of; Ki-Soo Park, Gyeongsang Natl Univ, Gyeongsangnam-do, Korea, Republic of; Changsoo Kim, Yonsei Univ, Seoul, Korea, Republic of

Background/Aim Multiple risk factors including dysipidemia, hypertension and hyperglycemia which cluster together are termed the metabolic syndrome. It means managing the metabolic syndrome is crucial to prevent cardiovascular disease (CVD). Several studies found that CVD is the common disease and the leading cause of on-duty death among firefighters. Although importance of understanding to investigate risk factors that causes CVD among firefighter has been emphasized, research about it is still behind. Thus, to understand risk factor of CVD among firefighters, this study was examined an association between metabolic syndrome and shift work among firefighters. **Methods** A total of 257 men firefighters were included from Firefighter Research Enhancement of Safety & Heath (FRESH) cohort in Korea. No history of CV related disease including hypertension and diabetes were selected. Weight circumference, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured. SBP and DBP were measured three times at interval of five minutes in resting and average of the three of SBP and DBP was used in this analysis. Fasting hyperglycaemia, Triglycerides and HDL cholesterol were analysed from blood sample collected from the participants. Metabolic syndrome, using the modified National Cholesterol Education Program (Adult Treatment Panel III) criteria, was defined. The information of shift works, smoking and alcohol consumption were self-reported from the participants and divided into three; no shift work, 24 hour shift work and 2 or 3 shifts works. To analysis the association between metabolic syndrome and shift works, logistic model was used, adjusting for age, BMI, smoking and alcohol. **Results** 26 (10.1%) participants out of 257 were metabolic syndrome in the participants group. The unadjusted prevalence of the metabolic syndrome by shift works is 8.2% in no shift work group, 7.1% in 24 hour shift work group and 15.9% in 2 or 3 shifts work group. Adjusted Odds Ratio (OR) with 95% CI for 24 hours shift work was 1.51 [0.35 6.45] and 4.77 [1.08 20.9] for 3 shifts works. **Conclusions** There is an association between metabolic syndrome and shift work in Korea firefighters, which implies shift work might be associated with CVD.

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P203

pNaktide Targeted to Adipocytes Inhibits Na/k-atpase Reactive Oxygen Species, Systemic Inflammation, and Obesity Development in Mice Fed a Western Diet (WD)

PrimaryAuthor.AuthorBlock:**Rebecca Martin**, Cameron Brickman, Jiang Liu, Komal Sodhi, Joseph I. Shapiro, Marshall Univ JCESOM, Huntington, WV

Introduction Obesity is a worldwide epidemic with many comorbidities. It has been demonstrated that oxidative stress can exacerbate obesity development. We have previously published that systemic administration of pNaKtide, a Na/K-ATPase signaling antagonist, was able to decrease oxidative stress and adipogenesis by blocking Na/K-ATPase signaling mediated amplification of oxidative stress.
Hypothesis Adipocyte dysfunction in mice fed western diet (WD) may be prevented by lentiviral-mediated adipocyte-specific delivery of pNaKtide.
Methods C57Bl6 mice were randomly divided into five groups: 1) normal chow 2) normal chow+lenti-adipo-pNaKtide 3) WD 4) WD+lenti-adipo-GFP and 5) WD+lenti-adipo-pNaKtide (n=6-8/group). Lentiviral constructs with pNaKtide driven by an adiponectin promoter were used to achieve pNaKtide expression specifically in adipose tissue. Groups 2 and 5 were given an intraperitoneal injection of lenti-adipo-pNaKtide and group 4 was given an intraperitoneal injection of lenti-adipo-GFP at beginning of the experiment and again at week 2; total time=12 weeks. Body weight was measured weekly. Glucose clearance was determined using an intraperitoneal glucose tolerance test before termination of the experiment. At sacrifice body weight, visceral and subcutaneous fat content of all mice were measured. Blood samples were collected for determination of inflammatory cytokines. Tissues were flash frozen and maintained at -80°C.
Results Lenti-adipo-pNaKtide significantly reduced WD-induced weight gain, and visceral and subcutaneous fat content. Lenti-adipo-pNaKtide reduced WD-induced changes in glucose tolerance and inflammatory markers TNFα, IL-6 and MCP-1 ($p<0.05$). An increase in cardiac hypertrophy in WD animals was attenuated with lenti-adipo-pNaKtide ($p<0.05$). Visceral fat of WD mice expressed higher levels of adipogenic markers PPARγ, FAS, and C/EBP. WD-induced Na/K-ATPase signaling was decreased.
Conclusion Collectively this study introduces the novel idea that adipocytes may have a systemic effect. Specifically targeting pNaKtide to the adipocytes with lenti-adipo-pNaKtide ameliorates this systemic effect. This new information is important in the development of new therapeutic targets for obesity.

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Gender-specific Relationships Between Plasma Levels of Endocannabinoids and Vagal and Sympathetic Control of Heart Rate in Normotensive Obese Older Adults

PrimaryAuthor.AuthorBlock:**Chris L. Schaich**, Hossam A. Shaltout, Wake Forest Univ Sch Med, Winston-Salem, NC; Alex Kovach, Megan Grabenauer, Brian F. Thomas, RTI Intl, Research Triangle Park, NC; Tina E. Brinkley, Barbara J. Nicklas, Debra I. Diz, Wake Forest Univ Sch Med, Winston-Salem, NC

Previous studies in obese individuals indicate higher circulating endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG) than in lean counterparts. However, the association between plasma endocannabinoids and autonomic control of blood pressure and heart rate has not been assessed in obesity. In a sample of normotensive, obese older adults we analyzed plasma content of the endocannabinoids AEA and total AG using mass spectrometry and examined correlations with various indices of spontaneous sympathovagal activity. Spontaneous baroreflex sensitivity (BRS) for heart rate control was calculated by spectral analysis of arterial pressure (AP) time (Sequence [Seq] Up, Seq Down and Seq All) and frequency (low-frequency [LF] and high-frequency [HF] α) domains from continuous resting AP recordings. In addition, time domain analysis was used to calculate heart rate variability (HRV) and blood pressure variability, indices of cardiac vagal tone and vascular sympathetic tone, respectively. The sample included 8 males and 17 females with a mean age of 68.4 ± 0.6 years, a mean body mass index of 35.0 ± 0.8 kg/m^2, and mean AP of 101.0 ± 2.2 mmHg. Across the complete sample, we report a significant inverse correlation between plasma AG content and HFα, an index of the vagally-mediated parasympathetic spontaneous BRS (r = -0.50, P < 0.05). We further report a significant inverse correlation between plasma AG and the vagal spontaneous BRS (Seq Up) in males (r = -0.87, P < 0.01) but not in females. However, in females but not males we found significant positive relationships between AEA and LFα, an index of sympathetic spontaneous BRS (r = 0.49, P < 0.05), and AEA and HRV (r = 0.50, P < 0.05). These results are consistent with a role for the endocannabinoid system to modulate autonomic control of the circulation in populations at risk for hypertension and cardiovascular disease, and suggest gender differences that have yet to be elucidated.

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P205

Short-term Western Diet Induced Hypertension in Female Rats is Associated With Vascular Epigenetic Modification

PrimaryAuthor.AuthorBlock:**Benjamin Kramer**, Kuo Zhang, Youhua Zhang, A. Martin Gerdes, Maria Alicia Carrillo-Sepulveda, NYIT Coll of Osteopathic Med, Old Westbury, NY

Over consumption of the western diet (WD) is a major contributor to the global epidemic of metabolic syndrome. Studies on the impact of WD on vascular complications related to hypertension have revealed contradictory results. Moreover, reports on the effect of WD on female cohorts is limited. Results from our lab have indicated that transient diabetic conditions increase vascular lysine acetylation levels, a post-translational epigenetic modification. Therefore, we hypothesized that short-term exposure to WD causes vascular endothelial dysfunction and hypertension in association with increased vascular lysine acetylation. Adult female Wistar rats were assigned either a control diet (13.4% fat, 56% carbohydrate, and 29.8% protein; n=4) or a WD (41% fat, 43% carbohydrates, and 17% protein;

n=6) for 17 weeks. Results showed the WD group exhibited increased body weight (376 ± 49 vs 305 ± 41g, p<0.05), periabdominal fat content (30 ±11 vs 11 ±2g, p<0.01), % body fat (8.1 ±1.0 vs 3.6 ±0.4%, p<0.01), and serum triglycerides levels (56 ± 13 vs 25 ± 4mg/dl, p<0.01) compared to controls. Blood glucose and free fatty acid levels did not differ between groups. Vascular reactivity studies, using wire myography, showed WD aortas had impaired acetylcholine-induced endothelial-dependent relaxation (83.5 ±4.2 vs 93.1 ±3.0%, p<0.001). Invasive left ventricular (LV) catheterization demonstrated that the WD group had increased systolic (147 ±12 vs 120 ±6mmHg, p<0.01) and diastolic blood pressures (105 ±9 vs 87± 4mmHg, p<0.01), and rate of LV relaxation (-dP/dt: 13.5 ±1.3 vs 9.4 ±2.4mmHg/ms, p<0.01) compared to controls. Histological orcein staining and morphometric analysis showed that WD aortas displayed severe elastic fiber derangement and medial thickening (150.6 ±13.5 vs 110.5 ±20.2µm, p<0.05). Lysine acetylation levels were significantly increased in WD vascular tissue (1.5-fold increase vs control, p<0.05). Additionally, levels of KAT2B/HAT, an acetyltransferase protein, were elevated in aortas from WD (2.0-fold increase vs control, n=2). Our results support our hypothesis that endothelial dysfunction and hypertension induced by WD in female rats correlates with increased epigenetic markers in vascular tissue.

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P206

Anti-hyperglycemic Effects of Angiotensin-II Type 2 Receptor Activation by C21 in High Sodium Diet Fed Obese Zucker Rats

PrimaryAuthor.AuthorBlock:**Sanket N Patel**, Quaisar Ali, Tahir Hussain, Univ of Houston Coll of Pharmacy, Houston, TX

Intake of high sodium diet (HSD) has been implicated in development of insulin resistance and obese subjects are salt-sensitive. Recently, we reported that stimulation of angiotensin-II type 2 receptor (AT_2R) by selective non-peptide agonist C21 prevents salt-sensitive hypertension and oxidative stress, however effect of AT_2R activation on insulin resistance and circulating lipids in obese animals fed HSD is unknown. Therefore, we investigated effects of AT_2R activation by C21 (1 mg/kg/day, s.c. by Alzet osmotic pump) on insulin resistance and circulating lipids in obese Zucker rats (OZR) fed HSD (4%) for 14 days. High-salt diet feeding significantly increased fasting plasma glucose (p<0.05) and insulin (p<0.05), and caused glucose intolerance as evident by oral glucose tolerance test (p<0.05). Concurrent treatment with C21 reduced plasma glucose (p<0.05) and insulin (p<0.05) and improved glucose tolerance (p<0.05). Circulating plasma free fatty acids (FFA) and triglycerides were unchanged in OZRs fed normal diet or HSD. Interestingly, however, C21 treatment lowered plasma FFA (p<0.05), but not triglycerides. Results suggest that AT_2R activation may prevent onset of hyperglycemia in obese animals fed HSD by improving insulin sensitivity, however, target organ involved in AT_2R-mediated effects remains to be explored.

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P207

Zeta Potential of Erythrocyte Membranes and Hypertension Characteristics in Patients With Metabolic Syndrome

PrimaryAuthor.AuthorBlock:**Mariya G. Sapacheva**, Mikhail V. Pisarev, Tatyana V. Koroleva, Valery I. Podzolkov, First Sechenov Moscow State Medical Univ, Moscow, Russian Federation

Aim: to study the relationship between total zeta potential of erythrocyte membranes (TZPEM) and duration, grade, and target organ damage/associated clinical conditions of hypertension in patients with metabolic syndrome (MS). **Material and methods**: 90 patients with metabolic syndrome (38 men and 62 women with mean age of 61.4±7.2 years and average hypertension duration of 7.19±5.4 years) were studied. Metabolic syndrome, hypertension grade and target organ damage were diagnosed according to the 2013 ESC Guidelines. The plasma TZPEM levels were detected by the Alcian cationic dye adsorption on the erythrocyte membrane. **Results**: to assess the relationship between hypertension characteristics and TZPEM the patients with MS were divided into groups according to the grade of hypertension, target organ damage/associated conditions and hypertension duration (below 5, 5-10, and more than 10 years). Hypertension was registered in all the patients in the main group. There were no significant differences in TZPEM between patient groups with various hypertension grades ($1.63\pm0.03x10^7$ in 1 grade hypertension, $1.58\pm0.05x10^7$ in 2 grade hypertension, and $1.60\pm0.04x10^7$ in 3 grade hypertension, $p>0.05$). Meanwhile, we found a significant reduction in TZPEM related to the target organ damage/associated conditions ($1.63\pm0.03 x10^7$, $1.60\pm0.04x10^7$ и $1.54\pm0.04x10^7$ in no target organ damage/target organ damage/associated clinical conditions, respectively, $p<0.05$). Also a significant reduction in TZPEM in association with the hypertension duration was revealed. The mean TZPEM was $1.65\pm0.04 x10^7$, $1.61\pm0.03x10^7$, and $1.56\pm0.05x10^7$ in patients with less than 5 years, 5-10 years, and more than 10 years duration of hypertension, respectively ($p<0.05$). A significant negative correlation between TZPEM and duration of hypertension was found in patients with MS ($r=-0.56$, $p< 0.05$).**Conclusion**: hypertension grade in patients with MS poorly correlates with TZPEM, whereas TZPEM was significantly lower with increasing duration of hypertension. TZPEM was also significantly lower in patients with target organ damage and/or associated clinical conditions which implies prognostic significance of TZPEM in patients with metabolic syndrome.

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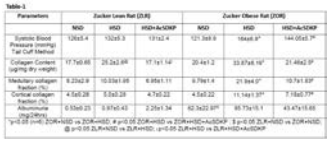
N-acetyl-seryl-aspartyl-lysyl-proline (acsdkp) Reduces High Salt- induced Hypertension and Kidney Damage in Obesity

PrimaryAuthor.AuthorBlock:**Mani Maheshwari**, Sumit Monu, Cesar Romero, Nitin Kumar, Tang-Dong Liao, ED Peterson, Oscar A Carretero, Henry Ford Health System, Detroit, MI

Introduction: Obesity increases the risk of salt sensitive hypertension and kidney damage. AcSDKP is a natural tetrapeptide with anti-inflammatory and antifibrotic properties but its

effect on obesity-related salt sensitive hypertension and renal damage is unknown. **Hypothesis**: AcSDKP prevents high-sodium diet (HSD) induced hypertension and kidney damage in obese rats. **Methods**: Eight-week-old Zucker obese rats (ZOR) and Zucker lean rats (ZLR) were treated with AcSDKP (1.6 mg/kg/day infused via osmotic minipumps) while maintained on either normal-sodium diet (NSD; 0.4%) or high-sodium diet (HSD; 4%) for 8 wk. Rats were divided into following six groups: ZOR+vehicle, ZOR+HSD, ZOR+HSD+AcSDKP, ZLR+vehicle, ZLR+ HSD, and ZLR+HSD+AcSDKP. Systolic blood pressure (SBP) was measured by tail cuff method. 24-hour urine collection was done to measure albuminuria as a marker of renal damage. Renal fibrosis (collagen content in the kidney) was measured by hydroxyproline assay. Medullary and cortical interstitial fibrosis was measured by picrosirius red staining. **Results** are summarized in Table 1. HSD increased SBP in ZOR but not in ZLR. AcSDKP treatment prevented HSD induced increase in BP in ZOR. HSD increased renal collagen content both in ZOR and ZLR and AcSDKP treatment decreased it. Increased interstitial fibrosis induced by high salt diet was confirmed by PSR staining in both cortical and medullary region and AcSDKP prevented this increase. Albuminuria in ZOR was significantly higher than ZLR, however neither high salt nor AcSDKP treatment was unable to normalize it. **Conclusion:** Chronic treatment with AcSDKP prevents high salt-induced hypertension and renal fibrosis in ZOR.



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P209

Mechanistic Target of Rapamycin Complex 1 (mTORC1) Signaling Generates Reactive Oxygen Species in Mouse Endothelial Cells via NFkB Signaling

PrimaryAuthor.AuthorBlock:**John J Reho**, Deng-Fu Guo, Andrew Olson, Kamal Rahmouni, Univ of Iowa, Iowa City, IA

Obesity is associated with vascular endothelial dysfunction and reactive oxygen species (ROS) signaling. We have previously demonstrated a novel role for the mechanistic target of rapamycin complex 1 (mTORC1) signaling pathway in the regulation of cardiovascular reactivity. In this study, we assessed the ability of mTORC1 signaling to generate ROS in mouse endothelial cells (MEC) and investigated the role of NF**κ**B signaling as a potential transcriptional regulator. We used an adenoviral construct of a constitutively active (CA) S6-kinase (Ad-CAS6K) to enhance mTORC1 signaling in MEC. Infection of MEC (48hrs) with the Ad-CAS6K resulted in increased mRNA levels of NADPH oxidase 1 (3.4±0.7 fold) and 2 (3.6±0.6 fold) and decreased superoxide dismutase 2 expression (0.5±0.2 fold) compared to an adenoviral GFP (Ad-GFP) control ($p<0.05$). Next, we used dihydroethidium staining as a measure of ROS signaling in MEC. Infection of MEC with the Ad-CAS6K enhanced ROS signaling compared to the Ad-GFP (1.5±0.1 fold; $p<0.05$). Conversely, infection with a dominant negative S6K construct (Ad-DNS6K) decreased ROS generation (0.6±0.1; $p<0.05$). Notably, infection of aortic rings of wildtype mice with the Ad-

CAS6K resulted in reduced acetylcholine induced vasorelaxation compared to Ad-GFP (Max. relaxation: 67±5 vs. 81±3%; $p<0.05$) with no change in relaxation evoked by sodium nitroprusside (Max. relaxation: 90±1% vs. 90±2%). The reduced endothelial-mediated vasorelaxation was rescued with Tempol (Max. relaxation: 74±2%; $p<0.05$) indicating ROS signaling as a mechanism of mTORC1 signaling induced endothelial dysfunction. Using immunoprecipitation assays, we uncovered a physical interaction between the mTOR subunit and the IKKβ subunit of the NFκB complex. We also found that blockade of the IKKβ subunit with BMS-345541 (300nM) prevented the increased ROS generation in MEC in response to the Ad-CAS6K (1.4±0.1 vs 0.8±0.1 fold; $p<0.05$) implicating NFκB in underlying mTORC1 signaling induced ROS generation. Our data demonstrate that enhanced mTORC1 signaling in endothelial cells impair endothelial function via ROS production and NFκB signaling complex and may play a critical role in the endothelial dysfunction associated with various conditions including obesity.

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P211

Intra-abdominal Lipectomy Normalizes Arterial Stiffness and Blood Pressure via Reduction in 20-HETE

PrimaryAuthor.AuthorBlock:**Amanda A Soler**, Brenda Hutcheson, Jenny Yang, New York Medical Coll, Valhalla, NY; Chastity Bradford, Tuskegee Univ, Tuskegee, AL; Frank F Zhang, Katie H Gotlinger, Michal L Schwartzman, Petra Rocic, New York Medical Coll, Valhalla, NY

Increased intra-abdominal (visceral) adipose tissue is a key feature of the metabolic syndrome affecting over 30% of the U.S. population. Expansion of visceral adipose tissue is linked to the development of hypertension and is a risk factor for cardiovascular disease that can ultimately lead to end-organ damage. While reduction in visceral adipose tissue volume offers cardioprotective effects, the cardiovascular mechanisms behind these beneficial effects remain unclear. In this study, we removed ~90% of visceral adipose tissue (=~5% body weight) by intra-abdominal lipectomy and assessed large arterial stiffness, large artery structural matrix components, and blood pressure in a metabolic syndrome rat model (JCR:LA-cp, JCR). Large artery stiffness was significantly elevated in JCR vs. normal (Sprague Dawley, SD) rats (75±2% JCR vs. SD (carotid)) with a concomitant significant increase in MMP12-dependent elastin degradation (3-6 fold vs. SD). Intra-abdominal lipectomy normalized large artery stiffness, blocked MMP12 activation and reduced elastin degradation in JCR animals (~75% (carotid) vs. untreated JCR). Likewise, hypertension in JCR animals was significantly attenuated by intra-abdominal lipectomy (MABP=156±3 mmHg JCR vs. 90±6 mmHg SD vs. 132±4 mmHg JCR+lipectomy). 20-hydroxyeicosatetraeonic acid (20-HETE), an arachidonic acid metabolite known to be a potent vasoconstrictor in resistance arteries, was significantly elevated in the visceral adipose tissue of JCR rats (~6 fold vs. SD). Intra-abdominal lipectomy normalized 20-HETE levels in JCR rats. Like intra-abdominal lipectomy, 20-HETE antagonists restored large artery elasticity, blocked MMP12 activation and elastin degradation, and significantly decreased blood pressure (125±3 mmHg JCR+20-HETE antagonists) in JCR rats. Thus, 20-HETE may be

an important adipokine that mediates the adverse effects of expanded visceral fat volume in the metabolic syndrome and its inhibition may provide a pharmacological approach for the management of central obesity-driven large artery stiffness and hypertension.

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P213

Metabolic and Cardiovascular Effects of Melanocortin-4 Receptors in Leptin Receptor-expressing Neurons

PrimaryAuthor.AuthorBlock:**Eva N Rodriguez Cruz**, Kamal Rahmouni, Huxing Cui, Univ of Iowa, Iowa City, IA

Studies in both humans and rodents have clearly established a role for melanocortin-4 receptor (MC4R) in energy homeostasis, sympathetic and cardiovascular function. Both MC4R-deficient humans and rodents develop severe obesity while keeping the sympathetic tone and blood pressure below or within the normal range, indicating that MC4R is required for obesity-associated sympathetic overdrive and hypertension. Indeed, sympathetic overdrive and cardiovascular side effect have been a major problem in developing anti-obesity drugs that target MC4R. Thus, better understanding the neural substrates that mediate distinct metabolic and sympathetic actions of MC4R is needed to provide a more specific target to treat obesity and associated hypertension. We have recently mapped the pattern of co-expression of MC4R and leptin receptor (LepR) and found that, throughout entire mouse brain, these two metabolically important receptors are uniquely co-expressed only in neurons of two brain regions, including the lateral hypothalamic area (LHA) and the periaqueductal gray (PAG). To specifically test the role of MC4R in LepR-expressing neurons, we generated mice with MC4R expression only in LepR-positive neurons by breeding Cre-dependently “reactivatable” MC4R-null mice (MC4R-TB) to LepR-Cre mice. We found that specific re-expression of MC4Rs only in LepR-positive neurons partially suppresses body weight gain especially in female mice (MC4R-TB 31.73 ± 2.2g vs. MC4R-TB::LepR-Cre 27.97 ± 1.8 g, $p=0.0013$). Direct multi-fiber recording of renal nerve activity (RSNA), however, revealed that neither baseline activity nor MC4R agonist-induced RSNA is significantly altered by restoration of MC4R signaling in LepR-positive neurons (change of RSNA in MC4R-TB 8.844 ± 3.05 vs. MC4R-TB::LepR-Cre 11.95 ± 2.7, $p>0.05$). Our results suggest that MC4R signaling in leptin-responsive neurons is sufficient to suppress body weight in female mice, but seemingly has no effect on sympathetic traffic to the kidney. Further investigation of the effect of MC4Rs in LepR-positive neurons on blood pressure regulation is underway.

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P214

Shotgun Metagenomic Analysis of Fecal Microbiome Reveals Profound Functional Differences in Patients with Hypertension

PrimaryAuthor.AuthorBlock:**Seungbum Kim**, Elaine M Richards, Yanfei Qi, Mohammed

Mohammed, Eileen M Handberg, Carl J Pepine, Mohan K Raizada, Univ of Florida, Gainesville, FL

Objectives: Recent studies have implicated changes of the gut pathology and altered microbiome in the animals of various hypertension models. However, these relationships in human hypertension are poorly understood. Thus, our objectives in this study was to test the hypothesis that microbiome from hypertensive patients (HTN) would be taxonomically and functionally differ from those of normotensive subjects. **Design and Method:** Fecal samples were collected from HTN (n=22, mean SBP 155.8±3.4mmHg) and reference subjects without hypertension (REF) (n=18, mean SBP 121.1±1.5mmHg) (see ClinicalTrials.gov, NCT02188381 for detailed protocol). Gut microbiomes were analyzed using shotgun metagenomics and the USEARCH6.1 algorithm for OTU clustering. Taxonomy was assigned with Metaphlan. Qiime, Phyloseq and Galaxy web applications were used to further analyze the data. **Results:** Two beta diversity measures, principal coordinates analysis and partial least squares discriminant analysis, showed significantly different microbiome composition between the two groups (ANOSIM p=0.012). A heatmap based on significantly enriched functional genes from each cohort (P<0.05) also showed clustering of samples by group. Metagenomic analysis showed that the butyrate kinase gene and the abundance of butyrate producing bacteria were negatively correlated with SBP (R^2=0.10 and P<0.05 for both). Additionally, functional analysis showed that HEME biosynthesis and hydroxyphenylacetate degradation were decreased in the HTN cohort (LDA score 2.15 and 1.95 respectively) suggesting inefficient nitric oxide synthesis and polyphenol digestion in HTN patients. **Conclusions:** 1) Significant difference in taxa of HTN from REF was observed. 2) Microbial genes linked to butyrate, nitric oxide and polyphenol were significantly decreased in HTN. Gene products of all these were shown to have important implications in cardiovascular diseases. Thus, our observations suggest that targeting of gut and its microbiota can offer a novel strategy for hypertension control.

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P215

NADPH Oxidase Dependent Oxidative Stress and Redox Sensitive MAPKs Contribute to the Mechanisms of Acupuncture in Rostral Ventrolateral Medulla of Spontaneously Hypertensive Rats

PrimaryAuthor.AuthorBlock:**Xuerui Wang**, Jingwen Yang, Caishuo Ji, Xianghong Zeng, Guangxia Shi, Beijing Hosp of Traditional Chinese Med affiliated to Capital Medical Univ, Beijing, China; Marc Fisher, Beth Israel Deaconess Medical Ctr, Dept of Neurology, and Harvard Medical Sch, Boston, MA; Cunzhi Liu, Beijing Hosp of Traditional Chinese Med affiliated to Capital Medical Univ, Beijing, China

NADPH Oxidase-Dependent Oxidative Stress and Redox-Sensitive MAPKs Contribute to The Mechanisms of Acupuncture in Rostral Ventrolateral Medulla of Spontaneously Hypertensive Rats.

Abstract

Oxidative stress in the rostral ventrolateral medulla (RVLM), where the sympathetic nervous control center is located, contributes to neural mechanisms of hypertension. Acupuncture was previously reported to

favorably affect high blood pressure. However, little is known about the effect of acupuncture on oxidative stress-modulated mechanisms in hypertension. This study was designed to evaluate the hypothesis that acupuncture exerts an antihypertensive effect via ameliorating oxidative stress and the redox-sensitive pathway in the RVLM of spontaneously hypertensive rats (SHRs). Two weeks of acupuncture reduced blood pressure and sympathetic nervous system activity in SHRs. Oxidative stress in the RVLM was alleviated by acupuncture, accompanied by a decrease in NADPH oxidase activity and expression of its subunits. Acupuncture significantly altered the mitogen-activated protein kinases (MAPKs) signaling pathway as assessed by pathway enrichment analysis in a gene chip assay. The phosphorylation of p38MAPK, extracellular signal-regulated protein kinase (ERK)1/2, but not Jun N-terminal kinase (JNK), were downregulated by acupuncture. Microinjection bilaterally of the superoxide dismutase mimetic tempol, NADPH oxidase inhibitor apocynin or diphenyleneiodonium chloride into the RVLM mimicked the antihypertensive effect of acupuncture. In contrast, the NADPH oxidase agonist TBCA abolished the beneficial effects of acupuncture. Furthermore, injection of capsaicin, or surgical sectioning of the sciatic nerve abolished the antihypertensive effect of acupuncture. We conclude that acupuncture decreases NADPH oxidase derived ROS and inactivates its downstream MAPKs in the RVLM to restore sympathetic vasomotor activity and decrease blood pressure. The neural transmission from the sensory sciatic nerve is involved in mechanism of acupuncture in treating hypertension.

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P216

Nicotinamide Nucleotide Transhydrogenase Regulates Mitochondrial Redox Balance and Endothelial Function in Response to Angiotensin II

PrimaryAuthor.AuthorBlock:Matthew Vetkoetter, Shashanka Rao K N, Xinggui Shen, Sibile Pardue, **David M Krzywanski**, LSU Health Sciences Ctr, Shreveport, LA

Hypertension (HTN) involves increased peripheral resistance to blood flow mediated by changes in vascular tone. Endothelial dysfunction is an initiating step in HTN and mitochondrial reactive oxygen species (ROS) contribute to endothelial dysfunction. Nicotinamide nucleotide transhydrogenase (NNT) is a critical enzyme in the regulation of mitochondrial NADPH levels and can impact mitochondrial redox status. We have demonstrated that the loss of NNT in C57Bl/6J mice is associated with a more severe hypertensive phenotype in response to Ang II characterized by mitochondrial and vascular dysfunction driven by vascular ROS production *in vivo* and *in vitro*. Little is known regarding the role of NNT human endothelial cells, but based on previous studies we hypothesize that the loss of NNT promotes mitochondrial and cellular ROS production and disrupts normal endothelial $^{\bullet}$NO function. In human aortic endothelial cells (HAEC) treatment with 500nM Ang II for two hours significantly increased NNT expression (225 $\pm$ 13%) and glutathione peroxidase 2 (Gpx2) activity (17.81 $\pm$ 0.75 vs 29.06 $\pm$ 2.53 nmol/min/ug protein) consistent with NNT's role in driving the mitochondrial antioxidant systems. Conversely, siRNA knockdown of NNT

inhibited Gpx2 activity (18.32 ± 1.17 vs 13.16 ± 0.7 nmol/min/ug protein) and increased mitochondrial superoxide production (0.12 ± 0.01 vs 0.17 ± 0.01 nmol/mg protein) in response to Ang II. These changes in the redox balance of the mitochondria contribute to mitochondrial dysfunction where the loss of NNT led to a marked decrease in the respiratory control ratio (RCR) of the mitochondria (6.42 ± 0.17 vs 3.83 ± 0.1) of Ang II treated cells. Ultimately, this shift to a pro-oxidative mitochondrial redox phenotype in these cells conspired to inhibit Ang II-induced •NO production (35.60 ± 1.28 vs 30.10 ± 1.24 pmol/mg protein). Our data indicate that the absence of NNT expression increases the steady state levels of mitochondrial ROS through increased ROS production and decreased antioxidant activity, which is contributes to reduced •NO production and endothelial dysfunction. These studies highlight NNT as a novel regulator of mitochondrial redox tone that may critically impact the ability of vascular ROS to contribute to the development of HTN.

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P217

Nox Compartmentalization, Protein Oxidation and ER Stress in Vascular Smooth Muscle Cells in Hypertension

PrimaryAuthor.AuthorBlock:**Livia L Camargo**, Augusto C Montezano, Adam Harvey, Sofia Tsiropoulou, Katie Hood, Zhenbo Cao, Richard Burchmore, Richard Hartley, Neil Bulleid, Rhian M Touyz, Univ of Glasgow, Glasgow, United Kingdom

In hypertension, activation of NADPH oxidases (Noxs) is associated with oxidative stress and vascular dysfunction. The exact role of each isoform in hypertension-associated vascular injury is still unclear. We investigated the compartmentalization of Noxs in VSMC from resistance arteries of Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHR). Expression of Nox1 and Nox4 was increased in SHR cells (96.6±28.7% and 48.2±21.2% vs WKY, $p<0.05$), as well as basal ROS levels measured by chemiluminescence (110.2±26.4% vs WKY, $p<0.05$) and amplex red (105.2±33.2% vs WKY, $p<0.05$). Phosphorylation of unfolded protein response activators, PERK and IRE1α, and expression of ER chaperone BiP were elevated in SHR cells ($p<0.05$ vs WKY), indicating activation of ER stress response. Immunoblotting after organelle fractionation demonstrated that Noxs are expressed in an organelle-specific manner, with Nox1, 2 and 4 present in plasma membrane, ER and nucleus, but not in mitochondria. In SHR cells, NoxA1ds (Nox1 inhibitor, 10μM) and GKT136901 (Nox1/4 inhibitor, 10μM) decreased AngII-induced ROS levels ($p<0.001$ vs Ctl). Additionally, mito-tempol (mitochondrial-targeted antioxidant, 50nM) and 4-PBA (ER stress inhibitor, 1mM) decreased basal ROS levels in SHR cells ($p<0.05$ vs Ctl). Furthermore, oxidation of the antioxidant enzymes Peroxiredoxins (Prx) was increased in SHRSP compared to WKY (2.51±0.14 vs 0.56±0.07, $p<0.001$). One-dimensional isoelectric focusing revealed that cytosolic Prx2 and mitochondrial Prx3 were more oxidized in SHRSP than WKY cells. Using a biotin-tagged dimedone-based probe (DCP-Bio) we identified oxidation of ER stress proteins BiP and IRE1. To investigate the effect of protein oxidation in vascular function, vascular reactivity was evaluated in isolated mesenteric arteries. Inhibition of general oxidation (DTT

1mM; Emax: 111.7±33.1) and peroxiredoxin (Conoidin A 10nM; Emax: 116.0±7.3) reduces vascular contraction in response to noradrenalin in WKY rats (Emax: 166.6±30.2; $p<0.05$). These findings suggest an important role for Nox1/4 in redox-dependent organelle dysfunction and post-translational modification of proteins, processes that may play an important role in vascular dysfunction in hypertension.

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P218

Role of UCP2 on Mitochondrial Dysfunction and Blood Pressure Regulation in the Renal Oxidative Stress-mediated Hypertension Associated With Dj-1 Depletion

PrimaryAuthor.AuthorBlock:Carmen De Miguel, Univ of Alabama at Birmingham, Birmingham, DC; William C Hamrick, Univ of Alabama at Birmingham, Birmingham, AL; Sudhar Jagarlamudi, Xiaobo Ma, Laureano Asico, Pedro Jose, **Santiago Cuevas**, The George Washington Univ, Washington, DC

DJ-1 and uncoupling protein 2 (UCP2) exert protective roles against mitochondrial (MT) oxidative stress. *DJ-1*$^{-/-}$ mice have increased systolic blood pressure (BP) (+30±3% vs WT, n=6). This study determined the mechanisms involved in the oxidative stress-mediated hypertension due to *DJ-1* germline deletion. There were no differences in sodium excretion, renal renin expression, NADPH oxidase activity and serum creatinine between *DJ-1*$^{-/-}$ and WT mice (n=5). However, renal expression of nitro-tyrosine was increased in *DJ-1*$^{-/-}$ mice (+176.8±31% vs WT mice, n=5). Tempol, a radical scavenger, normalized the BP (tempol: 118±2% vs 100±1% vs WT, n=4) and renal malondialdehyde (tempol: 160±23% vs 109±15% vs WT, n=4) in *DJ-1*$^{-/-}$ mice. Tempol-treated *DJ-1*$^{-/-}$ mice had higher serum nitrite/nitrate levels than placebo-treated (172±30% vs WT, n=4). Heat shock protein mtHSP60 was increased in *DJ-1*$^{-/-}$ mice (2.9±0.1-fold increase vs WT, n=4), indicating MT stress. However, there were no changes in the renal mRNA expression of mitophagy, MT fusion and MT biosynthesis markers indicating that MT function was not altered. Renal expression of *UCP2* was increased in *DJ-1*$^{-/-}$ mice (4.1±1.1-fold change vs WT, n=4), and was partially normalized by tempol (1.8±0.2-fold change vs WT, n=4), *UCP2* may have a protective role on MT function in this model. *UCP2* was selectively silenced via sub-capsular infusion of *UCP2* siRNA in the kidney (WT: 63%±7 vs control: *DJ-1*$^{-/-}$:60%±6 vs control; n=4). mRNA expression of mitophagy markers BNIP3 (-0.65±6-fold) and PINK1 (1.55±0.3-fold), MT fusion markers FIS1 (-0.29±0.03-fold) and NFN2 (1.42±0.06-fold), and MT biosynthesis marker PPRC1 (1.71±0.07-fold) were altered by *UCP2* silencing in *DJ-1*$^{-/-}$ mice (n=4). Renal-selective silencing of *UCP2* normalized BP in *DJ-1*$^{-/-}$ mice (*DJ-1*$^{-/-}$ mice: 122±5 vs 98±7 mmHg, n=4), and the serum nitrite and nitrate concentrations (-40±9% vs WT, n=4). In conclusion, deletion of *DJ-1* leads to oxidative stress-induced hypertension associated with down-regulation of NO synthesis. *UCP2* has protective properties against the development of MT dysfunction in MT oxidative stress conditions. However, excessive and chronic over expression of *UCP2* could have deleterious consequences on BP regulation.

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Vascular Cross-talk Between Redox and Calcium Signaling in Hypertension Involves Transient Receptor Potential Melastatin 2 Channel Activation

PrimaryAuthor.AuthorBlock:**Rhéure Alves-Lopes**, Augusto C Montezano, Karla B Neves, Aikaterini Anagnostopoulou, Silvia Lacchini, Rhian M Touyz, Univ of Glasgow, Glasgow, United Kingdom

The transient receptor potential melastatin 2 cation channel (TRPM2) is redox-sensitive and promotes Ca^{2+} influx after H_2O_2 activation through oxidative modification and PARP-ADPR-dependent mechanisms. TRPM2 also regulates Na^+ influx, and by increasing $[Na^+]i$ interferes with the Na^+-Ca^{2+} exchanger (NCX) inducing reverse mode action, promoting Ca^{2+} influx. These processes may be driven by Nox4-derived H_2O_2. We tested the hypothesis that vascular dysfunction in hypertension involves oxidative stress-induced TRPM2 activation through H_2O_2 production, which in turn promotes Ca^{2+} influx. Mesenteric arteries isolated from wildtype (WT), LinA3 (mice expressing human renin with Ang II-dependent hypertension), $Nox4^{-/-}$ and LinA3/$Nox4^{-/-}$ mice and vascular smooth muscle cells (VSMCs) from hypertensive and normotensive patients were used. Arteries from hypertensive LinA3 mice, exhibit increased U46619-induced vasoconstriction versus WT mice (Emax - LinA3 *vs* WT: 9.37 ± 0.51 *vs* 6.79 ± 0.29), an effect attenuated by olaparib (PARP-ADPR inhibitor) and 2-APB (TRPM2 blocker) and also increased mRNA expression (Fold change - related to control) of NOX4 (3.05 ± 0.30), TRPM2 (1.38 ± 0.24), NCX (1.973 ± 0.34) and salt inducible kinase 1 (1.833 ± 0.12) and sodium-potassium pump (1.43 ± 0.16), which are activated when intracellular levels of Na^+ rise beyond a critical point. These events seem to be regulated by NOX4, since they were not observed in mesenteric arteries from LinA3/$Nox4^{-/-}$ mice. Ang II-induced Ca^{2+} influx is potentiated in VSMCs from hypertensive patients (AUC-Ex490/Em535: normotensive: 15400±917.5 *vs* hypertensive - 22460±2388), a response followed by increased generation of O_2^- and H_2O_2 in cells from hypertensive patients. These ROS effects were attenuated by catalase, and 2-APB, 8-br and olaparib (TRPM2 inhibitors) and benzamil, KB-R7943 and YM244769 (NCX inhibitors). Our data indicate that TRPM2 ion channel activation contributes to redox-sensitive vascular dysfunction in hypertension. These findings suggest that dysregulation of TRPM2-NOX4-derived ROS and NCX may contribute to redox- and Ca^{2+} signalling important in vascular function in hypertension. TRPM2 may be a point of cross-talk between ROS and Ca^{2+} signalling.

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Perception of Blood Pressure Control and Satisfaction with Hypertension Care in the Rural Setting

PrimaryAuthor.AuthorBlock:**Harini Bejjanki**, Yan Gong, Univ of Florida, Gainesville, FL; David Willis, Heart of Florida Health Ctr, Ocala, FL; Kendra Siler-Marsiglio, WellFlorida Council,

Gainesville, FL; Eileen Handberg, Univ of Florida, Gainesville, FL; Daniel Haight, Univ of South Florida and Lakeland Regional Health, Lakeland, FL; Steven Smith, Carl Pepine, Rhonda Cooper-DeHoff, Univ of Florida, Gainesville, FL

Background: National Health and Nutrition Examination Survey (NHANES) data indicate only 50% with hypertension (HTN) have controlled blood pressure (BP) yet limited data are available regarding patient's knowledge of and satisfaction with their BP control.
Methods: We utilized MyHealthStory, an online health information exchange portal to identify a rural, diverse HTN population. Adult patients (≥ 18 yrs) were recruited from existing federally-qualified health center (FQHC) sites. The survey was sent via email to 5000 patients who voluntarily shared email addresses through MyHealthStory. Equal numbers of men and women of any race/ethnicity with a diagnosis of HTN and who consented to receive information about research in MyHealthStory were targeted. We developed the 21 question survey using Qualtrics. The first 300 patients who completed the survey were included. We compared a) patient-reported BP control (BP ≥ 140/90; or <140/90) and patient-reported satisfaction with BP control (yes=happy, no=unhappy) and b) patient reported BP control and perception of BP control (well controlled, uncontrolled); using McNemar's test for paired nominal data.
Results: Here we report data from the 238 completed surveys received from Caucasians (n=184, 61% female) and African Americans (n=54, 83% female). Among 117 Caucasians reporting BP, 51% (n=60) reported uncontrolled BP, 52% (n=61) reported perception of well controlled BP (p=0.49). A total of 77% (n=46) of patients who had uncontrolled BP reported satisfaction with their HTN care compared to 96% in those who had controlled BP (*P*<0.0001). Among 32 African Americans reporting BP, only 13% (n=4) reported controlled BP but 31% (n=10) reported perception of well controlled BP (p=0.034). Among the 4 African Americans whose BP was controlled, all 4 reported satisfaction with their HTN care, while among the 28 patients who reported uncontrolled BP, 23 (82%) reported satisfaction with their HTN care (*P*<0.0001).
Conclusions: Among hypertensive individuals, our survey data suggest there is a substantial disconnection between reported BP control, perception of BP control and satisfaction with HTN treatment, suggesting need for improvement in patient education surrounding HTN treatment and BP control.

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P221

Rural Living May Mean "Pressure Up": Investigating the Negative Impact of Rurality on Hypertension Control

PrimaryAuthor.AuthorBlock:**Rice J Zachary**, Emran Rouf, Scott and White Health Plan, Temple, TX

OBJECTIVES
To identify the impact of social determinants of health on adequate blood pressure (BP) control in a Medicare population.
METHODS
The population studied included 8308 adults aged 27 to 85 enrolled in a Medicare Cost Plan in Central Texas in 2016. We included Medicare members with at least one outpatient visit with a diagnosis of hypertension in the first six

months of 2016 and at least one outpatient visit with a diagnosis of hypertension in 2015. As part of a program evaluation, we combined clinical and socio-demographic data from claims, enrollment, and electronic medical records to estimate a multivariate logistic regression model and identify the clinical and non-clinical factors associated with poor BP control.
RESULTS
In 2016, over 74% (6172 of 8308) of Plan members were compliant with NCQA HEDIS age- and diabetes-adjusted BP control standards, a rate above the 2015 national average. Similarly, around 65% (5363 of 8308) of Plan members obtained an end-year target BP <140/90. After controlling for several clinical and demographic factors, a member living in a rural zip code was found to be 6.2% less likely (at $p<0.01$) to achieve a target BP <140/90 than if that member lived in an urban zip code. This negative association was strongest among members living in the most rural zip codes and was limited to Whites: on average, a White member living in a rural zip code was 6.9% less likely (at $p<0.01$) to have a target BP <140/90 than if he or she lived in an urban zip code.
CONCLUSION
The medical community has a limited understanding about how socio-geographic factors, such as rural living, impact BP control. Our analyses found that rural Whites were less likely than urban Whites to have BP <140/90, after controlling for comorbidities, hypertension severity, poverty, education, and other demographic factors. Even in our well-controlled Medicare population, system-wide hypertension control remains challenging due to cultural and geographic disparities. In such populations, in-depth qualitative analysis and creative patient outreach strategies may be necessary to improve hypertension control among the hardest to reach patients.

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P222

Prevalence of Hypotension in Adolescents

PrimaryAuthor.AuthorBlock:**Jyoti Bhatia-Barnes**, UT McGovern Medical Sch, Houston, TX; Monesha Gupta, Johns Hopkins All Children's Hosp, St. Petersburg, FL; Cynthia Bell, Josuha Samuels, UT McGovern Medical Sch, Houston, TX

Background
Hypotension is a widely used but poorly defined clinical entity. Currently accepted definitions of hypotension are the Pediatric Advanced Life Support (PALS) definition, the Brain Trauma Foundation definition, and the International Pediatric Sepsis Consensus Conference. However, its prevalence has not been defined.
Objective
To determine the prevalence of hypotension in school aged children.
Study Design
This study is a retrospective analysis of data from a series of cross-sectional school-based blood pressure screenings completed by the Houston Pediatric and Adolescent Hypertension Program (HPAHP) at the UTHealth at Houston. Screenings were performed in 21 urban and suburban middle and high schools in the Houston area from 2001 to 2012. We restricted the data set to include subjects with three systolic blood pressure (SBP) measurements performed using a Spacelabs 90217 oscillometric monitor, who did not report taking hypertensive medication, and were not missing demographic information. We defined hypotension based on 2 criteria: average 1st-3rd SBP less than the 5th percentile for height, age and gender. Within these definitions we also restricted the hypotension definition to subjects

with average SBP below 90mm Hg. Stata 13 SE was used for all data analysis.

Results

Of the total 22, 382 children enrolled, 15,114 children met inclusion criteria: 52% females, mean age 13.6 years (range 10.1-19.9). Racial distribution reflected that of Houston: 35% White, 32% Hispanic, 23% Black, 8% Asian, and 2% other/unknown. Seventy one (0.5%) children had an average SBP reading less than the 5th percentile and 112 (0.7%) had an average SBP less than the 5th percentile or 90 mm Hg. Compared to students without hypotension, students with hypotension defined by only the 5th percentile were older (14.3 vs 13.6 years, t-test p=0.0001); they also tended to be more female, though not significantly (61% vs 39%, Chi^2 p-value=0.134).

Conclusion

Although not as prevalent as hypertension in this age group (generally 2-5%), hypotension is a clinically significant entity that is seen in 0.5-0.7% of the general school-aged population.

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The ω-oxidation Pathway Underlies Hexadecanedioate Induced Blood Pressure Elevation

PrimaryAuthor.AuthorBlock:**Nora Hassan Alharbi**, Martin W. McBride, Sandosh Padmanabhan, Delyth Graham, Glasgow university, Glasgow, United Kingdom

Background: A novel pathway for blood pressure (BP) regulation involving the dicarboxylic acid hexadecanedioate (HEXA) was identified in a study associating BP and mortality outcomes with fasting blood metabolites. The functional role of HEXA on BP elevation and vascular reactivity was confirmed by oral administration of hexadecanedioic acid to WKY rats. This study characterized the metabolic effects of hexadecanedioic acid administration in WKY rats, and assessed hemodynamic changes after modulation of endogenous HEXA levels by perturbing the ω-oxidation pathway in the SHRSP rat.

Methods: WKY were treated orally with hexadecanedioic acid (250 mg/kg/day) or vehicle (n=5/group) for 3 weeks. Aorta, heart, brain, adipose, kidney, and liver were harvested and global metabolic profiles analysed by UPLC-MS/MS (Metabolon). The aldehyde dehydrogenase (ALDH) inhibitor disulfiram (25 mg/kg/day) or vehicle were administered to SHRSP rats for 14 days (n=7/group). BP was assessed by tail plethysmography and mesenteric arteries were used to assess vascular function by wire myography.

Results: Treatment with hexadecanedioic acid increased HEXA levels in all tissues except brain in WKY rats. Metabolomic analysis identified increased fatty acid (FAs) metabolites (e.g. acylcarnitines) in heart, long-chain FAs and ketone body (β-hydroxybutyric acid) in kidney, and dicarboxylate FAs in adipose tissue from hexadecandioic acid treated rats indicating an impairment of FA β-oxidation and a shift towards ω-oxidation. SHRSP rats showed a significant reduction in BP after treatment with disulfiram (ΔSBP, 20.7±4.6 mmHg; P=2.4x10^{-4}). Mesenteric resistance arteries from disulfiram treated SHRSP demonstrated a shift to the right in the contractile response curve to noradrenaline, indicating reduced vascular sensitivity compared to control vessels.

Conclusion: Administration of hexadecanedioic acid in addition to increasing BP impacts several metabolic readouts including changes in FA metabolism. Importantly, we demonstrate that inhibition of the ω-oxidation pathway enzyme,

ALDH, results in a significant lowering of BP in the hypertensive SHRSP rat. The ω-oxidation pathway is a target for further research to elucidate mechanisms by which HEXA affects BP.

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β-arrestin2 Stimulates SERCA2a SUMOylation and Activity in Cardiac Myocytes to Promote Contractility

PrimaryAuthor.AuthorBlock:**Katie A McCrink**, Jennifer Maning, Ava Brill, Angela Vu, Nova Southeastern Univ, Fort Lauderdale, FL; Walter J Koch, Temple Univ, Philadelphia, PA; Anastasios Lymperopoulos, Nova Southeastern Univ, Fort Lauderdale, FL

Background: Heart failure (HF) is the most lethal disease worldwide and new treatments are needed. The β_1-adrenergic receptor (AR) mediates the positive inotropy of catecholamines, partly via Sarco(Endo)plasmic Reticulum Ca^{2+}-ATPase (SERCA)-2a activation. Agonist-activated β_1ARs, however, are desensitized/downregulated in human HF due to the actions of the βarrestins (βarrestin1 and 2). βarrestins are GPCR adapter proteins and signal transducers. βarrestin1 is by far the predominant isoform in the heart and reduces contractility by desensitizing the β_1AR, whereas βarrestin2 is expressed at negligible levels and is beneficial post-myocardial infarction (MI), as it combats
inflammation and apoptosis. Herein, we sought to investigate whether cardiac βarrestin2 exerts any inotropic effects.
Methods: We used βarrestin knockout (KO) mouse hearts and also performed intra-cardiac adenoviral gene transfer of βarrestin2 (Adβarrestin2) in post-MI mice in vivo. For mechanistic signaling studies we used the cardiomyocyte cell line H9c2.
Results: SERCA2a SUMO (small ubiquitin-like modifier)ylation and activity and, consequently, cardiac contractility were increased in βarrestin1 KO`s vs. WT`s post-MI. The opposite was true for βarrestin2 KO`s post-MI. Additionally, βarrestin2, but not βarrestin1, was found to directly bind SERCA2a and induce its SUMOylation and activation in mouse hearts in vivo, as well as in cardiomyocytes in vitro acutely in response to β_1AR stimulation. Interestingly, βarrestin2 did not affect the classic β_1AR cAMP-dependent pro-contractile signaling pathway in cardiomyocytes, again contrary to βarrestin1. Importantly, and consistent with these findings, Adβarrestin2 gene transfer in post-MI mouse hearts in vivo resulted in enhanced cardiac function (post-MI ejection fraction of Adβarrestin2 vs. control (AdGFP) mice: 40.3$\pm$1.3% vs. 23.1$\pm$1.2%, respectively, $p<0.05$, n=5).
Conclusions: Cardiac β_1AR-activated βarrestin2, but not βarrestin1, promotes SERCA2a SUMOylation and activity, increasing cardiac contractility. Given also its anti-inflammatory and anti-apoptotic effects post-MI, cardiac-specific βarrestin2 gene transfer may be a novel and safe inotropic therapy for both acute and chronic HF.

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P225

Non-invasive Measurement of Renal Blood Flow (RBF) in Different Rat Models by Magnetic Resonance Imaging (MRI)

PrimaryAuthor.AuthorBlock:**Cesar A Romero**, Robert Knight, Glauber Cabral, Oscar A Carretero, Henry Ford Health System, Detroit, MI

Quantitative measure of RBF provides important information regarding renal physiology and pathology, in different animal's models. Arterial Spin Labelling-MRI (ASL-MRI) is a non-invasive method to measure blood flow without exogenous contrast media, using arterial water protons labeled by radiofrequency as an endogenous tracer. However, the low signal/noise radio, and the motion artifacts are a challenge for the acquisition of RBF in small animals. Our objective is evaluated the feasibility and reproducibility of the RBF measure by ASL-MRI in different hypertensive rats models. ASL-MRI images were obtained in Sprague-Dawley (SD) rats (200-300g) under inhalation anesthesia using a 7 Tesla Varian MRI system with a spin echo imaging sequence. After 4 days the MRI studies was repeated to evaluate reproducibility, using paired sample T-test and the test-retest reliability (TR) equation. RBF was also measured in in Dahl SS rats on regular chow and spontaneous hypertensive rats (SHR). Additionally we measure the RBF in a set of animals under unilateral nephrectomy (UNx) and renal arterial stenosis (RS) before and after the surgery. Table 1 shows the mean cortical RBF in different rat strains and models. Re-test analysis showed no relevant differences, being the means of differences 9.4±35 ml/min/100g tissue (p=0.58) in SD rats. The TR was 92.4±6%. UNx increase the RBF in 69.1% in comparison with sham group. (p<0.01). After the RS the blood pressure increased and the RBF decrease 56% (p<0.01) in comparison with sham group. ASL-MRI performed with navigator correction and respiratory gating is a feasible and reproducible non-invasive method to measure RBF in several rat models.

Table 1. RBF in several animals models (ml/min/100 gr tissue)

	Sprague-Dawley	Dahl SS	SHR
Right Kidney	198±33.7	221.5±56.7	182.8±31.6
Left Kidney	216.6±26.8	213.8±67	202.4±29.9

	Unx	RS
Sham	267.3±59	198±15
Surgery	456.6±34	86±7.2

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P226

Aggressive Blood Pressure Control Status Post-acute Aortic Dissection May be Associated With Acute Kidney Injury: A Pilot Study

PrimaryAuthor.AuthorBlock:**Charles Hopley**, T. Brett Reece, Willaim Hiatt, Univ of Colorado Sch of Med, Aurora, CO

Management of acute aortic dissection includes appropriate blood pressure control (120/80 mmHg per AHA guidelines) but this is not based on evidence. Excessive lowering of blood pressure may contribute to organ malperfusion. The kidneys are the most commonly affected organs, causing refractory hypertension and acute kidney injury (AKI). Recent landmark blood pressure trials with intensive pressure goals have a significant incidence of AKI. This pilot study tested the hypothesis that achieved systolic blood pressure less than 120 mmHg has a higher incidence of AKI than those treated systolic pressures > 120 mmHg. Methods: Patients were identified via surgical log. Retrospective chart review was performed on patients in the acute setting following dissection. Daily average blood pressures were calculated. Patients were included if they underwent surgical repair of aortic dissection and survived the first 24 hours. Patients were

excluded if they demonstrated shock physiology or required renal replacement therapy in the first 24 hours. Serum creatinine and clinical course (including anti-hypertensive regimen) were recorded. The primary endpoint was in-hospital occurrence of AKI (defined per KDIGO criteria) in the acute setting status post aortic dissection in patients receiving anti-hypertensive therapy. An unadjusted odds ratio was calculated. Data on potential confounders and covariates (baseline sCR, contrast exposure etc) were also collected. Results: From 2013-2017, 37 cases of surgically repaired aortic dissection were identified and 16 cases met inclusion/exclusion criteria. The incidence of AKI in patients treated to an average systolic blood pressure less than 120mmHg was 75% compared with 50% in those with pressure greater than 120 mmhg. OR =3.0 [95% CI 0.3612-24.9];p=0.3019. Covariates and potential confounders were balanced between groups but given the small numbers additional statistical adjustments were not performed. Conclusion The incidence of AKI was numerically higher in the group of patients achieving more intensive blood pressure targets. Our study was limited by small sample size and selection bias. Both of these issues can be addressed if we apply similar methodologies to address the same question on a lager sample size.

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Prostaglandin EP_4 Receptor Mediate Angiotensin II-induced Hypertension via Activation of PRR/ENaC Pathway

PrimaryAuthor.AuthorBlock:**Xiaohan Lu**, Fei Wang, Kevin Yang, Kexin Peng, Tianxin Yang, Univ of Utah, Salt Lake Cty, UT

Activation of COX-2/EP_4 pathway is shown to upregulate renal (pro) renin receptor (PRR) contributing angiotensin II (Ang II)-induced hypertension but the underlying mechanism remains unclear. Here, we tested the hypothesis that activation of EP_4 receptors increases ENaC expression/activity through PRR in the setting of Ang II treatment. Sprague-Dawley rats were infused for 2 weeks with vehicle, Ang II alone (100 ng/kg/min), or in combination with an EP_4 antagonist ONO-AE3-208 (ONO) (0.2 mg/kg/d). Following 14-d AngII treatment, 24-h urine volume and 24-h urinary Na^+ excretion both exhibited a significant increase which was completely blocked by ONO. Ang II infusion selectively elevated α-ENaC protein abundance (1.9-fold) in the renal medulla but not in the renal cortex, which was completely blocked by ONO, as assessed by immunoblotting. Interestingly, Ang II infusion elevated the cleaved γ-ENaC protein in both medulla and cortex, which was similarly abolished by ONO. In response to Ang II infusion, the increases in renal medullary mRNA were 1.7-fold for α-ENaC, 1.3-fold for β-ENaC, 2.4-fold for γ-ENaC as assessed by qRT-PCR; in contrast, none of the ENaC subunits showed obvious changes in the renal cortex. Ang II-induced increases in α-ENaC mRNA were reduced 41% by ONO with a modest effect on the response of β-ENaC and γ-ENaC. In cultured mpkCCD cells, Ang II treatment at 500 nM for 24 h induced a 2.5-fold increase of ENaC activity as assessed by epithelial volt-ohmmeter, which was completely blocked by ONO (10 μM) or a specific PRR inhibitor PRO20 (1.0 μM). To further determine the role of the EP4 receptor in the regulation of ENaC activity, the mpkCCD cells were treated with vehicle, CAY10580 alone (agonist of the EP_4 receptor, 10 μM), or in combination with PRO20 for 24 h. The ENaC

activity showed a 2.0-fold increase in response to CAY10580, which was nearly completely abolished by PRO20 (n = 6-8, p<0.05). CAY10580 induced a 2.1-fold increase in PRR protein abundance. Overall, these results suggest that activation of EP_4 induces PRR, leading enhancement of ENaC expression/activity, eventually contributing to increased blood pressure in response to Ang II treatment.

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Chronically Enhanced Fructose Intake Induces Salt Sensitive Hypertension in Mice and Increases NKCC2 Phosphorylation

PrimaryAuthor.AuthorBlock:**Kamal Mohamed-Ayed Kassem**, Pamela Harding, Pablo Ortiz, Henry Ford Hosp, Detroit, MI

High fructose consumption is implicated in hypertension and renal damage in humans. The mechanisms for the renal effects of fructose are not clear. In normal rats, a high fructose diet (20%) does not increase blood pressure, but rapidly (one week) induces a salt sensitive increase in blood pressure. NaCl reabsorption by the thick ascending limb (TAL) is mediated by the NKCC2 cotransporter. Higher NKCC2 activity is involved in salt sensitive hypertension, and its phosphorylation is increased by fructose in rats. We hypothesized that high fructose (20% drinking water), would induce NKCC2 phosphorylation and induce salt sensitive hypertension in normal C57/Bl6J mice. To test this, mice were given 20% fructose in drinking water and after two weeks they were treated for 10 more weeks with either fructose alone or fructose plus 4% Na diet. Tail cuff systolic blood pressure (SBP) was measured after 3 weeks of training. Total NKCC2 expression, GAPDH and NKCC2 phosphorylation at Thre96/101 (P-NKCC2) were measured by Western blot in freshly isolated TALs. Baseline SBP was 112 ± 3 mmHg. A transient increase to 125± 4 mmHg (p<0.05) was observed after 1 week on fructose which later decreased to 119 ± 3 mmHg (p=0.14) by week 2. At the end of week 2, high salt chow was added. BP remained normal for 3 weeks and then increased to 137 ± 11 mmHg (p<0.05) by week 4 on high salt, but not in fructose alone mice. After 10 weeks SBP remained elevated in the fructose plus high salt diet group (124 ± 3 vs 110 ± 5 mmHg; p<0.05). Water intake and urine volume was similar between the two high fructose groups. After 10 weeks, fasting glucose was higher in all mice with fructose (fructose + HS: 231± 8, fructose: 218 ± 7, normal diet: 122 ± 4 mg/dL, p<0.001). A fructose diet increased P-NKCC2 by 248 ± 82% (n=5, p<0.01) by one week and by 61 ± 21% (p<0.05) at week 12. The combination of high-fructose high salt diet increased P-NKCC2 by 226 ± 75% (p<0.05) at 12 weeks. Transcriptome profiling of isolated mouse TALs showed that 20% fructose (7 days) increased the expression of genes upstream of the SPAK and AMPK pathway. We conclude that 20% fructose in drinking water leads to a salt dependent increase in SBP, which appears to occur after mice develop abnormalities in glucose metabolism.

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P229

cGMP Stimulates Ubiquitination and Degradation of NKCC2 in Thick Ascending Limbs

PrimaryAuthor.AuthorBlock:**Gustavo R Ares Sr.**, Henry Ford Health System, Detroit, MI

NaCl reabsorption by the thick ascending limb (TAL) is mediated by the Na/K/2Cl cotransporter (NKCC2). Nitric oxide inhibits NaCl reabsorption in TALs by increasing the second messenger cyclic guanylate monophosphate (cGMP) and decreasing NKCC2 in the apical plasma membrane. Recently, we showed that internalized NKCC2 is constitutively degraded (0.33%/min). Protein degradation regulates channels and transporters activity along the nephron. However, whether NKCC2 is regulated by lysosomal degradation or ubiquitin-proteasomal system remains unknown. We hypothesized that internalized NKCC2 is degraded by the ubiquitin-proteasome system in a process stimulated by cGMP. TAL surface proteins were biotinylated and allowed to internalize. The biotin remaining in surface proteins was stripped away and only internalized NKCC2 measured by Western blot. cGMP enhanced the rate of disappearance of internalized NKCC2 by 83 % and this was blocked by a proteasomal (MG132) but not lysosomal (leupeptin) inhibitor (Control: 0.29 ± 0.04; cGMP: 0.53 ± 0.10 ; cGMP + MG132: 0.10 ± 0.10 ; cGMP + Leupeptin: 0.44 ± 0.06 %/min; $p< 0.05$). In general, protein degradation by the proteasomal system requires ubiquitination of the targeted protein. We found that NKCC2 inmuno-precipitated with ubiquitin. Proteasome inhibition induced the accumulation of ubiquitin-NKCC2 and this was enhanced by cGMP (MG132: 59 ± 14%, MG132+cGMP: 111 ± 25%; $p<0.05$). We concluded that internalized NKCC2 is degraded *via* the ubiquitin-proteasome pathway in a process stimulated by cGMP. cGMP-induced degradation of internalized NKCC2 may contribute to decreased NKCC2 trafficking to the apical membrane therefore decreasing NaCl reabsorption in TALs.

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P230

Uncoupling of NOS3 is Involved in Diminished Flow-induced No Production in Dahl Salt-sensitive Rat Thick Ascending Limbs

PrimaryAuthor.AuthorBlock:Nancy J Hong, **Agustin Gonzalez-Vicente**, Jeffrey L Garvin, Case Western Reserve Univ, Cleveland, OH

Increased luminal flow enhances nitric oxide (NO) production in thick ascending limbs (TALs). NO produced by NO synthase 3 (NOS3) inhibits Na transport. However, its effect on transport is reduced in Dahl salt-sensitive (SS) vs salt-resistant rats (SR). In Sprague-Dawley rat TALs, angiotensin II can acutely cause NOS3 to uncouple to produce superoxide (O_2^-) thereby reducing NO production. We hypothesized that flow-induced NO production is decreased in SS TALs and that this is due to NOS3 uncoupling. We measured flow-induced NO in isolated perfused TALs using the fluorescent dye DAF-FM and performed Western blots of renal medullary lysates. Flow-induced NO production was reduced 69% in TALs from SS (11±2 arbitrary units (AU)/min, n=6) vs SR (35±6 AU/min, n=8, $p < 0.008$). This difference between strains was not due to altered NOS3 expression (NOS3/GAPDH ratio of 0.91 ± 0.08

for SS vs 1.09 ± 0.08 for SR; n = 5 for each). The difference in flow-induced NO between strains was slightly reduced in the presence of the superoxide (O_2^-) scavenger tempol (19±2 vs 30±5 AU/min for SS and SR, respectively; n=9 for each strain, $p < 0.04$), suggesting that scavenging of NO by O_2^- plays only a minor role in the difference in flow-induced NO production between SS and SR thick ascending limbs. We next investigated whether NOS3 uncoupling could account for the difference between strains by using the fluorescent dye dihydroethidium to measure flow-induced O_2^- before and after treatment with the NOS inhibitor L-NAME. Blocking NOS3 reduced O_2^- production in SS TALs by 21±7%, from 38±5 to 30±5 AU/min (n=6, $p < 0.05$) whereas it had no effect in SR TALs (26±6 vs 28±3, n=5). We conclude that the diminished flow-induced NO in SS TALs is not due to differences in NOS3 expression nor acute flow-induced O_2^-, but rather in large part due to uncoupling of NOS3. Impaired flow-induced NO production in TALs could contribute to the Na retention associated with salt-sensitive hypertension.

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P231

Offspring of Captopril Treated Spontaneously Hypertensive Rats Have Lower Angiotensin II Type 1 Receptor Expression Which is Associated With Lower Blood Pressure

PrimaryAuthor.AuthorBlock:**J X Masjoan-Juncos**, Tang-Dong Liao, Ginette Bordcoch, Cesar A Romero, Oscar A Carretero, Henry Ford Hosp, Detroit, MI

It has been reported that SHR rats receiving angiotensin converting enzyme (ACE) inhibitor Captopril decrease blood pressure (BP) in at least two generation after the treatment was stopped. A decreased response to an intracerebroventricular infusion angiotensin I and angiotensin II in treated animals and their offspring was reported; however there is no reported mechanism that explains the changes observed in the untreated offspring of the Captopril treated animals. We hypothesize that captopril reduces angiotensin II type 1 receptor (AT1R) expression in CNS of the offspring of SHR rats treated with captopril. Animal groups are as follows: control animals, captopril treated animals, offspring of the control animals, offspring of the treated animals where the mother was removed from the treatment immediately after giving birth and Offspring of treated animals where the mother was removed from the treatment at weaning. BP was measured by intra-arterial method and Tail cuff. AT1R expression was measured in brain tissue using the posterior wall of the forth ventricle, as well as the top half of the brain stem. BP was different between treated groups and their offspring vs. control (Table 1). AT1R expression was significantly reduced in both offspring groups of the treated animals, when compared to control (Table 1). Therefore we conclude that captopril reduces blood pressure in the offspring of captopril treated SHR rats and that associates with a decrease in AT1R expression in CNS. Further research is necessary to determine the possible epigenetic mechanisms involved in AT1R reduction.

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Superoxide Increases Renin Activity, Renal Angiotensin II Type 1 Receptor (at1r) Function and Blood Pressure in Rats

PrimaryAuthor.AuthorBlock:**Xitao Wang**, Mohammad Asghar, Mohammad Saleem, Univ of Houston, Houston, TX

We examined role and mechanism of superoxide in renal AT1R function contributing to hypertension in Sprague-Dawley rats. Rats were divided into 4 groups and treated with 1. vehicle, 2. superoxide dismutase (SOD) inhibitor diethylthiocarbamate (DETC, 10mg/kg, i.p./daily for 2 weeks), 3. DETC+tempol (an SOD mimetic, 2 mM in drinking water), and 4. tempol only. Rats were sacrificed, blood plasma and kidneys obtained for biochemical studies. Superoxide [Fluorescence Unit (FU): control vs DETC vs DETC+tempol vs tempol, 0.10±0.006 vs 0.13±0.01 vs 0.09±0.007 vs 0.09±0.004] but not hydrogen peroxide levels increased in the renal tissues of DETC-rats than in vehicle-rats, which decreased in DETC+tempol rats. Nuclear levels of acetylated-Sp3 transcription factor, an index of its activity, (Density Unit: control vs DETC vs DETC+tempol vs tempol, 0.10±0.01 vs 0.17±0.02 vs 0.10±0.01 vs 0.13±0.02) and membranous AT1R proteins levels (Density Unit: control vs DETC vs DETC+tempol vs tempol, 0.98±0.32 vs 2.93±0.40 vs 0.99±0.24 vs 0.77±0.23) increased in DETC-rats, which were attenuated in DETC-tempol-rats. Moreover, plasma renin activity (FU: control vs DETC vs DETC+tempol vs tempol: 0.13±0.006 vs 0.19±0.04 vs 0.12±0.005 vs 0.13±0.01) increased in DETC-rats, which decreased in DETC+tempol-rats. Second set of rats underwent the same treatment as above, anesthetized, blood pressure (BP) and renal AT1R function (in terms of natriuresis) in response to AT1R blocker *candesartan* (10µg/kg, i.v. bolus) were determined. Systolic BP increased in DETC-rats than in vehicle-rats, which decreased in DETC+tempol-rats (mmHg: control vs DETC vs DETC+tempol vs tempol: 118.6±2.72 vs 136.7±2.96 vs 118.1±3.81 vs 123.1±6.88). Natriuresis in response to candesartan (cande) increased in vehicle-rats, which further increased in DETC-rats while decreased in DETC+tempol-rats (µmols/min: control vs control+cande vs DETC vs DETC+cande vs DETC+tempol vs DETC+tempol+cande vs tempol vs tempol+cande, 0.32±0.07 vs 0.58±0.10 vs 0.19±0.05 vs 1.06±0.34 vs 0.23±0.05 vs 0.49±0.14 vs 0.27±0.04 vs 0.66±0.11). Taken together, our results suggest that superoxide via Sp3 activation increases renin activity, membranous AT1R levels and function contributing to hypertension in rats.

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P233

Mas Receptor Deficiency Does Not Impair Cognitive Funcion of Vascular Dementia Model in the Presence of Angiotensin II Type 2 Receptor

PrimaryAuthor.AuthorBlock:**Akinori Higaki**, Masaki Mogi, Jun Iwanami, Li-Juan Min, Harumi Kan-no, Hui-Yu Bai, Bao-shuai Shan, Dept of Molecular Cardiovascular Biology and Pharmacology, Ehime Univ, Graduate Sch of Med, Toon City, Ehime Prefecture, Japan; Masayoshi Kukida, Dept of Cardiology, Pulmonology, Hypertension & Nephrology, Ehime Univ Graduate Sch of Med, Toon City, Ehime Prefecture, Japan; Toshihiro Yamauchi, Ehime Univ, Graduate Sch of Med, Toon City,

Ehime Prefecture, Japan; Takafumi Okura, Jitsuo Higaki, Dept of Cardiology, Pulmonology, Hypertension & Nephrology, Ehime Univ Graduate Sch of Med, Toon City, Ehime Prefecture, Japan; Masatsugu Horiuchi, Ehime Univ, Graduate Sch of Med, Toon City, Ehime Prefecture, Japan

Objective: Angiotensin (Ang) converting enzyme (ACE) 2/ Ang-(1-7)/Mas receptor axis has been considered as protective arm in the renin-angiotensin system and Ang-(1-7) is thought to interact with Ang II type 2 (AT_2) receptor according. Mas receptor is expressed highly in hippocampus and blood vessels in brain, but its actual function is still unclear. Thus, we examined the possible roles of Mas receptor in relation to the vascular cognitive impairment focusing on the interaction with AT_2 receptor. **Design and Methods:**Male 10-week-old C57BL6 mice (wild-type, WT), Mas1 receptor knockout mice (MasKO) and AT_2/Mas1 receptor double knockout mice (DKO) were subjected to bilateral carotid artery stenosis (BCAS) surgery. After six weeks from the treatment, we evaluated their cognitive function with Y-maze test and the Morris water maze test. **Results:** The cerebral blood flow (CBF) in each BCAS group was significantly reduced compared to its sham-operated counterparts (WT; 31.5±0.6 vs 28.0±0.8, MasKO; 31.7±0.8 vs 27.7±0.8, DKO; 33.0±0.5 vs 29.1±0.7). The alternation behavior (%) was significantly reduced in WT mice with BCAS compared to sham mice (69.6±3.5 vs 57.9±2.1), but there was no significant difference in MasKO and DKO mice (MasKO; 64.1±2.5 vs 63.1±2.5, DKO; 67.6±2.1 vs 61.1±4.0) in Y maze test. In the Morris water maze test, the mean arrival time at platform at day 5 (sec) was significantly higher in WT-BCAS mice than WT-sham mice (Sham; 20.9±4.6 vs BCAS; 47.3±6.5). In contrast to the results in WT, there was no significant difference in MasKO mice (Sham; 32.8±8.5 vs BCAS; 34.5±7.3). DKO-sham mice showed significantly lower spatial learning ability compared with WT-sham mice (DKO; 77.4±11.9 vs WT; 20.9±4.6). The total cell count in dentate gyrus area was significantly lower in WT-BCAS compared to WT-sham (sham; 255.7±7.0 vs BCAS; 209.4±5.4), but there was no significant change in MasKO mice(sham; 256.5±2.5 vs BCAS; 233.2±16.4). We could not see significant difference in the number of DCX-positive cells and the expressions of proinflammatory cytokines such as IL-6, TNF-α and MCP-1in all mouse groups. **Conclusion:**Mas receptor deficiency seems to be beneficial in vascular dementia on condition that AT_2 receptor exists.

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2-Methoxyestradiol Attenuates Angiotensin II Induced Hypertension: A Novel Paradigm

PrimaryAuthor.AuthorBlock:**Yong Zhang**, Benard Ogola, Laxmi Iyer, Vardan Karamyan, Thomas Thekkumkara, TTU Health Sciences Ctr, Amarillo, TX

Estrogen metabolite 2-methoxyestradiol (2ME2) has therapeutic potential in a number of cardiovascular disorders, including hypertension. However, specific or potential targets remains to be determined. In this study, animals were surgically implanted with PA-C40 telemetry in the intra-aortic vessel and 2ME2 (20 mg/kg/day, *i.p.* injection) effects in AngII infused Wistar-Koyoto (WKY) rats and spontaneously hypertensive rats (SHRs) were determined. In WKY rats 2ME2 treatment

resulted in significant reduction in AngII induced blood pressure (BP) compared to vehicle control (systolic; 143.69 ± 2.68 vs. 169.75 ± 1.93 mm Hg, diastolic; 99.91 ± 2.49 vs. 120.16 ± 1.25 mm Hg, and mean arterial pressure; 118.94 ± 2.52 vs. 142.73 ± 1.47 mm Hg). We observed a decrease in heart rate in 2ME2 exposed animals compared to control group (272.67 ± 9.88 vs. 329.12 ± 6.14 BPM). In addition, 2ME2 mediated reduction in workload of the heart when we calculated the rate pressure product or pressure rate index by 13.46 ± 2.32 indicating that 2ME2 can reduce AngII induced hemodynamic stress of the heart. [^{3}H]AngII binding studies showed that in kidney cortex AT1R expression was inhibited by 2ME2 treatment compared to control (2112 ± 71 vs. 3341 ± 248 DPM/mg protein). In a separate 5-week study, 2ME2-treated SHRs displayed significant reduction in diastolic BP (114.31 ± 0.66 vs. 123.28 ± 0.70 mm Hg) and mean arterial pressure (146.35 ± 0.67 vs. 151.54 ± 0.89 mm Hg) without significant change in systolic BP compare to the vehicle treated animals. Moreover, we found significant decrease in heart rate in 2ME2-treated animals (290.03 ± 2.69 vs. 312.92 ± 1.50 BPM). Analyses of kidney cortical tissue revealed downregulation of AT1R expression after 2ME2 treatments (20227 ± 2211 vs. 9515 ± 2115 DPM/mg protein). Consistent with the reduction in BP, the indicators of cardiac hypertrophy including heart weight (1.28 ± 0.08 vs. 0.99 ± 0.02 gram), cardiac collagen deposit and α-smooth muscle actin (α-SMC) positive myofibroblasts were decreased. The data demonstrate for the first time that 2ME2 control BP through downregulation of AT1R in kidney and heart rate, which may provide an insight to the mechanism(s) of cardioprotective effects of 2ME2 through the blockade of AngII induced hypertension.

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P235

Renin and Prorenin Uptake by Human Conditionally Immortalized Proximal Tubule Epithelial Cells Involves Megalin and Mannose 6-phosphate Receptor

PrimaryAuthor.AuthorBlock:**Alexandre G Martini**, Erasmus MC Univ, Rotterdam, Netherlands

Renin is filtered by the glomerulus, and subsequently reabsorbed in the proximal tubule in a megalin-dependent manner, as evidenced by a 20-40-fold rise in urinary renin in patients with Dent's disease or Lowe syndrome, i.e. disorders characterized by defective proximal tubular reabsorption. Remarkably, the reabsorption of filtered prorenin appears to be complete, as it can only be detected in urine of patients with Dent's disease or Lowe syndrome. To further investigate megalin-mediated renin and prorenin reabsorption, human conditionally immortalized proximal tubule epithelial cells (ciPTEC) were incubated at 37 ^{0}°C or 4 ^{0}°C with recombinant human renin or prorenin (1000 U/L), in the absence or presence of 10 mmol/L mannose 6-phosphate (M6P) to block (pro)renin binding and internalization via M6P receptors. Cell lysate (pro)renin levels were measured by immunoradiometric assay both before and after prorenin activation. At 37 ^{0}°C, cellular renin and prorenin tended to level off at 6 hours, reaching lysate levels of 75±18 and 100±54 pg/mg (mean±SEM), and all prorenin was detected as renin. Incubating ciPTEC at 4 ^{0}°C diminished the cellular accumulation of

renin and prorenin by ≈90%, and at this temperature prorenin remained in its inactive conformation. M6P blocked renin and prorenin uptake by >80% under all conditions. Importantly, M6P also concentration-dependently blocked the uptake of fluorescently labeled albumin (IC_{50} =≈ 3 mmol/L), to the same degree as the megalin inhibitor RAP-GST (IC_{50} =≈ 50 µg/mL). Since albumin is internalized in a megalin-dependent manner, these data indicate that M6P, on top of blocking M6P receptors, also interferes with the megalin-dependent uptake process. In conclusion, ciPTEC bind and internalize renin and prorenin in a temperature-dependent manner. This process involves megalin and/or M6P receptors, and results in prorenin activation at 37 ^{0}°C.

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P236

Site-1 Protease-derived Soluble (Pro) Renin Receptor Mediates Angiotensin II-induced Hypertension via Activation of Intrarenal Renin System

PrimaryAuthor.AuthorBlock:**Kexin Peng**, Sun Yat-sen Univ, Guangzhou, China; Fei Wang, Chuanming Xu, Xiaohan Lu, Tianxin Yang, Univ of Utah, Salt Lake City, UT

We have previously shown that collecting duct (CD)-specific deletion of (pro)renin receptor (PRR) attenuates angiotensin II (Ang II)-induced hypertension, accompanied with reduced soluble PRR (sPRR) that exerts antidiuretic action. Recent preliminary and published results demonstrated site-1portease (S1P) but not furin or ADMA19 as the predominant PPR cleavage enzyme. In the present study, we evaluated involvement of S1P-derived sPRR in Ang II-induced hypertension. By radiotelemetry, CD PRR KO mice exhibited reduced MAP on day 7 of Ang II infusion at 300 ng/kg/min as compared with floxed mice (MAP: 118±5 vs. 137±3 mmHg, N=5, p<0.05). Administration of sPRR-His, a histidine-tagged sPRR, at 120 µg/kg/d via i.v. infusion to CD PRR KO mice for additional 7 days largely restored the sensitivity to Ang II (MAP: 139±6 mmHg in sPRR-His +Ang II group vs. 116±5 mmHg in Ang II group, N = 4, p<0.05). The i.v. infusion was achieved via placement of a catheter in jugular vein with the other end connected to mimipump. In C57/BL6 mice, administration of a S1P inhibitor PF429242 (PF) via mini pump infusion at 30 mg/kg/d for 7 days attenuated Ang II-induced increases in MAP (day 7: 125±5 in Ang II+ PF group vs. 142±3 in Ang II group; N=6, p<0.05), urinary sPRR excretion (27±4 vs. 63±9 pg/24h; N=6, p<0.05). In parallel, urinary renin levels were elevated by Ang II, which was blunted by PF (renin activity: 0.17±0.03 in Ang II+PF vs. 0.80±0.081 in Ang II vs. 0.12±0.016 ng/24h in Control, N=6, p<0.01; active renin content: 15.2±2.7 vs. 236.0±23.2 vs. 5.6±1.3 ng/24h, N=6, p<0.01; prorenin content: 9.6±3.1 vs. 27.8±6.1 vs. 6.2±1.8 ng/24h, N=6, p<0.05; total renin content: 24.8±5.2 vs. 263.8±27.0 vs. 11.8±3.0 ng/24h, N=6, p<0.01). An intravenous infusion of sPRR-His counteracted the blood pressure-lowering effect of PF in Ang II-infused mice (MAP: 147±3 in PF+sPRR vs. 126±4 mmHg in PF; N=4, p<0.05). Together, these results suggest that S1P-derived sPRR contributes to Ang II-induced hypertension through activation of intrarenal renin-angiotensin system.

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P237

The At2 Receptor Agonist, C21, Can Also Stimulate Mas and Mrgd Receptors

PrimaryAuthor.AuthorBlock:**Anja Tetzner**, Maura Naughton, Kinga Gebolys, Univ Coll Cork, Dept of Pharmacology and Therapeutics, Cork, Ireland; Esther Sala, Óscar Villacañas, Intelligent Pharma, Barcelona, Spain; Thomas Walther, Univ Coll Cork, Dept of Pharmacology and Therapeutics, Cork, Ireland

Background: It is well accepted that Compound 21 (C21) is a highly selective non-peptide angiotensin AT2 receptor agonist. C21 as well as angiotensin (Ang)-(1-7) have cardiovascular protective effects and are opponents of the often detrimental Ang II within the renin-angiotensin system. Since the chemical structure of C21 is similar to the Mas receptor specific non-peptidic agonist AVE0991, and we could recently show that the AT2 receptor blocker, PD123319, can also block the effects of Ang-(1-7) at its natural receptors, Mas and MrgD, we tested whether C21 is also not AT2-specific but can stimulate the two Ang-(1-7) receptors, too. **Methods and Results:** Using cAMP as readout in receptor-transfected HEK293 cells, we generated pharmacological proof that Mas (EC_{50} = 1.995 x 10^{-12}M) and MrgD (EC_{50} = 2.958 x 10^{-9}M) are functional receptors for C21, whereby the three receptor blockers, A779, D-Pro7-Ang-(1-7), and PD123319 showed receptor-specific effects towards C21 signalling. Furthermore, C21 elevated the cAMP concentration in primary cells such as mesangial cells (EC_{50} = 1.12 x 10^{-6}M). However, significant increase in cAMP levels, but not in PKA activity, was still detectable in mesangial cells isolated from AT2-deficient mice, but completely blunted in Mas/MrgD-double knockouts. Finally, *in silico* modelling was performed to illustrate the structural similarities and differences between C21, AVE0991, and Ang-(1-7). **Conclusions:** Our results identify C21 as not being a specific AT2 receptor agonist but also interacting with the two Ang-(1-7) receptors, Mas and MrgD. Therefore, the partial overlap in beneficial effects of Ang-(1-7) and C21 might be based on the stimulation of the same receptors under specific pathophysiological circumstances. This also enforces the revisit of such publications which concluded on AT2 function by only using C21.

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P238

A New Fluorometric Method to Evaluate Efficiencies of Carboxy-terminal Phenylalanine Cleavage From Angiotensin II, Angiotensin III and Apelin Peptides

PrimaryAuthor.AuthorBlock:**Tilman Mueller**, Jan Wysocki, Peter D Serfozo, Pan Liu, Minghao Ye, Jing Jin, Daniel Batlle, Northwestern Univ, Chicago, IL

Angiotensin (Ang) II and apelins (AP) are intertwined by the actions of ACE2 that selectively cleaves the bond between proline and phenylalanine (Pro-Phe) at the carboxyl-termini of Ang II and AP leading to a conversion of Ang II (1-8) to Ang (1-7), and degradation of AP-13 and AP-36 peptides. Ang III (2-8) has been assumed - due to the presence of the Pro-Phe motif - but not yet experimentally demonstrated to be a substrate of ACE2. Measuring peptide substrates and products of these reactions are usually difficult using current ELISA assays due to their cross-reactivity. We sought to device a rapid and

simple screening method that would provide readout of peptide products formation from reactions involving exclusively the c-terminal Phe cleavage. To this end we used a quantitative fluorometric assay recently described by us to compare the relative strength of mouse (m) and human (h) recombinant ACE2 in regards to cleavage of the c-terminal Phe from Ang II, Ang III, AP-13 and AP-36. Phe cleavage of AP-36 by mrACE2 was as efficient as its cleavage of AP-13 (2063±106 vs. 1670±163 RFU/ng rACE2, NS, n=5 experiments). mrACE2 formed twice as much Phe from both AP-36 and AP-13 than from Ang II. We further found that rACE2 also removes Phe from Ang III (2-8) to form Ang (2-7). This reaction for both mrACE2 and hrACE2 was as efficient as the cleavage of Phe from Ang II to form Ang (1-7) (for mrACE2 1096±136 vs. 683±56 and for hrACE2 1289±92 vs. 1479±69 RFU/ng protein, NS, respectively). The new fluorometric method detects cleavage of single c-terminal Phe from different peptides which are substrates of ACE2. A novel finding using this approach is the ability of rACE2 to cleave Phe from Ang III (2-8) to form Ang (2-7). This method will allow comparisons of efficiencies of recombinant enzymes in cleaving Phe from variety of bioactive peptides from their c-terminal Pro-Phe motifs and as such can constitute a useful tool for screening carboxypeptidase activities.

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(Pro)Renin Receptor Contributes to Albumin Overload Nephropathy: Role of Site-1 Protease-Derived Soluble (Pro) Renin Receptor

PrimaryAuthor.AuthorBlock:**Hui Fang**, Chuanming Xu, Aihua Lu, Chang-Jiang Zou, Shiying Xie, Yanting Chen, Li Zhou, Mi Liu, Lei Wang, Weidong Wang, Tianxin Yang, Inst of Hypertension, Zhongshan Sch of Med, Guangzhou, China

(Pro) renin receptor (PRR) is a new member of the renin-angiotensin system (RAS) and its role in chronic kidney disease largely remains elusive. We tested the role of PRR in albumin overload (OA) nephropathy. Uninephretomized Sprague-Dawley rats were treated for 7 weeks with vehicle, bovine serum albumin (BSA) (5 g/kg/d via a single i.p. injection) or in combination with PRO20 (500 µg/kg/d via i.p. 3 times a day). The OA rat exhibited severe proteinuria (23-fold), interstitial fibrosis, and oxidative stress, accompanied with increases in urinary renin activity (3.6-fold), urinary angiotensinogen (AGT, 3.6-fold), urinary AngII(7.8-fold), renal ACE mRNA (2.4-fold), all of which were significantly attenuated by PRO20. Urinary soluble PRR (sPRR) as assessed by ELISA was increased 13.6-fold in the OA model. In cultured HK-2 cells, BSA treatment for 24 h decreased full-length PRR (76% reduction) and increased sPRR (8-fold). BSA also increased medium renin activity (3.0-fold), IL6 (2.9-fold for mRNA and 4.1-fold for medium protein) and IL8 (5.5-fold for mRNA and 2.6-fold for medium protein), all of which were attenuated by PRO20 or PRR siRNA. Although furin or ADAM19 was previously shown to mediate the generation of sPRR, inhibition of either one of these had no effect on BSA-induced cleavage of PRR. In contrast, a serine protease inhibitor AEBSF significantly suppressed the production of sPRR. Of serine proteases, proprotein convertases (PCs) mediates post-translational processing of proprotein to active protein and are of particular interest. Screening of 9 PCs led to the identification of site-1 protease (S1P) as the predominant cleavage enzyme. S1P inhibition with siRNA or PF-429242 reduced the sPRR

production by 47% and 87%, respectively. PF-429242 also reduced renin activity by 40%, in parallel with suppressed IL6 and IL8 levels. Administration of a recombinant sPRR reversed all the effects of S1P inhibition. Mutagenesis of the S1P but not furin cleavage site in PRR blocked the cleavage. Together, these results suggest that PRR mediates OA-induced renal injury at least in part through S1P-derived sPRR.

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P240

Decarboxylation of Ang-(1-7) to Ala^1-ang-(1-7) Leads to Major Changes in Pharmacodynamics

PrimaryAuthor.AuthorBlock:**Anja Tetzner**, Maura Naughton, Kinga Gebolys, Dept. Pharmacology and Therapeutics, Sch of Med and Sch of Pharmacy, Univ Coll Cork (UCC), Cork, Ireland; Esther Sala, Óscar Villacañas, Intelligent Pharma, Barcelona, Spain; Thomas Walther, Dept. Pharmacology and Therapeutics, Sch of Med and Sch of Pharmacy, Univ Coll Cork (UCC), Cork, Ireland

Background: Within the renin-angiotensin system, angiotensin (Ang)-(1-7) is cardiovascular protective, stimulates regeneration, and opposes the often detrimental effects of Ang II. We identified two receptors for the heptapeptide; the G protein-coupled receptors Mas and MrgD. Recently, a decarboxylated form of Ang-(1-7), Ala^1-Ang-(1-7) (Alamandine), has been described as having similar vasorelaxing effects as Ang-(1-7) but distinctively stimulating the MrgD receptor. **Methods and Results:** The aim of this study was to elucidate the consequences of the lack in the carboxyl group in amino acid one on intracellular signalling, to discover the receptor fingerprint for Ala^1-Ang-(1-7), and to characterize the consequences for pharmacodynamics. Therfore cAMP was used as the main readout in receptor-transfected HEK293 cells as well as primary cells (mesangial cells and HUVEC). Ala^1-Ang-(1-7) elevated cAMP concentrations in primary endothelial and mesangial cells. However, the dose-response curves clearly discriminated from the curves generated with Ang-(1-7) (EC_{50} = 1.1 x 10^{-8}M), with much lower EC_{50} (EC_{50} = 3.6 x 10^{-11}M) and bell-shape for Ala^1-Ang-(1-7). We provided pharmacological proof that both, Mas (EC_{50} = 6.31 x 10^{-12}M) and MrgD (EC_{50} = 3.98 x 10^{-13}M), are functional receptors for Ala^1-Ang-(1-7). Consequently, the heptapeptide failed to increase cAMP concentration in primary mesangial cells with genetic deficiency in both receptors. As for Ang-(1-7), the AT2 blocker PD123319 also blocked the Ala^1-Ang-(1-7) effects on Mas and MrgD receptors and in primary cells. The very distinct dose-response curves for both heptapeptides could be explained by *in silico* modelling, energy calculations, and an involvement of $G_{alpha\ i}$ for higher concentrations of Ala^1-Ang-(1-7). **Conclusions:** Our results identify Ala^1-Ang-(1-7) as a peptide with specific pharmacodynamic properties and build the basis for the design of more potent and efficient Ang-(1-7) analogues for therapeutic interventions in a rapidly growing number of diseases.

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Autophagic Flux is Diminished in Mesenteric Resistance Arteries of Spontaneously Hypertensive Rats

PrimaryAuthor.AuthorBlock:**Cameron G McCarthy**, Camilla F Wenceslau, R. Clinton Webb, Augusta Univ, Augusta, GA

Autophagy is the constitutively active catabolic process regulating protein quality control and energy homeostasis. However, dysregulation of this process can have detrimental effects on cellular function. In particular, insufficient autophagy has been proposed as a mechanism of cellular aging, as this leads to the accumulation of damaged macromolecules and organelles. Hypertension is a condition of vascular aging. In fact, many factors that contribute to the deterioration of vascular function as we age are exacerbated in clinical and experimental hypertension. Nonetheless, whether high blood pressure per se is the cause or effect of diminished autophagy remains to be clarified. We hypothesized that mesenteric resistance arteries (MRA) from spontaneously hypertensive rats (SHR) would have decreased autophagic flux as measured by conversion of microtubule-associated protein light chain 3 (LC3-I to LC3-II) compared to normotensive Wistar Kyoto rats (WKY). We observed that MRA from male 12-15 week old SHR have decreased basal expression both cytosolic LC3 (LC3-I) and phosphatidylethanolamine conjugated LC3 (LC3-II), and expression of these proteins are similarly decreased in SHR chronically treated with hydrochlorothiazide and reserpine (SHR+HCTZ/Res) [Arbitrary units (AU), LC3-1: WKY: 1.4±0.1, SHR: 1.1±0.1*, and SHR+HCTZ/Res: 0.7±0.1*; LC3-II: WKY: 1.4±0.1, SHR: 1.1±0.1*, and SHR+HCTZ/Res: 0.7±0.1*, *p<0.05 vs. WKY]. To understand autophagic flux, some MRA were incubated with lysosomal inhibitor chloroquine (CQ; 30 μM) for 2 hours. CQ incubation significantly increased LC3-II expression to a similar magnitude in WKY, SHR, and SHR+HCTZ/Res MRA (all *p<0.05 vs. basal). However, the percent increase in LC3-II expression after CQ incubation was significantly less in SHR compared to WKY, and SHR+HCTZ/Res was not different from either WKY or SHR (% increase from basal LC3-II, WKY: 546±187, SHR: 156±38*, and SHR+HCTZ/Res: 273±106, *p<0.05 vs. WKY). Overall, these data suggest that SHR have impaired autophagosome-lysosomal fusion, and this is not solely attributable to high blood pressure. Therefore, reconstituting autophagic activity could be a novel prophylactic or therapeutic measure against vascular aging in hypertension.

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Nicotinic Acetylcholine Receptor Subunit α7 is Protective Against Angiotensin II-induced Aneurysm and Hypertension

PrimaryAuthor.AuthorBlock:**Nayaab S Khan**, The Feinstein Inst for Medical Res, Manhasset, NY; Spyros Mavropoulos, Hofstra Northwell Sch of Med, Hempstead, NY; Kaie Ojamaa, The Feinstein Inst for Medical Res, Manhasset, NY

Alpha7 nicotinic acetylcholine receptor (α7 nAChR), an integral component of the cholinergic nervous system is known to mediate cholinergic anti-inflammatory activity in various disease models such as sepsis, stroke and neurocognitive disorders. We report for the first time that the α7 nAChR$^{-/-}$ deficient mouse serves as a novel model of hypertension and aneurysm formation. Seven month old male WT and α7

nAChR$^{-/-}$ mice weighing 28-33g were infused with low dose Ang II (350 ng/kg/min) or high dose (700 ng/kg/min) or vehicle for 15 days using mini-osmotic pumps (Alzet, model 2004) implanted subcutaneously. Blood pressure (BP) was recorded on day 0,3,7,10 and 14. Mice were euthanized on day 15. Heart and body weights were measured, histological analysis was performed on the aortas and immune profile of peripheral blood was analyzed by flow cytometry. High dose Ang II resulted in 70% mortality from aneurysm rupture in α7 nAChR$^{-/-}$ mice starting as early as the 4th day of infusion. While cardiac hypertrophy was not observed, low dose Ang II resulted in a sharp rise in blood pressure in α7 nAChR$^{-/-}$ beginning on the 3rd day to 167±3.7 mmHg compared to 138±3.3 mmHg in WT treated mice. On day14 of low dose treatment, BP in α7 nAChR$^{-/-}$ rose to 171±4.2 vs.135±3.1 in WT mice. No changes were observed in BP of untreated WT or α7 nAChR$^{-/-}$ animals. Histological analysis revealed high grade aneurysm in aortas of α7 nAChR$^{-/-}$ mice treated with low dose Ang II, demonstrating a prominent germinal center within the false lumen and fibrous desmoplastic stroma. Increased infiltration of CD11B^{+} monocytes, and myeloperoxidase^{+} stained neutrophils were observed in these aortas but not in the aortas of similarly treated WT mice. Flow cytometric analysis showed 27% ± 3.9 CD11B^{+}/CD45^{+} circulating monocytes and 48% ± 0.8 Ly6G^{+}/CD45^{+} neutrophils in α7 nAChR$^{-/-}$ vs. 19% ± 3 monocytes and 11.85% ± 2.9 neutrophils in WT mice. No differences in the levels of circulating immune cells were observed in untreated mice of either genotype. These data support a protective role of α7 nAChR in hypertension and aneurysm, potentially acting through its cholinergic anti-inflammatory activity. The α7 nAChR$^{-/-}$ mouse may serve as a new genetic model of aneurysm relevant in studies of the human disease.

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Smooth Muscle Cullin-3 Deficiency Causes Severe Early Onset Hypertension and Nitric Oxide Resistance

PrimaryAuthor.AuthorBlock:**Larry N Agbor**, Anand Nair, Jing Wu, Deborah R Davis, Henry L Keen, Frederick W Quelle, Univ of Iowa, Iowa City, IA; James A McCormick, Oregon Health and Science Univ, Portland, OR; Jeffrey D Singer, Portland State Univ, Portland, OR; Curt D Sigmund, Univ of Iowa, Iowa City, IA

Mutations in *Cullin-3* (*Cul3*) resulting in exon 9 skipping (CUL3α9) cause human hypertension. We demonstrated that selective expression of CUL3α9 in smooth muscle causes arterial stiffness and hypertension. We hypothesized that deletion of CUL3 in smooth muscle causes severe hypertension. Mice carrying a conditional allele of CUL3 were bred with mice expressing a tamoxifen-inducible CRE-recombinase driven by a smooth muscle promoter. Mice were administered tamoxifen *i.p.* (75 mg/kg) for 5 consecutive days to generate smooth muscle CUL3 knockout (S-CUL3KO). CUL3 protein was undetectable whereas Cullin-1 protein was preserved in aorta from S-CUL3KO mice. We assessed vascular function in the cerebral basilar artery and aorta using pressurized and wire myograph, respectively. Blood pressure (BP) was measured by radiotelemetry. S-CUL3KO mice exhibited significantly increased systolic BP (SBP) at 2 weeks and 4 weeks post tamoxifen compared to corn oil controls (2 wks SBP mmHg: 145±1 vs 115±2, $p<0.001$; 4 weeks SBP: 169±1 vs 115±3,

p<0.001). Pulse wave velocity was also increased in S-CUL3KO mice (3.7±0.1 m/s vs 2.2±0.1, P<0.001), suggesting increased arterial stiffness. Aorta from S-CUL3KO mice exhibited severely impaired vasorelaxation to acetylcholine (ACh) compared to controls (at 100 µM: 1.0±3% vs 77±5%, p<0.0001), and to the nitric oxide donor sodium nitroprusside (SNP) (at 100 µM: 15±4% vs 96±1%, p<0.001). In agreement with data from aorta, cerebral basilar artery from S-CUL3KO mice also exhibited significant impairment to ACh- and SNP-mediated vasorelaxation. Conversely, S-CUL3KO aorta retained an ability to relax in response to a cGMP analogue (8-pCPT-cGMP, at 100 µM: 8-pCPT-cGMP 70±3%) and to the heme-independent soluble guanylate cyclase activator (BAY 58-2667, at 10 µM: BAY 58-2667 94±2%), indicating that downstream mechanisms controlled by cGMP remain intact in the absence of CUL3. Captopril (120 mg/kg/day) was sufficient to normalized BP in S-CUL3KO mice to pre-tamoxifen levels (change in SBP mmHg: -50.2±1.9 S-CUL3KO vs -25.0±2 control, P<0.0001). We conclude that smooth muscle CUL3 is a major BP determinant, and identifying novel CUL3 substrates in smooth muscle would be beneficial as therapeutic targets in the treatment of hypertension.

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P244

Characterization of Exosomes From Vascular Endothelial and Smooth Muscle Cells

PrimaryAuthor.AuthorBlock:**Michael Boyer**, Satoru Eguchi, Victor Rizzo, Ariel Baggette, Rosario Scalia, Temple Univ, Philadelphia, PA

Exosomes are small nanovesicles derived from endosomal compartments termed multivesicular bodies. These multivesicular bodies fuse with the plasma membrane, release exosomes into extracellular space, allowing them to transport their cargo of functional nucleic acids and proteins. Despite their well-documented existence, not much is known about exosomes in vascular biology. The luminal surface of the intima consists of a confluent monolayer of endothelial cells (ECs), while the medial layer is characterized by presence of vascular smooth muscle cells (VSMCs). We characterized the exosomes released by these cell types in culture to better understand the packaging of these vesicles and their capacity to participate in cell-to-cell communication. Rat aortic ECs exosomes were generated in serum-free media for 48 hours while rat aortic VSMC exosomes were generated in serum-free media for 72 hours. Media harvested from both was ultracentrifuged at 10,000xg to remove microparticles followed by centrifugation of the supernatant at 100,000xg to pellet exosomes. Exosome pellet was re-suspended in PBS, 0.22

µm filtered and spun at 100,000xg to remove contaminating particles. Transmission electron microscopic analysis of VSMC exosomes confirmed particle morphology with diameters ranging from 60-150 nm. Particle size and concentration was determined using NTA NanoSight. The modal size of EC exosomes and VSMC exosomes were 107.2±5.4 nm and 116.6±7.5 nm, respectively. Western blotting analysis was performed to characterize common and specific markers for vascular exosomes. We confirmed common exosome markers such as CD81, CD63, caveolin-1, and flotillin-1 in both populations. ECs specifically expressed VE-cadherin and TSG-101 whereas VSMCs expressed syntenin-1 and smooth muscle actin. Cell-to-cell signaling *in vitro* assays revealed that EC-derived exosomes can stimulate hypoxia-inducible factor 1-alpha expression in VSMCs in a dose-dependent and time-dependent manner. Maximal induction was seen at 6 hours of stimulation and using $5x10^8$ particles/mL. From these data, we conclude that exosomes are released from these cell types and play a potential role in cell-to-cell communication to maintain homeostasis and/or promote pathology.

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P245

Microrna-145-5p, a Potential Therapeutic Target of Regulating Vascular Calcification

PrimaryAuthor.AuthorBlock:**Abu S Ahmed**, Matilda H Sheng, David J Baylink, Loma Linda Univ, Loma Linda, CA

Vascular calcification (VC) occurs in most otherwise normal individuals over 60 years of age and is intimately linked to hypertension with severe cardiovascular complications, resulting in considerable morbidity and sometimes death. VC is characterized by the deposition of bone tissue within the vessel wall. The cause of VC is unknown although considerable progress has been made in characterizing its pathogenesis. There is direct or indirect evidence for several factors which could contribute to VC including, vitamin K deficiency, magnesium deficiency increased parathyroid hormone, increased serum phosphate, increased BMP activity and microRNAs (a small non-coding RNA molecules). The BMP pathway deserves attention because it is the sine qua non of osteoblast differentiation. With respect to microRNAs, these also could be relevant to the cause of VC because microRNAs are known to influence VC, and importantly, microRNAs can change with age. Here, we used an *in vitro* model to mimic VC using osteogenic medium in a vascular smooth muscle cell (VSMC) line. From microRNA analysis using quantitative real-time PCR we identified a potential key microRNA, miR-145-5p involved in vascular calcification. Specifically, we found that in our *in vitro* model of VC has a significant decrease in miR-145-5p expression. In silico analysis revealed that miR-145-5p may bind and regulate SMAD5, which mediates the action of BMP. Accordingly, we found an increase in SMAD5 expression at the mRNA level in VC condition *in vitro*. Based on the foregoing, we now propose the hypothesis that in young normal individuals, miR-145-5p normally suppresses SMAD5 activity in vasculature; whereas, in aging, miR-145-5p expression is impaired which would result in increasing SMAD5 gene expression, as well as its

phosphorylation, which in turn would eventually lead to VC. Thus, miR-145-5p might be a potential therapeutic target to regulate VC in the elderly.

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Soluble (Pro)renin Receptor Induces Endothelial Cell Injury via Activation of Nox4/NF-κB Pathway

PrimaryAuthor.AuthorBlock:**Ziwei Fu**, Jiajia Hu, Fei Wang, Jiahui Su, Xiaohan Lu, Sun Yat-sen Univ, Guangzhou, China; Tianxin Yang, Univ of Utah and Veterans Affairs Medical Ctr, Salt Lake City, UT

Circulating soluble (pro)renin receptor (sPRR), the cleavage product of the extracellular domain of PRR, is elevated in patients with cardiovascular diseases, highlighting its value as a disease biomarker. We explored a potential biological effect of sPRR in vascular endothelial cells. In primary human umbilical vein endothelial cells (HUVECs), a 2-h treatment with a recombinant histidine-tagged sPRR (sPRR-His) at 50 nM decreased IκBα protein by 55%, associated with 1.5-fold increase in nuclear fraction protein abundance of NF-κB p65 as assessed by immunoblotting. By immunostaining, the increased p65 was localized to the nucleus. The NF-κB p65 transcription factor activity assay on the isolated nuclear fractions showed that the activity increased 1.9-fold after 2-h sPRR-His treatment. At 3 h of sPRR-His treatment, mRNA expression was increased 3.8-fold for IL-6, 30.8-fold for IL-8, 17.3-fold for VCAM-1, and 4.2-fold for ICAM-1 as assessed by qRT-PCR; ELISA detected a 2.6-fold increase in medium IL-6 and 3.7-fold increase in medium IL-8. Moreover, enhanced apoptosis was evidenced by a 2.9-fold increase of cleaved caspase 3 protein and a 4-fold increase in the apoptotic cell number, as assessed by immunoblotting and annexin V-FITC/PI double staining flow cytometry, respectively. In addition, sPRR-His induced a 69% decrease in phospho-eNOS (Ser1177) protein abundance. At 2 h, sPRR-His induced a 2.0-fold increase in Nox4 protein and a 2.8-fold increase in medium H2O2 as assessed by immunoblotting and ROS-Glo H2O2 assay, respectively. A Nox1/4 inhibitor GKT137892 and NADPH oxidase inhibitor apocynin completely abolished sPRR-His-induced NF-κB activation, cytokine production, and apoptosis, similar to the effect of NF-κB inhibition with PDTC. Overall, the present study for the first time reports that sPRR serves as a pro-inflammatory and pro-apoptotic mediator in vascular endothelial cells through activation of Nox4/NF-κB. These findings support PRR/sPRR as a potential target for treatment of cardiovascular disease.

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P247

Triiodothyronine Ameliorates Endothelial Dysfunction in Rats Following Acute Myocardial Infarction

PrimaryAuthor.AuthorBlock:**Kuo Zhang**, Yi-Da Tang, Fuwai Hosp, Beijing, China; Youhua Zhang, Ying Li, Olga Savinova, Kaie Ojamaa, A. Martin Gerdes, Maria Alicia Carrillo Sepulveda, New York Inst of Technology, Old Westbury, NY

Background: Low thyroid hormone (TH) function is recognized as a significant contributor in the pathogenesis after acute myocardial infarction (MI). Endothelial dysfunction contributes significantly to the poor prognosis of MI. We hypothesize that long-term treatment with low dose T3 improves endothelial function and cardiac contractile activity compared to beta blocker, the current recommended therapy for MI.
Methods: Adult female Sprague-Dawley rats were subjected to left anterior descending coronary artery ligation (MI) or sham surgeries. Survivors were randomly assigned to vehicle (MI, n=11), T3 (MI+T3, n=11) and metoprolol (MI+Meto, n=11). Vehicle, T3 (5 ug/kg/day) and metoprolol (2 mg/kg/day) were supplied in drinking water ad libitum immediately following MI for 2 months. Heart function and LV hemodynamics were measured. Isolated thoracic aortic rings were used to test relaxation response to acetylcholine (ACh) in a wire myograph. The maximal effect elicited by ACh (E_{max}) and the sensitivity to ACh (pEC_{50}) were analyzed. One-way ANOVA with Bonferroni correction was used for multiple comparisons.
Results: Serum concentration of free and total T3 were normal in all the experimental groups. T3 and metoprolol improved LV contractile function measured by fractional shortening (21.88±2.06 vs 17.88±1.23%, p<0.01, T3 vs MI; 21.12±3.88 vs 17.88±1.23%, p<0.05, Meto vs MI; 46.86±1.84% for sham) and LV +d*p*/d*t* (7307±1128 vs 5479±810 mmHg/s, p<0.01, T3 vs MI; 7022±695 vs 5479±810 mmHg/s, p<0.05; Meto vs MI; 9160±1881 mmHg/s for sham). Aortas from vehicle-treated group exhibited a marked impairment of endothelial-dependent relaxation measured by pEC_{50} (6.65±0.22 vs 7.19±0.16, p<0.001, MI vs Sham), which was significantly improved in the T3 treated group (6.96±0.22 vs. 6.65±0.22, p<0.01, T3 vs MI) but not in metoprolol group (6.85±0.21 versus 6.65±0.22, p=0.22, Meto vs MI). T3 and metoprolol increased maximal relaxation measured by E_{max} (90.56±3.55 vs 79.50±3.98%, p<0.001, T3 vs MI; 89.81±6.75% vs 79.50±3.98%, P<0.001, Meto vs MI; 96.93±1.91% for sham).
Conclusion: Long-term treatment with a physiological dose of T3 following MI is equally effective as metoprolol on LV function while improving endothelial function as an additional benefit.

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P248

Novel Role of Axl Kinase in Endothelial Cell Proliferation and Pulmonary Arterial Hypertension

PrimaryAuthor.AuthorBlock:**Anastasia Gorelova**, Sanghamitra Sahoo, Patrick J Pagano, Univ of Pittsburgh, Pittsburgh, PA

Pulmonary arterial hypertension (PAH) is a poorly characterized disease of unclear etiology that affects individuals of all ages. Vascular remodeling and increase in pulmonary artery (PA) and right ventricle (RV) pressures are two major culprits in RV failure and death in PAH. Recent advances in the study of PAH suggest that endothelial cell proliferation is an early instigator of this hallmark remodeling. We postulated that Axl receptor tyrosine kinase (implicated in pro-proliferative and pro-survival signaling in cancerous cells) could mediate endothelial proliferation and thus hemodynamic changes occurring in PAH. Using immunofluorescent microscopy of lung microvessels of human PAH vs. non-PAH, we

observed Axl expression on intimal endothelial cells but not medial smooth muscle cells. Furthermore, digitized microscopy revealed that Axl tended to increase on the endothelium of PAH vessels (1.65±0.15-fold vs. non-PAH; n=3-4; *p=0.057*). To address the role of Axl *in vivo*, an Axl inhibitor R428 was employed in a mouse model of pulmonary hypertension. C57Bl/6 mice were subjected to hypoxia at pO_2=10% and VEGF receptor antagonist SU5416 (Su/Ch) or normoxia (Norm) for 3 wks. Indeed, Su/Ch caused a significant rise in lung Axl protein and mRNA (7.1±0.4- and 2.4±0.5-fold, Su/Ch vs. Norm, protein and mRNA, respectively; n=3-6; p<0.01). As predicted, RV pressure (RVP) rose from 27±0.5 to 43±1.8 mmHg (Norm vs. Su/Ch; n=6; p<0.01). However, we did not observe a decrease in RVP with twice-daily gavage of 75 mg/kg R428 (43±1.4 mmHg, Su/Ch + R428; n=6). A similar pattern was observed with mean PA pressure (18.4±0.3 and 28.7±1.2 mmHg, Norm vs. Su/Ch, p<0.01; 28.7±0.9 mmHg, Su/Ch + R428), RV resistance (1403±256 vs. 2703±464 Wood units, Norm vs. Su/Ch, n/s; vs. 3610±625 Wood units, Su/Ch + R428) and Fulton index (0.26±0.01 and 0.34±0.02, Norm vs. Su/Ch, p<0.05; 0.38±0.02, Su/Ch + R428). In conclusion, our preliminary results demonstrate upregulated Axl expression in the endothelium of PAH patients and in lungs of PH mice and suggest that Axl kinase may play a novel role in pulmonary vascular endothelial proliferation and remodeling in PAH. It remains to be determined whether drug bioavailability or severity of disease precluded an ameliorative effect of an Axl inhibitor.

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P249

Effects of Lifestyle Changes on Metabolism and Early Vascular Lesion in Healthy People

PrimaryAuthor.AuthorBlock:**Xu Fengcheng**, Yu Chaoping, Liu Tianhu, Pidu District People's Hosp, Chengdu, China

Objective Through propaganda and education on lifestyle change, we study the effects on metabolism and vascular lesions in healthy people.**Methods** the healthy subjects that conform to the requirements, through propaganda and education on vascular health, through moderate exercise, proper control of starchy foods, low salt, low fat diet, reduce smoking and other lifestyle changes, compare changes in weight, renal function, fasting blood glucose, blood lipids and ankle brachial index (ABI), cardio ankle vascular index(CAVI) before and after lifestyle changes. **Results** After lifestyle changed, the subjects' body mass index [(23.13±3.18)kg/m^2 *vs* (22.67±3.36)kg/m^2], ABI[1.11±0.08 *vs* 1.09±0.09], CAVI[(7.14±1.13) *vs* (7. 01±1.18)], serum creatinine[(84.31±22.41)umol/L *vs* (79.92±23.64)umol/L], blood uric acid[(337.79±102.17)umol/L *vs* (328.12±88.33)umol/L], low density lipoprotein cholesterol[(2.49±0.65) mmol/L *vs* (2.37±0.69) mmol/L],all have good changes.Conclusion Healthy lifestyle is good for metabolism and early vascular lesions, can improve metabolic disorder and slow the occurrence of arteriosclerosis.

Table 1 Results of blood pressure and metabolic indexes before and after lifestyle change

	Before intervention	After intervention	t	P
BMI	23.13±3.18	22.67±3.36	3.1650	**0.0015**
SBP	128.12±14.21	128.42±14.59	0.1273	0.8749
DBP	80.95±9.79	80.26±9.88	1.6686	0.0952
BUN	5.00±1.43	4.96±1.53	0.3170	0.7535
CREA	84.31±22.41	79.92±23.64	4.2106	**0.0001**
UA	337.79±102.17	328.12±88.33	2.208	**0.0282**
TG	1.51±0.95	1.48±1.04	0.6713	0.5019
CHOL	4.48±0.86	4.46±0.81	0.4401	0.6553
HDL-C	1.48±0.49	1.52±0.38	1.4371	0.1537
LDL-C	2.49±0.65	2.37±0.69	3.8850	**0.0001**
FBG	4.94±0.55	4.76±0.68	5.5642	**0.00001**
CAVI	7.14±1.13	7.01±1.18	2.42	**0.02**
ABI	1.11±0.08	1.09±0.09	5.6946	**0.000001**

* P<0.05, ** P<0.01

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P250

Comparison of Clinical Variables Independently Associated With Large Artery Stiffness and Microvascular Dysfunction in African-American Diabetic Patients

PrimaryAuthor.AuthorBlock:**Ayobami Eluwole**, Ajibola Adedayo, Fasika Tedla, Arye Kremer, Nicole Mastrogiovanni, Mohammed Afzal, Carl Rosenberg, Paul Dreizen, John Larossa, Mohamed Boutjdir, Mary Ann Banerji, Clinton Brown, Moro Salifu, Ahmed Bakilah, Jason Lazar, Downstate Medical Ctr, Brooklyn, NY

RATIONALE Diabetes mellitus is a risk factor for large and small vessel disease and portends a disproportionate morbidity and mortality burden in African Americans. Diabetes is often accompanied by cardiovascular risk factors that predispose to arteriopathy, it's been shown to impair large and small vessel function prior to onset of target organ damage. Factors contributing to subclinical vascular dysfunction have not been well studied in this population. The objective of this study was to compare clinical variables associated with large artery stiffness and microvascular dysfunction. METHODS A total of 141 patients with diabetes were recruited from medical clinics over a 6 month period. Medical information was obtained via interview and medical record review including laboratory results. Pooled Cohort Score was calculated for each subject. Microvascular function was assessed by vascular reactivity index (VRI), which assesses changes in digital temperature before and after release of arterial cuff occlusion (VENDYS 5000BC DTM system (Endothelix, Inc.). Large artery stiffness was assessed by carotid-femoral pulse wave velocity (PWV) using applanation tonometry (Sphygmocor, Atcor Inc.). RESULTS Mean age was 60+8 years, 64% were female. 80% had hypertension, 90% had dyslipidemia and 15% had chronic kidney disease. Mean HbA1C levels were 8.1+2.2%. On univariate analysis, Pooled-cohort-score was significantly correlated with PWV (r=.25, p=.003) but not with VRI (r=.02, p=.78). Neither PWV nor VRI was significantly correlated with HbA1c. On multivariate analysis, PWV was independently associated with age, gender, creatinine level and waist circumference but not with traditional risk factors (R2=.26, p<.001 for model). VRI was not significantly correlated with any of the clinical or laboratory measures. CONCLUSIONS Large artery stiffness is independently associated with age, female gender, renal function and waist circumference but not with poorer glycemic control. In contrast, microvascular dysfunction was not associated with any of the clinical or laboratory measures. The role of metabolic risk factors in the differential progression of subclinical large and small artery vessel dysfunction and outcomes merit further study.

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P251

Relationship Between Reactive Hyperemia Index in Patients with Coronary Artery Disease With the Cardiac Function and Prognosis

PrimaryAuthor.AuthorBlock:**Liu Tianhu**, Yu Chaoping, Xu Fengcheng, Pidu District People's Hosp, Chengdu, China

Objective To investigate the relationship between reactive hyperemia index(RHI) in patients with coronary heart disease (CHD) with the cardiac function and prognosis, intervention so as to provide guidance for coronary heart disease severity and prognosis assessment. **Methods** 500 cases of volunteers had coronary artery angiography by Judkins method in our hospital. coronary angiography showing one or more quarantine branch of coronary artery stenosis lower than 50% or more were taken as the standard for coronary heart disease diagnosis, and the volunteers were divided into CHD group (n=81) and health group (n=419). RHI and left ventricular ejection fraction (LVEF) of two groups were detected. The CHD group were followed up for 1 year and survival prognosis and cardiovascular events prognosis of the patients were statistically analyzed and the relationship between RHI and LVEF, cardiovascular events rate and mortality were analyzed. **Results** Compared with health group, RHI and LVEF of CHD group were lower (*P*<0. 05). RHI of patient in CHD group with LVEF ≥ 50% were higher than that of patient with LVEF < 50% (*P*<0. 05). Pearson correlation analysis results showed that RHI and LVEF of CHD patients were positively correlated (r=0.827, *P*<0. 05). Coronary heart disease group were followed up for 1 year and the cardiovascular events rates and mortality rates were 28.40% and 9.88% respectively, and RHI and LVEF of patient with cardiovascular events were lower than that of patients without coronary heart disease, and RHI and LVEF of death patients were also lower than that of survived patients (*P*<0. 05). Spearman unconditionally correlation analysis results showed that the RHI and cardiovascular events and mortality in patients with CHD are negatively correlated (r=-0.794, -0.762, *P*<0. 05). **Conclusion** RHI in CHD patients is lower and closely related to the cardiac function and prognosis, this may be related to RHI reflecting endothelial function and endothelial function damage of CHD associating with disease development, therefore, RHI may be reference indicators of disease severity and prognosis assessment of CHD.

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Hemochromatosis Induce Arterial Stiffness and Endothelial Dysfunction and They Can Be Effectively Reversed by Phlebotomy

PrimaryAuthor.AuthorBlock:**Mariano Duarte**, Analia Aquieri, Javier Coyle, Esteban Gonzalez Ballerga, Hosp de Clinicas Facultad de Medicina UBA, Buenos Aires, Argentina; Eleonora Savio Galimberti, Lankenau Inst for Medical Res, Philadelphia, PA; Carlos Reyes Toso, Catedra de Fisiologia Facultad de Medicina UBA, Buenos Aires, Argentina

Hereditary Hemochromatosis (HH) is a genetically determined disease where iron is excessively stored in tissues, causing organ injury and failure. An increase of cardiovascular risk (CVR) has been reported in HH. Part of the iron overload induces vascular fibrosis and endothelial dysfunction (ED). Therefore, arterial wall emerge as a novel organ target organ in HH. Carotid-femoral pulse wave velocity (PWV-CF) assesses arterial stiffness (AS), and has predictive value for CV events. PWV variation pre- and post-induced transient ischemia of brachial artery (PWV-CB) has been validated to evaluate ED. We hypothetized that patients with HH have an increase in PWV-CF and PWV-

CB due to iron overload, and that phlebotomy by reducing iron levels decreases arterial stiffness and restores endothelial function. To test this hypothesis we assessed PWV-CF and PWV-CB in 52 patients with HH before and after phlebotomy and compare against control. PWV-CF and PWV-CB variations were assessed using Complior System (Artech-Medical, Francia). PWV-CF was significantly increased in HH patients compared to control group (8.5±1.7 m/s vs. 6.4±0.8 m/s, P<0.001, n=52/30 patients/group), suggesting increased AS. Changes in PWV-CB after transient ischemia were reduced in HH patients (0.3%) compared to controls (8.2%) (P<0.001), suggesting ED. Phlebotomy significantly reduced PWV-CF in HH patients (Post-phlebotomy: 6.5±0.8 m/s vs. Pre-phlebotomy: 8.5±1.7 m/s, P<0.001) back to control values (6.4±0.8 m/s). Changes in PWV-CB variation were partially reduced post - phlebotomy. (PWV-CB variation was 0.3 % in HH pre-phlebotomy vs. - 5.7 % in HH post-phlebotomy). These results showed a significantly increased AS and ED in HH group suggesting that arterial wall represents a novel target in HH and could may the nexus to cardiovascular disease. Both parameters (AS and ED) were significantly improved after phlebotomy, Our findings supports future research including more patients with HH and others iron overload disorders.

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Relation Between Microvascular Function and Large Artery Stiffness in African American Diabetic Patients

PrimaryAuthor.AuthorBlock:**Ajibola M Adedayo**, Ayobami Eluwole, Fasika Tedla, Arye Kremer, Nicole Mastrogiovanni, Mohammed Afzal, Carl Rosenberg, Paul Dreizen, John LaRosa, Mohamed Boutjdir, MaryAnn Banerji, Clinton Brown, Moro Salifu, Ahmed Bakillah, Jason Lazar, SUNY Downstate Medical Ctr, Brooklyn, NY

RATIONALE
Diabetes is highly prevalent among African Americans and poses a higher risk for vascular complications in this population. Although socioeconomic factors are well known to influence outcomes, true biologic differences in risk factor vulnerability have been suggested. Vascular complications have been traditionally viewed as either macrovascular (myocardial infarction and stroke) or microvascular (retinopathy, nephropathy, and neuropathy). Better glycemic control is known to improve microvascular but not macrovascular complications. In recent years, there has been a growing appreciation that microvascular dysfunction may promote large artery disease and vice versa. Given this notion of vascular “cross-talk” and since subclinical dysfunction is known to precede target organ damage, the objective of this study was to determine whether subclinical microvascular dysfunction is related to large artery stiffness.
METHODS
A total of 141 patients with type II diabetes were recruited from our outpatient clinics over a 6 month period. Medical information was obtained via patient interview and electronic medical record review including laboratory results. Microvascular function was assessed by the vascular reactivity index (VRI), which

assesses changes in digital temperature before and after release of arterial cuff occlusion (VENDYS 5000BC DTM system Endothelix, Inc.). Large artery stiffness was assessed by carotid-femoral pulse wave velocity (PWV) using applanation tonometry (Sphygmocor, Atcor Inc.).
RESULTS
Mean age was 60±8 years, 64% were female. 80% had hypertension and 90% had dyslipidemia. 15% had chronic kidney disease. Mean HbA1C levels were 8.1±2.2%. For the entire group, VRI was significantly correlated with PWV (r=.27, p=.002). On multivariate analysis, VRI was independently associated with PWV (β=-1.0, p=.001) and a trend towards an association with HbA1c (β =.07, p=.09) after adjusting for traditional cardiovascular risk factors.
CONCLUSIONS
Among African Americans with diabetes, subclinical microvascular dysfunction is significantly correlated to large artery stiffness and possibly to glycemic control. Further study is needed to clarify mediating factors of these relationships.

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P254

Apolipoprotein Expression Changes in the Dahl Salt Sensitive Rat Model of Spontaneous Superimposed Preeclampsia

PrimaryAuthor.AuthorBlock:**Kenji J Maeda**, Ashley C Johnson, Michael R Garrett, Jennifer M Sasser, Univ of Mississippi Medical Ctr, Jackson, MS

Preeclampsia (PE) is a multifactorial pregnancy-specific syndrome with the main characteristic feature of the mother developing hypertension and systemic organ dysfunction after 20 weeks of gestation. Worldwide, PE accounts for up to 8% of pregnancy complications and is considered to be a leading cause of maternal and fetal morbidity and mortality. Currently, there is little progress in developing treatments for PE, nor are there robust biomarkers for early detection. Using the Dahl salt sensitive (S) rat, a model of spontaneous superimposed preeclampsia, and the Sprague Dawley (SD) rat, normal pregnancy control, we previously performed a microarray study (Affymetrix Rat 2.0ST GeneChip) on gestational day 14 placental samples which identified several genes in the apolipoprotein family to be temporarily dysregulated between the Dahl S and SD rats. The goal of the current study was to validate these microarray findings and determine if these changes persist through late pregnancy. We performed quantitative real-time-PCR analysis (BioRad PrimePCR Custom Array) and confirmed significant increases in expression of various isoforms of apolipoproteins in the Dahl S rat compared to that of the SD on gestational day 14 (Table). However, there were no significant differences between the two strains on gestational day 20, indicating a potential role of these apolipoproteins earlier in PE development. Our data are consistent with patient studies suggesting apolipoproteins may be involved in PE, serve as potential biomarkers for early detection, and be a target for therapeutic interventions.

Table: Gene Expression in Placentae of Dahl S Compared to SD
Normalized Relative Regulation (p-value)

Apolipoprotein	Dahl S	
	Day 14 (n=5)	Day 20 (n=6)
Apoa2	+ 89.5 (<0.05)	+ 1.08 (0.28)
Apoa4	+ 27.1 (<0.05)	−1.49 (0.34)
Apob	+ 30.2 (<0.05)	−1.26 (0.25)
Apoc2	+ 52.5 (<0.05)	−1.42 (0.21)
Apom	+ 22.0 (<0.05)	−4.70 (0.90)

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P256

Vasopressin Infusion During Pregnancy in Mice Induces Early Histological Placental Phenotypes of Preeclampsia

PrimaryAuthor.AuthorBlock:**Jeremy A Sandgren**, Katherine N Gibson-Corley, Danny W Linggonegoro, Katherine J Perschbacher, Shao Yang Zhang, Sabrina M Scroggins, Guorui Deng, Nicole A Pearson, Donna A Santillan, Gary L Pierce, Mark K Santillan, Curt D Sigmund, Justin L Grobe, Univ of Iowa, Iowa City, IA

Human preeclampsia (PE) is associated with elevated secretion of arginine vasopressin (AVP), and chronic infusion of AVP into pregnant mice is sufficient to model PE by causing hypertension, renal glomerular endotheliosis, proteinuria, fetal placental hypoxia, and growth restriction. Early stages of PE are associated with defective trophoblast invasion of maternal spiral arteries, leading to decreased artery diameter and placental oxygenation. AVP infusion (24 ng/hr, sc) into pregnant mice caused placental hypoxia (chromatin-bound HIF1α on gestational day (GD)17.5; saline n=5, 0.31±0.01; AVP n=5, 0.34±0.01 AU, p<0.05) and reduced placental growth factor mRNA (n=18, 1.0 (0.7-1.3) vs n=21, 0.3 (0.2-0.4) fold (1 se), p<0.05). Therefore, we performed histological analyses of placentas collected from mice at GD12.5 infused with saline or AVP, with the hypothesis that AVP leads to early placental PE phenotypes. Similar to effects on GD17.5, this preliminary cohort demonstrated increased urine protein content (n=7, 27±3 vs n=13, 44±3, g/L p<0.05) and mid-gestational systolic blood pressure (-7.6±3.1 vs +3.5±2.2, mmHg p<0.05), similar fetal (75±7 vs 78±4 mg, p=0.75) and placental (83±10 vs 81±4 mg, p=0.80) masses, and similar changes in heart rate (+37±16 vs +39±12 bpm, p=0.93). GD12.5 placentas were then stained with haemotoxylin and eosin or immunostained for cytokeratin-8 to examine morphological changes induced by AVP. AVP infusion had no significant effects on labyrinth (saline n=3, 647±25 vs AVP n=7, 602±35 um, p=0.45), spongiotrophoblast (342±19 vs 363±24 um, p=0.61), or decidua (622±47 vs 520±47 um, p=0.24) layer thicknesses. AVP caused a reduction in average maximum spiral artery diameter (171±28 vs 121±8 um, p<0.05) and a trend toward reduced total spiral artery number (8.4±2.9 vs 4.9±0.7, p=0.13), but no difference in the maximum invasion depth of CK8-positive trophoblasts (310±50 vs 286±37 um, p=0.72). We conclude that AVP infusion is sufficient to induce cardinal mid-gestational features of PE in pregnant mice, including reduced spiral artery diameter. Such morphological changes may be associated with the placental hypoxia and reduced placental growth factor expression in model.

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P257

Investigating Angiotensin II Infusion as a Model of Superimposed Preeclampsia in Pregnant Stroke Prone Spontaneously Hypertensive Rats

PrimaryAuthor.AuthorBlock:**Hannah L Morgan**, Elaine Butler, Elisabeth Beattie, Martin W McBride, Delyth Graham, Univ of Glasgow, Glasgow, United Kingdom

Background

Hypertensive disorders of pregnancy are a leading cause of maternal mortality worldwide. Chronic hypertension with superimposed preeclampsia is becoming more prevalent in modern society. Animal models are vital for understanding this condition. Our aim was to induce a preeclamptic phenotype in pregnant stroke prone spontaneously hypertensive (SHRSP) rats by angiotensin II (AngII) infusion.

Methods

AngII, at a dose of 500ng/kg/min or 1000ng/kg/min, was administered by minipump in pregnant SHRSP rats from gestational day (GD)10. Cardiovascular and renal changes were monitored using radiotelemetry, echocardiography and weekly urine collections. GD18 *ex vivo* uterine artery function and structure were assessed using myography.

Results

Both AngII doses resulted in an immediate and sustained increase in systolic blood pressure compared to vehicle control (*172.5±5.0mmHg; 500ng/kg/min and 204.1±5.6mmHg; 1000ng/kg/min vs 159.0±0.4mmHg; p<0.001*). Cardiac output increased over pregnancy in the vehicle group (*53±6L/min vs 81±5L/min; p<0.01*), however this trend was not observed in 500ng/kg/min treatment group (*60±6L/min vs 56±11L/min*) and was reversed in 1000ng/kg/min treatment group (*60±6L/min vs 36±6L/min; p<0.01*). GD14 urinary albumin:creatinine ratios were significantly increased in both treatment groups (*1.3±0.2; 500ng/kg/min, 2.9±0.1; 1000ng/kg/min vs 0.5±0.2; vehicle; p<0.05, p<0.001*). Uterine arteries in the 1000ng/kg/min treatment group demonstrated significantly reduced cross-sectional area (*$4.8x10^4±32μm^2$ vs $7.6x10^4±16μm^2$; p<0.01*). Both treatment groups had a reduced vasorelaxation to $2x10^5$M carbachol (*93.6±3.9%; 500ng/kg/min, 84.7±4.3%; 1000ng/kg/min vs 56.9±21.1%; vehicle; p<0.05*) and contractile response to $2x10^5$M noradrenaline demonstrated an

increased trend in both treatment groups (*66.9±16.1KPa; 500ng/kg/min, 61.8±6.2KPa; 1000ng/kg/min vs 45.1±5.7KPa; vehicle*).

Conclusion

This study demonstrates that AngII infusion in pregnant SHRSP rats can mimic specific haemodynamic, cardiac and urinary profiles common to preeclamptic women. This rodent model of superimposed preeclampsia will be crucial for detailed investigation of underlying causes and treatment options for this condition.

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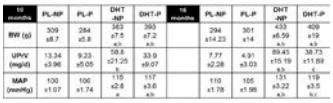
P259

Characterization of Hyperandrogenemic Female Rats With History of Pregnancy

PrimaryAuthor.AuthorBlock:**Chetan N Patil**, Carolina Dalmasso, Rodrigo O Maranon, Jane F. Reckelhoff, U of Mississippi Medical Ctr, Jackson, MS

Women with polycystic ovarian syndrome (PCOS) experience hyperandrogenemia, elevated MAP, irregular menses, and difficulty becoming pregnant. As a result many PCOS women undergo assisted fertilization. The long-term cardiovascular consequences of pregnancy in PCOS women are not clear. We tested the hypothesis that a single pregnancy attenuates cardio-renal parameters in hyperandrogenemic female rats, a model of PCOS. Female SD rats were implanted with Dihydrotestosterone (DHT, 7.5mg/90d; replaced every 85 d) or placebo pellets, beginning at 6 wks of age. At 3 months females were paired with SD males. After delivery and lactation, females were divided into placebo (PL) or DHT rats with (P) or without pregnancy (NP) and allowed to age to 10 mos (still estrous cycling) or 16 mos (post cycling) (n=5-8/grp). At 10 mos, body weight (BW), proteinuria (UPrV), and MAP were higher in DHT than PL rats, and pregnancy history had little effect. At 16 mos, BW was higher in DHT than PL rats, and was similar in NP and P groups. UPrV and MAP were also higher in DHT groups than PL, but were significantly attenuated in DHT-P vs DHT-NP. These data suggest that pregnancy in women with PCOS may be cardiovascular protective with aging. The mechanisms remain to be determined.



10 months	PL-NP	PL-P	DHT-NP	DHT-P	16 months	PL-NP	PL-P	DHT-NP	DHT-P
BW (g)	309 ±8.7	284 ±5.8	369 ±7.5 a,c	393 ±7.2 a,b		294 ±14.23	301 ±14	433 ±6.59 a,c	409 ±19 a,c
UPrV (mg/d)	13.34 ±3.96	9.23 ±5.05	58.8 ±21.25 a	33.9 ±9.07		7.77 ±2.28	4.91 ±3.03	89.45 ±15.19 a,c	38.73 ±11.89 c
MAP (mmHg)	100 ±1.07	106 ±1.74	119 ±2.8 a	107 ±3.6 a,b		110 ±1.78	105 ±1.98	131 ±3.22 a,b	119 ±3.5 b,c

a, p<0.05 vs PL-NP; b, p<0.05 vs PL-P; c, p<0.05 vs DHT-NP, mean ± SEM, two-way ANOVA.

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P260

Placental Ischemia-stimulated TH17 Cells Induce Preeclampsia-associated Cytolytic Natural Killer Cells During Pregnancy

PrimaryAuthor.AuthorBlock:**Denise C Cornelius**, Corbin A Shields, Maggie L McCalmon, Tarek Ibrahim, Babbette LaMarca, Univ of Mississippi Medical Ctr, Jackson, MS

Preeclampsia (PE), a hypertensive disorder of pregnancy, is associated with increased circulating T_H17 cells (T_H17s), IL-17 and cytolytic

Natural Killer cells (cNKs). Although the stimulus for cNKs in PE is currently unknown, recent *in vitro* studies suggest that IL-17 induces proliferation and cytotoxic activity in human NK cells. We have previously demonstrated that placental ischemia (PI)-stimulated T_H17s mediate pathophysiology in pregnant rats similar to that seen in PE. In this study we tested the hypothesis that PI-stimulated T_H17s induce cNKs and their associated cytokines, IFNγ and TNFα, and cytolytic proteins, perforin and granzyme B. T_H17s were injected i.p. into NP rats on gestation day (GD) 12. Total NK cells (tNKs) and cNKs in blood and placental lymphocytes were quantified via flow cytometry. MAP, pup and placental weight, tissue ROS, cytokines, perforin and granzyme B were measured. As previously shown circulating T_H17s and IL-17, MAP, IUGR, and placental and renal ROS were significantly increased after T_H17 adoptive transfer. MAP was 100.6±1.7 mmHg (n=7) in NP rats and 119.4±2.6 mmHg (n=7) in NP recipients of T_H17s (NP+T_H17, $p< 0.05$). Placental tNKs (% gated) were significantly increased in NP+T_H17 (19.7±4.6%) compared to NP rats (5.4±2.0%; $p< 0.05$). Importantly, placental cNKs were significantly increased in response to T_H17s (NP: 2.9±0.9% vs NP+T_H17: 14.9 ±4.0%, $p< 0.05$). Serum and placental IFNγ significantly increased from 5.8±3.3 pg/mL and 1.1±0.6 pg/mL/mg, respectively in NP to 193±26.12 pg/mL and 3.9±0.6 pg/mL/mg in NP+T_H17 ($p< 0.05$). Serum and placental TNFα increased from 0.9 pg/mL and 0.1 pg/mL/mg in NP to 4.5 pg/mL and 0.2 pg/mL/mg in NP+T_H17 ($p< 0.05$). Placental perforin was significantly increased from 0.3 pg/mL/mg in NP to 1.6 pg/mL/mg in NP+T_H17 ($p< 0.05$). Serum perforin, and serum and placental granzyme B were comparable between NP vs NP+T_H17. These data suggest a role for PI-stimulated T_H17s to induce a cytotoxic phenotype in NKs during pregnancy and identifies stimulation of cNKs as a new mechanism by which the T_H17/IL-17 pathway may mediate pathophysiology during PE. Further investigation into this innovative pathway may identify novel targets with therapeutic potential to improve outcomes associated with PE.

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P261

Vasopressin System Components are Dysregulated in Human Preeclamptic Placenta

PrimaryAuthor.AuthorBlock:**Guorui Deng**, Katherine J Perschbacher, Jeremy A Sandgren, Nanmeng Yu, Sabrina M Scroggins, Eric J Devor, Donna A Santillan, Katherine N Gibson-Corley, Curt D Sigmund, Anne E Kwitek, Mark K Santillan, Justin L Grobe, Univ of Iowa, Iowa City, IA

Preeclampsia (PE) is a cardiovascular disorder of late pregnancy characterized by pregnancy-specific onset of hypertension and proteinuria. Although initiating events leading to PE development remain unclear, emerging studies suggest arginine vasopressin (AVP) signaling may contribute to PE pathogenesis, as elevated circulating copeptin (a stable biomarker of AVP secretion) levels precede the onset of human PE, and chronic infusion of AVP into wildtype mice phenocopies PE. Here, we tested the hypothesis that AVP signaling is dysregulated in

PE placenta by performing *in-silico* reanalysis of a publically available gene expression dataset derived from placentas of human PE and normal pregnancies (GSE75010). Significant increases in expression [log_2 values] of *AVP* (Con: 6.96 ± 0.02, PE: 7.03 ± 0.17, p=0.005) and its receptors *AVPR1a* (Con: 5.73 ± 0.02, PE: 5.80 ± 0.02, p=0.03) and *OXTR* (Con: 6.58 ± 0.02, PE: 6.65 ± 0.02, p=0.003) were observed in PE placentas (n=80) compared to normal placentas (n=77). Preeclamptic placentas also displayed an enrichment in a hypoxic gene signature, as shown by gene set enrichment analyses (NES: 1.75, FDR q<0.001, FWER p <0.001), suggesting that these placentas exhibited increased hypoxia-related signaling. Interestingly, *AVPR1a* expression as well as *OXTR* is positively and significantly correlated with the expression of two hypoxia-inducible genes *HK2* and *DDIT4* (*AVPR1a* v *HK2*: r^2=0.04, p=0.02, *AVPR1a* v *DDIT4*: r^2=0.06, p=0.003, *OXTR* v *HK2*: r^2=0.05, p=0.007, *OXTR* v *DDIT4*: r^2=0.04, p=0.008). To further elucidate the role hypoxia may play in promoting AVP signaling, HTR8/SVNeo cells (immortalized human first trimester trophoblasts) were treated with the hypoxia mimetic dimethyloxallyl glycine (DMOG). DMOG incubation did not significantly alter the expression of *AVP* and *AVPR1a*. Unexpectedly, a marked reduction in *OXTR* expression was observed upon DMOG treatment. In summary, these data suggest that 1) components of the AVP signaling pathway are aberrantly expressed in human PE placentas, 2) PE placentas exhibit a gene expression signature consistent with increased canonical hypoxia signaling, and 3) hypoxia may not be the cause of elevated placental *AVP*, *AVPR1a*, and *OXTR* expression in human PE.

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P262

Vasopressin Receptors Regulate Immune Responses in Preeclampsia

PrimaryAuthor.AuthorBlock:**Sabrina M. Scroggins**, Donna A. Santillan, Daffney T. Gomendoza, Lindsay K. Gravatt, Kathryn J. Huber-Keener, Katherine J. Perschbacher, Jeremy A. Sandgren, Eric J. Devor, Justin L. Grobe, Mark K. Santillan, Univ of Iowa, Iowa City, IA

The pathogenesis of preeclampsia (PE) involves imbalanced T helper (T_H) cell populations and resultant changes in pro- and anti-inflammatory cytokine release. Elevated secretion of arginine vasopressin (AVP) precedes the development of symptoms in PE in humans, and chronic infusion of AVP (24 ng/hr s.c.) throughout gestation is sufficient to initiate cardiovascular, renal, and immune phenotypes of PE in wild-type C57BL/6J mice. We hypothesize that increased AVP signaling may mediate the immune changes observed in PE. AVP infusion throughout gestation in mice resulted in increased pro-inflammatory IFNγ (T_H1) in the maternal plasma (N=7, $p<0.05$) and IL-17 (T_H17) in the placenta (N≥10, $p<0.001$). The T_H2-associated anti-inflammatory cytokines IL-4 and IL-10 were decreased in maternal kidney (IL-4: N=8, $p<0.05$; IL-10: N=5, $p<0.01$) and fetal kidney (IL-4: N=5, $p<0.05$; IL-10: N=5, $p<0.05$) of AVP-infused dams. **To elucidate the receptor dependency of these effects,** AVP-infused dams were simultaneously treated with chronic infusion of AVP V_{1A} and/or V_2 receptor antagonists (22 ng/hr s.c). Combined blockade of V_{1a}+V_2 receptors by conivaptan, as well as specifically blocking V_2 by tolvaptan, corrected AVP induced reductions in IL-4 in maternal (conivaptan: N=5, $p<0.05$; tolvaptan: N=5, $p<0.0.0001$) and fetal kidneys (conivaptan: N=5, $p<0.0001$; tolvaptan: N=5, $p<0.05$). These data implicate V_2 as the receptor involved in kidney IL-4 deficiency in PE, whereby blockade of V_2 restores IL-4, preventing inflammation. Combined blockade of V_{1A}+V_2 receptors by conivaptan corrected the placental loss of IL-4 (N=5, $p<0.05$), whereas blockade of either receptor alone was insufficient, suggesting loss of placental IL-4 via AVP is mediated by both V_{1a} and V_2 receptors. In contrast, increased placental IL-17 was only corrected by selective blockade of V_{1A} by relcovaptan (N=5, $p<0.05$), suggesting a novel role for V1a receptor in pro-inflammatory placental IL-17 production in PE. Collectively these results demonstrate the sufficiency of AVP to induce the immune changes typical of PE, and support a dominant role for V_{1A} in the induction of pro-inflammatory IL-17 (T_H17) release versus a dominant role for V_2 receptors in the suppression of anti-inflammatory IL-4 (T_H2) release.

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P263

Interleukin-17 Infusion Promotes Cerebral Edema and Increased Blood Brain Barrier Permeability in Pregnant Rats

PrimaryAuthor.AuthorBlock:**Junie P Warrington**, Jeremy Duncan, Heather A Drummond, Joey P Granger, Michael J Ryan, Univ of Mississippi Medical Ctr, Jackson, MS

While cerebrovascular abnormalities such as cerebral edema and increased blood-brain barrier (BBB) permeability are a common complication of (pre)eclampsia, the exact mechanisms underlying these abnormalities remain unclear. One potential mechanism could

be increased Interleukin-17 (IL-17), a pro-inflammatory cytokine that is increased in both preeclampsia patients and the rat model of placental ischemia created by reducing uterine perfusion pressure. While IL-17 has been linked to the pathogenesis of preeclampsia, its effect on cerebrovascular function during pregnancy is unknown. Thus, we tested the hypothesis that increasing circulating IL-17 during pregnancy promotes regional increases in brain water content and BBB permeability. Recombinant mouse IL-17 (150 pg/day) was infused via mini-osmotic pump (i.p) from gestational day 14 to 19 in pregnant rats (n = 5-7 per group). Brain water content was assessed using the dry weight to wet weight ratio and BBB permeability was assessed by quantifying Evans blue extravasation into the anterior cerebrum, hippocampus, cortex, striatum, posterior cerebrum, and cerebrum. As reported previously, IL-17 infusion resulted in a significant increase in mean arterial pressure (119 ± 3 vs. 104 ± 4 mmHg in normal pregnant group; p = 0.010). Water content tended to be increased in the cortex (80.5 ± 0.2 vs. 79.5 ± 0.4%, p = 0.054) and striatum (77.6 ± 0.8 vs. 75.1 ± 1.4%, p = 0.062) and was significantly increased in the posterior cerebrum (79.7 ± 0.4 vs. 77.8 ± 0.5%, p = 0.007) and cerebrum (79.8 ± 0.3 vs. 78.8 ± 0.1%, p = 0.005) of the IL-17 treated group. BBB permeability was increased only in the cortex (0.017 ± 0.002 vs 0.012 ± 0.002, p = 0.046) of rats receiving IL-17 infusion. These data suggest that increased IL-17 during pregnancy contributes to edema formation and increased BBB permeability in the cerebral cortex and may be a therapeutic target in preeclampsia. Future studies will determine whether reducing IL-17 levels in placental ischemic rats will prevent these cerebrovascular changes.

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P264

Endothelial-specific Interference With PPARγ Activity in Offspring Born From AVP-induced Preeclamptic Pregnancies Has Cardio-renal and Metabolic Consequences

PrimaryAuthor.AuthorBlock:**Anand R Nair**, Masashi Mukohda, Larry N Agbor, Ko-Ting Lu, Jing Wu, Jeremy A Sandgren, Justin L Grobe, Curt D Sigmund, Univ of Iowa, Iowa City, IA

Peroxisome proliferator-activated receptor gamma (PPARγ) is a ligand-activated transcription factor known to regulate metabolic and vascular function. Mutations in PPARγ result in hypertension, and synthetic agonists of PPARγ reduce blood pressure. Previously we found that mice expressing dominant-negative (DN) PPARγ driven by an endothelium-specific promoter (E-DN) exhibit vascular dysfunction. Preeclampsia (PE) is a hypertensive disorder of pregnancy which carries cardiovascular and metabolic risk to offspring. PE is associated with vascular dysfunction, and we therefore hypothesized a role for endothelial PPARγ in the pathogenesis of PE and its sequelae. C57BL/6J dams were bred with E-DN sires, and symptoms of PE were induced by the infusion of vasopressin (AVP, 24 ng/hr sc) throughout gestation. We assessed phenotypes of PE first in pregnant dams, and then in offspring as adults. Compared to saline infusion (SAL), AVP elevated maternal blood pressure (SBP: 116±3 vs 107±3, $p<0.05$) at gestational day (GD) 14-15 and urine protein (70±6 vs 27±4 mg/mL, $p<0.05$) at GD17. Offspring from these pregnancies were phenotyped in adulthood to assess cardiovascular and metabolic function. Data were stratified to sex, genotype, and maternal

exposure to AVP vs SAL. Systolic blood pressure in adult male and female offspring born to AVP-infused pregnancies was similar to mice born to SAL pregnancies. At 20 weeks of age, vasorelaxation responses to acetylcholine were not different in offspring exposed to PE compared to mice born from SAL pregnancies. However, urinary protein levels were significantly elevated in both male (58±13 vs 32±5 mg/ml, $p<0.05$) and female (38±3 vs 25±2 mg/ml, $p<0.05$) adult E-DN born to PE pregnancies compared to E-DN controls born from SAL pregnancies. Male E-DN offspring exposed to PE showed significantly increased gain in body weight over time compared to male NT exposed to PE (ΔBW: 20±8 vs 14±2 g). These data highlight the impact of *in utero* exposure to elevated AVP upon cardiovascular function in the mother, and the adverse renal and metabolic consequences of PE upon offspring. Moreover, our data suggests that interference with endothelial PPARγ in pups born from PE pregnancies increases the risk for renal and metabolic dysfunction.

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P265

Placental Ischemia Causes Renal Mitochondrial Impairment in Reduced Uterine Perfusion Pressure Rats

PrimaryAuthor.AuthorBlock:**Venkata Ramana Vaka**, Kristen M McMaster, Mark W Cunningham Jr, Tarek Ibrahim, Lorena M Amaral, Nathan E Usry, Babbette LaMarca, Univ of Mississippi Medical Ctr, Jackson, MS

Introduction: Preeclampsia (PE) is characterized by new onset hypertension in pregnancy and is associated with renal dysfunction, proteinuria and is believed to be initiated by placental ischemia. Mitochondrial dysfunction is an important source of reactive oxygen species (ROS) generation. To better understand the role of mitochondrial dysfunction in hypertension during PE we examined renal mitochondrial expression in the kidney of RUPP rats. **Methods:** Female Sprague Dawley rats were dived into two groups; normal pregnant (NP) and RUPP rats. On gestational day (GD) 14, RUPP surgery was performed, GD18 carotid catheters were inserted, and GD19 conscious blood pressure (MAP) was measured, renal mitochondria were isolated for respiration and analysis. Respiration measurements were performed on intact isolated mitochondria under glutamate/malate as complex I substrate using Oroboros Oxygraph-2K. Oxidative phosphorylation was analyzed by western blot using total Oxphos cocktail antibody and VDAC. **Results:** MAP was elevated in RUPP (n=8) vs NP rats (n=10) (125±6 vs. 99±2 mmHg, $p<0.05$). RUPP (n=3) renal mitochondria show reduced expression of Complex I (0.5±0.02 vs 0.8±0.09, $p<0.05$) and Complex II (0.7±0.03 vs 1±0.08, $p<0.05$) compared to NP mitochondria. State 3 (624±112 vs 878±9 pmol/sec/mg, $p=0.22$) and maximal (496±87vs 748±75 pmol/sec/mg, $p=0.14$) respiration rates trended towards reduction in RUPPs (n=4) compared to NP (n=2).

Conclusion: Reduced expression of Complex I and II expression with reduced respiratory rates indicate mitochondrial impairment in the RUPP kidneys. Although, renal mitochondrial mediated oxidative stress may be one mechanism of hypertension in the RUPP rat model of PE, further exploration of oxidative stress in the kidney is necessary to fully understand the contribution of mitochondrial mediated oxidative stress to pathology of PE.

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P266

Immunological Characterization of the Dahl SS Model of Superimposed Preeclampsia

PrimaryAuthor.AuthorBlock:**Erin B. Taylor**, Eric M. George, Michael R. Garrett, Michael J. Ryan, Jennifer M. Sasser, Univ of MS Medical Ctr, Jackson, MS

Patients with existing hypertension and renal disease have an increased risk for developing superimposed preeclampsia. The Dahl SS rat has been shown to be a model of superimposed spontaneous preeclampsia characterized by exacerbated hypertension, increased urinary protein excretion, and increased fetal demise. Because of the underlying immune system dysfunction present in preeclamptic pregnancies in humans, we hypothesized that the Dahl SS rat would also have an altered immune status. In the present study, Dahl SS rats fed a normal (0.3%) salt diet were compared to normal pregnant Sprague Dawley (NP) rats. On gestational day 19, spleens and placentas were collected for flow cytometric analyses. Splenic $CD3^+CD4^+$ T cells were analyzed for cytokine production after polyclonal stimulation with phorbol myristate acetate and calcium ionophore to elicit a primary cytokine response. Dahl SS rats had increased percentages of inflammatory $TNF\alpha^+$ T cells as compared to NP rats (4.8±0.3% vs. 1.9±0.4%, $p<0.001$), but there were no significant differences in percentages of IFN-γ^+, IL-4^+, or IL-17^+ splenic T cells. Analyses of placental lymphocytes revealed that Dahl SS rats had increased percentages of placental $CD3^+CD4^+$ T cells as compared to NP rats (24.3±1.2% vs. 7.6±1.3% , $p<0.0001$) as well as $CD3^+CD8^+$ T cells (9.2±0.5% vs. 4.9±0.4% , $p<0.001$), but there was no difference in $CD45RA^+$ B cells. For comparison, we also assessed placental leukocytes in the established reduced uterine perfusion pressure (RUPP) model that mimics characteristics of preeclampsia. Previous studies have reported that pregnant Sprague Dawley rats with surgically induced RUPP have aberrant immune system function which contributes to the pathophysiology of the preeclamptic phenotype in this model. While placentas from RUPP rats had an increase in placental $CD3^+CD4^+$ T cells as compared to NP rats (14.9±1.9% vs. 7.6±1.3% $p<0.001$), similar to pregnant Dahl SS, the percentages of $CD3^+CD8^+$ T cells and $CD45RA^+$ B cells were not significantly different. Taken together, these data demonstrate an association between proinflammatory T cells and superimposed preeclampsia, and suggest that different immunological mechanisms may contribute to the pathogenesis of preeclampsia in different models.

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P267

Placental Ischemia Causes Cardiac Structural and Functional Abnormalities in the Reduced Uterine Perfusion Pressure Rat Model

PrimaryAuthor.AuthorBlock:**Bhavisha Bakrania**, Joey P. Granger, Univ of Mississippi Med. Ctr, Jackson, MS

Preeclampsia (PE) is a disorder prevalent in 5-7% of pregnancies. It is characterized by maternal hypertension and endothelial dysfunction. While it has been shown that systolic and diastolic function is impaired in women with PE, the exact mechanisms responsible for the cardiac dysfunction in PE have yet to be fully elucidated. PE is thought to develop in response to placental ischemia and ultimately the release of anti-angiogenic and pro-inflammatory factors into the maternal circulation. However, the impact of placental ischemia on cardiac function is unclear. Therefore the aim of this study was to assess cardiac structure and function in response to placental ischemia utilizing the Reduced Uterine Perfusion Pressure (RUPP) rat model. Briefly in this model, silver clips are placed on the abdominal aorta and branches of the ovarian arteries on gestational day (GD) 14 to induce placental ischemia. For this study, the RUPP (*n*=8) group underwent surgery on GD 14, and both normal pregnant (NP, *n*=8) and RUPP rats had carotid catheters placed on GD 18. Blood pressure and echocardiography measurements, and tissue harvest were performed on GD 19. The RUPP group had significantly increased mean arterial pressure compared to the NP group on GD 19 (123±3 vs. 97±2 mmHg, P<0.01). RUPP rats had lower mean left ventricular ejection fraction (60±2 vs. 78±2 %, P<0.01) and fractional shortening (46±3 vs. 56±1 %, P=0.05), in addition to cardiac hypertrophy (0.97±0.04 vs. 0.91±0.02 g, P=0.02). These data were accompanied by increased cardiomyocyte surface area (348±36 vs. 289±23 μm^2, P=0.03). In conclusion, this study shows that the RUPP rat develops cardiac structural and functional abnormalities after only five days of placental ischemia. Furthermore, these data suggest that the RUPP model could be useful in investigating and understanding the mechanisms which underpin these cardiac changes in PE patients.

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P268

Overexpression of miR-210 Induces Preeclampsia-like Symptoms in Pregnant Mice by Attenuating the STAT6/IL-4 Pathway

PrimaryAuthor.AuthorBlock:**Kelsey R. Bounds**, Valorie L. Chiasson, Rachel M. Engelbrecht, Lu J. Pan, Baylor Scott & White HL, Temple, TX; Piyali Chatterjee, Baylor Scott & White HL/Texas A&M HSC, Temple, TX

Preeclampsia (PE) is a pregnancy-related hypertensive disorder that affects 5-8% of all pregnancies worldwide. Placental microRNA (miRNA) expression is known to be dysregulated during PE which includes miR-210. However, the role of miR-210 in the development of PE is still unknown. We have previously demonstrated STAT6, a transcription factor, to be a direct target of miR-210. In addition, STAT6 modulates levels of IL-4, an anti-inflammatory cytokine which is known to be beneficial during pregnancy. Given that the

STAT6/IL-4 pathway is important for normal pregnancy, we hypothesized that an overexpression of miR-210 in mice will contribute to PE-like symptoms by inhibiting the STAT6/IL-4 pathway. Systolic blood pressures (SBP) were measured using the tail-cuff method on both WT (C57Bl/6J) and miR-210 TG mice. The miR-210 TG mice exhibited increased SBP compared to the WT mice (WT: 95±2 mmHg, miR-210 TG: 115±2.4 mmHg, $p<0.05$). The miR-210 TG mice also displayed endothelial dysfunction measured by aortic vascular reactivity using wire myography (WT: 93% miR-210 TG: 68% relaxation, $p<0.05$ vs. WT) and a significant increase in proteinuria (4 fold, $p<0.05$ vs. WT). Furthermore, the miR-210 TG mice exhibit placental necrosis and a significant increase in the number of malformed pups. Placental STAT6 levels decreased in miR-210 TG compared to WT mice (2.6 fold, $p<0.05$ vs. WT) as determined by immunoblotting. In addition, qRT-PCR analysis of the miR-210 TG placentas exhibited a (4.8 fold, $p<0.05$ vs. WT) decrease in IL-4 expression. Immunoblotting and immunohistochemistry (IHC) studies showed a decrease in IL-4 levels (2.4 fold, $p<0.05$ vs. WT) and immunoreactivity in the miR-210 TG placentas as well as. IHC studies in human placentas also showed a decrease in both IL-4 and STAT6. Based on our results, miR-210 overexpression induces PE-like symptoms including hypertension, endothelial dysfunction, proteinuria, and malformed pups in pregnant mice. In addition, miR-210 overexpression attenuated the STAT6/IL-4 anti-inflammatory pathway. Our data suggests that either targeting miR-210 or the STAT6/1L-4 pathway could be a potential therapeutic in mitigating the symptoms of PE.

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P269

Circulating Factors in Response to Placental Ischemia Cause Vascular Endothelial Mitochondrial Oxidative Stress

PrimaryAuthor.AuthorBlock:**Venkata Ramana Vaka**, Kristen M McMaster, Mark W Cunningham Jr, Tarek Ibrahim, Lorena M Amaral, Denise Cornelius, Babbette LaMarca, Univ of Mississippi Medical Ctr, Jackson, MS

Introduction: Preeclampsia (PE) is associated with placental ischemia, new onset hypertension, oxidative stress and endothelial dysfunction. Factors linking placental ischemia with endothelial dysfunction and hypertension are not completely understood. Mitochondrial (mt) dysfunction (dys) is a major source of reactive oxygen species (ROS) and we have shown that placental ischemia causes oxidative stress in RUPP rats. We hypothesize that circulating factors in RUPP rats cause vascular endothelial mt dys and mt ROS as a contributor to endothelial dysfunction and hypertension during pregnancy. **Methods:** Female Sprague Dawley rats were dived into two groups; normal pregnant (NP) and RUPP rats. On gestational day (GD) 14, RUPP surgery was performed, GD18 carotid catheters were inserted, and GD19 conscious blood pressure (MAP) was measured. GD 19 placentas were collected and mitochondria were isolated for respiration and ROS measurements. Mt ROS was measured spectrophotometrically in HUVECs incubated with 10% serum from NP or RUPP rats using MitoSox Red. **Results:** MAP was elevated in RUPP (n=9) compared to NP rats (n=9) (122±2 vs. 104±2 mmHg, $p<0.05$). State 3 (313±16 vs 423±15 pmol/sec/mg, $p<0.05$) and maximal (244±13 vs 300±11 pmol/sec/mg, $p<0.05$) respiration rates were significantly reduced in placental mitochondria from RUPP (n=7) vs NP

(n=8) rats. RUPP placental mitochondria show 35-fold increase in ROS production compared NP mitochondria ($p<0.05$). HUVECs incubated with RUPP (n=7) serum showed significantly increased ROS vs NP (n=7) serum (9±3 vs 3±1, % gated, $p=0.05$).**Conclusion:** Reduced placental mitochondrial respiration and increased mt ROS support the hypothesis that mt dys and mt ROS occurs in response to placental ischemia. Importantly, increased ROS from endothelial cells in response to RUPP serum indicate the importance of circulating factors to cause vascular mt dyst and mt ROS.

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P270

First Trimester Elevation in Circulating Endothelin-1 and Arterial Stiffness are Predictive of Late Pregnancy Preeclampsia

PrimaryAuthor.AuthorBlock:**Alexandria M. Betz**, Gary L. Pierce, Donna A. Santillan, Eric J. Devor, Sabrina M. Scroggins, Debra S. Brandt, Amy K. Stroud, Kellie M. Thomas, Seth W. Holwerda, Allison L. O'Deen, Lyndsey E. DuBose, Justin L. Grobe, Curt D. Sigmund, Mark K. Santillan, Univ of Iowa, Iowa City, IA

Preeclampsia (PE) is characterized by late pregnancy hypertension and proteinuria. PE causes significant morbidity for the maternal-fetal unit. Circulating endothelin-1 (ET-1), a potent vasoconstrictor, is elevated at the time of diagnosis of human PE. In addition, women with PE demonstrate arterial stiffness as early as the end of the first trimester. However, it is unknown if arterial stiffness is associated with a first trimester elevation in ET-1 and post-delivery placental ET-1. We hypothesized that 1) first trimester plasma ET-1 is elevated and is associated with arterial stiffness in women who develop PE; 2) first trimester ET-1 is predictive of PE; and 3) placental ET-1 is increased in PE. To address these questions, we performed a nested case-control study in women at risk for PE. First trimester plasma ET-1 was measured via ELISA; aortic stiffness and carotid beta-stiffness (CβS) were measured by carotid-femoral pulse-wave velocity (CFPWV) and carotid tonometry/ultrasound, respectively. While the maternal age of controls (n=126; age 30 ± 0.45 years) and PE (n=15; age 31 ± 1.3 years) were similar, the PE group had a higher first trimester BMI (35 ± 3 vs. 29 ± 1 kg/m^2, $p = 0.01$), systolic (125 ± 2 vs. 113 ± 1 mmHg, $p< 0.01$) and diastolic blood pressure (68 ± 2 vs. 60 ± 1 mmHg, $p< 0.01$) compared with controls. In addition, first trimester plasma ET-1 (2.7 ± 0.4 vs. 2.0 ± 0.2 pg/mL, $p < 0.01$), CFPWV (7.2 ± 0.5 vs. 6.1 ± 0.2 m/s, $p = 0.016$), and CβS (8.4 ± 1.9 vs. 6.3 ± 0.3, $p = 0.055$) were higher in the PE group. Consistent with previous studies, third trimester plasma ET-1 was elevated in the PE group (2.9 ± 1.1 vs. 1.6 ± 0.1 pg/mL, $p < 0.01$) which paralleled a 2.5 fold increase in placental decidual ET-1 mRNA ($p < 0.0001$). ROC analyses showed that first trimester plasma ET-1 (AUC=0.71, $p < 0.001$) and CFPWV (AUC=0.70, $p=0.014$) were predictive of PE. This study supports the novel concept that elevated ET-1 in preeclampsia begins early in the first trimester and is associated with premature arterial stiffness. Further, these novel data suggest that ET-1 may play an important role in the first trimester prediction and pathogenesis of preeclampsia.

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P271

Interleukin-4 Supplementation Improves Chronic Inflammation and Hypertension in Response to Placental Ischemia During Pregnancy

PrimaryAuthor.AuthorBlock:**Lorena M Amaral**, Jesse Cottrell, Ashlyn Harmon, Denise C Cornelius, Mark W Cunningham Jr, Medical Ctr-UMMC, Jackson, MS; Gerd Wallukat, Florian Herse, Elios Clinic, Charite, Campus-Buch & Max-Delbrueck Ctr, Berlin, Germany; Tarek Ibrahim, Ralf Dechend, Babbette LaMarca, Medical Ctr-UMMC, Jackson, MS

Preeclampsia (PE), new onset hypertension in response to placental ischemia during pregnancy, is associated with chronic inflammation characterized by elevated tumor necrosis factor (TNF-α), interleukin-6 (IL-6), agonistic autoantibody to the Ang II type 1 receptor (AT1-AA). In addition, evidence for a shift towards proinflammatory CD4+TH1 vs CD4+TH2 profile exist in PE. Therefore we tested the hypothesis that IL-4, a cytokine essential for TH2 differentiation and proliferation, would improve pregnancy outcomes in response to placental ischemia (RUPP) by shifting the immune profile toward CD4+TH2. Interleukin-4, 600 ng/day, was administered via a mini-osmotic pump on day 14 of gestation into normal pregnant (NP) rats and into RUPP rats immediately following the reduced uterine perfusion pressure (RUPP) procedure and carotid catheters were inserted on gestation day 18**.** Blood pressure (MAP), TNF-α, IL-6, AT1-AA and circulating TH2 cells were measured on GD 19. MAP in NP rats (n=10) was 96 $\pm$ 2, 101 $\pm$ 2 in NP+IL-4 rats (n=14), 130 $\pm$ 4 in RUPP rats (n=6), which improved to 108 $\pm$ 2 mmHg in RUPP+IL-4 rats (n=15), $p< 0.05$. Plasma levels of TNF-α and IL-6 were 25 $\pm$ 6, 29$\pm$ 3 in NP rats (n=7-8/group), 116 $\pm$30, 224$\pm$ 62 in RUPP rats (n=9-10/group), which decreased to 23$\pm$ 5 and 135$\pm$48 pg/mL in RUPP+IL-4 rats (n=5-7/group), $p<0.05$. Importantly, plasma levels of AT1-AA were 0.3$\pm$0.4 in NP rats (n=8), 0.3$\pm$0.2 in NP+IL-4 rats (n=5), 18$\pm$0.3 in RUPP rats (n=9), which were blunted to 4$\pm$1 bpm in RUPP+IL-4 rats (n=14), $p<0.05$. Circulating TH2 cells were 17.3 ± 2.7 in NP rats (n=3), 9.0 ± 2.0 in RUPP rats (n=5), which improved to 13.4 ± 3.2 % gated in RUPP+IL-4 rats (n=9). This study illustrates that administration of IL-4 decreases TNF-α, IL-6, AT1-AA, possibly by increasing TH2/IL-4 in RUPP rats, which ultimately led to a reduction in hypertension in response to placental ischemia of pregnancy.

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P272

Preeclamptic Placental CD4+ T Cells Cause Preeclamptic Like Features in Normal Pregnant Nude Athymic Rats

PrimaryAuthor.AuthorBlock:**Tarek Ibrahim**, Ashlyn C. Harmon, Denise C. Cornelius, Lorena Amaral, Mark Cunningham, Kedra Wallace, Basbette Lamarca, Univ of MS Medical Ctr, Jackson, MS

Preeclampsia (PE), new onset hypertension during pregnancy, is associated with a proinflammatory profile compared to normal pregnancy (NP). We hypothesize that $CD4^+$ T cells play an important role to cause much of the pathophysiology associated with PE. To determine if $CD4^+$ T cells isolated from PE patients cause PE symptoms during pregnancy compared to those collected from NP women, $CD4^+$ T cells were isolated from placentas of both PE and NP women using magnetic separation with anti-CD4 antibodies, cultured in TexMACS medium with IL-2 at 37°C in 5% CO_2, and injected intraperitoneally into pregnant, nude-athymic rats on day 12 of gestation. On day 18, carotid catheters were implanted and on day 19 MAP was measured and blood and tissues were collected. MAP was 125±3 mmHg in rats with NP T cells but increased to 140±9 mmHg in rats with PE T cells. Significant differences in circulating cytokines TNF-α, IL-6, IL-17 and sFlt-1 were found with PE vs NP $CD4^+$ T cells (TNF-α- PE=142.4 pg/mL, NP=79.4 pg/mL; IL-6 - PE=311.6 pg/mL, NP=277.8 pg/mL; IL-17-PE= 7.054 pg/mL, NP=3.185 pg/mL; sFlt-1-PE=90.7 pg/mL, NP=58.2 pg/mL. However, there was no difference in Isoprostane levels or IL-2 between the two groups (Isoprostane-PE=3628 pg/mL, NP=3061 pg/mL; IL-2-PE=125 pg/mL, NP=125 pg/mL). ROS in tissues was measured using chemiluminescence and no difference was found in placenta while renal ROS showed an increased with PE $CD4^+$ T cells vs NP $CD4^+$ T cells (ROS-PE=5528 RLU/min/mg protein, NP=3372 RLU/min/mg protein). In addition, no difference in ET-1 mRNA was found in the placenta of nude rats receiving PE CD4+T cells, however, renal cortical ET-1 mRNA expression was increased 4.5 fold in rats with PE CD4+ T cells compared to those receiving to NP CD4+ T cells. These data indicate an important role for placental PE $CD4^+$ T cells to cause inflammation and stimulate renal vasoactive pathways that may contribute to hypertension during pregnancy. This research is funded by R01HD067541-06

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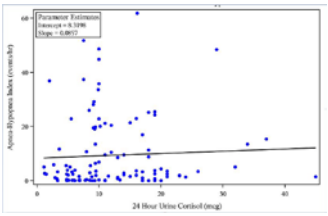
P273

Association Between 24-hr Urinary Cortisol and Severity of Obstructive Sleep Apnea in Patients With Resistant Hypertension

PrimaryAuthor.AuthorBlock:**Badhma Valaiyapathi**, Mohammed Siddiqui, Eric Judd, Maria El Hachem, Suzanne Oparil, David A Calhoun, Tanja Dudenbostel, Univ of Alabama at Birmingham, Birmingham, AL

Background: Plasma cortisol levels have been found to be negatively associated with severity of obstructive sleep apnea (OSA) based on morning salivary cortisol levels. Studies on pediatric patients have suggested that salivary cortisol can be used as a biomarker for assessment of OSA severity and to analyze prognosis following treatment with continuous positive airway pressure (CPAP). We hypothesized that 24-hr urinary cortisol levels are associated with severity of OSA in patients

with resistant hypertension (HTN).
Methods: In this prospective study, 197 patients were recruited from University of Alabama at Birmingham resistant HTN clinic. Office blood pressure (BP), diagnostic polysomnogram (PSG), 24-hr urine analysis for cortisol were done in these patients as part of study protocol. Patients with a diagnosis of OSA who were already treated with CPAP were excluded.
Results: Out of 116 patients who were analyzed based on inclusion criteria, 53.5% were female, 62.1% were African American and the mean baseline values were age 57.4 ± 10.6 in years, body mass index 34.9 ± 7.4 kg/m², 24-hr urinary cortisol 12.1 ± 7.7 mcg, apnea-hypopnea index (AHI) 9.4 ± 13.0 events/hr. Regression analysis was done between 24-hr urinary cortisol and AHI, and the Pearson correlation coefficient was 0.0498 (p = 0.602). There was no significant correlation between 24-hr urinary cortisol level and increasing AHI.
Conclusion: In this cross-sectional study, 24-hr urinary cortisol level was not significantly associated with increasing AHI, indicating that 24-hr urinary cortisol may not play a role in the severity of OSA in patients with resistant HTN.



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P274

Double Hit! A Unique Case of Resistant Hypertension

PrimaryAuthor.AuthorBlock:**Kristen DeCarlo**, Nidhi Agrawal, NYU Sch of Med, New York, NY

Case Description: A 52 year-old woman with obesity, HLD, diet-controlled diabetes was referred for resistant hypertension. Her blood pressure (BP) was uncontrolled (systolic 145-200 mmHg, diastolic 95-135 mmHg) on 5 medications. Physical exam revealed a systolic ejection murmur at the base. An ECHO showed normal systolic function and moderate LVH. Plasma aldosterone was 20.7 ng/dL (normal 3-15 g/dL), plasma renin was 0.50 ng/mL/h (normal 0.65-5.0 ng/mL/h), and aldosterone:renin ratio was 41.4 [ng/dL]/[ng/mL/h], meeting criteria for Primary Aldosteronism (PA). Saline infusion test was confirmatory for PA. CT scan of the adrenals did not reveal an adenoma. Subsequent Adrenal Venous Sampling confirmed bilateral Idiopathic Adrenal Hyperplasia (IAH; right and left adrenal vein selectivity index of 42.6, 9.2 respectively; lateralization index was 1.3). She was normokalemic (3.7-4.4 mEq/L), total calcium (Ca) was 11.2 mg/dL (normal Ca 8.0-10.4), and creatinine was 1.4 mg/dL (GFR 47 mL/min/1.73m^2). Intact parathyroid hormone (iPTH) was 131.2 pg/mL, phosphorus was 2.4 mg/dL, and 25-hydroxyvitamin D was 30.8 ng/mL, suggesting primary hyperparathyroidism (PPTH). Neck ultrasound was concerning for PTH adenoma and sestamibi scan localized a left adenoma. DXA scan and renal ultrasound were normal. IAH was treated medically with spironolactone. Her BP remained elevated (systolic 138-172 mmHg, diastolic 92-100 mmHg). She underwent parathyroidectomy and histology confirmed an adenoma. Post-operative iPTH and Ca normalized (49.5 pg/mL, 9.7 mg/dL respectively). Postoperative BP

measurements improved (systolic 123-138 mmHg, diastolic 75-86 mmHg). **Discussion:** Evidence shows that a hyperfunctioning parathyroid gland may contribute to maintaining hyperaldosteronism in PA. This is based on in-vitro studies showing PTH increased aldosterone secretion from adrenocortical cells in a concentration dependent manner. Gene expression and immunohistochemistry studies show PTH receptors in aldosterone-producing adenomas and MR receptors in parathyroid cells, suggesting a bi-directional relationship. The significance of this case is in the potential for further understanding of the pathophysiology of common causes of secondary hypertension.

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P275

Peripheral 18-oxocortisol Concentration Reflects Intra-tumoral Activities of Both CYP11B1 and CYP11B2 in Aldosterone-producing Adenoma

PrimaryAuthor.AuthorBlock:**Yuta Tezuka**, Yuto Yamazaki, Tohoku Univ Hosp, Sendai, Japan; Masaaki Kitada, Tohoku Univ Graduate Sch of Med, Sendai, Japan; Masahiro Nezu, Ryo Morimoto, Masataka Kudo, Yasuhiro Igarashi, Yasuhiro Nakamura, Sadayoshi Ito, Tohoku Univ Hosp, Sendai, Japan; Mari Dezawa, Tohoku Univ Graduate Sch of Med, Sendai, Japan; Hironobu Sasano, Fumitoshi Satoh, Tohoku Univ Hosp, Sendai, Japan

Background: The 18-oxocortisol (18oxoF) is an expected biomarker for detection of patients with aldosterone-producing adenoma (APA) among patients with primary aldosteronism. However, the mechanism of 18oxoF synthesis in APA and differences of the production amount among APAs remain unclear. **Objective:** To examine the associations between peripheral 18oxoF concentration (p18oxoF) and clinical or pathological parameters in APA cases. **Method:** We included 48 APA cases who were diagnosed in our center. The p18oxoF and tissue steroid levels were measured by highly sensitive LC-MS/MS measurement. We performed immunohistochemical (IHC) staining of 11B-hydroxylase (CYP11B1), aldosterone synthase (CYP11B2) and 17α-hydroxylase (c17) for all the cases. The IHC images were analyzed by HALO digital image software. Moreover, we performed immunofluorescence staining to evaluate the number of CYP11B1/B2 double positive cells (B1/B2 cells). **Result:** The characteristics of 48 cases were as follows; Age 51.7 ±11.8 years; Male 54.2% (26/48); Plasma aldosterone (A) 52.2 ±45.8 ng/dl; Plasma renin activity 0.35 ±0.43 ng/ml/h; Serum cortisol (F) 10.0 ±3.5 μg/dl; Cross-sectional area (CSA) of APA 63.9 ±52.7 mm^2. The p18oxoF showed a lognormal distribution from 0.19 to 183.13 ng/dl (Median; 11.92 ng/dl). The p18oxoF, a derivative of F, was positively correlated with plasma A ($r=0.728$, $P<0.0001$) but not serum F (P=N.S.). However, there were significant correlations between p18oxoF and intra-tumoral A or F levels in 15 frozen APA tissues which were available (A: $r=0.750$, $P<0.01$, F: $r=0.575$, $P<0.05$). On the other hand, p18oxoF was positively correlated with CSA ($r=0.734$, $P<0.0001$). Divided them into low and high groups based on p18oxoF, high group had larger CSA (36.1 ±37.9 vs 91.8 ±51.2 mm^2, $P<0.0001$) and immunoreactive positive areas of CYP11B1 (7.9 ±12.5 vs 17.9 ±18.4 mm^2, $P=0.003$), CYP11B2 (9.0 ±8.8 vs 20.7 ±9.9 mm^2, $P<0.0001$) and c17 (6.1 ±10.1 vs 12.6 ±7.6 mm^2, $P=0.0004$) than low group. High group also had more B1/B2 cells than low group (106.5 ±93.1 vs 49.3 ±59.3 /mm^2, $P=0.009$). **Conclusion:** These

results suggest 18oxoF synthesis could be influenced by the size of APA, which reflects intra-tumoral activities of not only CYP11B2 but also CYP11B1, a rate-limiting enzyme of F.

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P276

(Pro)Renin Receptor-dependent Renal Generation of Aldosterone Contributes to ENaC Activation During Angiotensin II-induced Hypertension

PrimaryAuthor.AuthorBlock:**Fei Wang**, Kexin Peng, Xiaohan Lu, Univ of Utah and Veterans Affairs Medical Ctr, Salt Lake Cty, UT; Kevin Yang, Adam Nau, Univ of Utah, Salt Lake Cty, UT; Tianxin Yang, Univ of Utah and Veterans Affairs Medical Ctr, Salt Lake Cty, UT

(Pro)renin receptor (PRR) has emerged as a novel regulator of ENaC under various physiological and pathophysiological conditions including Ang II-induced hypertension. However, the mechanism for ENaC-activating action of PRR remains incompletely understood. The present study attempted to test renal-derived aldosterone (Aldo) as a potential mediator of PRR signaling in the setting of Ang II treatment. In normal Sprague-Dawley rats with intact adrenal glands, a 7-d minipump infusion of Ang II at 100 ng/kg/min induced a 3.2-fold increase in urinary Aldo excretion and a 1.8-fold increase in plasma Aldo, as assessed by ELISA. Following intramedullary delivery of a PRR decoy peptide PRO20 (120 μg/kg/d) (IM PRO20), Ang II-induced increases in urinary Aldo was reduced by 75% without affecting plasma Aldo, in parallel with attenuated hypertension (radiotelemetry-measured MAP on day 7: 132.6 ± 5.8 mmHg in Ang II+IMPRO20 vs. 157.1 ± 6.2 mmHg in Ang II). Following adrenalectomy (ADX), urinary Aldo was decreased from 9.3 ± 1.2 ng/24h to an unbeatable level under basal condition; it was elevated to 0.96 ± 0.05 ng/24h by Ang II and became undetectable again by IM PRO20, as assessed by mass spectrometry. Similar results were obtained by ELISA. In parallel, urinary Na+/K+ ratio in ADX rats was decreased by Ang II infusion and blunted by IM PRO20. Furthermore, Ang II infusion induced a 1.3-fold increase in urinary Aldo excretion and this increase was reduced by 60% in CD PRR KO mice, correlating to the changes in MAP. In cultured mpkCCD cells exposed to 10 nM prorenin, amiloride-sensitive transepithelial Na+ transport, measured by using epithelial volt-ohmmeter, was increased 2.1-fold at 6 h that sustained at 24 h, which was suppressed by 86%
following treatment with the mineralocorticoid receptor antagonist eplerenone. Medium Aldo was increased by 2.6-fold following exposure to 10 nM prorenin for 24 h. Overall, these results suggest that activation of PRR results in local generation of Aldo that likely contributes to increased ENaC activity and thus hypertension during Ang II treatment.

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P277

Mineralocorticoid Receptor Signaling Regulates TRPV4-mediated Vasodilation of Parenchymal Arterioles

PrimaryAuthor.AuthorBlock:**Janice M Diaz-Otero**, Ting-Chieh Yen, William F Jackson, Anne M Dorrance, Michigan State Univ, East Lansing, MI

Hypertension impairs endothelium-dependent dilation of the parenchymal arterioles (PA) that regulate white matter perfusion. In the periphery, mineralocorticoid receptor (MR) activation facilitates hypertension-associated artery dysfunction; the role of MR signaling in PA dysfunction has not been defined. We hypothesized that Angiotensin II (AngII)-hypertension impairs TRPV4-mediated dilation in an MR-dependent manner. 16 week old male C57bl/6 mice were treated with AngII (800ng/kg/min) ± the MR antagonist, eplerenone (EPL; 100mg/kg/day) for 4 weeks; Sham mice served as control. PAs were isolated and studied by pressure myography. Data are presented as mean ± SEM; n=4-6 per group. AngII increased systolic blood pressure, an effect not blunted by EPL (Sham:147 ± 3; AngII: 177* ± 3; AngII+EPL: 182* ± 6mmHg; *=p<0.05 vs. Sham). However, EPL prevented the increased myogenic tone (31±1 vs 48*±4 vs 35±3 % tone; *=p<0.05 vs. Sham) and the impaired carbachol (CbCh)-induced dilation (51±3 vs 24*±2 vs 49±3 %dilation; Sham vs. AngII vs. AngII+EPL; *=p<0.05 vs. Sham) caused by AngII infusion. CbCh-induced dilation was unaltered by L-NAME (10^{-5}M) and indomethacin (10^{-4}M) in all groups (Sham: 51±3 vs. 53±2; AngII:24±2 vs. 28±2; AngII+EPL 49±3 vs. 47±3% dilation; CbCh vs. CbCh+L-NAME+Indomethacin) implicating endothelium-derived hyperpolarization (EDH). In contrast, the TRPV4 antagonist, GSK2193874 (GSK2, 10^{-7}M) blunted the CbCh-induced dilation in all groups (Sham: 51±3 vs 13*±8; AngII: 24±2 vs 7*±2; AngII+EPL: 49±3 vs 16*±4 % dilation; CbCh vs CbCh+GSK2 ;*=p<0.05). In the AngII and AngII+EPL mice, TRPV4 inhibition reduced myogenic tone (AngII: 16±3; AngII+EPL: 17±6 %tone loss; p<0.05), an effect that was not observed in the Sham mice (2±3 %tone loss). AngII infusion decreased cerebral perfusion; EPL prevented this decrease (Sham: 938±32; AngII:596*±53; AngII+EPL:793±36 perfusion units; *=p<0.05 vs. Sham). MR activation regulates TRPV4 signaling in PAs during AngII-infusion, independent of hypertension. Impaired TRPV4 function plays a critical role in AngII-hypertension-associated changes in PA reactivity and may increase the risk of small vessel disease.

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P278

Identification of Differentially Expressed Aortic Genes in Brown Norway Introgressed Chromosome 2 Segments into Hypertensive Dahl Salt Sensitive Rats

PrimaryAuthor.AuthorBlock:**Olga Berillo**, Sofiane Ouerd, Ku-Geng Huo, Asia Rehman, Lady Davis Inst, Montreal, QC, Canada; Chantal Richer, Daniel Sinnett, Sainte-Justine Univ Hosp, Montreal, QC, Canada; Anne E Kwitek, Dept of Internal Med, Univ of Iowa, Iowa, IA; Pierre Paradis, Ernesto L Schiffrin, Lady Davis Inst, Montreal, QC, Canada

Background: Rat chromosome (RNO) 2 introgression from normotensive Brown

Norway (BN) rats into hypertensive Dahl salt sensitive (SS) background (consomic SB2) reduced vascular inflammation. We hypothesized that the BN-RNO2 contains genes that reduce vascular inflammation, which could be identified using microRNA (miRNA) and total RNA expression profiling in aorta of congenic rats containing different portions of BN-RNO2 on the SS background. **Methods and results:** Twelve-to-13-week-old male SS rats and congenic rats containing the distal portion of BN-RNO2 (SB2a), the middle segment (SB2b) and the proximal segment (SB2e) on the SS background, fed a normal-salt diet, were studied. Systolic blood pressure (SBP) was measured by telemetry. SBP was lower in SB2a and SB2b but not SB2e compared to SS (125±3, 127±6, 138±4 vs 146±2 mm Hg, $P<0.05$). Total RNA was extracted from aorta and used to construct libraries for small and total RNA sequencing using Illumina HiSeq-2500. The bioinformatics pipeline included: FastQC for quality control, STAR for genome alignment to *Rattus norvegicus* release-86, mirdeep2 for miRNA annotation and counting, Htseq-count for mRNA and long non-coding RNA annotation and counting; R for differential expression analysis. Differentially expressed miRNAs and genes (mRNA and non-coding RNA) were identified in SB2a vs SS (miRNAs: 3 up and 2 down, genes: 1 up and 3 down), SB2b vs SS (miRNAs: 2 up and 3 down, genes: 67 up and 112 down) and SB2e vs SS (miRNAs: 29 up and 25 down, genes: 12 up and 35 down), with FDR<0.05. Differentially expressed genes encoded within different BN-RNO2 congenic portions were identified in SB2a vs SS (2 down), SB2b vs SS (14 up and 18 down) and SB2e vs SS (1 down). **Conclusions and perspectives:** Differentially expressed BN-RNO2 encoded genes were identified in aorta of congenic SB2a, SB2b and SB2e rats. Whether these genes play a role in inflammation or vascular injury remains to be determined.

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P279

Mapping of Chromosome 2 Differentially Expressed Aortic Genes Linked to Vascular Inflammation Using Congenic Rats Fed a High-salt Diet

PrimaryAuthor.AuthorBlock:**Olga Berillo**, Sofiane Ouerd, Ku-Geng Huo, Lady Davis Inst for Medical Res, Montreal, QC, Canada; Chantal Richer, Res Ctr, CHU Ste-Justine, Montreal, QC, Canada; Daniel Sinnett, Res Ctr, CHU Ste-Justine, Univ de Montréal, Montreal, QC, Canada; Anne E. Kwitek, Univ of Iowa, Iowa, IA; Pierre Paradis, Lady Davis Inst for Medical Res, Montreal, QC, Canada; Ernesto L. Schiffrin, Lady Davis Inst for Medical Res, Sir Mortimer B. Davis-Jewish General Hosp, McGill Univ, Montreal, QC, Canada

Background: Three congenic rat strains (SB2a, SB2b and SB2e) were created by chromosome (Chr) 2 fragment introgression from normotensive Brown Norway (BN) rats into hypertensive Dahl salt sensitive (SS) background. SB2a and SB2b rats fed a normal-salt diet presented reduced blood pressure (BP) and inflammation when compared to SS rats. We hypothesized that BN-Chr2 contains antihypertensive and anti-inflammatory genes that could prevent high-salt diet (HSD)-induced BP elevation and vascular injury in SB2a and SB2b rats. These genes will be identified using microRNA (miRNA) and total RNA expression profiling analysis in aorta of congenic rats fed a HSD.Methods and results: Four-to-6 week-old male SS, SS, SB2a and SB2b rats were fed a HSD

(4% NaCl) for 8 weeks or until they developed a stroke as manifested by seizures. Systolic blood pressure (SBP) was measured by telemetry. Systolic BP was higher in SB2b but not SB2a when compared to SS (185±8, 167±7 vs 168±5 mm Hg). Total RNA was extracted from aorta and used to construct libraries for small and total RNA sequencing using Illumina HiSeq-2500. The bioinformatics pipeline included: FastQC for quality control, STAR for genome (Rattus norvegicus, release-86) alignment, mirdeep2 for miRNA annotation and counting, Htseq-count for mRNA and long non-coding RNA annotation and counting; R for differential expression analysis. Differentially expressed miRNAs and genes (mRNAs and non-coding RNAs) were identified in SB2a vs SS (miRNAs: 11 up and 10 down; genes: 92 up and 91 down) and in SB2b vs SS (miRNAs: 3 up and 2 down; genes: 10 up and 13 down) with FDR<0.05. Differentially expressed genes encoded within different BN-Chr2 congenic portions were identified in SB2a vs SS (genes: 7 up and 2 down) and SB2b vs SS (genes: 6 up and 4 down).Conclusions and perspectives: Differentially expressed BN-Chr2 encoded genes were identified in aorta of congenic SB2a and SB2b rats fed HSD. Whether these genes play a role in HSD-induced BP elevation and vascular inflammation remains to be determined.

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P280

Butyric Acid Protects Against Aldosterone-salt-induced Hypertension and Renal Injury via Suppression of (Pro) Renin Receptor

PrimaryAuthor.AuthorBlock:**Kexin Peng**, Sun Yat-sen Univ, Guangzhou, China; Long Zhao, Chuanming Xu, Univ of Utah, Salt Lake City, UT; Fei Wang, Tianxin Yang, Univ of Utah, salt lake city, UT

Butyric acid (BA), a short-chain fatty acid derived from gut microbiota, exhibits beneficial effects on cardiovascular disease. We examined the potential antihypertensive and renal protective action of BA in a mouse model of aldosterone (Aldo)-salt-induced hypertension. C57/BL6 mice were subjected to Aldo-salt protocol consisting of minipump infusion of Aldo at 0.2 mg/kg/d plus 1% NaCl as drinking fluid, or in combination with minipump infusion of BA at 60 mg/kg/d. A 3-wk BA treatment lowered radio telemetry-measured MAP (146±3.8 vs. 133±2.9 mmHg, N=9, p<0.05), cardiac hypertrophy (6.7±0.32 vs. 5.4±0.28 mg/g body weight, N=9, p<0.05), 24-h urinary albumin (434±41vs. 218±22 μg/24h, N=9, p<0.01), the kidney hypertrophy (9.9±0.58 vs. 7.9±0.38 mg/g body weight, N=9, p<0.05), polyuria (19.5 ± 2.5 vs. 8.7±1.4 ml/24h, N=6, p<0.01), renal 8-isoprostane (0.74±0.14 vs. 0.35±0.05 pg/mg, N=6, p<0.05), and urinary TBARS (276±43 vs. 133±032 nmol/24h, N=9, p<0.05). The hematocrit was decreased by Aldo-salt, which was reversed by BA (Control: 50.0±0.4% vs. Aldo-salt: 46.2±0.8% vs. Aldo-salt + BA: 49.6±1.0%, all N=9, all p<0.05). Recent study has identified (pro) renin receptor as a key regulator of blood pressure. To examine the underlying mechanism of BA on Aldo-salt induced effect, we detected full length (pro) renin receptor (fPRR) and soluble PRR (sPRR) expression in vivo and in vitro in this study. The renal expression of fPRR and sPRR were increased 1.75-fold and 2.67-fold by Aldo-salt treatment, respectively, and these increases were both blocked by BA (1.75±0.08 vs. 0.93±0.14, N=6, p<0.01; 2.67±0.23 vs. 0.92±0.29, N=6, p<0.01, respectively). The Aldo-salt induced urinary sPRR excretion was also

blunted by BA (Control: 1533±73 vs. Aldo-salt: 4800±365 vs. Aldo-salt + BA: 2615±138 pg/24h, all N=6, all p<0.05). In cultured mpkCCD cells, Aldo treatment induced protein expression of fPRR and sPRR, which were blocked by BA (fPRR: 1.25±0.09 vs. 0.73±0.08, N=5, p<0.01; sPRR: 2.33±0.35 vs. 1.03±0.15, N=5, p<0.01). Our results suggest that BA protected against Aldo-salt-induced hypertension and renal injury likely through suppression of PRR.

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P281

Urinary Metabolites Associated With Blood Pressure on a Low- or High-sodium Diet

PrimaryAuthor.AuthorBlock:**Hong Xue**, Medical Coll of Wisconsin, Milwaukee, WI; Yuan Cheng, Haiying Song, Shenzhen Second People's Hosp, Shenzhen, China; Xiaoqing Pan, Yifei Wan, Tao Wang, Medical Coll of Wisconsin, Milwaukee, WI; Zhongmin Tian, Entai Hou, Xi'an Jiaotong Univ, Xi'an, China; Ian R Lanza, Mayo Clinic, Rochester, MN; Pengyuan Liu, Yong Liu, Purushottam W Laud, Kristie Usa, Medical Coll of Wisconsin, Milwaukee, WI; Yongcheng He, Shenzhen Second People's Hosp, Shenzhen, China; Mingyu Liang, Medical Coll of Wisconsin, Milwaukee, WI

Dietary salt intake has significant effects on arterial blood pressure and the development of hypertension. Mechanisms underlying salt-dependent changes in blood pressure remain poorly understood. The goal of the present study was to utilize urine samples from the Dietary Approaches to Stop Hypertension (DASH)-Sodium trial and a targeted metabolomic approach to identify associations of urinary metabolites with blood pressure phenotypes on low- or high-sodium intake. We examined urinary levels of metabolites in 103 participants of the DASH-Sodium trial after 30 days on a defined diet containing high sodium (targeting 150 mmol sodium intake per day) or low sodium (50 mmol sodium per day). Systolic or diastolic pressure was 10 or more mmHg higher on the high-sodium intake in 51 of the 103 participants. Targeted chromatography/mass spectrometry analysis was performed in 24-h urine samples for 10 metabolites related to the tricarboxylic acid cycle and 47 amino metabolites. Urinary metabolite levels improved the prediction of classification of blood pressure salt-sensitivity based on race, age and sex. Random forest and generalized linear mixed model analyses identified significant (false discovery rate <0.05) associations between 24 h excretions of β-aminoisobutyric acid, cystine, citrulline, homocysteine and lysine and systolic blood pressure and between cystine and diastolic blood pressure. The differences of 24 h excretion of homocysteine between low- and high-sodium intakes were significantly associated with the differences in diastolic blood pressure. These associations were significant with or without considering demographic factors. Treatment with β-aminoisobutyric acid significantly attenuated high-salt-induced hypertension in Dahl salt-sensitive rats. These findings support the presence of new mechanisms of blood pressure regulation involving metabolic intermediaries.

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P282

Low Dose LNAME Causes Salt Sensitive Hypertension via Activation of NCC

PrimaryAuthor.AuthorBlock:**Conghui Wang**, Reheman Latapati, Sayoko Ogura, Suangsuang Koid, Fumiko Mori, The Univ of Tokyo, Dept of Internal Med, Tokyo, Japan; Tastuo Shimosawa, Professor and Chair Intl Univ of Health and Welfare, Sch of Med Mita Hosp, IUHW, Tokyo, Japan

Background:
Blood pressure is regulated both by vascular resistance and blood volume. Our group recently clarified norepinephrine activates sodium channel (sodium chloride cotransporter: NCC) via epigenetic modulation (Nat Med 2011). The accumulating data strongly suggest that vasoactive substances are not only act on vasculature but also effect on sodium homeostasis. We hypothesized that blockade of nitric oxide synthesis by L-NAME has a direct effect on sodium channels in the kidney.
Method:
C57BL/6J Mice were treated with 8%NaCl (high salt; HS) or 8%NaCl +L-NAME (0.7mg/10ml). Blood pressure was measured by telemetry. After 4 weeks' treatment, the function of sodium channel was monitored by pharmacologically and molecular biologically. mDCT cell was used to observe the direct effect of L-NAME on NCC.
Result:
Low dose L-NAME or high salt loading alone did not change blood pressure in the C57BL/6J mouse, but L-NAME shifted the pressure natriuresis curve toward the right and induced salt-sensitive hypertension in HS group. No significant changes were observed in morphological changes in the kidney. However, significant changes of sodium and chloride excretion were observed after hydrochlorothiazide challenge test but not in amiloride test, which was consistent with increases in membrane NCC. This highly suggested NCC, not ENaC was activated by L-NAME to cause salt-sensitive hypertension. To determine whether L-NAME active NCC directly or not, we treated mDCT cells with L-NAME. The membrane NCC expression was increased in a time and dose depended manner.
Conclusion:
Low dose L-NAME inappropriately and directly activate NCC in both vivo and vitro, and finally induces salt-sensitive hypertension.

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P283

Effects of High Salt Diet on Vascular Function and Renal Injury in a Novel Mouse Model of Neurogenic Hypertension

PrimaryAuthor.AuthorBlock:**Pablo Nakagawa**, Larry N Agbor, Anand Nair, Javier Gomez, Chunyan Hu, Jing Wu, Curt D Sigmund, Univ of Iowa, Iowa City, IA

In the brain, transcription of the renin gene is initiated from an alternative promoter within exon 1b resulting in the expression of Renin-b, a renin isoform lacking the signal peptide and first third of the prosegment. The specific deletion of Renin-b paradoxically induces neurogenic hypertension concomitant with increased brain renin-angiotensin system and renal sympathetic nerve activation. High dietary sodium intake has been associated with hypertension, endothelial dysfunction, and renal complications especially

in salt-sensitive populations. We hypothesized that deletion of Renin-b causes vascular dysfunction and induces renal damage. In addition, high dietary sodium intake further exacerbates end-organ damage in Renin-b deficient mice (Ren-b KO). To evaluate vascular function, acetycholine (Ach) and nitroprusside (SNP)-induced dose-dependent vasorelaxation was assessed in aorta, carotid and basilar arteries isolated from Ren-b KO or littermate controls fed normal or high sodium diet (4% NaCl) for 4 weeks. Urinary protein excretion was measured at baseline and after 4 weeks on high sodium diet in control and Ren-b KO mice. At baseline, Ren-b KO exhibited impaired Ach-induced vasorelaxation in the aorta (logEC50 control: 0.88mM vs Ren-b KO: 1.1mM; p = 0.014; n=4) while SNP dose-response curve was unaltered. Urinary protein levels were increased in Ren-b KO (control: 11.0±3.7 vs Ren-b KO: 45.8±9.3 mg/day; p = 0.007; n=6-8). In control mice, 4 weeks high sodium diet increased urinary protein levels compared to baseline (baseline: 11.0±3.7 vs high salt: 31.6±7.4 mg/day); however high salt diet did not worsen proteinuria in Ren-b KO (baseline: 45.8±9.3 vs high salt: 44.1±4.5 mg/day). Similarly, Ren-b KO fed high sodium diet did not exhibit higher degrees of vascular dysfunction in comparison with control mice on high sodium diet. Our data indicates that Renin-b deletion promotes end-organ damage, likely as a consequence of high blood pressure or sympathetic nerve activation or both. Surprisingly, high sodium diet failed to exacerbate vascular dysfunction or proteinuria in Ren-b KO. Studies are in progress to evaluate the blood pressure response to high sodium diet in Ren-b KO mice to assess if Ren-b KO mice are salt sensitive.

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P284

Neuron Specific (Pro)renin Receptor Knockout Reduces Sodium Appetite and Attenuates the Development of DOCA-salt Induced Hypertension

PrimaryAuthor.AuthorBlock:**Fatima Trebak**, Univ of Nevada Reno, Reno, NV; Wencheng Li, Wake Forest Univ, Winston-Salem, NC; Yumei Feng, Univ of Nevada Reno, Reno, NV

The (pro) renin receptor (PRR) is a key component of the renin-angiotensin system that is highly expressed in the brain. We previously showed that the neuronal PRR deletion attenuates deoxycorticosterone acetate (DOCA)-salt induced hypertension. However, the mechanism underlying remains unclear. To test our hypothesis that PRR is involved in the regulation of sodium appetite during DOCA-salt hypertension, we used a neuron-specific PRR knockout (PRRKO) mouse model generated using the Cre-LoxP system. The PRRKO and their wildtype controls (WT) were implanted with 50 mg DOCA pellet with

free access to regular water and 0.9 % saline as the drinking solution. Blood pressure (BP) was monitored by telemetry system in conscious free moving mice. The fluid intake and urine output were monitored along 21 days of DOCA-Salt treatment. The BP is significantly lower in PRRKO compared with WT mice following 21 days of DOCA-salt treatment (112 ± 2 vs. 134 ± 7 mmHg, P= 0.0186). Interestingly, we found that saline intake (27.8 ± 1.8 vs. 15.9 ± 1.2 ml/day, P=0.0007) and total fluid intake (31.1 ± 1.9 vs. 21.1 ± 1.4 ml/day, P=0.003) were higher; while the regular water intake was lower (3.4 ± 0.6 vs. 5.2 ± 0.3 ml/day, P=0.03) in WT compared to PRRKO mice. PRR deletion in the neurons reduced sodium appetite presented as the ratio of saline intake over total fluid intake (0.75 ± 0.016 vs. 0.89 ± 0.019, P=0.0005), as well as total sodium intake (2.45 ± 0.19 vs. 4.28 ± 0.28 mmol/day, P=0.0007) compared with WT mice at the end of the protocol. In addition, the urinary sodium excretion was lower (13.3±1.17 vs. 20±1.17 mmol/day, P<0.0001), but not potassium excretion (0.64 ± 0.028 vs. 0.56 ± 0.05 mmol/day, P=0.1291) in PRRKO compared with WT mice; however, there is no difference in urine sodium and potassium concentrations. Furthermore, plasma vasopressin level (19.0 ± 2.7 vs. 33.6 ± 2.7 pg/ml, P=0.0037) is lower in the PRRKO compared with WT mice at the end of DOCA-salt treatment. In summary, PRR deletion in the neurons reduced sodium appetite, circulating vasopressin level, and attenuated the development of DOCA-salt induced hypertension. Taken together, the present findings suggest that PRR regulates the BP and plays a key role in salt-sensitive hypertension, at least in part, by modulating sodium appetite.

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P285

Hydralazine Attenuates the Development of Hypertension in the Female Dahl Salt-sensitive Rat in a T Cell-independent Manner

PrimaryAuthor.AuthorBlock:**Amrita Vijay Pai**, Crystal A West, Aline Souza, Parnika S Kadam, Emma J. Pollner, David A. West Jr., Hong Ji, Xie Wu, Kathryn Sandberg, Georgetown Univ, Washington, DC

Introduction: Several studies in Dahl salt-sensitive (**DS**) rats suggest that T cells play a role in salt-sensitive hypertension. To further investigate the role of T cells, we compared T cell profiles in hypertensive DS and normotensive Dahl salt-resistant (**DR**) rats as well as in DS rats treated with hydralazine (**HYD**) to attenuate the development of hypertension.
Methods: Mean arterial pressure (**MAP**) was measured by telemetry in DS rats (n=13) from 1 to 4.5 months (**mo**) of age. At 1.5 mo, all of the DR (n=8) and half of the DS rats were treated with vehicle (VEH, n=7). The other half of the DS rats (n=6) received HYD (25 mg/kg/day) in the drinking water. At 4.5 mo, renal T helper (**Th**) and cytotoxic (**Tc**) cells were assessed by multicolor flow cytometry.
Results: In the DS kidney, the frequency of $CD4^+$ Th cells [(%): DS-VEH, 76±1.2* vs. DR-VEH, 55±0.7; *p<0.0001; n=7-8/group] was higher while the frequency of $CD8^+$ Tc cells [(%): DS-VEH, 14±1.2* vs. DR-VEH, 35±1; *p<0.0001; n=7-8/group] was lower compared to DR rats. 10 weeks of HYD treatment attenuated the age-associated increase in MAP observed in DS rats [p<0.0001, Two-Way ANOVA (time, treatment); MAP (mmHg): DS-VEH, 157±4 vs. DS-HYD, 133±3; *p<0.0004; n=6-7/group]. HYD had no effect on the frequency of $CD4^+$ [(%):77±1.5] or $CD8^+$ [(%):15.5±0.9] T cells in the kidney of DS rats [($CD4^+$): DS-VEH vs. DS-HYD, p=0.83; ($CD8^+$):

DS-VEH vs. DS-HYD, p=0.5; n=7-8/group]. In summary, the ratio of Th ($CD4^+$) to Tc ($CD8^+$) cells is higher in the kidney of DS compared to DR rats and HYD had no effect on the T cell profile in the DS rat kidney under conditions in which the MAP was attenuated by 20 mm Hg. **Conclusions:** These findings indicate the DS rat has more active Th cells in the kidney compared to the DR rat. Our study also suggests that vasodilators can attenuate the development of hypertension in the DS rat in a Th- and Tc-independent manner.

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P286

Circulating Lipoxin A4 Causes Resolution of Inflammation via Estrogen Receptor Activation, While Mitochondria N-Formyl Peptides Induce Inflammation via Formyl Peptide Receptor in Hypertension

PrimaryAuthor.AuthorBlock:**Camilla Ferreira Wenceslau**, Cameron G. McCarthy, Theodora Szasz, R. Clinton Webb, Augusta Univ, Augusta, GA

Recently, we observed that mitochondrial components have inflammatogenic proprieties and that their fragments are elevated in the circulation of spontaneously hypertensive rats (SHR). Also, infusion of mitochondrial N-formyl peptides (F-MITs) into rats induces inflammation and vascular dysfunction via formyl peptide receptor (FPR) activation. In contrast, Lipoxin A4 (LXA4), a mediator of resolution of inflammation, also binds FPR. However, LXA4-FPR-dependent effects seem to be opposite from F-MITs. We first hypothesized that LXA4 is decreased in SHR. We collected plasma from male SHR and Wistar Kyoto rats (WKY) (12 weeks old) and LXA4 was extracted via hydrophobic, reverse-phase, silica-based column. Our initial hypothesis was refuted, since LXA4 was increased in SHR (WKY: 0.33±0.05 vs. SHR: 0.79±0.08 ng/ml, $p<0.05$). As a result, we inhibited LXA4 synthesis in SHR with a 5-lipoxygenase (5-LOX) inhibitor Zileuton (10mg/kg/day i.p.) for 10 days. 5-LOX blockade did not change blood pressure (BP), but did cause a deterioration of vascular function in intrarenal and mesenteric resistance arteries in SHR. On the other hand, the blockade of FPR in SHR for 10 days with Cyclosporin H (CsH) and WRW4 (0.1 mg/kg/day) reduced BP (telemetry: SHR+control: 151±7.2 vs. SHR+CsH+WRW4: 138±0.4 mmHg, $p<0.05$) and left ventricle mass. FPR blockade also ameliorated acetylcholine-induced relaxation of intrarenal (Emax: SHR+control: 10±5 vs. SHR+CsH+WRW4: 28±6 %, $p<0.05$) and mesenteric resistance arteries (Emax: SHR+vehicle: 74±10 vs. SHR+CsH+WRW4: 99±0.6 %, $p<0.05$). From these data, we suggest that LXA4 does not act on FPR to promote resolution of inflammation. We believe that another factor binds FPR (e.g., F-MITs) and leads to vascular damage in SHR. In support of this, we observed that LXA4 phosphorylates estrogen receptor (ER) $alpha^{Ser118}$ in aortic vascular smooth muscle cells (VSMC) and ER antagonist ICI-182 (100nM), but not FPR antagonist, abolishes these responses. On the other hand, FMITs increased phosphoERK 1/2 in VSMC and FPR antagonists blocked this increase. In conclusion, FMITs and LXA4 have opposite effects in SHR. LXA4 induces resolution of inflammation, possibly via ER activation, and F-MITs lead to damage via FPR activation.

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P287

Hypertension and Danger-associated Molecular Patterns

PrimaryAuthor.AuthorBlock:**Tyler D Bammert**, Monika R Fleshner, Caitlin A Dow, Grace M Lincenberg, Jared J Greiner, Univ of Colorado, Boulder, CO; Brian L Stauffer, Univ of Colorado Anschutz Medical Ctr, Denver, CO; Christopher A DeSouza, Univ of Colorado, Boulder, CO

Recent advances in immunobiology have established the importance of endogenous self-molecules or danger-associated molecular patterns (DAMPs) in the initiation of various inflammatory processes as well as the development and progression of atherosclerotic cardiovascular disease (CVD). High-mobility group box 1 (HMGB1) and heat shock protein 72 (Hsp72) are prototypical DAMPs that initiate sterile inflammation, drive inflammatory processes and promote atherogenesis. Hypertension is associated with increased inflammatory burden. The mechanisms underlying blood pressure-related inflammatory stress are not fully understood. It is currently unknown whether DAMPs are dysregulated with elevated blood pressure. Accordingly, the aim of this ongoing study is to determine the influence of hypertension, independent of other risk factors, on circulating expression of HMGB1 and Hsp72. To date, 20 sedentary, middle-aged adults have been studied: 10 normotensive (6M/4F; age: 58±2 yr; BMI: 28.0±1.5 kg/m^2; BP: 112/68±2/1 mmHg) and 10 hypertensive (5M/5F; age: 59±1 yr; BMI: 28.1±1.3 kg/m^2; BP: 151/92±2/1 mmHg). All subjects were non-smokers, normolipidemic, normoglycemic, non-medicated and free of overt CVD. Plasma concentrations of HMGB1 and Hsp72 were determined by enzyme immunoassay. Circulating concentrations of both HMGB1 (92.2±4.4 vs 69.5±4.3 ng/mL) and Hsp72 (1.6±0.4 vs 0.8±0.1 ng/mL) were significantly higher (35% and 100%, respectively) in the hypertensive compared with normotensive group. HMGB1 concentration was strongly and positively associated with both systolic ($r=0.60$; $p<0.01$) and diastolic ($r=0.57$; $p<0.01$) blood pressure; whereas Hsp72 was positively correlated with systolic ($r=050$; $p<0.01$) blood pressure only. Elevated circulating concentrations of HMGB1 and Hsp72 are consistent with activation of sterile inflammation pathways that, in turn, promote a hyper-inflammatory state. These initial results suggest that dysregulation of DAMPs may contribute mechanistically to the heightened inflammatory burden associated with hypertension and deserve further study.

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P288

Systemic Administration of an Angiotensin Type-2 Receptor Agonist Decreases Renal Regulatory T Cells

PrimaryAuthor.AuthorBlock:**Ellen E Gillis**, Jennifer C. Sullivan, Augusta Univ, Augusta, GA

There is increasing evidence supporting a critical role of the immune system in the development of hypertension. Our lab has previously reported sex differences in the renal T cell profile in both Spontaneously Hypertensive Rats (SHR) and Angiotensin II (Ang II) models of hypertension, with females having more anti-inflammatory regulatory T cells (Tregs) than males. Ang II has a well-defined role in the activation of pro-inflammatory T cells in hypertension via the angiotensin type-1 receptor (AT1R). Less is known about the role of the angiotensin type-2 receptor (AT2R) in the regulation of immune cells, although the AT2R has been shown to be cardioprotective and AT2R expression is greater in females than males. Based on the potential anti-hypertensive role of AT2Rs, we hypothesized that administration of an AT2R agonist, Compound 21 (C21), would increase renal Tregs, and this increase would be greater in females due to greater AT2R expression. Male and female SHR (10 weeks of age, n=3-4) were implanted with telemetry units for continuous monitoring of mean arterial pressure (MAP). Following 10 days of recovery, baseline MAP was recorded for 5 days. Rats were then divided into the following treatment groups: surgical controls, low dose C21 (150 ng/kg/min, sc by osmotic minipump), high dose C21 (300 ng/kg/min, sc by osmotic minipump). Kidneys were harvested after 2 weeks of treatment and flow cytometry was performed on whole kidney homogenates. MAP was not altered by C21 treatment in males (137±4 vs 134±4 vs 134±4 mmHg; n.s.) or females (128±2 vs 136±5 vs 134±4 mmHg; n.s.). Interestingly, despite having no effect on MAP, there was a significant decrease in renal $CD3^+CD4^+FoxP3^+$ Tregs in females following both low and high doses of C21 (data expressed as % $CD3^+CD4^+$ cells: 6±0.6 vs 3±0.6 vs 3.5±1.3 %, respectively; p=0.02). Tregs decrease in males following the high dose of C21 only (data expressed as % $CD3^+CD4^+$ cells: 3.3±0.3 vs 3.3±0.5 vs 1.7±0.7 %, respectively; p=0.05). Total $CD3^+$ T cells, $CD3^+CD4^+$ T cells, and Th17 cells were not altered by C21 treatment. In conclusion, AT2R activation suppresses renal Tregs, and females are more sensitive than males. These data suggest a novel role for AT2R regulation in the kidney in hypertension.

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P289

Cardio-protective Peptide Ac-SDKP is Highly Concentrated in Lymph Nodes

PrimaryAuthor.AuthorBlock:**Cesar A Romero**, Sonal Vaid, Nitin Kumar, Tang-Dong Liao, Oscar A Carretero, Henry Ford Health System, Detroit, MI

N-acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP) is a natural peptide released from its precursor Thymosin β4 (Tβ4) by meprin-α and the prolyl oligopeptidase enzymes (POP) in sequential steps. Ac-SDKP is hydrolyzed by the angiotensin converting enzyme (ACE). Ac-SDKP has anti-inflammatory and anti-fibrotic effects in heart, aorta and kidney and part of the beneficial effects of ACE inhibitors has been ascribed to the increased levels of Ac-SDKP. Ac-SDKP is present in spleen and thymus, however, the presence of Ac-SDKP in the lymph node has not been studied. We hypothesized that Tβ4, Ac-SDKP and its releasing enzymes are present in lymph node. Ac-SDKP concentration was evaluated in Sprague-Dawley (SD) rat tissues.

Tβ4 as well as Meprin-α and POP enzymes were measured in the lymph node, thymus, spleen and other tissues obtained from SD rats. Additionally, Ac-SDKP content was measured in different cell populations of lymph node obtained through cell sorting. Lymph node showed the highest Ac-SDKP concentration (181±18.3 ng/mg of protein), followed by the testis (104.3±6.5, thymus (54.2±1.8) and spleen (44.9±4.7) in SD rats (P<0.001 lymph node vs. testis, thymus and spleen). Meprin-α and POP activity were present in lymph node, spleen and thymus. Tβ4 and Meprin-α immunostaining were found to be positive in multinucleated giant cells in the cortical region of lymph node and along the septums; it was also found in the follicular region and germinal center. POP staining was found positive in the cortical region. In the lymph node, Ac-SDKP concentration was higher in Macrophages (CD45+CD68+) in comparison with T lymphocytes (CD45+CD3+) (150±110 pmol/100,000 cell vs. 0.3±0.1pmol/100,000cell, respectively). We conclude that in lymph node, Ac-SDKP is highly concentrated and that all the components of Tβ4/Ac-SDKP system are present. Macrophages could be the main source of Ac-SDKP in lymph node. The presence of Ac-SDKP in lymph node may have important implications in understanding inflammation and target organ damage in cardiovascular diseases.

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P290

Unilateral Cervical Vagotomy Protects Against Renal Injury and Hypertension in a Mouse Model of Lupus Hypertension

PrimaryAuthor.AuthorBlock:**Grace S Pham**, Sarika Chaudhari, Daniel L Fancher, Tanya B Michaels, Luke D'Cunha, Keisa W Mathis, UNTHSC, Fort Worth, TX

Chronic renal inflammation has been implicated in hypertension. The vagus nerve controls inflammation by immunoregulatory mechanisms like the hypothalamic-pituitary-adrenal axis and the cholinergic anti-inflammatory pathway. It is not clear whether changes in vagal activity affect inflammation downstream in the kidney. We hypothesized that decreasing vagal neurotransmission would impede these anti-inflammatory mechanisms, exacerbate renal inflammation and promote hypertension. To study this, we used a mouse model of systemic lupus erythematosus (SLE) that develops hypertension after the onset of autoimmunity. Female SLE (*NZBWF1)* and control *(NZW)* mice received right unilateral cervical vagotomy (Vx) or sham surgery (n=4-6/group) and 3 weeks later had catheters implanted to measure blood pressure. Sham-operated SLE mice had elevated plasma double-stranded DNA autoantibodies ($3.8 \times 10^5 \pm 1.3 \times 10^4$ vs. $1.1 \times 10^5 \pm 5.0 \times 10^4$ activity units; all $p<0.05$ unless otherwise noted), a hallmark of the disease; enlarged spleens (239.1 ± 80.4 vs. 124.1 ± 11.4 mg); and an elevated splenic CD3+CD4+ T cell population (flow cytometry; 21.9 ± 2.1 vs. 14.6 ± 2.8%) relative to controls. Vagotomy did not alter disease severity ($3.2 \times 10^5 \pm 3.5 \times 10^4$; p = 0.20), but prevented splenomegaly (119.7 ± 24.2 mg) and reduced CD3+CD4+ T cells (14.7 ± 0.7) in SLE mice. SLE mice trended toward higher renal cortical TNF-α than controls ($3.5 \times 10^6 \pm 1.7 \times 10^6$ vs. 7.6×10^4; normalized to total protein; p = 0.22), although SLE/Vx mice had lower concentrations than SLE/sham mice ($4.9 \times 10^5 \pm 1.3 \times 10^5$ vs. $3.5 \times 10^6 \pm 1.7 \times 10^6$). SLE mice had higher albumin excretion rates (AER) than controls ($1.2 \times 10^4 \pm 3.6 \times 10^3$ vs. $2.0 \times 10^1 \pm 4.8$ μg/day) and Vx decreased AER in SLE mice ($1.2 \times 10^4 \pm 3.6 \times$

10^3). SLE mice were hypertensive compared to controls (158 ± 10 mmHg vs. 108 ± 4 mmHg) and vagotomy protected SLE mice from hypertension (133 ± 4). In sum, these data counter our initial hypothesis—unilateral vagotomy reduced renal inflammation and halted the progression of SLE hypertension. The vagus nerves have myriad functions and perhaps other neuroimmune interactions compensate for the ligation of one nerve. Future studies will investigate whether our findings may be due to long-term adaptation following unilateral vagotomy.

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P291

Sodium-independent Dietary Effects on Renal Immune Cell Infiltration in Salt-sensitive Hypertension

PrimaryAuthor.AuthorBlock:**Justine M Abais-Battad**, Hayley Lund, Mingyu Liang, Pengyuan Liu, David L Mattson, Medical Coll of Wisconsin, Milwaukee, WI

Recent studies have shown sodium-independent dietary effects to be important in the development of Dahl SS hypertension and renal disease. Dahl SS/JrHsdMcwiCrl (SS/Crl) rats fed a grain-based diet (Teklad 5L2F) were less susceptible to salt-induced blood pressure elevation (116.5±1.2 vs 141.9±14.4 mmHg) and albuminuria (21.7±3.5 vs 162.9±22.3 mg/day) compared to SS/JrHsdMcwi (SS/Mcw) rats fed a 4.0% high salt casein-based diet (AIN-76A) for 3 weeks (n=6-8/group). Given the role of immunity in hypertension, SS/Crl rats displayed significantly less total CD45+ leukocyte infiltration in the kidney than SS/Mcw (6.1±0.9 vs 11.6±2.6 $x10^6$ cells/kidney). Reductions were observed in all subsequent immune cell populations, including CD3+ T cells (6.8±0.8 vs 14.4±2.2 $x10^5$), CD45R+ B cells (1.5±0.4 vs 6.7±2.2 $x10^5$), and CD11b/c+ antigen presenting cells (5.0±0.7 vs 9.2±2.1 $x10^6$ cells/kidney) in SS/Crl versus SS/Mcw. The SS/Crl immune system appears to be less activated, as demonstrated by a respective 44.8% and 66.8% reduction in CD4+CD25+ and CD8+CD25+ T cells, as well as a 40.4% decrease in TNFα-producing CD4+ T cells. Upon taking a discovery approach, RNA sequencing data (n=4 pools of 3 rats each) revealed genes related to hematopoietic cell lineage, the complement and coagulation cascade, B cell signaling, and primary immunodeficiency. Specifically, RNA expression of components of the NLRP3 inflammasome pathway were upregulated, including TLR4 (44.0%), NLRP3 (33.8%) and caspase-1 (26.4%), with a similar trend for IL-1β (25.4%), in the renal medulla of SS/Mcw rats fed high salt. This coincided with increased protein expression of NLRP3 (1.5-fold) and ASC (3.2-fold), together demonstrating increased NLRP3 inflammasome mRNA and protein expression in SS/Mcw rats after high salt challenge. Interestingly, RNA expression of these NLRP3 inflammasome proteins were significantly reduced in SS/Crl versus SS/Mcw rats fed high salt, including TLR4 (39.2%), NLRP3 (31.2%), caspase-1 (32.8%) and IL-1β (39.2%). These data indicate that the NLRP3 inflammasome may be a key mechanistic step in determining how these non-sodium components of the diet affect immunity, and warrant further studies to elucidate its role on salt-sensitive hypertension and renal damage.

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P292

Reversal of Cardiac Remodeling After Surgical Intervention Leads to Distinct Cardiac Function Outcomes

PrimaryAuthor.AuthorBlock:**Leonardo Jensen**, Amanda Almeida Silva, Nataly Cunha Oliveira, Leandro Eziquiel de Souza, Maria Claudia Costa Irigoyen, Heart Inst, São Paulo, Brazil

Hypertension is the leading cause of cardiovascular diseases Nevertheless diagnosed hypertension can be controlled and monitored with adequate drug regimens. Yet once cardiac hypertrophy is established, a number of patients develop diastolic cardiac dysfunction resulting from cardiac remodeling with fibrosis and cell death. The present study aims to test the hypothesis that animals with aortic stenosis for five weeks can restore cardiac function after the removal of the aortic constriction. Male mice with 10 weeks of age were randomized in three groups **Sham**, **Rem**, and **Tac**. After five weeks of the establishment of cardiac hypertrophy the aortic constriction was removed and the animals were followed for additional five weeks **Rem**. The positive control group **Tac** remained with the clip until ten weeks. Echocardiography was performed after 72 hours of the removal of the five weeks stenosis **Rem** and at the end of ten weeks. We also used cutoff points based on sham group for the ejection fraction **EF≥45%** and the ratio of E wave per e' **E/e'≤35**. The Rem group was separated into two outcomes according to the presence of dysfunction. Results were expressed as mean ± standard error of the mean SEM. Statistical analyses were performed and $p \leq 0.05$ were considered statistically significant. Septum thickness increased after five weeks of stenosis in both Rem 0.812±0.01mm and Tac 0.764±0.02mm groups as compared to sham group 0.675±0.01mm $p \leq 0.05$. Left ventricle mass was significantly different in Sham vs Tac 73.98±2.40mg vs 111.79±11.81mg $p<0.0001$ After 10 weeks the REM group did not present any hypertrophy markers. Among 18 animals of Rem group, 9 had reduced EF compared to sham after 5 weeks 36.97±1.87%vs52,74±4.17% $p<0.0001$ and after 10 weeks 49±1.13%vs40±1.06% $p<0.0001$ constituting an occurrence of systolic problems of 50%. When we analyzed E/e' 13 animals presented diastolic dysfunction and an increased ratio compared to sham animals 41.07±1.12vs30.79±1.95 $p<0.05$ at 5 weeks. Therefore, the occurrence of diastolic dysfunction reached 72%. The proposed model induced hypertrophy. Despite that, only half the Rem animals had a reduction in ejection fraction, whereas most developed diastolic dysfunction. These findings open a new path for the investigation of these distinct outcomes

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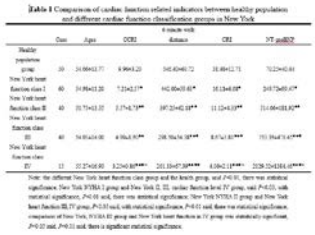
P293

The Correlations Between NT-proBNP and Cardiac Reserve by Phonocardiogram Exercise Test

PrimaryAuthor.AuthorBlock:**Yu Chaoping**, Xu Fengcheng, Liu Tianhu, Pidu District People's Hosp, Chengdu, China

Objective To investigate the correlations between NT-proBNP and Cardiac contractility reserve index(CCRI) and Cardiac reserve index(CRI) and find more theoretical and clinical evidence that CRI, CCRI can reflect the degree of heart failure,promote the use of them in the Primary hospitals and community health service centers. **Methods** According to the inclusion and exclusion criteria,155 cases were included in the study, 60 volunteers without cardiovascular disease were selected as the control group, all of them were classed by NYHA functional classification, phonocardiogra were recorded by phonocardiogram exercise test (PCGET) before and after 6 minutes walk test, measured S1 amplitude and heart rate, calculated CCRI, CRI, detected NT-proBNP, and analysed the CCRI, CRI, and NT proBNP correlations. **Results** Compared with the healthy group, the NT-proBNP, CCRI, CRI, 6 minutes walking distance were significantly different in different NYHA FC, Moreover, with the increase of NYHA FC level, CRI, CCRI gradually decreased, 6 minutes walk distance gradually shortened, NT-proBNP gradually increased, the differences were statistically significant. **Conclusion** The CCRI, CRI and NT-proBNP have good correlations, so they can reflect the classification of cardiac function, they are facilitate, economic, no invasive, rapid, they are worth using widely in primary hospitals and community health service centers.



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P294

Leptin Deficiency Reduces Cardiac Reserve Independently of Increased Body Weight

PrimaryAuthor.AuthorBlock:**Fabio N Gava**, John E Hall, Jussara M do Carmo, UMMC, Jackson, MS

Obesity is associated with structural and functional changes in the heart and abnormal cardiovascular responses to exercise in humans and experimental animals. We and others have demonstrated that specific deletion of cardiomyocyte leptin receptors causes cardiac dysfunction associated with decreased energy production in the heart. However, the importance of leptin in normal cardiac reserve during increased stress is still unknown. In this study we examined if leptin deficiency, independently of obesity, alters cardiovascular responses to a stress test induced by dobutamine in 20 week old non-obese ob/ob mice (n=4, 30±2g) that were pair-weighted to match the body weight of wild type (WT) control mice (n=5, 31±3g), and obese ob/ob mice fed ad-lib (n=4, 65±4g). An additional group of non-obese ob/ob mice (n=4) was infused with leptin via osmotic minipump (4 µg/kg/min, IP) for 7 days. Mice were instrumented with for continuous infusion of saline and progressively increasing doses of

dobutamine (2-12 ng/g/min, 2 min at each dose). Long and short axis left ventricle dimensions were obtained before and one minute after each dose of dobutamine using a 30 MHZ transducer (VEVO2100®). Baseline heart rate (HR) was similar in obese ob/ob (431±24 bpm) and WT control (421±28 bpm) mice, but significantly reduced in non-obese ob/ob (356±28 bpm) mice. Compared to WT mice, non-obese ob/ob mice had no significantly differences in baseline ejection fraction (EF) (76±4 vs. 66±4%) but higher EF was observed in obese ob/ob (83±3%) mice. Dobutamine infusion increased average delta HR by 42±7, 104±16 and 37±11 bpm, and EF by 24.8±1.2, 4.9±0.5 and 33.3±0.8 % in WT, non-obese ob/ob and obese ob/ob mice, respectively. Chronic leptin replacement restored EF response to dobutamine in non-obese ob/ob mice (93±2 vs. 94±2% in WT mice). These results show that leptin deficiency leads to reduced myocardial reserve independent of changes in body weight, and leptin replacement in non-obese ob/ob mice completely restored EF response to cardiovascular stress test.

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P295

High Dietary Fat Intake Attenuates Pressure Overload-induced Cardiac Remodelling in a Spontaneous Hypertensive Rat Model

PrimaryAuthor.AuthorBlock:**Eugene McNamara**, Univ Coll Dublin, Dublin, Ireland; Chris Watson, Queen's Univ of Belfast, Belfast, United Kingdom; Liam Shiels, Adam Russell-Hallinan, Univ Coll Dublin, Dublin, Ireland; Mark Ledwidge, St Vincents Univ Hosp, Dublin, Ireland; John Baugh, Univ Coll Dublin, Dublin, Ireland

Heart failure with preserved ejection fraction (HFpEF) is emerging as a metabolic disease. HFpEF initiation and progression is associated with the pro-inflammatory state found in conditions such as hypertension, obesity and diabetes. Although the role of dietary fat in the genesis of metabolic disorders and tissue inflammation has been characterised, dietary fat induced cardiac pathology and inflammation in the context of pressure-overload is less well defined. In this study, we reveal the cardioprotective effects of saturated fat intake on cardiac phenotype and cardiac inflammation in an established rat model of hypertension. Ten week old male normotensive Wistar (WKY), control rats (n=10), and matched SHR (n=20), were split into low-10%kcal and high-45%kcal fat chow. After 16 wks of diet intake, bodyweight profiles were similar among all groups (p=NS). Unconscious tail-cuff plethysmography confirmed elevated arterial pressure (>140mmHg) in both SHR dietary groups, (SBP in 45%kcal; 154.4 ± 21.7 mmHg, $p<0.05$ and 10%kcal; 151.4 ± 31.5 mmHg, p<NS). Endpoint echocardiography revealed increased LVMi (normalised: tibia length) in 10%kcal, which was ameliorated in 45%kcal (respectively; 24.7 ± 2.2 mg/mm vs. WKY, $p<0.05$ and 22.5 ± 1.3 mg/mm, vs. WKY, p=NS). Furthermore, anterior wall thickness in both systole and diastole was greater in 10%kcal fed animals ($p<0.01$ vs. WKY). Left ventricular remodelling was exaggerated in 10%kcal vs. 45%kcal, with significant increases in cardiomyocyte cross-sectional area and collagen deposition (vs. WKY control). In SHR groups, cardiac $CD68^+$ macrophage infiltration was increased (10%kcal; 122.5 cells/mm^2, & 45%kcal; 151.1 cells/mm^2, $p<0.05$ vs. WKY).

Alterations in markers of metabolic dysfunction was evident with increased total cholesterol (10%kcal, 21.7 ± 0.52 mg/dL, $p<0.01$ vs. WKY and 45%kcal; 22.01 ± 0.83 mg/dL, $p<0.001$ vs. WKY), and glycated hemoglobin (HbA1c) in high-fat fed rats vs. 10%kcal control ($p<0.05$). In summary, dietary fat intake may prevent heart failure development, despite cardiac inflammation and dysfunctional metabolism. Collectively, our data show long-term saturated fat intake attenuates pathological cardiac fibrosis and hypertrophy in a rat model of chronic pressure-overload.

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P296

Deletion of Endothelial Sirt3 Causes Diastolic Dysfunction

PrimaryAuthor.AuthorBlock:Xiaochen He, Heng Zeng, **Jian Xiong X Chen**, Univ of Mississippi Medical Ctr, Jackson, MS

The morbidity of heart failure with preserved ejection fraction (HFpEF) increases with advanced age and is associated with endothelial dysfunction. In the present study, we analyzed the cardiac function and coronary microvascular function in endothelial specific SIRT3 knockout (ECKO) mice and global SIRT3 KO mice, as well as endothelial glycolytic metabolism and angiogenesis *in vitro*. We found that SIRT3 ECKO mice developed left ventricle (LV) diastolic dysfunction, as evidenced by prolonged isovolumic relaxation time (IVRT) and reduction of coronary flow reserve. Western blot analysis revealed a significant decrease in glycolytic enzyme PFKFB3 in SIRT3 KO-ECs, accompanied by a dramatic reduction in basal glycolysis, glycolytic reserve and glycolytic capacity. Moreover, SIRT3 KO-ECs exhibited higher oxygen consumption rate and more prominent production of reactive oxygen species (ROS) than WT-ECs. SIRT3 KO-ECs exhibited less potent angiogenic capabilities as indicated by decreased network formation and migration. In contrast, global knockout of SIRT3 in mice resulted in progressive deterioration of LV function, as evidenced by a decrease in ejection fraction and fraction shortening at 12 months of age. SIRT3 KO mice also exhibited reduction of ANP and ERK-1/2, and increased ROS production, gp91(phox), caspase-3 and Wnt7 in the heart. In conclusion, we demonstrated that ablation of SIRT3 in ECs caused an HFpEF phenotype with impaired glucose metabolism and angiogenesis. Global SIRT3 KO mice, however, exhibited an HFrEF phenotype. Our data suggest cell-specific effects of SIRT3 deletion on cardiac function.

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P297

The Deleterious Role of Prostaglandin E2 EP3 Receptor in Ang II-induced Hypertension

PrimaryAuthor.AuthorBlock:**Timothy Bryson**, Henry Ford Health System/Wayne State Univ Sch of Med, Detroit, MI; Jiang Xu, Henry Ford Health System, Detroit, MI; Pamela Harding, Henry Ford Health System/Wayne State Univ Sch of Med, Detroit, MI

High blood pressure (BP) is a major risk factor for heart and renal disease. Prostaglandin E2

(PGE2) is a product of the arachidonic acid cascade and is known to mediate inflammation and have vasodilatory effects. Previous data from our lab have shown that PGE2 reduces cardiac contractility when signaling through its EP3 receptor subtype and that EP3 expression increases in the left ventricle of mice subjected to Angiotensin II (Ang II) hypertension. We therefore hypothesized that EP3 activation would worsen BP in the Ang II hypertension model and would also decrease cardiac function. To test our hypothesis, we treated 10-12 wk. old C57Bl/6 mice with either Ang II (1.4 mg/kg/d) or vehicle via osmotic mini pump and simultaneously treated them with the EP3 agonist, Sulprostone (80 μg/kg/d, S.C.), or vehicle for 2 weeks. As expected, Ang II infusion increased BP significantly (115 mmHg ± 4.8 vs. 172 mmHg ± 12.1; $p< 0.005$). After 2 weeks, however, there was no difference in BP between the Ang II group and the Ang II + Sulp. group. Echocardiography in conscious animals demonstrated that treatment with Ang II + Sulp. group reduced shortening fraction (SF) from 61.28 ± 1.82 % to 56.71 ± 0.91 %; ($p<0.05$) whereas Ang II treatment alone did not affect SF. This suggests that EP3 activation combined with Ang II infusion may reduce cardiac function in as little as 2 weeks. We then repeated this protocol using the EP3 inhibitor, L798, 106 (40 μg/kg/d). Remarkably, the increase in BP with Ang II was almost completely abolished when animals received the inhibitor (168.3 mmHg ± 6.5 for Ang II group vs. 117.9 mmHg ± 17.1 for Ang II + L798, 106 group; $p< 0.05$) without changes in cardiac function. Since the effects on BP are independent of changes in cardiac function, we hypothesize the effects are on total peripheral resistance and future experiments will examine the vasculature to identify possible mechanism(s). In conclusion, EP3 receptor activation did not worsen BP after Ang II infusion but treatment with the EP3 inhibitor completely normalized blood pressure. This suggests that there is commonality between the EP3 and Ang II signaling pathways. We also propose that EP3 receptor activation is deleterious in an Ang II model since treatment with Sulprostone reduces cardiac function after only 2 weeks.

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P298

Excretion of Urinary Marinobufagenin And Angiogenic Factors in Pregnancies With Increased Risk for Preeclampsia: A Pilot Study

PrimaryAuthor.AuthorBlock:Nathan Drever, Baylor Scott & White HL/TAMUHSC, Temple, TX; Hong Wang, John Wages, Bo Yu, Panorama Res Inc., Sunnyvale, CA; Syeda H Afroze, TAMUHSC, Temple, TX; Ram Kalagiri, Baylor Scott & White HL/TAMUHSC, Temple, TX; James W Larrick, Panorama Res Inc., Sunnyvale, CA; Thomas J Kuehl, **Mohammad N Uddin**, Baylor Scott & White HL/TAMUHSC, Temple, TX

Background: Previous work has shown an increase in urinary excretion of marinobufagenin (MBG) in patients with preeclampsia (preE) compared to normal pregnancy at delivery. MBG is a novel urinary marker for preE; however, it is unknown how MBG levels fluctuate during gestation. **Hypothesis:** Shifts in MBG and angiogenic factors during early pregnancy identify cytotrophoblast's dysfunction, contributing to reduced vascular development with impaired perfusion and preE. **Methods:** In this study, we prospectively enrolled two groups of patients; 50 patients in each group total of 100 patients for serial collection of urine and serum during each trimester and at delivery. These groups

are: (1) Pregnant women at risk for development of preE; and (2) Pregnant women at low risk for development of preE. Risks were calculated for each pregnancy using the sum of relative risk values. Urine samples from 100 subjects (100 in first trimester, 67 in second trimester, 67 third trimester, and 67 at delivery) were assayed using ELISA for MBG (Panorama Research, Inc.), sENG, VEGF, PIGF, sFlt-1 expressed as pg/mg creatinine and the calculated uFP (log of 10x sFLT-1/PIGF ratio). The angiogenic factors were analyzed by commercially available kits. **Results:** Of 100 subjects, 50 had no risk for preE and 50 had relative risks on the average of 6.6 with a range of 1.4 to 16.6. There were no associations between relative risk and any of the 6 angiogenic measures during the first trimester ($p > 0.36$) and second trimester ($p > 0.22$) using linear regression. However, during the second trimester, the high risk cohort had lower PIGF levels than the no risk cohort ($p < 0.05$ using Mann-Whitney U test). Comparing the serial values of 6 angiogenic measures in 67 subjects during the first, second, third trimesters and at deliveries, only MBG and PIGF varied significantly ($p < 0.05$) with MBG. **Conclusions:** MBG increases with progression of pregnancy and the levels of MBG are the highest during delivery. We conclude that MBG levels increase during normal pregnancy, however, the differential levels of MBG in normal and preE pregnancies are now underway. Relationships to development of preE symptoms and MBG levels are pending with the addition of patients and additional sample.

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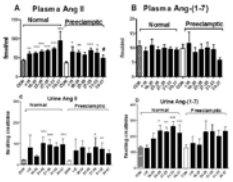
P299

Longitudinal Study of Angiotensins in Normal and Preeclamptic Pregnancy

PrimaryAuthor.AuthorBlock:**K. Bridget Brosnihan**, Wake Forest Sch of Med, Winston Salem, NC; David C Merrill, Aurora Medical Group, West Allis, WI; Liliya C Yamaleyeva, Wake Forest Sch of Med, Winston Salem, NC; Kai Chen, Aurora Medical Group, West Allis, WI; JaNee Joyner, Courtney Gruver, Kristen Lanier, Carol Moorefield, Wake Forest Sch of Med, Winston Salem, NC

To address whether differential regulation of the renin-angiotensin system (RAS) occurs in preelampsia, we performed an extensive analysis of the time course of circulating and urinary profiles of the vasoconstrictor (Ang II) and the vasodilator [Ang-(1-7)] components in pregnant and preeclamptic women. Plasma and spot urine samples were collected prospectively from nulliparous normal pregnant (n=67) and preeclamptic (n=19) women between the ages of 18-40, at <16, 16-20, monthly between 24-36 weeks of gestation, and a control sample at six weeks post-delivery (CON). Mean blood pressure ($p<0.001$) was highest at 31-33 and 35-37 weeks of gestation in preeclamptic compared to normal pregnant women, and associated with mild proteinuria. Plasma Ang II was elevated in normal pregnant subjects at < 16 weeks of gestation and maintained at the higher levels over gestation (Fig A). In preeclamptic subjects plasma Ang II was elevated during gestation, but at 35-37 weeks plasma Ang II was reduced compared with normal pregnant subjects. Plasma Ang-(1-7) was unchanged in both normal and preeclamptic subjects (Fig B). Urinary Ang II was increased at 23-37 weeks of gestation in normal subjects and 27-33 in preeclamptic subjects (Fig C); urinary Ang-(1-7) was elevated in normal pregnant subjects (Fig D) but not in preeclamptic women.

The activation of the RAS, particularly Ang II throughout normal gestation may contribute to the maintenance of vascular tone during normal pregnancy. However higher sensitivity to Ang II in preeclampsia may be potentiated by the higher circulating and urinary levels of Ang II, unopposed by local renal Ang-(1-7), and thus contribute to the development of preeclampsia.



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P300

Fetal Status Evaluation Using Photoacoustics and 3D Power Doppler Ultrasound

PrimaryAuthor.AuthorBlock:**Liliya M Yamaleyeva**, K. Bridget Brosnihan, Tiffaney Bledsoe, Wake Forest Sch Med, Winston Salem, NC

Pregnancy complications can seriously impact fetal well-being. However the tools used for the evaluation of fetal oxygenation in clinic are limited to the monitoring of fetal heart rate or invasive techniques (fetal scalp pH and PO_2). Therefore, the development of methods enabling accurate, non-invasive and in real-time assessment of fetal status throughout pregnancy is warranted. In this study we tested the combination of photoacoustic imaging (PAI) and 3D power Doppler for the assessment of fetal distress. PAI combines optical contrast of photoacoustic laser technology with high spatial resolution of ultrasound. Power Doppler is a sensitive technique for the detection of blood flow. We hypothesize that a combination of PAI with 3D power Doppler can non-invasively and in real time establish fetal oxygenation, volume, and tissue vascularity. Pregnant C57Bl/6 mice were infused with nitric oxide synthase inhibitor, L-NAME via osmotic minipumps (50 mg/kg/day; days 11 to 14 of gestation) to induce the hypertensive phenotype. At day 14 of gestation, systolic blood pressures were higher in L-NAME-treated vs. untreated C57Bl/6 mice although these values did not reach hypertensive levels (90.8±3.1 vs. 104.4±3.7 mmHg, $p<0.05$, n=4-5). Fetal weights were lower in the L-NAME-infused mice versus controls (0.12±0.01 vs. 0.17±0.01 g fetal weight per cm tibia length, $p<0.05$, n=5-6). Fetal liver sO2 was lower in L-NAME-infused mice (47.5±2.1 vs. 56.1±1.6 %, n=4, $p<0.05$), while no differences were found in fetal brain sO2. Total fetal volume was lower in L-NAME-treated mice (213.8±26.5 vs. 399±45.1 %, n=5, $p<0.05$) and positively correlated with fetal body weights obtained postmortem in study groups. Fetal tissue vascularity was also lower in L-NAME-infused mice possibly due to limited vascular branching or blood flow (40.8±1.5 vs. 47.7±1.8%, $p<0.05$). These changes were associated with a compensatory increase in fetal heart rate in L-NAME-treated mice (170.7±9.1 vs. 135.8±5.7 %, $p<0.05$). We conclude that a combination of PAI with 3D power Doppler provides valuable information about fetal oxygenation and growth in association with tissue vascularity thus permitting non-invasive, in real-time analysis of fetal well-being.

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P301

Preeclampsia and Gestational Hypertension: Does sEng Levels Make the Important Difference Preeclampsia and Gestational Hypertension: Does sEng Levels Make the Important Difference

PrimaryAuthor.AuthorBlock:**Athar H Siddiqui**, Rahul Negi, Sch of Medical Sciences, Univ of Hyderabad, Hyderabad, India; Vemanamanda Haritha, Nuzhat Aziz, Fernandez Hosp Educational & Res Fndn, Hyderabad, India

The origin of Preeclampsia (PE), a multi-systemic disorder of pregnancy, lies to a great extent on the balance of the anti-angiogenic and angiogenic proteins during pregnancy. In the present study, we have determined the levels of sFlt-1 and sEng levels (anti-angiogenic protein) and Placental Growth Factor, PlGF (angiogenic Protein) in the serum of the patients with hypertensive disorder of pregnancy (including Gestational Hypertension, GH and PE) and Normotensive (NT) pregnancy. The levels of sFlt-1 were found to be significantly higher in the 3rd trimester and at term in the hypertensive pregnancy (GH and PE), compared to the NT pregnancy, (8.67±1.93 ng/ml, n=13 vs. 3.95±.37 ng/ml, n=111 at the 3rd trimester and 14.90±2.68 ng/ml, n=15 vs. 6.58±.42 ng/ml, n=111 at term). The levels of sFlt-1 were considerably higher in the PE samples compared to the GH samples, but not significant. Similarly the levels of sEng were found to be significantly higher in the 3rd trimester and at term in the hypertensive pregnancy (GH and PE), compared to the NT pregnancy (11.18±1.51 ng/ml, n=15 vs. 7.86±.25 ng/ml, n=106 at the 3rd trimester and 12.68±1.70 ng/ml, n=14 vs. 10.01±.42 ng/ml, n=106 at the term). The levels of sEng were, however, found to be significantly higher in all the trimester and at term in the PE samples compared to the NT Samples (6.49±1.14 ng/ml, n=7 vs 4.21±.28 ng/ml, n=106, 6.89±2.30 ng/ml, n=7 vs. 3.76±.25 ng/ml, n=106, 13.10±2.12 ng/ml, n=7 vs. 7.86±.37 ng/ml, n=106, 14.93±2.27 ng/ml, n=7, vs. 10.01±.42 ng/ml, n=106 at the 1st, 2nd, 3rd trimesters and term respectively), but not in the GH samples. The angiogenic protein, PlGF was found to be significantly lower in the hypertensive conditions of pregnancy (PE and GH) samples compared to the NT samples, only in the 3rd trimester (440±48.58 pg/ml, n=15 vs 1278±104 pg/ml, n=119), although its levels were considerably decreased in the 2nd trimester and at term, but not significant. Within the hypertensive conditions of pregnancy, the blood pressure increase at term was more in the PE patients compared to the GH patients (42±5.1/25±3.3 mm Hg vs. 18±3.7/13±3.3 mm Hg, $p<0.05$) compared to the BP recorded at the 1st trimester. The results of the present study indicate a more potent role of sEng in the development of PE.

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P302

Nicotinamide Ameliorates IgA Nephropathy and Pregnancy Outcome in Pregnant Grouped-ddy Mice

PrimaryAuthor.AuthorBlock:Tomofumi Fushima, Akiyo Sekimoto, Yuji Oe, Emiko Sato, Hiroshi Sato, Sadayoshi Ito, **Nobuyuki Takahashi**, Tohoku Univ, Sendai, Japan

Objective: IgA nephropathy (IgAN) affects women in childbearing ages. We have reported that nicotinamide (Nam) ameliorates a preeclampsia (PE)-like condition and pregnancy outcome in PE mouse models (*PNAS* 2016, AJP 2017). Our preliminary data show that pregnant grouped ddY (gddY) mice, a model of IgAN, have PE-like phenotype and adverse pregnancy outcome. The aim of the present study is to clarify whether Nam ameliorates IgAN and pregnancy outcome in pregnant gddY mice. **Design and Method:** Pregnant gddY mice 9-11 weeks of age were divided into four groups (gddY, gddY+Nam, gddY+sFlt-1, and gddY+sFlt-1+Nam). PE-like phenotype was induced by administering sFlt-1 adenovirus ($4x10^8$ pfu) at 14.5 dpc. Nam 500 mg/kg/day was administered daily using gavage from 12.5 dpc. Age-matched pregnant slc:ddY mice were used as a control. **Results:** Compared with control ddY mice, gddY mice showed higher SBP and urinary albumin excretion (Fig. 1), and smaller litter size and fetal weights. Moreover, gddY+sFlt-1 mice showed severe endotheliosis and poor pregnancy outcome. Nam alleviated hypertension, albuminuria (Fig. 1), mesangial expansion and endotheliosis in gddY and gddY+sFlt-1 mice. In addition, Nam prolonged pregnancy period of gddy+sFlt-1 mice. Fetal weights from gddY+Nam mice, and gddY+sFlt-1+Nam mice were larger than those of gddY and gddY+sFlt-1 mice. **Conclusions:** Nam alleviated maternal hypertension, proteinuria and fetal growth restriction in pregnant gddY and gddY+sFlt-1 mice. Nam could help treat maternal IgAN with pregnancy and PE with IgAN.

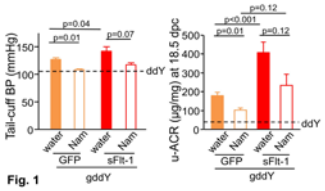

Fig. 1

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P303

Endothelin Modulates Local Complement Activation in Placental Ischemia-induced Hypertension

PrimaryAuthor.AuthorBlock:**Jean F Regal**, Cameron R Wing, Luke McCutcheon, Jeffrey S Gilbert, Univ of Minnesota Medical Sch, Duluth campus, Duluth, MN; Sherry D Fleming, Div of Biology, Kansas State Univ, Manhattan, KS

Preeclampsia is characterized by hypertension, reduced placental perfusion, intrauterine growth restriction and increased activation of complement, part of innate immunity. Using the Reduced Uterine Perfusion Pressure (RUPP) model of placental ischemia-induced hypertension in rat, our previous work demonstrated the importance of complement activation in mediating maternal hypertension. Previous studies also demonstrated endothelin A receptor (ET_A) plays a significant role in RUPP-induced hypertension, and RUPP-induced systemic complement activation occurs even with ET_A antagonism with atrasentan (ATR). To further probe mechanistic connections between complement and endothelin, we hypothesized that ET_A regulates local complement activation

(C3 deposition). Dams received drinking water with 50 or 75 ug/ml ATR from gestation day (GD)13-19 corresponding to estimated average 5.5-6.6 and 8.0-9.3 mg/kg/day, respectively. On GD14 rats underwent Sham or RUPP surgery with clip placement on abdominal aorta and uterine arteries to decrease placental perfusion. As previously reported, RUPP increased mean arterial pressure (MAP) compared to Sham, and ATR attenuated RUPP-induced hypertension but not RUPP-induced placental C3 deposition (immunohistochemistry). Importantly, in Sham animals ATR increased C3 deposition and ATR 75 markedly decreased message for membrane bound placental complement regulators Crry, CD55 and CD59 that normally control complement activation. These data suggest that endothelin binding ET_A modulates endogenous complement regulators to influence and prevent excess pathological complement activation in placenta during pregnancy.

Treatment	MAP (mm Hg)	Placenta C3	Fold change of message from Sham		
			Crry	CD55	CD59
Sham	94±3	0.46±0.22	1.00±0.16	1.00±0.21	1.00±0.17
Sham ATR 50	91±3	2.0±0.01#	1.33±0.31	1.20±0.19	1.63±0.12#
Sham ATR 75	85±2#	1.38±0.47#	0.38±0.17#	0.30±0.10#	0.43±0.17#
RUPP	109±5*	1.75±0.3*	1.01±0.23	1.87±0.55*	0.87±0.18
RUPP ATR 50	100±3#	1.50±0.29	1.33±0.30	1.62±0.39	1.68±0.22#
RUPP ATR 75	92±3#	1.0±0.58	0.82±0.30	0.69±0.29#	0.59±0.35

n=4-13; *p<0.05 vs Sham; #p<0.05 comparing ATR to Sham or RUPP controls

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Motivia®, a Cardiovascular Disease Prevention Program, Decreases Hospitalization Length in Patients With High Risk for Developing Cardiovascular Event. Retrospective Analysis From 2010-2015

PrimaryAuthor.AuthorBlock:**Hernan A Perez**, Blossom DMO, Cordoba, Argentina; Natalia Jorgensen, Univ ISALUD, Buenos Aires, Argentina; Santiago Torales, Blossom, Buenos Aires, Argentina; Andrés Chianalino, Dept of Hosp Cost Analysis OSLyF Cordoba, Cordoba, Argentina; Luis J. Armando, Blossom DMO, Cordoba, Argentina

Objective: Cardiovascular events (CE) due to atherosclerosis are preventable but the identification of adults at high risk (HR) is required. Burden of atherosclerosis is not considered for categorization of HR patients (HRPts), even though only fractions of CE are predicted by Framingham risk factors (FRS). Our objective was to investigate the effectiveness of a program (Motivia®) designed for detection and monitoring of HRPts in a health insurance program. Design and Method: Motivia® is a program where patients are categorized based on the cardiovascular risk score using our previously reported algorithm, FRS combined with the Total Plaque Area (TPA) determination, and treatment is based on the post-test score. In this retrospective analysis (2010-2015) only HRPtes (based on TPA+FRS) were included. After categorization, Pts were referred to Motivia® high risk medical attention physician (MHP) or primary attention physician (PP) to perform evaluation and monitoring. Each Pt was seen at least each 3 months to achieve control of risk factors. The outcome measured were, time to first hospitalization for acute ischemic syndrome, coronary revascularization procedure, stroke, diabetes complication, and heart failure. We used models of survival (Log Rank test and Cox proportional hazards model) for the analysis. Outcome of these 2 groups were compared. Pts signed informed consent and the study was approved by the Blossom DMO Argentina ethics committee. Results: The analysis showed a significant reduction in the overall rate of CVE from 1.37% to 1.03% in both groups while in > 64 years old the reduction was greater (from 4.51% to 2.45%). However, after age, sex and pretreatment risk correction, we did not observe difference in the likelihood to have a cardiovascular event. Nevertheless, interms of hospitality length, patients under

MHP had shorter stay than PP (2.3 vs. 3.5 days/bed). Conclusions: Motivia® program, based on intensive treatment using TPA determination may benefit patients and health insurance by decreasing hospitality length.

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Intensive Follow-up of High Cardiometabolic Risk Patients. Eight Years Experience

PrimaryAuthor.AuthorBlock:Hernán A. Perez, Blossom DMO, Córdoba, Argentina; **Nestor H Garcia**, CONICET, La Calera, Argentina; Luis J. Armando, Blossom DMO, Córdoba, Argentina

We sought to evaluate the effect of a model of care of patients (pts) with chronic diseases (MCC) in the control of risk factors (RF) of pts with high cardiovascular risk (CVD). Thus, we run a Prospective community-based descriptive study of uncontrolled intervention. The latter was an MCC that consisted of: CV risk stratification with treatment allocation, performance awards, inclusion of chronic condition management software (MoviHealth®), educational and self-monitoring tools, weight reduction, chat with health agent and recreational activities. Pts were evaluated from 2008-2015 and included >18years volunteering from a local Health Service. We first determined Framingham Score (FRS) using body mass index (BMI). Those with a FRS<10% continued with a family doctor (FD), those with a FRS>10% a total plaque area of carotid atherosclerosis was determined for re-stratification (p-Test FRS). Pts with p-Test FRS>10% were followed by the MCC team to manage their CVD, whereas the others were monitored by FD. We established 2 rewarding programs when patient had RF controlled (extra payment for the physician and free medication for the pts). Follow-up was supported by MoviHealth®. We defined pts with controlled RF when HbA1c <7%, Total cholesterol < 200mg/dl, BP <130/70 mmHg. Recommendations of the Argentinean Society of Internal Medicine were followed for each pathology. Retrospectively, the incidence of CV events was evaluated. Results: In 2008, 1317 pts were evaluated (27% of the population), reaching 72% in 2015 (table). The inclusion of hypertensive (HTA) and diabetic (DM) subjects increased the number of pts with controlled HTA from 48.1% with SBP<140 mmHg to 77.4% in 2015; and from 24.1% with SBP<130 mmHg in 2010 vs vs 48.3% in 2015. As for HbA1c there was 8.4± 1.8% in 2010 vs 7.1±1.4% in 2015; and in 2015, 76% of pts had HbA1c<8%. In 2010, 66.1% had LDL<120mg/dl vs 71.8% in 2015. Also, 70% of DM had a LDL<100mg/dl (2015). The BMI did not change. The retrospective analysis of CV events showed a reduction of 48% from 1.90% in 2011 to 0.99% in 2015. Thus, we conclude that implementing and sustaining MCC is effective for the control of RF and possibly the decrease of CV events. Efforts through health providers are necessary to optimize control of risk factors in our community.

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P306

Maternal High Fat Diet Acts on the Brain to Induce Baroreflex Dysfunction and Sensitization of Angiotensin II-induced Hypertension in Adult Offspring

PrimaryAuthor.AuthorBlock:Yu-Ping Zhang, Hebei North Univ, Zhangjiakou City, China; Yan-Li Huo, Zhi-Qin Fang, Xue-Fang Wang, Jian-Dong Li, Hai-Ping Wang, Hebei North Univ, Zhangjiakou, China; Wei Peng, Hebei North Universiety, Zhangjiakou, China; Alan Kim Johnson, **Baojian Xue**, Univ of Iowa, Iowa City, IA

Accumulating evidence indicates that maternal high fat diet (HFD) is associated with metabolic syndrome and cardiovascular disease in adult offspring. The present study tested the hypothesis that maternal HFD modulates the brain renin-angiotensin system (RAS), oxidative stress and proinflammatory cytokines that alters angiotensin II and tumor necrosis factor-α (TNF-α) actions to sensitize the angiotensin II-elicited hypertensive response in adult offspring. All offspring were cross-fostered by dams on the same or opposite diet to yield 4 groups: offspring from normal fat control diet (CD)-fed dams suckled by CD-fed dams (OCC) or by HFD-fed dams (OCH) and offspring from HFD-fed dams fed HFD suckled by CD-fed dams (OHC) or by HFD-fed dams (OHH). RT-PCR analyses of the lamina terminalis (LT) and paraventricular nucleus (PVN) indicated upregulation of mRNA expression of several RAS components, NADPH oxidase and proinflammatory cytokines in 10-week old male offspring of dams fed HFD during either pregnancy, lactation or both (OHC, OCH and OHH). These offspring also showed decreased baroreflex sensitivity and increased pressor responses to intracerebroventricular microinjection of either angiotensin II or TNF-α. Furthermore, chronic systemic infusion of angiotensin II resulted in enhanced upregulation of mRNA expression of RAS components, NADPH oxidase and proinflammatory cytokines in the LT and PVN and an augmented hypertensive response in the OHC, OCH and OHH groups when compared to the OCC. The results suggest that maternal HFD blunts baroreflex function and enhances pressor responses to angiotensin II or proinflammaroty cytokines through upregulation of the brain RAS, oxidative stress and inflammation.

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P307

pNaKtide Attenuates Dyslipidemia and Atherosclerosis by Blocking Na/K-ATPase/Reactive Oxygen Species Amplification in ApoE -/- Mice

PrimaryAuthor.AuthorBlock:**Hibba Chaudhry**, Alexandra Nichols, Athar Nawab, Dana Sharma, Komal Sodhi, Marshall Univ, Huntington, WV

Background: We have previously reported that the α1 subunit of the sodium potassium adenosine triphosphatase (Na/K-ATPase) acts as an amplifier for reactive oxygen species (ROS) in addition to its ion pumping function. We have also shown that blockade of this amplification with a novel peptide, pNaKtide, ameliorates oxidative stress and obesity in mice subjected to a high-fat diet.**Hypothesis:** Given the importance of oxidative stress in the pathophysiology of atherosclerosis, we chose to examine whether pNaKtide might be effective in ameliorating dyslipidemia and atherosclerosis

in ApoE -/- mice.**Methods:** pNaKtide was administered in ApoE -/- mouse fed western diet. 25 mg/Kg pNaKtide was administered intraperitoneally once every 7 days. Lipid profile, glucose insulin levels, and ROS levels were measured. Aortas were dissected and quantification of aortic lesions was done.**Results:** Our results show that pNaKtide improved glucose tolerance and HOMA-IR scores in ApoE-/- mice fed a western diet ($p<0.05$). Also, pNaKtide administered to these mice significantly decreased plasma ALT, triglycerides, FFA, and LDL levels. Further, our results show that ApoE -/- mice fed a western diet had decreased plasma HDL levels and this decrease was reversed by pNaKtide. Plasma ROS levels were significantly attenuated by pNaKtide treatment. Mice fed a western diet had increased plaque size. Plaque size was significantly decreased by pNaKtide treatment.**Conclusion:** This study suggests that the Na/K-ATPase/ROS signaling cascade is a possible mechanism for the development of dyslipidemia and atherosclerosis associated with the metabolic syndrome phenotype and pNaKtide presents a potential novel treatment for these pathologies.

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Rgs2 Promotes Uterine Blood Flow During Pregnancy

PrimaryAuthor.AuthorBlock:Jennifer N Koch, Elizabeth A. Owens, Shelby Dahlen, Jie Li, **Patrick Osei Owusu**, DREXEL UNIVERSITY COLLEGE OF MED, Philadelphia, PA

Regulators of G protein signaling (RGS) proteins are crucial in mediating vascular smooth muscle contraction via the regulation of heterotrimeric G proteins, affecting blood pressure and arterial blood flow. Previous studies by others and us showed that RGS2 deficiency augments vascular tone and impairs uterine blood flow (UBF) in non-pregnant mice, and that an *Rgs2* loss-of-function mutation is linked to preeclampsia in humans; however, the mechanisms are unclear. Here, we tested the hypothesis that increased RGS2 expression and/or function facilitates placental perfusion by promoting vasodilation and UBF. We determined gene expression throughout pregnancy and post-partum period by real-time qPCR, while uterine blood flow and blood pressure were examined by ultrasound and carotid artery catheterization, respectively, under anesthesia. RGS2 expression decreased markedly by pregnancy day 10 (0.049 ± 0.013 vs. 0.023 ± 0.017) but returned to non-pregnancy level by day 15 (0.049 ± 0.013 vs. 0.041 ± 0.008,) in wild type mice. The pattern of changes in impedance to UBF mimicked gene expression profile in WT mice; in contrast, impedance remained elevated in *Rgs2-/-* mice at pregnancy day 15 (RI; WT: 0.516 ± 0.027, vs. RGS2-/-: 0.714 ± 0.020). Systemic blood pressure was similar between WT and Rgst2-/- mice at all stages of pregnancy. The results together indicate that RGS2 promotes uterine perfusion during pregnancy independently of its blood pressure effects. These findings are clinically relevant as selective targeting of G protein signaling could improve utero-placental hypoperfusion during pregnancy and prevent the development of pregnancy complications such as preeclampsia.

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P309

Alterations In Cardiac Structure And Function Caused By Preeclampsia

PrimaryAuthor.AuthorBlock:**Kristin Kraeker**, Florian Herse, Dominik N. Mueller, Max-Delbrück-Ctr for Molecular Med, Berlin, Germany; Ralf Dechend, Charité, Berlin, Germany; Nadine Haase, Max-Delbrück-Ctr for Molecular Med, Berlin, Germany

Preeclampsia is a major cause of maternal and fetal mortality and morbidity and since 2011 the American Heart Association mentioned the history of preeclampsia as a risk factor for later cardiovascular disease. To investigate the cardiovascular impact of preeclampsia, we use a transgenic rat model with females harbouring the gene for human angiotensinogen and males for human renin. After mating the female rat shows typical symptoms of preeclampsia at the end of the second trimester, like high blood pressure, proteinuria or growth restriction of the fetus. With echocardiography analysis a mild, but significant reduced systolic ejection fraction (EF 67.78 ±3.4 vs. 57.11 ±1.8) indicates functional changes. Speckle trackle analysis allows to trace the myocardium over the whole cardiac cycle and gives more sensitive results. A significant decrease in longitudinal (GLS -20.4 ±0.9 vs. -15.5 ±0.6), radial (GRS 26.5 ±4.1 vs. 15.6 ±2.4) and circumferential strain (GCS -22.5 ±0.7 vs. -15.7 ±0.9) and all global strain rates (GLSR -4.9 ±0.3 vs. -3.1 ±0.2; GRSR 4.8 ±0.6 vs. 3.0 ±0.4; GCSR -5.1 ±0.3 vs. -3.8 ±0.3) was measured and describes a reduced myocardial deformation in relation to its original shape and within the speed at which this occurs. This is consistent with a higher relative wall thickness (RWT 0.1875 ±0.007 vs. 0.2205 ±0.009) and an increased perivascular fibrosis (PF 2.58 ±1.4 vs. 3.72 ±1.7) in preeclamptic hearts at the end of pregnancy. This demonstrates extensive alterations in structure and function of maternal hearts caused by preeclamptic pregnancy. *(Values mean ±SEM)*

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P310

Atuplex Formulated With Small Interfering RNA For Soluble Fms-like Tyrosine Kinase-1 (sFLT1) Selectively Silences Maternal And Placental sFLT1 In A Preeclamptic Rat Model

PrimaryAuthor.AuthorBlock:**Nadine Haase**, Kristin Kräker, Max-Delbrueck Ctr for Molecular Med, Berlin, Germany; Ute Schaeper, Jens Endruschat, Silence Therapeutics, Berlin, Germany; Dominik N. Müller, Max-Delbrueck Ctr for Molecular Med, Berlin, Germany; Ralf Dechend, Charité, Berlin, Germany

Preeclampsia is a pregnancy-related disorder characterized by hypertension and proteinuria, affecting about 5-8% of pregnancies. It is a major cause of fetal and maternal morbidity/mortality. The disease is most probably multifactorial and caused by several placental dysfunctions. As an important mediator, sFLT1 is induced in the placenta and in the serum of preeclamptic women, acting as a decoy receptor for placenta like growth factors (PLGF) and inducing an antiangiogenic balance. RNAi mediated gene specific silencing could represent a new therapeutic option in preeclampsia management. We tested sFLT1-specific small interfering RNA (siRNA) formulated with the liposomal siRNA delivery system AtuPLEX for the ability to ameliorate symptoms without deteriorating fetal health in

an established rat model of preeclampsia. Transgenic rats expressing human angiotensinogen (hAGT) and renin (hREN) were crossed to produce a model of preeclampsia (PE rat) in the dams. Beginning on day 7 of gestation, transgenic hAGT dams were dosed intravenously with 2.8 mg/kg siRNA every third day through gestational day 19. Mean blood pressure was continuously recorded by radiotelemetry and 24 hour urine samples were collected in metabolic cages at day 17/18 of gestation. Rats were euthanized at day 21 of gestation. Biodistribution experiments showed that sFLT1 siRNA formulated with AtuPLEX delivers siRNA to the placenta but not to the embryo. In PE rats, our treatment was able to decrease sFLT1 mRNA expression in placenta, especially in the labyrinth layer. Circulating sFLT1 was also reduced by FLT1 siRNA treatment but did not show statistical significance. Silencing of sFLT1 did not prevent blood pressure increase in the last third of pregnancy in our preeclamptic rat model (159 ± 5 mmHg vehicle vs. 152 ± 2 mmHg sFLT1 siRNA). Proteinuria was not ameliorated (5712 ± 2038 µg/d vehicle vs. 3585 ± 1301 µg/d sFLT1 siRNA). In addition, sFLT1 siRNA had no influence on IUGR, measured by brain to liver weight ratio. AtuPLEX formulated siRNA specifically silenced maternal and placental sFLT1. The mode of delivery appears to be safe for fetal health. However, this sFLT1 knock down showed no protective effect on the preeclamptic phenotype in this Ang-II-based model.

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P311

Intracellular Renin Angiotensin Aldosterone System inMesangial Cells Under the Stimulus of Aldosterone

PrimaryAuthor.AuthorBlock:Danielle Stoll, Univ Federal De Sao Paulo, Sao Paulo, Brazil; Rodrigo E Yokota, Danielle S Aragão, Univ Federal De Sao Paulo, São Paulo, Brazil; **Dulce E Casarini**, Univ Federal De Sao Paulo, Sao Paulo, Brazil

Mesangial cells (MCs) are a local target for aldosterone (aldo) action in the modulation of the renin angiotensin aldosterone system (RAAS).Its molecular mechanisms is far from clear, and are extremely important in understanding renal pathologies. We investigated if the effect of aldosterone stimulus in supra, physiological and sub physiological doses was able to modulate the intracrine human MCs RAAS. We performed viability assays, western blot, immunofluorescence, ACE and ACE2 activities. As a result, we have obtained that the ACE activity was increased after MCs treatment with aldosterone 1 and 10 nM when compared with 0.1nM after 72h. Previous cells treatment with spirolactone followed by aldo reduced ACE catalytic activity but not ACE2. ACE and ACE 2 expression increased after 72h of MCs treatment with aldo 10 nM. The immunofluorescence showed that ACE was overexpressed in MCs after 72h with 10nM aldosterone treatment being similar to control group, differing from the treatment with spirolactone + aldo stimulation that decreased ACE expression, internalized Angiotensin II and changed the AT1 receptor localization. The same treatment increased MAS receptor production in MCs after 72h of aldosterone treatment with 10, 1.0, 0.1 and 0.01nM , but not with spirolactone + aldo treatment that inhibited receptor synthesis. ACE2 was detected in cell nucleus with 24h of treatment with aldo

and in cytoplasm after 72h, being reduced after the treatment with spirolactone+aldo. Ang 1-7 was localized at cell nucleus and in cytoplasm after treatment with aldo 24h and 72h, decreasing with spirolactone + aldo treatment. Immunofluorescence results demonstrated that MCs pre-treated with spironolactone decreased ACE, ACE2 and MAS receptor expression, and altered ANG II localization, suggesting that spironolactone acts on the mineralocorticoid receptor internalizing ANG II into the nucleus, probably by AT1 and AT2 receptors pathways. These results demonstrated that aldosterone can modulate the local RAAS in mesangial cells suggesting that its physiological concentrations are necessary for the feasibility of these cells while high doses are cytotoxic and altered the local system.

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P313

Age and Response of Plasma Aldosterone to Saline Infusion in Primary Hypertension

PrimaryAuthor.AuthorBlock:**Cristiana Catena**, Gianluca Colussi, Marileda Novello, Nicole Bertin, Gabriele Brosolo, Andrea Palomba, Francesca Spagnol, Leonardo A Sechi, Internal Med, Udine, Italy

Objective. In patients with primary hypertension, plasma aldosterone levels are significantly decreased by an intravenous saline load (IVSL), but this response is variable among patients and related to the ability of salt to modulate aldosterone production. Previous studies have reported that activity of the renin-angiotensin-aldosterone system is related with age. The aim of this study was to investigate whether age might affect the aldosterone response to an IVSL. Design and method. In 124 hypertensive patients (48±13 y, 71 males) who were washed-out of antihypertensive drugs for 2 weeks, we measured renin and PA levels before and after an IVSL (2 lt. saline in 4 h). In all patients secondary causes of hypertension were excluded. For statistical purposes patients were subdivided into two groups based on age (< or > 60 y). Results. Twenty-three of 124 patients (18%) were had 60 y of age or more (range 61-77) and the remaining were younger than 60 y (19-60 y). No differences were found between the two groups for gender, body mass index, serum potassium and creatinine clearance. Urinary sodium excretion was comparable in patients older (159±83) or younger (159±66) than 60 y. Baseline PA (76 [49-121] pg/ml) and renin (5.0 [1.9-9.8] mcIU/ml) levels were significantly lower in patients older than 60 y than in those younger than 60 y (115 [77-162] pg/ml, P=0.019; 10.7 [5.1-20.8] mcIU/ml, P=0.026). As expected IVSL decreased significantly (P<0.001) both plasma renin and aldosterone levels. Post IVSL renin was 3.0 [1.1-4.9] mcIU/ml in patients older than 60 y and 6.2 [3.0-12.5] mcIU/ml younger than 60 y. Following IVSL, PA decreased by 87% (10 [10-13] pg/ml) in patients older than 60 y and by 76% (28 [10-49] pg/ml; P=0.003) in patients younger than 60 y. The change in PA induced by IVSL was significantly and inversely related to age (r=-0.185, P=0.039). Conclusions. PA levels are lower in hypertensive patients older than 60 y. This difference becomes more relevant after IVSL suggesting greater response of the renin-angiotensin system to volume expansion. This should be taken into account in the diagnostic work-out of hypertensive patients.

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P314

Effect of Sex on Blood Pressure and Endothelial Dysfunction in the Novel BPH2 Mouse Model of Hypertension

PrimaryAuthor.AuthorBlock:**Sean P Didion**, Univ of Mississippi Med Ctr, Jackson, MS

Very little is known regarding blood pressure and endothelial function between the sexes in the hypertensive BPH2 mouse. Thus, the first goal was determine whether blood pressure and endothelial function are significantly different between male and female BPH2 mice. Information regarding the role of the renin-angiotensin system in the BPH2 mouse is also limited; therefore the second goal was to determine the role of the renin-angiotensin system by treating BPH2 mice with captopril for 4 weeks. Systolic blood pressure (SBP) was significantly elevated ($P<0.05$) and yet comparable ($P>0.05$) in male and female BPH2 mice and averaged 140±3 and 136±3 mmHg, respectively, whereas, in control mice SBP averaged 112±4 mmHg. Endothelial responses to acetylcholine in carotid artery were markedly impaired ($P<0.05$) and to a similar degree in male and female BPH2 mice as compared to controls. Captopril treatment was associated with a significant ($P<0.05$) reduction in blood pressure of 35±7 and 43±4 mmHg in male and female BPH2 mice, respectively. Captopril also resulted in an improvement of endothelial responses in male and female BPH2 mice. These findings demonstrate that male and female BPH2 mice are equally hypertensive and both sexes are characterized by endothelial dysfunction. In addition, the renin-angiotensin system may contribute to both hypertension and endothelial dysfunction in this model. Taken together, our data define the BPH2 mouse as an important model to compare and contrast the effects of hypertension between the sexes. Supported by NIH HL-107632.

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P315

Gender Differences of Aortic Wave Reflection and Influence of Menopause on Central Blood Pressure in Patients With Arterial Hypertension

PrimaryAuthor.AuthorBlock:Valeria Costa-Hong, Henrique C Muela, Thiago A Macedo, Allan R Sales, **Luiz A Bortolotto**, Heart Inst (InCor) of HCFMUSP, São Paulo, Brazil

Background: Evidences suggest that central hemodynamics indexes are independent predictors of future cardiovascular events and all-cause mortality. Multiple factors have been pointed to have potential influence on central aortic function: height, heart rate, left ventricular ejection duration and blood pressure level. Data related to the influence of gender and postmenopausal status on aortic wave form reflection is scarce. **Methods:** In a cross sectional study, 122 hypertensive patients (52 men and 70 women) were studied. Hypertension was defined as blood pressure (BP) levels ≥140/90 mmHg or use of antihypertensive drugs. Central arterial pressure, augmentation index (AIx) and augmentation index normalized to 75bpm (Alx75) were obtained using applanation tonometry. Menopause and postmenopause

history were accessed by a direct questionnaire. Postmenopause was defined as at least one year since last menstruation. Patients were paired by age, gender and menopausal status and 4 groups were compared: group 1 (young men, ≤ 48y), group 2 (young women, ≤4 8y), group 3 (older men, > 48y) and group 4 (older women, > 48y). **Results:** Height and weight were significantly lower in women than in men at the same age. Conversely, Alx (32.7±9.8% vs. 20.1±11.7%, p<0.01), Alx75 (29.6±6.7% vs. 18.3±9.4%, p<0.01) and central systolic blood pressure (136±30 vs. 125±23 mmHg, p=0.03) were higher in women than men. The menopausal women had the worst indexes of aortic wave reflection. **Conclusion:** Women patients had both higher reflected aortic pressure wave form and central blood pressure indexes and these findings were worsened by the menopausal status.

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P316

Gonad Dependent Changes in Kallikrein Kinin System Behavior and Renal Electrolyte Excretion After Rectifying Outer Medulla K^+ Channel Blockade in Spontaneously Hypertensive Rats

PrimaryAuthor.AuthorBlock:**Dario Guevara**, Elisabet M Oddo, Alejandro F Celìa, Martin Solernó, Luis A Di Ciano, Fernando R Ibarra, Elvira E Arrizurieta, Pablo J Azurmendi, IIM A Lanari, UBA, Buenos Aires, Argentina

The kallikrein kinin system (KKS) is involved in blood pressure (BP) regulation by a mechanism not completely defined. Our previous reports have shown that high K^+ intake or prepuberal gonadectomy (Gx) diminish BP with a simultaneous increase in urine kallikrein activity (UKa) and plasma aldosterone (PA) levels, revealing a link between those systems. Thus, since K^+ may be involved in the regulation of KKS, we explored the rectifying outer medulla K^+ (ROMK) channel blockade using glibenclamide (Gli) in different gonad contexts.Spontaneously hypertensive rats of both sexes, half of them Gx at weaning, were studied at 12 weeks of age (n = 32). Glucose solution (4%) with or without Gli (10 mg/kg bwt) was orally administered in the last 3 days of the experiment. We analyzed BP, glomerular filtration rate, PA, daily urine Na^+ and K^+ excretion and UKa. Renal cortex kallikrein activity (RKa) and UKa were determined by colorimetric assay. Renal mRNA levels of Kcjn1 (ROMK), Atp1α1 (Na^+K^+Atpase) and Klk1 (kallikrein 1) genes were determined by quantitative real time PCR. Urine Na^+/K^+ increased after Gli treatment (0.55 ± 0.03 *vs* 1.34 ± 0.30, $p<0.05$) due to a K^+ excretion decrease in intact male and ovariectomized rats and to a Na^+ excretion increase in intact female. These changes were concomitant with increased GFR within the normal range (0.51 ± 0.06 *vs* 0.76 ± 0.06 ml/min/100g bwt $p < 0.01$) and no differences in BP and PA among groups. After Gli treatment, renal cortex Klk1 and RKa levels increased in intact males (297 and 137 %, $p < 0.05$, respectively), while in orchidectomized group Klk1 and UKa levels increased (179 and 230 %, $p < 0.05$; respectively). Kcjn1 and Atp1α1 mRNA levels decreased in renal medulla of all groups (1.29 ± 0.28 *vs* 0.32 ± 0.11 and 0.94 ± 0.23 *vs* 0.20 ± 0.02, $p < 0.01$, respectively). Taken together, gonad dependent changes were seen in urine Na^+ and K^+ excretion and KKS behavior after ROMK blockade without changes in the BP and aldosterone. Moreover, the repression in Kcjn1 and Atp1α1 genes could be related to the observed ion transport changes.

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P317

Sex Differences in the Hemodynamic and Sympathetic Responses to Centrally Administered Tumor Necrosis Factor-α in Rats

PrimaryAuthor.AuthorBlock:**Shun-Guang Wei**, Yang Yu, Robert B. Felder, Univ of Iowa Carver Coll of Med, Iowa City, IA

Introduction: Accumulating evidence indicates that sex differences exist in the clinical and experimental outcomes of various cardiovascular diseases. In addition to its protective effect on renin-angiotensin system activity, estrogen has an anti-inflammatory influence. The central actions of pro-inflammatory cytokines (PICs) contribute significantly to cardiovascular and autonomic dysfunction in hypertension and heart failure. In male adult rat, central administration of PICs induces substantial increases in blood pressure (BP), heart rate (HR) and renal sympathetic nerve activity (RSNA), and blocking PICs reduces sympathetic excitation in experimental models of hypertension and heart failure. Whether PICs have similar central sympatho-excitatory effects in the female rat remains unknown. **Hypothesis:** We hypothesized that female rats may be protected from the central cardiovascular and autonomic effects of PICs. **Methods:** Urethane anesthetized male and female Sprague Dawley rats (10-12 weeks) underwent an intracerebrovascular (ICV) injection of the prototypical PIC tumor necrosis factor-α (TNF-α, 100 ng). BP (mmHg), HR (beats/min) and RSNA (% change) responses were continuously recorded for 4-5 hours. **Results:** In male rats (n=6), ICV TNF-α induced a dramatic and long-lasting increase (*p<0.001 vs. baseline) in BP (23.1 ± 2.5*), HR (82 ± 8*) and RSNA (109.5 ± 4.3 %*), that began within 20-30 mins and peaked at 90-120 mins after ICV injection. In the female rats (n=6), ICV TNF-α elicited significantly (p<0.05) smaller increases (*p<0.001 vs. baseline) in BP (14.8 ± 1.8*), HR (55 ± 6*) and RSNA (78.5 ± 6.3*), compared with the male rats. **Conclusion:** These data demonstrate a sex difference in the cardiovascular and sympathetic responses to centrally administered PICs. Whether the observed differences can be explained by an estrogen effect on TNF-α signaling per se or by an estrogen effect on TNF-α-induced renin-angiotensin activity remains to be determined. However, a reduced response of female rats to central inflammation may be an important contributor to sex differences in pathophysiology of hypertension and heart failure.

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P318

Signaling Pathways Triggered by the Mas Receptor

PrimaryAuthor.AuthorBlock:Valeria Burghi, Univ of Buenos Aires, Sch of Pharmacy and Biochemistry, Dept of Biological Chemistry, IQUIFIB, Buenos Aires, Argentina; Emiliana B Echeverria, Univ of Buenos Aires, Sch of Pharmacy and Biochemistry, ININFA, Buenos Aires, Argentina; Diego T Quiroga, Marina C Munoz, Univ of Buenos Aires, Sch of Pharmacy and Biochemistry, Dept of Biological Chemistry,

IQUIFIB, Buenos Aires, Argentina; Natalia C Fernandez, Univ of Buenos Aires, Sch of Pharmacy and Biochemistry, ININFA, Buenos Aires, Argentina; **Fernando P Dominici**, Univ of Buenos Aires, Sch of Pharmacy and Biochemistry, Dept of Biological Chemistry, IQUIFIB, Buenos Aires, Argentina

Mas receptor (MasR) is a class A Orphan G-protein-coupled receptor (GPCR). Although angiotensin-(1-7) [Ang-(1-7)] has been reported as its putative ligand, the intracellular signaling pathways activated by the MasR remain only partially characterized. In this study we examined MasR-dependent activation of G protein-mediated and ERK mediated signaling pathways. With that aim in human lung carcinoma A549 cells we evaluated Gq-coupled activity by monitoring changes in intracellular Ca^{2+} levels after Ang(1-7) stimulation. Incubation with 100nM Ang-(1-7) failed to increase Ca^{2+} levels either in endogenously expressing or MasR overexpressing A549 cells. On the other hand, transfection of HEK293T cells with a wild-type MasR (wt-MasR) construct resulted in a significant decrease in basal cAMP levels ($p<0.05$) that depended on the amount of wt-MasR protein expressed but was not observed when increasing amounts of mutant MasR lacking the PDZ binding motif were expressed. Pretreatment of wt-MasR expressing cells with pertussis toxin restored basal cAMP levels ($p<0.05$) whereas no effect was observed in mock-transfected cells. Also, cAMP production after forskolin stimulation was lower in cells expressing wt-MasR than in control cells. These results indicate a high level of constitutive receptor activity towards cAMP modulation involving Gαi-protein. Treatment with Ang-(1-7) increased p-ERK levels in a concentration-dependent manner in both wt-MasR and in mock-transfected HEK293T cells. In view of these results we are analyzing whether ERK activation is mediated through Ang-(1-7) binding to endogenously expressed receptors different from MasR. As MasR has been suggested to participate in cardiovascular and renal functions, comprehensive pharmacological characterization of MasR signaling is essential for developing clinical therapeutics targeting MasR function.

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P319

5-HT7 Receptors Mediate 5-HT-induced Hypotension

PrimaryAuthor.AuthorBlock:**Stephanie W Watts**, Bridget M Seitz, Gregory D Fink, Michigan State Univ, East Lansing, MI

Serotonin (5-HT, 5-hydroxytrypamine) lowers blood pressure in the conscious rat when given chronically (25 µg/kg/min; $\geq$24 hours). This same dose of 5-HT nearly normalizes the mean arterial blood pressure of hypertensive rats, including the deoxycorticosterone acetate (DOCA) salt hypertensive rat (baseline =166$\pm$9 vs 112$\pm$3 mm Hg). We have found that 5-HT causes venodilation through activation of the 5-HT_7 receptor and presently test the hypothesis that the hypotension stimulated by 5-HT *in vivo* is 5-HT_7 receptor-dependent. Two approaches were made. First, male Sprague Dawley rats were instrumented with radiotelemeters for measurement of blood pressures, HR and activity. Through a venous line, the 5-HT_7 receptor antagonist SB269970 (3 mg/kg, i.v.) was given hours prior to implantation of all rats with an Alzet pump delivering 5-HT (as above). SB269970 abolished the fall in blood pressure

during 24 hours of 5-HT-infusion when compared to vehicle (vehicle = -18.73±2.0 vs SB269970 = -.7±1.51 mm Hg; N=6). Second, we created a global 5-HT_7 receptor knockout (KO) rat through CRISPR-Cas9 technology using a Sprague Dawley background. Genotyping validated creation of deletion of exon 1-2 and/or indel formation on exon 1 and exon 2 of the 5-HT_7 receptor gene. In female KO (N=4) vs WT (N=4), baseline mean arterial blood pressure in radiotelemeter-implanted rats was significantly elevated (KO=111±1 mm Hg; WT=103±2 mm Hg; $p<0.05$). Infusion of the same dose of 5-HT (25 µg/kg/min) which lowered mean pressure in the WT rats (baseline: 103.5±1.85 to 89.9±3.06 mm Hg 24 hours) did not lower blood pressure in the KO at 24 hours (111.7±1.1 to 117.5±1.0 mm Hg) nor through 6 more days of infusion. Blood pressure reduction in the WT was accompanied with an elevation in heart rate (410±8 to 474±5 bpm; $p<0.05$) that was not observed in the 5-HT_7 receptor KO (387±10 to 395±4.3 bpm; $p>0.05$). Thus, pharmacological and molecular work powerfully support the necessity of the 5-HT_7 receptor for 5-HT-induced hypotension. The significance of this study capitalizes on the unique pharmacology of the 5HT_7 receptor and provides potential insight into the development of new treatment for CV diseases.

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Cooperative Signaling and Membrane Recruitment of the Dopamine-1 (D_1R) and Angiotensin Type 2 (AT_2R) Receptors in Human Renal Proximal Tubule Cells (RPTC)

PrimaryAuthor.AuthorBlock:**John J Gildea**, Micah J Mallory, Robin A Felder, The Univ of Virginia, Charlottesville, VA

The D_1R and AT_2R are interdependent natriuretic receptors that are critical for the regulation of renal sodium balance and are implicated in both hypertension and salt-sensitivity. D_1R and AT_2R stimulation with the D_1R agonist fenoldopam (FEN) and Angiotensin III (AngIII), selectively increases cAMP and cGMP, respectively, leading to D_1R and AT_2R recruitment to the cell surface (measured by fluorescent extracellular receptor epitope specific antibodies). The interdependence of the D_1R and AT_2R recruitment was investigated following AT_2R stimulation with AngIII (10 nmol/L 1 hour) which leads to D_1R cell surface recruitment using a normally D_1R/Gs coupled cell line (i22) (VEH 8209±863, AngIII 19485±3425 RFU, n=6, $p<0.05$) but not in a D_1R uncoupled from Gs cell line (i19). These data were verified by increasing intracellular sodium with monensin (100 µM, 1 hr) and measuring cell surface binding with a fluorescent labelled D_1R agonist SKF83566 (10 nmol/L, 1 hr, i22 1.67±0.7 fold, n=5, $p<0.0001$, i19, NS). The importance of dopamine in regulating these receptor actions was shown by stimulating the AT_2R with AngIII and measuring D_1R recruitment followed by blockade of amino acid decarboxylase using either carbidopa or benzeraside (100 µM each, 1 hour). Additionally, FEN stimulated D_1R surface recruitment is blocked by the AT_2R inhibitor PD123319 (1 µM, 1 hour) and the coupling defect is fully rescued by 8Br-cGMP (1 mmol/L, 1 hour), a cell permeable second messenger

normally thought to signal specifically through the AT_2R. We then show that FEN stimulation leads to increased production of AngIII only in the D_1R/Gs coupled i22 cell line (5.82±0.43 fold, n=6, p<0.05) but not in i19 the D_1R/Gs uncoupled cell line and this production was blocked with an aminopeptidase A inhibitor, EC-33 (10 µmol/L, 1 hour). Addition of 8Br-cAMP (1mmol/L 1 hour) not only leads to an increase in AngIII production, but also cGMP production (4.33±0.36 fold, n=6, p<0.05) that is blocked by PD123319 (potent, selective, non-peptide angiotensin AT_2R antagonist, 1 µM, 1 hour). In summary we not only show that dopaminergic stimulation leads to increased AngIII production in a D_1R coupled manner, but also that AngIII stimulation of AT_2R leads to dopaminergic activation in a D_1R coupled manner.

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Clozapine-induced Hypertension: Role of the Dopamine Type 4 Receptor (D4R) in Human Renal Proximal Tubule Cells (RPTC)

PrimaryAuthor.AuthorBlock:Peng Xu, The Univ of Virginia, Charlottesville, VA; Dianna L Kelly, Univ of Maryland, Baltimore, MD; Christopher Kitchen, The Univ of Maryland, Baltimore, MD; John J Gildea, Katie A Schiermeyer, The Univ of Virginia, Charlottesville, VA; Pedro A Jose, George Washington Univ, Washington DC, DC; Sarah M Norman, Robert P McMahon, The Univ of Maryland, Baltimore, MD; Z Wang, George Washington Univ, Washington DC, DC; **Robin Felder**, The Univ of Maryland, Charlottesville, VA

Clozapine (CLZ), a potent D_4R antagonist that is an effective antipsychotic drug for the treatment of schizophrenia, is associated with hypertension. The hypertensive side effect of CLZ may be through inhibition of the D_4R because germline deletion of D_4R in mice causes hypertension. The hypertension caused by germline deletion of D_4R may, in part, be due to increased renal expression of the angiotensin type 1 receptor (AT_1R) and renal expression of sodium exchangers, transporters, and pumps, e.g., NHE3, NKCC2, NCC, and Na^+K^+/ATPase, α subunit. CLZ administered subcutaneously (20mg/kg/day/x3 days, n=5) in mice induced hypertension, shifted the pressure-natriuresis plot to the right, and increased the renal expressions of NCC and Na^+K^+/ATPase, α subunit, relative to vehicle-treated mice (n=5). Activation of D_4R decreased AT_1R expression and Na^+K^+/ATPase activity in rat renal proximal tubule cells (RPTCs). Therefore, we studied two groups: subjects 18 to 75 years old with a DSM-IV diagnosis of schizophrenia or schizoaffective disorder treated with CLZ for >2 months (CLZ, N=32) and untreated healthy controls (**HC**, N=20) without psychiatric illness. There were no significant differences in blood pressure (BP) between the two groups since more than half of the CLZ participants received antihypertensive medication. We studied RPTCs cultured from the participant's urine (CLZ, N=18; **HC,** N=9) and treated those cells with 100 nM CLZ. CLZ decreased D_4R expression in both **HC** and CLZ groups (**HC**, VEH 1.04±0.17 vs CLZ 0.73±0.09, n=9, paired t-test, P<0.05; CLZ, VEH 1.02±0.09 vs CLZ 0.75±0.06, n=16, paired t-test, P<0.01). CLZ also increased reactive oxygen species (Rosstar550 assay) in the **HC** but not CLZ-treated group (**HC**, VEH 0.92±0.07 vs CLZ 1.02±0.09, n=8, **paired t-test**, P<0.05; CLZ VEH 1.09±0.18 vs CLZ 1.07±0.18, n=14). The density of plasma membrane AT_1R tended to be higher in CLZ-treated hypertensive (CLX HP) than the CLZ-treated normotensive participants (CLZ NP): (**HC**, 0.28±0.08 n=8; CLZ NP, 0.10±0.02 n=4; CLZ

HP, 0.81±0.31 n=7). Thus, CLZ may induce hypertension through a D_4R/AT_1R-mediated mechanism similar to that reported in D_4R knockout mice. Additional studies will determine if CLZ increases sodium transport in human RPTCs.

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P322

Interaction of Shroom3 With Fyn Impacts Phosphorylation of Nephrin Causing Proteinuria With Foot Process Effacement

PrimaryAuthor.AuthorBlock:**Madhav C Menon**, Nimrod Philippe, Chengguo Wei, Ruijie Liu, Weijia Zhang, Zhengzi Li, John C He, Barbara Murphy, Mount Sinai Sch of Med, New York, NY

In the translational GoCAR study, we identified that a CKD-associated SHROOM3-SNP, and tubular *Shroom3* expression correlated with the development of fibrosis in renal allografts. We showed that SHROOM3 facilitated TGF-B signaling suggesting its potential role as a therapeutic target. However, recent data suggest a *protective* role for SHROOM3 in proteinuria and glomerular development. To study the role of SHROOM3 in adult glomeruli, we used doxycycline-inducible (DOX), shRNA-mediated SHROOM3 knockdown, Podocin- and tubular-specific (PAX8)-RTTA mice, comparing these to non-transgenic DOX-fed littermates. After 2wks of DOX, adult Podo-RTTA mice developed significant albuminuria compared to littermates. Albuminuria was reversible on DOX-withdrawal, and reappeared on re-initiation. No podocyte loss [WT1 stain] was seen in these mice(8-wks DOX). EM revealed >50% foot process effacement. PAX8-RTTA mice did not show proteinuria. Glomerular RNA- seq identifed intracellular signaling/Small GTPAse signaling/integrin signaling/ actin-cytoskeleton among downregulated Gene-ontology terms in knockdown mice vs Controls. To identify protein interacting partners of SHROOM3, we performed mass spectrometry on protein lysates of 293-T cells overexpressing SHROOM3 immunoprecipitated (IP) with either anti-V5, -SHROOM3 or IgG. Among 491 unique interactions, we confirmed SHROOM3 as the top ranking protein. Interestingly, FYN - a src-kinase - was a top ranking candidate. Podocyte FYN is crucial for NPHS1-phosphorylation, and FYN-deficient mice show identical proteinuria phenotype. In human podocytes, we confirmed the interaction of endogenous SHROOM3 and FYN by IP. Glomerular protein extracts of Shroom3-knockdown mice showed decreased phosphorylation of FYN, and NPHS1. In human allografts from GoCAR, we identifed a corresponding reduced albuminuria (>1year post-transplant) associated with homozygosity of the risk allele in the donor. In summary, Podocyte-specific SHROOM3 knockdown causes a reversible proteinuria phenotype in adult mice, by interacting with FYN, a mechanism distinct from its effect on renal fibrosis in allografts.

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P325

6β-hydroxytestosterone, a Cytochrome P450 1B1 Metabolite of Testosterone, Contributes to Angiotensin II-Induced Abdominal Aortic Aneurysms in Hyperlipidemic Male Mice

PrimaryAuthor.AuthorBlock:**Kamalika Mukherjee**, Ajeeth K Pingili, Ahmad N Dhodi, Kafait U Malik, Univ. of Tennessee Health Science Ctr, Memphis, TN

It is well established that men have a higher risk of developing cardiovascular diseases including hypertension and abdominal aortic aneurysms (AAAs) than do premenopausal women of the same age. Angiotensin II (Ang II) induces abdominal aortic aneurysms (AAA) to a greater degree in male than in female mice, and castration protects male mice against Ang II-induced AAAs. Previously we reported that cytochrome P450 (CYP) 1B1 contributes to Ang II-induced hypertension as well as AAAs in male mice. Recently we have shown that CYP1B1-testosterone derived metabolite 6β-hydroxytestosterone (6β-OHT), mediates Ang II-induced hypertension and cardiac and renal fibrosis in male mice. The current study was conducted to determine the contribution of CYP 1B1-generated 6β-OHT to Ang II-induced AAAs in male mice. Sixteen-week old intact or castrated male, *ApoE$^{-/-}$/ Cyp1b1$^{+/+}$* (ApoE KO) and *ApoE$^{-/-}$ / Cyp1b1$^{-/-}$* (DKO) mice were infused with 700 ng/kg/min Ang II or its vehicle (s.c.) with osmotic minipumps for 28 days. These mice were also injected with 6β-OHT (15 µg/g body weight, i.p. every 3rd day) or its vehicle for the duration of the experiment. The abdominal aortas were analyzed for development of AAAs (a 50% increase in external abdominal aortic diameter). Ang II significantly increased (P<0.05) the incidence as well as severity of AAAs in intact ApoE KO mice (66.7% incidence; 8 of 12), compared to 0% (0 of 5) incidence in vehicle-treated mice, which were minimized in castrated ApoE KO (0% incidence; 0 of 9) and intact DKO (0% incidence; 0 of 9) mice. Administration of 6β-OHT restored the incidence and severity of AAAs in Ang II-infused castrated ApoE KO (44.4% incidence; 4 of 9) and intact DKO (62.5% incidence; 10 of 16) mice. In contrast, treatment with testosterone failed to increase the incidence and severity of Ang II-induced AAAs in the intact DKO mice (10% incidence; 1 of 10). Histological analysis of sections of the abdominal aortas confirmed the above results, along with disruption of elastin fibers, a pathological hallmark of AAAs. These data suggest that CYP1B1-generated testosterone metabolite, 6β-OHT, contributes to Ang II-induced AAAs in hyperlipidemic male mice. Therefore, inhibitors of CYP1B1 could be useful in the treatment of AAAs in males with hyperlipidemia.

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P326

Effects of D_1R/AT_2R Coupling on Aminopeptidase N (APN) in Human Renal Proximal Tubule Cells (RPTC)

PrimaryAuthor.AuthorBlock:**Micah J Mallory**, John J Gildea, Peng Xu, Robin A Felder, UNIVERSITY OF VIRGINIA Dept of Pathology, Charlottesville, VA

Dopamine receptor type 1 (D_1R) and angiotensin receptor type 2 (AT_2R) uncoupling from their intracellular second messengers are implicated in both salt sensitivity of blood pressure (SS) and hypertension. Angiotensin III (Ang III) and dopamine (DA) work cooperatively through binding to the interdependent D_1R and

AT_2R to regulate renal sodium balance through autocrine signaling pathways. Aminopeptidase N (APN) is a renin-angiotensin system (RAS) enzyme localized in RPTC responsible for converting the natriuretic peptide AngIII into the anti-natriuretic agonist angiotensin IV (AngIV). The expression of APN (measured by a fluorescently labelled CD13 antibody) was investigated after increasing intracellular salt concentration with both the ionophore monensin (100 µM, 1hr.) and NaCl buffer solution (50 mmol/L, 1 hr.) in known G protein coupled (i22) and G protein uncoupled (i19) RPTC. Increasing intracellular sodium resulted in a more dramatic decrease in overall APN expression in uncoupled cells (i19 vehicle (VEH) 1.00 ± 0.03, Mon 0.87 ± 0.01 fold, n=3, $p<0.05$) compared to a slight decrease in coupled cells (i22 VEH 1.00 ± 0.01, NaCl 0.96 ± 0.01 fold, n=6, $p<0.05$). Two unknown coupling urine cell lines (RMC031-202 and RMC031-6) were evaluated alongside these known cells in an attempt to classify each as coupled or uncoupled. RMC031-202 replicated i22 (coupled) trends while RMC031-6 closely replicated i19 (uncoupled) data. Moreover, baseline APN expression comparison (normalized to i22) between the cell lines reveled a significantly elevated baseline for known and suspected uncoupled cells (i19 1.69 ± 0.047, RMC031-6 2.01 ± 0.004 fold over i22, n=3, $p<0.05$). Known (i22) and suspected (RMC031-202) normally coupled cells had equivalent baselines ($p>0.05$). An uncoupling defect may result in decreased APN regulation in RPTC causing higher baseline levels of APN expression. These increased levels of APN, in turn, lead not only to amplified production of the anti-natriuretic agonist AngIV but also reduced potential for cooperative natriuretic AngIII/DA autocrine signaling thus disrupting the dopaminergic system's ability to suppress RAS and maintain proper sodium balance.

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P327

Inter-individual Difference in Sensitivity of Aversive Salt Taste

PrimaryAuthor.AuthorBlock:**Minoru Isomura**, Shamane Univ, Matsue, Japan; Toru Nabika, Shamane Univ, Izumo, Japan

Salt is an indispensable nutrient, but excessive amount of salt intake becomes harmful. In order to prevent excessive intake of salt, there is a mechanism to feel high concentration salt as an aversive taste. In recent years, it has clarified that high concentration salt stimulates different taste buds from those sensed by low concentration of salt. In this study, we analyzed relationship between inter-individual difference in sensitivity of aversive salt taste and amounts of daily salt intake. After obtained written informed consent, 1,254 individuals who came annual health checkups were recruited for this study. To test aversive salt taste, five different saline solutions (0.25%, 0.5%, 1%, 1.5%, 2%) were prepared. They started drinking of salt solution from low to high concentration of solutions. In each trial of drinking the salt solution, they were asked whether they were capable of drinking it. When they felt to avoid drinking the salt solution, salt concentration of the solution was recorded as sensitivity of aversive salt taste. Amount of daily salt intake was estimated by using spot urine and by dietary questionnaire. As a result, 784(63%) and 267(21 %) of individuals felt up to the 1.5% and 2% of salt solution as aversive salt taste, respectively. Hence, their sensitivities of aversive salt taste were 1.5% or 2% of salt

solutions, respectively. However, resting 203(16%) of individuals showed capability to drink 2% of salt solution. Although we compared sensitivities of aversive salt taste of each individual and daily salt intake that estimated by urine, there were no difference between them. However, daily salt intake that estimated by dietary questionnaire showed clear relationship, in which individuals with the high sensitivities of aversive salt taste consumed the more amount of salt.

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P328

Angiotensin II Inhibits the High Salt-induced Production of Sphigosine-1-phosphate in the Renal Medulla

PrimaryAuthor.AuthorBlock:Weili Wang, Junping Hu, Pin-Lan Li, Justin L Poklis, **Ningjun Li**, Virginia Commonwealth Univ, Richmond, VA

We have previously shown that sphigosine-1-phosphate (S1P) produces natriuretic effects via activation of S1P receptor 1 in the renal medulla and that this natriuretic effect may be through inhibition of epithelial sodium channel. The present study examined the expression of the enzymes that produce S1P in the renal medullary tissue and tested the hypothesis that angiotensin II (ANG II) reduces the expression of S1P-producing enzyme and thereby the levels of S1P in the renal medulla. Male adult C56BL/6 mice, 10-12 weeks old, were treated with a low salt diet (LS, 0.4%), high salt diet (HS, 4% NaCl) or HS + ANG II (600ng/kg/min, S.C.) for 10 days. A high salt diet increased the level of S1P, whereas ANG II significantly inhibited the HS-induced increase of S1P levels in the renal medullary tissue. The levels of S1P were 6.6 ± 0.34, 11.4 ± 1.33 and 3.5 ± 0.49 pmol/mg of tissue in LS, HS and HS + ANG II group, respectively. There were no difference in the levels of sphingosine kinase 1 (SPHK1), the enzyme that produces S1P by phosphorylating sphingosine, among the different groups of mice by Western blot analysis. However, a high salt diet increased the protein levels of acid ceramidase (ACDase), an upstream enzyme that produces sphingosine, the substrate for SPHK1. This HS-induced increase in ACDase was inhibited by ANG II. The relative protein levels of ACDase were 1.0 ± 0.07, 1.4 ± 0.07 and 0.17 ± 0.11 in LS, HS and HS + ANG II group, respectively. These results demonstrated that a high salt diet increased the levels of S1P in the renal medulla, probably by increasing the level of one of the S1P-producing enzymes ACDase, and that ANG II reduced the levels of ACDase and S1P in the renal medulla. Given the diuretic effect of S1P, ANG II-induced reduction of S1P production in the renal medulla may be a mechanism contributing to the sodium retention and hypertension associated with excessive ANG II. (Support: NIH grant HL89563)

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P329

Sympathetically Alpha-1 Adrenoceptor Mediated Regulation of the NCC in Rat Models of Salt Sensitive and Neurogenic Hypertension

PrimaryAuthor.AuthorBlock:**Richard D Wainford**, Alissa A Frame, Boston Univ Sch Med, Boston, MA

Hypothesis Excess SNS release of norepinephrine (NE) increases NCC activity, via an α1 adrenoceptor pathway, to drive the development and maintenance of salt-sensitive and neurogenic hypertension (HTN).
Methods Male Sprague-Dawley (SD) rats receiving a continuous s.c. saline or NE (600ng/min) infusion alone or in combination with terazosin (10mg/kg/day) were fed a 14-day 0.6% (NS) or 4% NaCl (HS) diet. Separate groups of male SD rats received a continuous s.c. NE infusion and 28-day NS or HS intake, on day 14 of HS a sub group of rats were switched to a co NE-terazosin s.c. infusion. Groups of male SHR rats received s.c. saline or terazosin for 14 days. Endpoint measurements (day 14 or 28) were basal MAP and NCC activity (peak natriuresis to iv hydrochlorothiazide (HCTZ; 2mg/kg) infusion and phosphoNCCT58 immunoblotting) and expression (via immunoblotting) was assessed (N=4/gp).
Results NE infused SD rats exhibit HTN and fail to suppress NCC expression and activity during HS-intake. α1-adreoceptor antagonism (confirmed pharmacologically) abolished the salt-sensitive component of NE HTN and restored dietary sodium evoked suppression of the NCC in NE infused SD rats. Critically, α_1-adrenoceptor antagonism lowers BP and reduces NCC activity in established SHR HTN and restores sodium-evoked suppression of NCC activity and abolishes the salt sensitivity of BP in established NE-evoked SS HTN.
Conclusion SNS activation of the NCC by NE occurs in rat models of neurogenic and SS HTN. Our data demonstrates antagonism of α_1-adrenoceptors lowers BP and NCC activity in established SS and neurogenic HTN and suggests α_1-antagonists as a therapeutic option in sympathetically mediated HTN.

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Customization of Low-sodium Diet and Its Efficacy

PrimaryAuthor.AuthorBlock:**Srividya Kidambi**, Andrea Moosreiner, Merrill Rubens, Brittaney Obi, Michael Widlansky, Sudhi Tyagi, Venkata Puppala, Andreas Beyer, Allen Cowley, David Mattson, Theodore Kotchen, Mingyu Liang, Medical Coll of Wisconsin, Milwaukee, WI

Introduction: Lack of adherence with a low-sodium (Na) diet remains a challenge for many patients. Most research studies use standardized diets for subjects which may decrease adherence and hence result in sub-optimal lowering of blood pressure (BP). We hypothesized that adoption of a customized low-Na diet will result in high rates of adherence, lower BP, and improve vascular function. **Methods:** Post-menopausal women (50-65 years) and men (30-65 years) with baseline BP ≥ 130/80 mm Hg or history of HTN were recruited. Subjects were placed on a 2-week 1200mg Na/day diet (after a 3-day run-in period during which subjects were given 2400 mg of Na/day in addition to their regular diet). Subjects were off all BP medications 1 week prior to the start of the diet and during 2 weeks of study diet. All food was prepared by a bio-nutritionist with a customized menu according to food logs kept by the subjects. Two 24h urine Na measurements were taken to ensure compliance with the diet. In addition to BPs, conduit (brachial artery flow-mediated dilation (FMD)) and micro (~ 200 μm extracted from

gluteal adipose tissue biopsies) vessel functions were evaluated before and after the diet period to assess in vivo and in vitro vascular function respectively. **Results:** Seventeen subjects (9 African-American, 7 Caucasian, and 1 Hispanic) with mean BPs of 145 ± 14/87 ± 6 mm Hg (± SD) before starting the low-Na diet were recruited. During the diet period, subjects consumed an average of 1000 mg of Na/day (66% reduction from baseline Na intake). Excluding 2 subjects in whom the 24h urine Na did not drop (instead increased) after 2-weeks indicating non-adherence, the average SBP/DBP reductions were -11 ± 14 (-7%, p=0.06)/-8 ± 10 mm Hg (-9%, p=0.04). Average urine Na levels were 230 ± 83 (before) and 71 ± 82 (after) mmol/24hr. Brachial artery FMD improved from 7.3 ± 3.2 to 9.6 ± 2.3 % (p=0.04) after 2 weeks. Maximal Arteriolar FMD (endothelial dependent) trended toward an increase from 54 ± 18 to 77 ± 13 % and smooth muscle dependent dilation to papaverine was not effected. **Conclusions:** The adoption of a customized low-Na diet can result in high rates of dietary adherence (88% in the current study) and lower BPs. In addition, vascular function improves within 2 weeks on a low-Na diet.

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P331

Angiotensin-salt Hypertension Requires Ouabain-sensitive Na^+ Pumps

PrimaryAuthor.AuthorBlock:Ling Chen, Univ Maryland Med Sci, Baltimore, MD; Jerry B. Lingrel, Univ of Cincinnati Coll of Med, Cincinnati, OH; John M. Hamlyn, **Mordecai P Blaustein**, Univ Maryland Med Sci, Baltimore, MD

Dietary salt is a major factor in the pathogenesis of essential hypertension (EH), but the underlying links are unresolved. Animal models indicate that angiotensin (Ang) II and high dietary salt (HS) are convergent signals that act via the brain to elevate blood pressure (BP). Low-dose sc Ang II+HS is a common model for EH. We tested the Na^+ pump ouabain binding site's role in this model because it is crucial in some other hypertension models (e.g., ACTH and Nedd4-2-knockout +HS). Mice that express Na^+ pumps with a mutant, ouabain-resistant α2 catalytic subunit ($\alpha2^{R/R}$; cation transport is normal), and wild type (WT), ouabain sensitive controls ($\alpha2^{S/S}$) were studied. [80-90% of rodent artery myocyte Na^+ pumps are ouabain-resistant ($\alpha1^{R/R}$); only 10-20% are α2.] BP was measured by telemetry. First, 3 basal 24 hr BPs were recorded. Osmotic 4-week minipumps were then implanted sc in all mice to deliver vehicle (saline; Expt. #1,3), or 400 (Expt. #1,2) or 800 (Expt. #3) ng/kg/min Ang II; simultaneously, in Expt. #2, the diet was switched from 0.4% (standard) to 2% NaCl (HS). BPs were monitored every 3-4 days for up to 4 weeks. Also, in Expt. #2, on day 21, all mice received 2 ip injections, 4 hrs apart, of 10 mg/kg DigiFab, Fab fragments that immuno-neutralize ouabain, while BP was continuously monitored; on day 23, the mice received 2 ip injections of CroFab, anti-crotalus toxin ('control') Fab fragments. **Results**: 1. Basal mean BP (MBP) was 10±2 mm Hg higher in $\alpha2^{R/R}$ than in WT mice (P<0.01; n=21 & 29; ANOVA). 2. In WT mice, 400 ng/kg/min sc Ang II and Ang II+HS raised MBP by 15±1 and 34±1 mm Hg, respectively (P<0.01; n=7-8; ANOVA). 3. The MPB elevation in Ang II+HS $\alpha2^{R/R}$ (17±2 mm Hg) was only half that in WT mice (P<0.01; n=7 each; ANOVA). 4. DigiFab rapidly (<1 hr) reduced MBP by 14±2 mm Hg in Ang II+HS

hypertensive WT mice (P<0.001; n=7; T-test), but not in $\alpha2^{R/R}$ mice (P<0.01; n=7 each; ANOVA); CroFab did not lower MBP in either strain. 5. 800 ng/kg/min sc Ang II elevated systolic BP by 55±3 mm Hg in WT mice, but by only 37±3 mm Hg in $\alpha2^{R/R}$ mice (P<0.05; n=3-5; ANOVA). **Conclusions:** Ouabain-sensitive α2 Na^+ pumps and their endogenous ligand are both required for full expression of low-dose Ang II-salt hypertension. Ouabain-sensitive α2 pumps apparently also contribute to high-dose Ang II-hypertension.

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P332

Dietary Salt Intake and Blood Pressure Control in Hypertensive Individuals Under Antihypertensive Treatment During 7 Years

PrimaryAuthor.AuthorBlock:**Kazuto Ohno**, Hiroyuki Takase, Masashi Machii, Daishi Nonaka, Enshu Honspital, Hamamatsu, Japan; Tomonori Sugiura, Nobuyuki Ohte, Nagoya City Univ Graduate Sch of Medical Sciences, Nagoya, Japan; Yasuaki Dohi, Nagoya Gakuin Univ, Seto, Japan

Purpose: Excess salt intake is one of the most important causes of hypertension. Salt restriction is a key strategy in the management of hypertension and, thus, should be instructed for hypertensive patients under medical treatment. We investigated recent changes in dietary salt intake and blood pressure (BP) levels in hypertensive patients. **Methods:** Total of 12422 hypertensive subjects (male 71.0% [8814 of 12422], 64.6±9.2 year-old) under medical treatment who visited our hospital for a physical checkup from 2010 to 2016 were enrolled. They were divided into 3 groups according to the number of antihypertensive drugs prescribed (1, 2 and ≥3 drugs). Cross-sectional analyses were performed using data in each year and changes during the 7 years were investigated. Individual salt intake was estimated using a spot urine by a previously reported method. **Results:** BP levels and the accomplishment rate of the target BP (<140/90mmHg) were improved in each group during the 7 years without significant difference among the groups (Overall 2010 to 2016; BP 132.7±13.6/80.0±8.9 to 128.8±13.7/76.3±9.6 mmHg and accomplishment ratio 65.6 [968 of 1475] to 76.4% [1433 of 1875]). However, individual salt intake was gradually increased in all groups (2010 to 2016 in 1, 2, and ≥3 drugs; 11.7±3.7 to 12.2±4.0, 11.9±3.7 to 12.7±3.9, and 12.2±3.9 to 12.9±4.1 g/day, respectively) and the accomplishment rate of salt restriction (<6g/day) was significantly reduced in subjects with increased number of antihypertensive drugs (3.5 [225 of 6435], 2.8 [125 of 4564], and 2.3% [33 of 1423] in groups with 1, 2, and ≥3 drugs, respectively). The accomplishment rate of the target BP was significantly higher in patients who achieved salt restriction than in those who did not achieve salt restriction in all groups (Over all; 80.2 [307 of 383] vs. 73.3% [8829 of 12039]). **Conclusions:** The control of BP in individuals with antihypertensive medications was improved in the last 7 years. However, salt restriction has not been successfully achieved especially in hypertensive patients with multiple antihypertensive medications. Excess salt intake may induce resistance to antihypertensive treatment and,

thus, increases the number of antihypertensive drugs for BP control.

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Repeated Measurementof Casual Urine Na/K Ratio May Provide Useful Information to Screen Early Stage Chronic Kidney Disease Patients With Higher Sodium and Lower Potassium Intake

PrimaryAuthor.AuthorBlock:Yuka Okuyama, **Haruhito A Uchida**, Okayama Univ Grad Sch Med, Okayama, Japan; Toshiyuki Iwahori, Shiga Univ of Medical Science, Otsu, Japan; Ryoko Umebayashi, Ayako Kato, Okayama Univ Grad Sch Med, Okayama, Japan; Katsuyuki Miura, Hirotsugu Ueshima, Shiga Univ of Medical Science, Otsu, Japan; Jun Wada, Okayama Univ Grad Sch Med, Okayama, Japan

Objective: Lowering sodium-to-potassium ratio has been reported to benefit people for hypertension prevention and control in epidemiological studies. Four to seven repeated measurements of casual urine sodium-to-potassium ratio is known to provide high correlation and good agreement quality with less bias to estimate 7-day 24-hour urinary Na/K ratio in normotensive and hypertensive individuals. However, little is known about urinary Na/K ratio in patients with chronic kidney disease (CKD). The aim of this study was to clarify the relationship of the repeated measurement of casual and 24-hour urinary sodium-to-potassium ratio in patients with CKD.
Design and method: A total of 61 inpatients with CKD, 31 in stage 1-3 (eGFR ≥ 30 ml/min/1.73m^2) and 30 in stage 4-5 (eGFR < 30 ml/min/1.73m^2), aged 20 to 85 under low-sodium diet (NaCl 6 g/day) were recruited in Okayama University hospital. Sodium-to-potassium ratio in casual urine at 4 points/day (first void after rising, each urine after breakfast, lunch or dinner) for 2 days and 2-day 24-hr urine at the same day were measured. Correlation and the quality of agreement by Bland and Altman between casual urine and 24-hour urine samples were analyzed.
Results: Mean 24-hour Na and K excretion was lower in participants in stage 4-5 (Na: 87.5 mmol/24h, K: 18.8 mmol/24h) than in participants in stage 1-3 (Na: 99.0 mmol/24h, K: 26.1 mmol/24h), whereas mean 24-hour urine Na/K ratio was higher in participants in stage 4-5 (5.1) than in participants in stage 1-3 (4.1). Casual urine Na/K ratio was strongly correlated with 2-day 24-hour urinary Na/K ratio by sampling 2 casual urine specimens per day for 2 days in participants in stage 1-3 (r = 0.69-0.78), but not in stage 4-5 (r = 0.12-0.19). The bias for mean Na/K ratio between 2-day 24-hour urine and sampling 2 casual urine per day for 2 days in participants in stage 1-3 ranged from -0.86 to 0.16, and the quality of agreement for the mean of this casual urine sampling was similar to that of all 8 points of casual urine samples for estimating 2-day 24-hour values.
Conclusion: Repeated casual urine Na/K ratio measurement may provide good estimate of 24-hour urine Na/K ratio, in stage 1-3 CKD patients as well as normotensive and hypertensive people; however, not in stage 4-5 CKD patients.

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Persistent Adrenomedullin Derivative Inhibits Development of Hypertension

PrimaryAuthor.AuthorBlock:**Sayaka Nagata**, Motoo Yamasaki, Kazuo Kitamura, Univ of Miyazaki, Miyazaki, Japan

Human adrenomedullin (hAM) consists of a 52 amino acid peptide that is amidated and has a disulfide bond. The bioactive peptide hAM has a variety of physiological functions, such as vasodilatation, hormone secretion, neurotransmission, embryogenesis, wound healing and immunoregulation. hAM has shown several therapeutic effects in experimental models of various diseases, including ischemic heart disease, inflammatory bowel disease, stroke and retinochoroidal disease. However, these therapies required continuous administration of hAM as the half-life of native hAM is quite short in blood. To resolve these issues, the aim of this study was to synthesize novel hAM derivative, and to examine its effect in spontaneously hypertensive rats (SHR). First, we conjugated the hAM N-terminal with 60kDa polyethylene glycol (PEG) (60 kDa PEG-hAM). To demonstrate that PEG binds covalently to α-amino residue of the N-terminal tyrosine of hAM, 60 kDa PEG-hAM was digested with cyanogen bromide and the AM(6-52) thus obtained was confirmed by ion exchange chromatography and MALDI-MS. Next, to determine plasma hAM concentrations, either 60 kDa PEG-hAM (10 nmol/kg) or native hAM was administrated subcutaneously with Wistar rat. We compared hAM concentrations in the peripheral blood of rats after injection with either 60 kDa PEG-hAM or native hAM. The hAM concentrations in the 60 kDa PEG-hAM group after 1, 7 and 10 days were 2600, 740 and 280 pM, respectively. In contrast, the hAM concentrations in the native hAM group after 1 day was 6.7pM, not detect 7 and 10 days. In addition, 60kDa PEG-hAM stimulated cAMP production in cultured human embryonic kidney cells expressing a specific AM receptor. Finally, we compared blood pressor after administrated subcutaneously with either 60 kDa PEG-hAM (20 nmol/kg) or saline in SHR with high salt diet. After 9 days, a single subcutaneous administration of 60 kDa PEG-hAM inhibited development of hypertension with SHR. In summary, the plasma half-life of 60 kDa PEG-hAM was much longer than native hAM, and subcutaneous injection of 60 kDa PEG-hAM in a single dose inhibited development of hypertension with SHR. Therefore, these results suggest that 60 kDa PEG-hAM is a possible therapeutic agent for the treatment of hypertension.

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P335

Greater Reduction in Sympathetic Tone following ET_B Receptor Blockade in Rats Lacking the Clock Gene Bmal1 during High Salt Diet

PrimaryAuthor.AuthorBlock:**Jermaine G Johnston**, Bryan K Becker, Chunhua Jin, David M Pollock, Univ of Alabama Birmingham, Birmingham, AL

The absence of diurnal oscillations in blood pressure is associated with increased cardiovascular morbidity and mortality. The clock gene *Bmal1* plays important roles in diurnal cardiovascular control as mice lacking *Bmal1* have lower blood pressure and lack a diurnal rhythm. Our lab has previously reported a global *Bmal1* knockout rat model that lacks a night-day difference in sodium excretion. Due to the importance of endothelin signaling in sodium homeostasis and autonomic tone, we sought to characterize the hemodynamic and autonomic responses of our *Bmal1* knockout (KO) rat to high salt diet and endothelin receptor blockade. Male rats homozygous for the *Bmal1* mutation (KO, n = 4) and wild type (WT, n = 7) littermate controls were implanted with telemetry transmitters to record blood pressure. After a recovery period of at least one week, the rats were placed on 7 days each of normal salt (0.49% NaCl) diet, high salt (4.0% NaCl) diet, followed by high salt diet containing the specific ET_B receptor antagonist A192621 (10 mg/kg/day, p.o.). Rats were placed in metabolic cages for the last three days of each diet. Surprisingly, KO rats had a similar night-day difference in mean arterial pressure (MAP) as WT during normal salt diet (6.3 ± 0.4 vs. 6.9 ± 0.9 mmHg; respectively), high salt diet (7.1 ± 0.1 vs. 5.4 ± 0.9 mmHg; respectively), and high salt + A192621 (5.4 ± 0.4 vs. 4.8 ± 1.1 mmHg; respectively). KO and WT rats had similar 24-hr MAP during normal salt diet (104.1 ± 3.3 vs. 107.3 ± 1.2 mmHg; respectively), high salt diet (113.8 ± 4.1 vs. 114.0 ± 1.4 mmHg; respectively), and high salt + A192621 (136.3 ± 8.6 vs. 133.4 ± 3.1 mmHg; respectively). Despite these similar blood pressure responses to high salt diet and ET_B antagonism, KO rats had a significantly greater reduction in vasomotor sympathetic to parasympathetic tone compared to WT rats as demonstrated by low frequency to high frequency (LF/HF) analysis of diastolic blood pressure variability (-0.9 ± 0.3 vs. 0.1 ± 0.2 ΔLF/HF relative to normal salt; respectively; p = 0.01). These results indicate that lack of *Bmal1* may result in greater ET_B receptor mediated vasomotor sympathetic tone in rats fed a high salt diet and that factors other than *Bmal1* may be influential in circadian control of blood pressure in rats.

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P336

Excreted Living Renal Proximal Tubule Cells (RPTC) Demonstrate Increased Sodium and Bicarbonate Transport in Inverse Salt Sensitive Individuals Using a Patch Clamp Instrument

PrimaryAuthor.AuthorBlock:**Peng Xu**, Katherine A Schiermeyer, John J Gildea, Hanh T Tran, UNIVERSITY OF VIRGINIA Dept of Pathology, Charlottesville, VA; Robert M Carey, UNIVERSITY OF VIRGINIA Dept of Med, Charlottesville, VA; Robin A Felder, UNIVERSITY OF VIRGINIA Dept of Pathology, Charlottesville, VA

In our previous clinical studies of salt sensitivity of blood pressure, we have demonstrated that approximately 11% of study participants have a

paradoxical increase in ≥7mm of Hg blood pressure on a low NaCl diet (5 meq/day) vs a high NaCl diet (300 meq/day). We define these participants as having inverse salt sensitivity (ISS) while salt resistant (SR) participants demonstrate ≤7mm Hg. RPTCs from 2 ISS and 2 salt resistance (SR) participants were patch clamped and ramp currents were recorded using a voltage ramp protocol from -100 to +45 mV within 200 ms in at least 5 experiments for each cell line. Activity of sodium transporters was probed by exchanging an 80 mM Na^+ with a 140 mM Na^+ buffer. Na^+ transport was significantly increased during the low salt to high salt exchange (ISS1: 80mM Na+ 90.6 ± 17.78 pA VS 140 mM Na, 192.8 ± 47.67 pA, n=9, P<0.05 paired t-test; ISS2: 126.5 ± 41.14 pA VS 198.3 ± 47.45 pA, n=6 P<0.05 paired t-test). The fold change in current from 80 to 140 mm Na^+ buffer in ISS cells was significantly increased when compared to salt resistant (SR) (normal) participants indicating ISS cells have more sodium channel activity than SR cells (ISS 2.07 ± 0.01, n=2 VS SR 1.3 ± 0.1, n=2, P<0.05, t-test). Activity of bicarbonate transporters was probed by exchanging 25mM Na gluconate with 25mM NaHCO3. A similar trend was found for bicarbonate transport which showed increased current when we exchanged the Na^+ gluconate to NaHCO3, while SR cells didn't show any difference when switching Na^+ gluconate to NaHCO3 (ISS1: Gluconate 461.5 ± 226.6 pA VS 1932 ± 658.7 pA, n=8, P<0.05 paired t-test; ISS2: 284.1 ± 83.02 pA VS 1102 ± 295.4 pA, n=6, P<0.05 paired t-test; SR1: 144.1 ± 20.93 pA VS 164.7 ± 27.22 pA, n=12; SR2: 256.5 ± 38.67 pA VS 277.8 ± 38.16 pA, n=5). Comparing bicarbonate**/**gluconate ratios, ISS cells demonstrated an increased ratio over the SR cells (ISS 8.65 ± 2.35, n=2 VS SR 1.11 ± 0.01, n=2, p=0.08) indicating a sodium bicarbonate transporter is hyperactive in ISS cells. Many transporters can be contribute to ISS, therefore we will selectively silence the sodium and bicarbonate transporters to determine which one(s) contribute to the etiology of ISS which then could be targeted for therapeutic intervention.

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Sodium-independent Elevations of Blood Pressure are Accompanied by Immune Cell Infiltration in the Kidney and Renal Damage in Dahl SS Rats

PrimaryAuthor.AuthorBlock:Brittany M Wade, **David L Mattson**, Medical Coll of Wisconsin, Milwaukee, WI

Infiltration of immune cells in the kidney is driven by elevated renal perfusion pressure and amplifies sodium-sensitive hypertension and renal injury in Dahl Salt Sensitive (SS) rats fed a high salt diet. The present studies were performed to determine the importance of immunity in the development of sodium-independent hypertension and renal damage in SS rats and SS lacking T- and B-lymphocytes due to a null mutation in the Rag1 gene (SS-Rag1^{em1Mcwi}). The baseline level of mean arterial pressure (MAP) was not different between groups, and the continuous infusion of AngII (5 ng/kg/min, iv) to SS and SS-Rag1^{em1Mcwi} fed low salt (0.4% NaCl) led to a significantly greater increase in MAP in SS (190±3 mmHg) than in SS-Rag1^{em1Mcwi} (177±3 mmHg) after 12 days of infusion (n=9 rats/group). Renal damage, as assessed by albumin excretion rate, was significantly increased after 12 days of AngII infusion in the SS (from 32±4 to 81±9 mg/day) and in the Rag1 mutants (from 12±2 to 51±8 mg/day). Compared to vehicle-infused rats,

kidneys of AngII-treated SS (n=4-5/group) had increased CD45+ total leukocytes (1.8±0.2 vs 4.4±1.2 x 10^6 cells/kidney), including CD11b/c+ macrophages/monocytes and CD3+ T Cells. Upon cessation of the AngII infusion, MAP in the SS-Rag1^{em1Mcwi} significantly decreased to a level not different from control values (135±5 vs 128±3 mmHg). In contrast, though MAP decreased in the SS when AngII infusion was stopped, blood pressure remained at a level greater than control values throughout the 9-day recovery period (157±8 vs 129±2 mmHg). Albumin excretion rate also tended to decrease in both SS and SS-Rag1^{em1Mcwi} rats following the return to saline infusion, but the reversibility was not complete. Despite the maintenance of elevated pressure in the SS following AngII withdrawal, there was a significant and complete reversal in the number of CD45+, CD11b/c+, and CD3+ cells in the SS kidneys. The present data indicate that immune cells amplify sodium-independent hypertension and the development of renal damage in the SS rat, but also indicate that factors in addition to renal perfusion pressure may mediate immune cell infiltration into the kidney in hypertension.

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P338

Long Term End Organ Inflammation and Dysfunction in Mice After Angiotensin II Induced Hypertension

PrimaryAuthor.AuthorBlock:**Wei Chen**, Liang Xiao, David G Harrison, Vanderbilt Univ, Nashville, TN

Hypertension is associated with vascular and renal inflammation leading to organ dysfunction and injury. Although reduction of hypertensive stimuli or antihypertensive therapy can lower blood pressure, it is unclear if inflammation persists beyond the initial hypertensive stimulus. We sought to examine the hypothesis that a short-term hypertensive insult leads to ongoing inflammation and end organ dysfunction. C57BL/6 mice received a subcutaneous infusion of angiotensin II (490 ng/kg/min) or a sham infusion via osmotic minipumps for two weeks. The minipumps were then removed, and the mice were allowed to recover for two months. To evaluate renal function, mice received a challenge of normal saline equal to 10% of each body weight via intraperitoneal injection and the urine excreted in the subsequent 4 hours was measured. Whereas sham-treated mice excreted 88±7%, mice that had received ang II 2 months earlier excreted only 51±9% of the injected volume ($p<0.05$). Moreover, albuminuria was doubled in the mice that had received prior ang II infusion (0.55 ± 0.1 vs 0.26 ± 0.1 μg/ml, $p < 0.05$). After sacrifice, the renal and aortic samples of both groups of mice were analyzed by flow cytometry. We found that the numbers of total leukocytes ($CD45^+$), total T lymphocytes ($CD3^+$) and monocytes/macrophages ($F4/80^+$) were 4 to 5 times higher in aortas and 45 to 70% higher in the kidneys even after two months following ang II infusion compared to sham-treated mice. We have previously shown that isolevuglandin-protein adducts in antigen presenting cells are immunogenic, and we found that these were persistently in $MerTK^+/CD64^+$ macrophages in the aorta mice that had received ang II 2 months earlier. There was also striking perivascular fibrosis in mice that had received prior ang II, but not in sham infused mice. These data indicate that a persistent inflammatory process, accompanied with renal dysfunction and aortic fibrosis continues for a prolonged period of

hypertension. Efforts to ameliorate this might reduce the long-term risk renal and vascular disease.

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P339

Genetically Induced Renal Lymphangiogenesis Prevents the Development of L-NAME Hypertension in Mice

PrimaryAuthor.AuthorBlock:**Catalina A Lopez Gelston**, Dakshnapriya Balasubbramanian, Gabriella R Abouelkheir, Alexandra H Lopez, Kayla R Hudson, Eric R Johnson, Victoria C Garza, Reem A Daboul, Joseph M Rutkowski, Brett M Mitchell, Texas A&M Univ Health Science Ctr, College Station, TX

In humans and experimental animals, persistent immune system activation, accumulation of immune cells in the kidney, and subsequent inflammation plays an essential role in the development of hypertension (HTN). To reduce inflammation, lymphatic vessels drain extracellular fluid from the interstitium and traffic immune cells to draining lymph nodes. However, little is known about the connection between hypertension and renal lymphatic vessels. We hypothesized that renal lymphatic vessel density would increase in mice with L-NAME HTN and that genetically induced renal lymphangiogenesis would prevent this increase in blood pressure. L-NAME (0.5 mg/mL) was administered in the drinking water for two weeks and caused HTN (SBP: 153±3 vs. 103±3 mmHg; $p<0.05$) and renal lymphatic vessel dilation compared to control mice. Kidneys from mice with L-NAME HTN had significantly increased gene expression of the lymphangiogenic marker *Vegfc*, macrophage marker *Adgre1* (F4/80), dendritic cell marker *Cd11c*, Th1 cell marker *Tbx21*, and the pro-inflammatory cytokine *Il6*. Blood pressure decreased after a two-week washout period following L-NAME (SBP: 113±2 mmHg) which was associated with a decrease in renal gene expression of *Adgre1* (F4/80) and *Cd11c*, however renal lymphatic vessels remained dilated. To determine if augmenting renal lymphatic vessel density prior to L-NAME treatment would prevent HTN, we used transgenic mice that in response to doxycycline undergo kidney-specific VEGF-D overexpression (KidVD+ mice) and renal lymphangiogenesis. Doxycycline (200 mg/L) was administered in the drinking water of KidVD+ and KidVD- mice for four weeks with L-NAME being added during the final three weeks. Starting doxycycline one week prior to L-NAME prevented HTN in KidVD+ mice while slightly decreasing SBP in KidVD- mice (SBP: 112±4 vs. 134±2 mmHg; $p<0.05$). Renal gene expression of the Th17 cell marker *Rorc* was decreased and the lymphatic chemokine markers *Ccl21* and *Ccl19* were increased significantly in KidVD+ mice. These data together demonstrate that L-NAME HTN can alter the size of renal lymphatic vessels and genetically augmenting renal lymphatic vessel density prior to L-NAME can prevent the development of HTN.

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Immunomodulatory Effect of Rapamycin on the Expression of Anti-inflammatory Cytokines in Npr1 Gene-deleted Mice

PrimaryAuthor.AuthorBlock:Venkateswara R Gogulamudi, Umadevi Subramanian, Meaghan Bloodworth, **Kailash N Pandey**, Tulane SOM Physiology, New Orleans, LA

Disruption of natriuretic peptide receptor-A (NPRA) gene (*Npr1*) activates the pro-inflammatory responses, which contributes to the pathogenesis of hypertension and end-organ damage. The objective of this study was to determine the kinetic responses of pro- and anti-inflammatory cytokines in *Npr1* gene-knockout (KO) mice. *Npr1* 0-copy ($Npr1^{-/-}$), 1-copy ($Npr1^{+/-}$), and 2-copy ($Npr1^{+/+}$) mice were pre-treated with rapamycin and multiplex analyses were done to assess cytokines levels. Pro-inflammatory cytokine, IFN-γ protein levels in plasma and kidney of 0-copy and 1-copy mice were markedly higher compared with 2-copy mice (76±1; 49±2 vs 32±1.3 pg/ml; 79±2; 29±1 vs. 27±0.5 pg/mg, respectively). Similarly, IL-6 levels in plasma and kidney were significantly elevated in 0-copy and 1-copy mice than 2-copy mice (52±1; 27±0.5 vs.12±0.4 pg/ml; 49±1.5; 38±2 vs.18±1.1 pg/mg, respectively). Interestingly, anti-inflammatory cytokine IL-5 protein levels in plasma and kidneys were significantly down-regulated in 0-copy and 1-copy mice than 2-copy mice (6±0.8; 5±0.1 vs. 12±0.5 pg/ml; 6±0.6; 9±0.4 vs. 28±0.5 pg/mg). IL-10 levels in plasma and kidney of 0-copy and 1-copy mice were also significantly decreased than 2-copy mice (22±0.4; 39±1 vs. 62±3 pg/ml; 14±1.7; 36±0.2 vs. 52±3 pg/mg). Rapamycin significantly reduced the levels of IFN- γ in plasma and kidney of 0-copy (50%, 35%) and 1-copy (63%, 55%) mice and IL-6 level in 0-copy (60%, 38%) and in 1-copy (40%, 26%) mice compared with 2-copy mice. In contrast, rapamycin treatment significantly elevated IL-5 levels in plasma and kidneys of 0-copy (68%, 77%) and 1-copy (61%, 64%) mice and IL-10 levels in 0-copy (80%, 78%) and 1-copy (47%, 25%) mice than 2-copy mice. A significant decrease in blood pressure occurred in rapamycin-treated 0-copy (19±4 mmHg) and 1-copy (12±3 mmHg) mice than untreated control mice. The results demonstrate that the expression of pro-inflammatory cytokines is greatly upregulated in *Npr1* KO mice compared with 2-copy mice and rapamycin serves as the immune modulator of anti-inflammatory cytokines in these animals. The present findings implicate that rapamycin might act as an anti-inflammatory drug for the treatment of pathophysiology of hypertension-associated inflammation.

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Renal Denervation Prevents Cholinergic Mediated Hypertension and Renal Macrophage Infiltration

PrimaryAuthor.AuthorBlock:Nandita Raikwar, Jason Ratcliff, Chantal Allamargot, Mark W Chapleau, Francois M Abboud, **Sailesh C Harwani**, Univ of Iowa Hosp, Iowa City, IA

In vivo cholinergic activation with nicotine induces renal infiltration of M1 inflammatory CD161a+/CD68+ macrophages and the development of hypertension. Renal denervation has been proposed as a treatment for essential hypertension. Based on this we hypothesized that the renal sympathetic nerves

play a role in these processes. Bilateral renal denervation was performed surgically in 3-4 week old Spontaneously Hypertensive Rats (SHR), a genetic model of essential hypertension. Following one week recovery, animals received subcutaneous infusion of nicotine (15mg/kg/day) via osmotic pumps for 2 weeks. Blood pressure was measured by tail-cuff and kidneys harvested on completion of infusion. Prior to nicotine infusion, at baseline, there was no difference between the systolic blood pressures of the sham treated (n=7) and renal denervation (n=10) groups, 132 ± 4 vs 128 ± 3 mmHg, respectively ($p>0.05$). In contrast, nicotine infusion significantly raised the systolic blood pressure in the sham treated group (159 ± 3mmHg), but not in the renal denervation group (135 ± 5 mmHg) ($p<0.001$). Moreover, nicotine infusion induced a significantly greater infiltration of inflammatory CD161a+ immune cells and CD161a+/CD68+ inflammatory macrophages into the renal medulla of the sham treated group (n=4) (13 ± 2 cells/hpf and 5 ±1 cells/hpf, respectively), compared to renal denervation(n=4) (2 ± 1 cells/hpf and 2 ± 1 cells/hpf) ($p<0.001$). We conclude that renal sympathetic innervation is required for the nicotine-induced migration of inflammatory CD161a+ immune cells and CD161a+/CD68+ inflammatory macrophages into the renal medulla and that renal denervation prevents the cholinergic-induced renal inflammation and hypertension in this model.

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P342

Sirt 1 on Endothelial Dysfunction in Small Arteries From Obese Patients

PrimaryAuthor.AuthorBlock:**Agostino Virdis**, Stefano Masi, Monica Nannipieri, Daniela Guarino, Univ of Pisa, Pisa, Italy; Marco Anselmino, Dept of General Surgery, Azienda Ospedaliero-Univria Pisana, Pisa, Pisa, Italy; Rocchina Colucci, Dept of Pharmacology, Univ of Padova, Padova, Italy; Emiliano Duranti, Corrado Blandizzi, Stefano Taddei, Univ of Pisa, Pisa, Italy

Obesity is associated with endothelial dysfunction, characterised by a reduced nitric oxide (NO) bioavailability due to increased reactive oxygen species (ROS). Sirtuins, and more specifically Sirt-1, are enzymatic proteins involved in regulation of glucose metabolism, inflammation and intracellular levels of ROS. This study aimed to determine the role of Sirt-1 in regulating NO bioavailability of small resistance arteries isolated from subcutaneous tissue of obese patients. 10 subjects (5 with severe obesity, Ob; 5 normal weight controls, Ctrl) underwent biopsy of subcutaneous adipose tissue during laparoscopic bariatric surgery. Function of small arteries was assessed with pressure micromyography. Endothelial-dependent vasodilation (VDep) and NO production was assessed by acetylcholine (ACh, 0,001-100µM), with and without pre-incubation with L-NAME (100µM). The influence of sirtuins on NO bioavailability was assessed repeating Ach with a selective Sirt-1 agonist (SRT-1720, 1µM), alone or plus L-NAME. Ob showed a reduced response to Ach vs Ctrl ($P<0.001$), associated with a reduced inhibition of L-NAME on Ach (Ob: $P=0.002$; Ctrl: $P<0.001$). SRT-1720 improved the VDed induced by Ach ($P<0.001$) in Ob, although it did not reach values from Ctrl. The simultaneous incubation with L-NAME and SRT-1720 before Ach stimulation abolished the

VDed obtained with the incubation of SRT-1720 in the Ob group (SRT-1720 vs SRT-1720+L-NAME: P<0.001). In small arteries of Ob, stimulation of Sirt-1 activity partially restores endothelial function due to an improved NO bioavailability.

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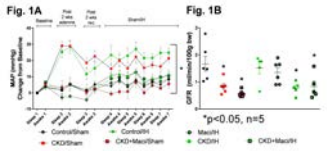
A Dual Endothelin A/B Receptor Blocker in a Rat Model of Sleep Apnea and Chronic Kidney Disease

PrimaryAuthor.AuthorBlock:**Laura V Gonzalez Bosc**, Wieslawa Giermakowska, Carolyn E Pace, Adelaeda Barrera, Perenkita Mendiola, David T Jones, Minerva Murphy, Nancy L Kanagy, Univ of New Mexico, Albuquerque, NM

Obstructive sleep apnea (OSA) is characterized by recurrent episodes of pharyngeal collapse during sleep resulting in intermittent hypoxia (IH) and sleep fragmentation. OSA affects 5% to 20% of the US population, is associated with high incidence of hypertension, and is a prognostic indicator of accelerated renal failure. More than 20 million people in the US have chronic kidney disease (CKD). In rodents, endothelin-1 (ET-1) contributes to IH-induced hypertension, and ET-1 levels inversely correlate with GFR in end-stage CKD patients. These findings provide the rationale to test the hypothesis that a dual ET receptor antagonist will attenuate the development of hypertension and renal dysfunction in a combined rat model of IH and CKD. Male Sprague Dawley rats received one of three diets: A) control, B) 0.2% adenine, C) 0.2% adenine + 30 mg/kg/day of macitentan (dual ET_A/ET_B receptor antagonist, Actelion Pharmaceuticals) for 2 weeks followed by 2 weeks of recovery (regular chow or chow+macitentan). Rats were then exposed to sham or IH (20 short exposures/hr to 5% O_2 and 5% CO_2 7 hr/day during sleep) for 4 weeks. Changes in mean arterial blood pressure (MAP) recorded by telemetry are in **Figure 1A** and estimated glomerular filtration rate **in Figure 1B**. In summary, macitentan prevents increases in blood pressure caused by CKD, IH and by the combination of CKD+IH. However, it does not improve kidney function. Our data suggest that macitentan could be an effective antihypertensive in CKD patients with irreversible kidney damage as a way to protect the heart, brain and eyes from elevated arterial pressure but it does not reverse toxin-induced tubule atrophy in our experimental conditions.



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P344

Nuclear Factor E2-related Factor 2 (nrf2) Causes Early Microvascular Endothelial Dysfunction and Increased Adma by Activation of the Cox2/tp Receptor Pathway in Mice Infused With Angiotensin II for Three Days

PrimaryAuthor.AuthorBlock:**Dan Wang**, Georgetown Univ Medical Ctr, Washington, DC; Xueqin Hao, Henan Univ of Science and Technology, Luoyang, China; Cheng Wang, The Third Hosp of Sun Yat-sen Univ, Gungzhou, China; William J Welch, Georgetown Univ Medical Ctr, Washington, DC; James Tomlinson, James James, MRC Clinical Sciences Ctr, Imperial Coll London, London, United Kingdom; Christopher S Wilcox, Georgetown Univ Medical Ctr, Washington, DC

Background: Activation of Nrf2 by tert-buthylhydroquinone (tBHQ) prevents hypertension, oxidative stress, endothelial dysfunction and ADMA in mice infused with angiotensin II (Ang II) for 12 days. However, Nrf2 activation with bardoxolone methyl increased cardiovascular events in diabetic patients within one week. Since we found that tBHQ activated cyclooxygenase (COX)-2 within 3 days, we tested the hypothesis that short-term tBHQ administration to Ang II infused mice increases microvascular dysfunction via COX dependent of PGs and TxA_2 that activate thromboxane-prostanoid receptors (TP-Rs). **Methods:** Mesenteric resistance arterioles(MRAs)were isolated from mice infused for 3 days with ANG II (400 ng/kg/min) or vehicle and given oral tBHQ (0.1% of water) or vehicle (n=6 mice/group). Endothelial derived relaxation factor (EDRF) was assessed by a myograph and NO and ROS by RatioMaster™. **Results:** Compared to vehicle, Ang II infused mice given tBHQ had increased (P<0.05) conscious mean arterial pressure (135±6 vs 115±7 mmHg), urinary 8-Iso prostane (1.5±0.4 vs 1.1±0.2ng/mg creatinine), and decreased EDRF (17± 3 vs 23± 2%) and NO (0.23 ± 0.02 vs 0.35 ± 0.02 Δunits), and enhanced (P<0.05,) cellular ROS (0.27 ± 0.02 vs 0.12 ± 0.03 Δunits), mitochondria ROS (0.24 ± 0.02 vs 0.1 ± 0.02 Δunit) and microvascular asymmetric dimethylarginine (ADMA, 77 ± 6 vs 55 ± 7 nmol/mg protein). All these effects of tBHQ were prevented in COX-1 knockout mice drinking parecoxib (COX-2 inhibitor) and in TPR and Nrf2 knockout mice. **Conclusions:** Activation of Nrf2 increases short term Ang II-induced increases in BP, oxidative stress, ADMA and endothelial dysfunction. These depend on signaling via COX 1 + 2 and TP receptors. Our studies reveal novel dual effects of Nrf2 in Ang II infused mice: increased BP, ROS, ADMA and endothelial dysfunction within 3 days secondary to activation of COX/TPR signaling follow by the opposite effects of Nrf2 on BP, ADMA and vascular function within 12 days. Therefore, blockade of COX-2/TPRs pathway during initiation of Nrf2 therapy may prevent the devastating early adverse effects that have prevented its use in hypertension or diabetes.

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P345

Overexpression of Mitochondrial Deacetylase Sirt3 Protects Endothelial Function and Attenuates Hypertension While Sirt3 Depletion Increases Oxidative Stress and Endothelial Dysfunction Due to SOD2 Hyperacetylation

PrimaryAuthor.AuthorBlock:Anna E Dikalova, Hana A Itani, Arvind K Pandey, David G

Harrison, **Sergey I Dikalov**, Vanderbilt Univ Medical Ctr, Nashville, TN

We have recently reported SOD2 hyperacetylation and reduced Sirt3 level in human subjects with essential hypertension. We hypothesized that diminished Sirt3 expression promotes endothelial dysfunction and hypertension while Sirt3 overexpression protects endothelial function and attenuates hypertension. Indeed, hypertension was markedly increased in Sirt3 knockout ($Sirt3^{-/-}$) in response to angiotensin II (0.7 mg/kg/day) compared with wild-type C57Bl/6J mice. Sirt3 depletion caused SOD2 inactivation due to SOD2 hyperacetylation, increased mitochondrial $O_2^{\bullet-}$ and diminished endothelial nitric oxide. Angiotensin II infusion in wild-type mice was associated with Sirt3 inactivation and SOD2 hyperacetylation in aorta and kidney. To test the specific role of Sirt3 in vasculature we have generated tamoxifen-inducible endothelium specific Sirt3 knockout mice ($Ec^{Sirt3\ KO}$) and tamoxifen-inducible smooth muscle specific Sirt3 knockout mice ($Smc^{Sirt3\ KO}$). Deletion of Sirt3 in smooth muscle exacerbated hypertension (165 mm Hg vs 155 mm Hg in wild-type) and significantly increased mortality in angiotensin II infused $Smc^{Sirt3\ KO}$ mice (30% vs 3% in wild-type) which was associated with higher rate of aortic aneurysm. $Ec^{Sirt3\ KO}$ mice had elevated basal blood pressure by 12 mm Hg and hypertension was exacerbated in $Ec^{Sirt3\ KO}$ mice accompanied by impaired vascular relaxation and reduced endothelial nitric oxide. Treatment of angiotensin II-infused $Sirt3^{-/-}$ mice with SOD2 mimetic mitoTEMPO rescued endothelial-dependent relaxation and reduced blood pressure. We tested if Sirt3 overexpression protects endothelial function and attenuates angiotensin II-induced hypertension. These new mice were obtained by crossing the EIIa-cre with Sirt3flox mice resulting in constitutively increased Sirt3 in the whole body. Sirt3 overexpression abolished angiotensin II induced impairment of vasorelaxation and attenuated development of hypertension. Our data suggest that diminished Sirt3 activity leads to SOD2 hyperacetylation and contributes to the pathogenesis of hypertension. It is conceivable that Sirt3 agonists and SOD2 mimetics may have therapeutic potential in cardiovascular disease.

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P346

Diurnal Variation of Serum Uric Acid Levels and Corresponding Variations of Oxidative Stress Makers in Patients With Hypertension and Stable Coronary Artery Disease

PrimaryAuthor.AuthorBlock:**Megumi Shimizu**, Dept of Cardiovascular Med, Juntendo Univ Sch of Med, Tokyo, Japan; Takatoshi Kasai, Shoichiro Yatsu, Azusa Murata, Dept of Cardiology, Juntendo Univ, Graduate Sch of Med, Tokyo, Japan; Hiroki Matsumoto, Dept of Cardiovascular Med, Juntendo Univ Sch of Med, Cardiovascular Respiratory Sleep Med, Juntendo Univ Graduate Sch of Med, Tokyo, Japan; Jun Shitara, Dept of Cardiovascular Med, Juntendo Univ Sch of Med, Tokyo, Japan; Takao Kato, Tobu Chiiki Hosp, Tokyo, Japan; Shoko Suda, Dept of Cardiovascular Med, Juntendo Univ Sch of Med, Cardiovascular Respiratory Sleep Med, Juntendo Univ Graduate Sch of Med, Tokyo, Japan; Masaru Hiki, Dept of Cardiovascular Med, Juntendo Univ Sch of Med, Tokyo, Japan; Ryo Naito, Juntendo Univ Urayasu Hosp, Tokyo, Japan; Kazunori Shimada, Hiroyuki

Daida, Dept of Cardiovascular Med, Juntendo Univ Sch of Med, Tokyo, Japan

[Background] Hyperuricemia has been known as a risk factor of hypertension, stroke and coronary artery disease (CAD). Uric acid (UA) itself has antioxidative activity, but it is also reported that UA can also stimulate oxidative stress. In healthy subjects, it has been shown that UA has diurnal variation; elevate in the early morning. However, it is unclear whether such diurnal variation is observed and whether corresponding diurnal variation of oxidative stress can also be observed even in patients with CAD. Therefore, we investigated presence of diurnal variation of serum levels of UA, oxidative stress makers in patients with hypertension and CAD. [Method] We measured serum levels of UA, NOx, and urinary levels of 8-OHdG, serum at 6 p.m., 6 a.m., 12 p.m. in 26 men with CAD. We excluded patients who was taking drugs which can affect UA levels. [Result] Overall 20 patients were enrolled. Serum UA levels were 5.67±0.99 mg/dl at 6 p.m., 5.86±0.99 mg/dl at 6 a.m., and 5.73±0.99 mg/dl at 12 p.m., indicating obvious diurnal variation (P=0.0004). Serum UA levels increased significantly from 6 p.m. to 6 a.m. (P=0.002) and decreased significantly from 6 a.m. to 12 p.m. (P=0.002). Urinary level of 8-OHdG were 13.96±4.95 ng/mlCr at 6 p.m., 19.47±9.20 ng/mlCr at 6 a.m., and 16.19±5.52 ng/mlCr at 12 p.m., indicting corresponding diurnal variation to the serum UA level (P=0.013). Similarly, urinary levels of 8-OHdG increased significantly from 6 p.m. to 6 a.m. (P=0.008) and tend to decrease from 6 a.m. to 12 p.m. (P=0.57). Serum NOx levels were 13.19±6.60μM at 6 p.m., 12.11±6.02μM at 6 a.m. and 15.08±6.61μM at 12 p.m. and showed reciprocal diurnal variation to the serum UA and urinary 8-OHdG levels (p=0.0028). [Discussion] Serum UA levels showed diurnal variation even in patients with hypertension and CAD. Considering the fact that oxidative stress makers showed corresponding diurnal variations, diurnal variation of serum UA level may play a role in the pathogenesis of CAD.

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P347

γδ T Cells Drive $CD4^+$ And $CD8^+$ T Cell Activation In Hypertension

PrimaryAuthor.AuthorBlock:**Antoine Caillon**, Pierre Paradis, Ernesto L. Schiffrin, MCGILL UNIVERSITY, Montreal, QC, Canada

Objective: Both innate (monocyte/macrophages) and adaptive immune cells (T lymphocytes) have been shown to play a role in the development of vascular injury in hypertension. Recently, we demonstrated that a small subset of “innate-like” T lymphocytes, expressing the γ/δ T cell receptor (TCR) rather than the αβ TCR, plays a key role in hypertension and vascular injury. We demonstrated an increased number and activation ($CD69^+$) of γδ T cells during the development of hypertension caused by angiotensin (Ang) II infusion, and that deficiency in γδ T cells prevented Ang II-induced hypertension, resistance artery endothelial dysfunction and spleen T-cell activation in mice.

We hypothesized that γδ T cells mediate activation of other T cells in hypertension.
Method and Results: Fourteen to 15-week old male C57BL/6 wild-type (WT) mice were infused with Ang II (490 ng/kg/min, SC) for 3, 7 and 14 days (n=5-7) and spleen T cell profile was determined by flow cytometry. A correlation was demonstrated between the frequency (FREQ) and the number (#) of activated $CD69^+$γδ T cells and $CD4^+CD69^+$ T cells (FREQ: r=0.41, *P*<0.05 and #: r=0.58, *P*<0.001) and $CD8^+CD69^+$ T cells (FREQ: r=0.36, *P*<0.05 and #: r=0.50, *P*<0.01). We also demonstrated a high correlation between the # of $CD69^+$ γδ T cells expressing CD27, a marker of interferon-γ expressing cells and a member of the T-T interaction molecules, with $CD4^+CD69^+$ (r=0.88, *P*<0.001) and $CD8^+CD69^+$ (r=0.81, *P*<0.01) T cells after 7 days of Ang II infusion.
Conclusion: This study demonstrated an association between $CD27^+CD69^+$ γδ T cells and activated T cells. These results suggest that γδ T cells drive activation of other T cells in Ang II-induced hypertension. Targeting γδ T cells may contribute to reduce inflammation in hypertension.

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P348

Immunosuppression with Cyclophosphamide Attenuates Renal Injury but Not Hypertension in an Experimental Model of Autoimmune Disease

PrimaryAuthor.AuthorBlock:**Victoria L Wolf**, Erin B Taylor, Michael J Ryan, Univ of Mississippi Medical Ctr, Jackson, MS

Cardiovascular disease is the major cause of mortality among patients with the autoimmune disorder systemic lupus erythematosus (SLE). Our laboratory previously showed that immunosuppression with mycophenolate mofetil, a common therapy in patients with SLE, attenuates the development of hypertension in an experimental model of SLE. Cyclophosphamide is another common therapy for patients with SLE that has contributed to improved disease management; however, its impact on the development of hypertension associated with SLE is not clear. We tested whether treatment with cyclophosphamide (25 mg/kg, once/week, IP injection) for four weeks attenuates hypertension in an established female mouse model of SLE with hypertension (30 week old NZBWF1 females). Plasma anti-dsDNA IgG levels, pathogenic for the disease, were lower in cyclophosphamide-treated SLE mice compared to vehicle-treated SLE mice (161 ± 43, n=19 vs 501 ± 114, n=21, p<0.05 units/mL), suggesting efficacy of the therapy to suppress aberrant immune system function. Mean arterial pressure (MAP) was assessed by carotid artery catheters in conscious mice. Treatment did not attenuate the development of hypertension (136 ± 3 mmHg, n=15) when compared to vehicle treated SLE mice (131 ± 4 mmHg, n=13, p=0.69]; however, urinary albumin excretion (72.2 ± 68 mg/day, n=21, mg/day) was lower in cyclophosphamide treated animals (0.06 ± 0.02 mg/day, n=13). Corresponding with the reduction in autoantibodies, preliminary data suggest that cyclophosphamide treatment lowered circulating $CD45R^+$ B cells (15.87 ± 8.49%, n=10 vs 26.96 ± 4.72%, n=8). Paradoxically, circulating $CD11b^+Ly6G^+$ neutrophils were increased in cyclophosphamide treated SLE mice compared to vehicle treated (39.26 ± 4.92%, n=10 vs 20.58 ± 6.01%, n=8, p=0.06). These data suggest that cyclophosphamide treatment attenuates autoantibody production and renal disease during SLE, but that the potential to impact

MAP may be blunted by the increase in circulating neutrophils.

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P349

Plasma Corin Levels Reflect Dynamic Changes in Cardiac Expression Induced by Experimental Acute Myocardial Infarction

PrimaryAuthor.AuthorBlock:**Dong Wang**, Inna P. Gladysheva, Ryan D. Sullivan, Tai-hwang Michael Fan, Radhika M. Mehta, Ranjana Tripathi, Yao Sun, Guy L. Reed, Univ of Tennessee, Memphis, TN

Introduction: Corin is a cardiac membrane protease that activates pro-ANP and pro-BNP. Changes in circulating corin levels have been linked to poor clinical outcomes following acute myocardial infarction (AMI) in some, but not all studies and the pathophysiologic mechanisms responsible for these changes are not understood. **Hypothesis:** We examined the hypothesis that plasma corin levels are closely correlated with alterations in cardiac corin expression related to acute ischemic injury. **Methods:** AMI was induced by left anterior descending coronary artery ligation. Hearts and plasma were assessed at 3 hrs, 24 hrs and 3 days post AMI. We measured plasma corin, troponin T levels, cardiac corin, infarct size, ANP and BNP expression by ELISA, qRT-PCR and histology staining. Cardiac function was assessed by echocardiography. Data represent means ± SE of n = 7-9 mice per group. **Results:** Plasma corin levels were significantly increased at 3 hrs (1090±237.50 pg/ml, P<0.05), 24 hrs (1666±214.50 pg/ml, P<0.001), and 3 days (1221±185.90 pg/ml, P<0.01P<0.01) post-AMI with a “rise-and-fall” pattern similar as troponin T when compared to non-MI group (553.9±96.13 pg/ml). In contrast, at the same time-points, cardiac corin expression dropped by 5% (P>0.05), 69% (P<0.001) and 65% (P<0.001) as measured by real time PCR and 30% (P<0.05), 76% (P<0.001) and 75% (P<0.001) by immunohistology. Transcript levels of pro-ANP increased at 24 hrs (20%) and 3 days (3.8-fold, P<0.001) vs. non-MI mice. Transcript levels of pro-BNP also increased 3.2-fold at 24 hrs (P<0.001) and 1.8-fold at 3 days (P<0.01) post MI. EF% and FS% consistently dropped by 36% (P<0.01), 60% (P<0.001) and 68% (P<0.001) and 42% (P<0.01), 63% (P<0.001) and 72% (P<0.001) respectively. Plasma corin levels were negatively correlated with cardiac corin (P<0.01), EF% and FS% (P<0.05) and positively correlated with infarct sizes (P<0.01). **Conclusions:** Plasma corin levels markedly increase and then decline following AMI, reflecting dynamic changes of cardiac corin expression related to acute cardiomyocyte injury. As such, plasma corin levels may serve as a valuable marker to indicate the severity of acute myocardial injury and, over a longer term, expression by the remaining viable cardiomyocyte mass.

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P350

Abnormal VCG and Hi-Res Parameters of P and QRS Complex in Hypertensive Patients Without/with Paroxysmal Atrial Fibrillation

PrimaryAuthor.AuthorBlock:**Slavomira Filipova F**, Faculty of Med, Slovak Med Univ and Natl Inst of Cardiovascular Diseases, Bratislava, Slovakia; Anna Vachulova F, Dana Skultetyova, Peter Hlivak M, Natl Inst of Cardiovascular Diseases, Bratislava, Slovakia; Ludovit Gaspar M, Faculty of Med, Comenius Univ, Bratislava, Slovakia

AIM Today we have not the established and single clinical mode to identifying hypertensive (HT) patients in risk for paroxysmal atrial fibrillation (pAF). Some non-invasive VCG and high-resolution VCG (Hi-Res) for P and QRS loops are available for ECG/VCG measurements in clinical practice via routinely used ECG/VCG equipment (General Electric). It is possible, that especially P wave and P loop values can reflect the abnormal status in atrial myocardium prior the pAF onset. MATERIAL AND METHOD We studied 276 HT patients in sinus rhythm: group I (n=133, without documented pAF), group II (n=129, with well-documented pAF) and group III (n=14, patients after successful radiofrequency ablation for AF or atrial flutter). ECG parameters were evaluated: (1) heart rate in SR; (2) VCG P loop and QRS loop non-filtered/filtered duration: nPd, fPd, nQRSd, fQRSd; (3) other Hi-Res P and QRS parameters: HFLAd, RMS(40)v; (4) angle between axes P-QRS and QRS-T loops; (5) echoCG parameters: LA dimension, LV ejection fraction, width of IVS ad posterior wall. RESULTS In group II a III the non-filtered parameters (nPd, nQRSd) and filtered parameters (fPd, fQRSd) were significantly longer than in group I (for nPd : 135.9 ms, 145.1 ms vs. 129.0 ms, $p<0.05$; for nQRsd : 104.2 ms, 110.0 ms vs. 99.0 ms, $p<0.01$; for fPd: 143.0 ms, 154.9 ms vs. 133.0 ms, $p<0.005$; for fQRSd 119.7 ms, 125.9 ms vs. 113.0 ms, $p<0.005$). P loop axis analysis is significantly higher in loop II and III vs. group I (+48.2 gr., +53.4 gr. Vs. 48 gr., $p<0.01$). Angle P-QRS is significantly wider in group II and III vs. group I (38.7 gr., 42.1 gr. Vs. 25.0 gr, $p= 0.005$. EchoCG parameters were not significantly different (LA dimensions for groups I,II,III: 39.8, 42.7 and 42.0 mm, n.s.; LVEF for groups I,II,III> 59.7, 57.8 and 58.1, ns.). CONCLUSIONS HT patients with verified pAF in documentation have more abnormal P and QRS wave/loop parameters than HT patients without history of pAF. According to our results, the most informative ECG and VCG factors for possible future pAF are: fPd, fQRSd, angle between loop axes P-QRS. ECG/VCG parameters (non-filtered and especially after filtration via to Hi-Res analysis) have potential to improve the risk stratification for possible future pAF.

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Cornell Product in Electrocardiogram is More Strongly Related to LV Regional Wall Motion Than Sokolow-Lyon Voltage

PrimaryAuthor.AuthorBlock:**Joji Ishikawa**, Tokyo Metropolitan Geriatric HP, Tokyo, Japan; Yuko Yamanaka, Jichi Medical Univ, Tochigi, Japan; Shintaro Watanabe, Sano Kosei General Hosp, Tochigi, Japan; Kazumasa Harada, Tokyo Metropolitan Geriatric HP, Tokyo, Japan

Backgrounds: Left ventricular hypertrophy (LVH) evaluated by Cornell product in electrocardiogram predicted future stroke events greater than LVH by Sokolow-Lyon voltage. Therefore, we evaluated whether Cornell product was related to regional myocardial wall motion greater than Sokolow-Lyon voltage. **Methods:** We enrolled 288 hypertensive patients who were performed echocardiography for evaluating target organ damage. Cornell product was calculated as follows; R in aVL lead + S in V3 lead (0.6 mV added in female)×QRS duration; Sokolow-Lyon voltage, R in V5 lead + S in lead V1. We evaluated left ventricular mass index (LVMI), diastolic function of septal E/e', and [longitudinal (GLS), radial (RS) and circumferential (CS)] strain using Altida (Toshiba, Japan). **Results**: Mean age was 63.4±13.2 years (male 47.9 %). There were 65.2 % of patients with antihypertensive medication. Both Cornell product, and Sokolow-Lyon voltage were related to LVMI ($r=0.392$, $p<0.001$ and $r=0.315$, $p<0.001$) and E/e' ($r=0.260$, $p<0.001$; $r=0.264$, $p<0.001$), independently in multivariate linear regression analysis (both $p<0.05$). On the other hand, Cornell product was more strongly related, compared to Sokolow-Lyon voltage, to GLS (beta=0.200, P=0.005; beta=0.098, P=0.161), inner per outer ratio of RS (beta=-0.163, P=0.031; beta=0.117, P=0.116), and inner per outer ratio of CS (beta=0.148, P=0.044; beta=0.078, P=0.279). **Conclusions**: Both Cornell product and Sokolow-Lyon voltage were independently related to LVMI and diastolic function measured by tissue Doppler image; however, Cornell product was more strongly related to left ventricular regional wall motion of global longitudinal strain and trasmurality of radial and circumferential strain, than Sokolow-Lyon voltage.

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Prostaglandin E2 Reduces Mitochondrial Function in Adult Mouse Cardiomyocytes

PrimaryAuthor.AuthorBlock:**Pamela Harding**, Henry Ford Hosp, Detroit, MI; Timothy D Bryson, Henry Ford Hosp/Wayne State Univ, Detroit, MI; Indrani Datta, Yun Wang, Albert Levin, Henry Ford Hosp, Detroit, MI

Hypertension is a leading cause of heart failure and both conditions are characterized by increased prostaglandin E2 (PGE2) which signals through 4 receptor subtypes (EP1-EP4) to elicit diverse physiologic effects. We previously reported that cardiomyocyte-specific deletion of the EP4 receptor results in a phenotype of dilated cardiomyopathy in male mice that is characterized by reduced ejection fraction. Subsequent gene array on left ventricles from these mice, coupled with Ingenuity Pathway Analysis (IPA) demonstrated that genes differentiating WT mice and EP4 KO mice with low ejection fraction were significantly overrepresented in mitochondrial ($p=2.51 \times 10^{-28}$) and oxidative phosphorylation ($p=3.16 \times 10^{-}$

[30]) pathways. We therefore hypothesized that PGE2 could reduce mitochondrial function. To test this hypothesis, we used isolated mouse cardiomyocytes (AVM) from 16-18 week old male C57Bl/6 mice and treated them with 1 μM PGE2 for various times. Mitochondrial gene expression was examined using a RT-profiler kit for mitochondrial energy metabolism, complex I activity with a spectrophotometric assay, ATP levels with a bioluminescence assay and mitochondrial membrane potential using JC-1 staining. Treatment of AVM with PGE2 for 4 hrs reduced expression of multiple genes from mitochondrial pathways including sub units of mitochondrial NADH dehydrogenase ubiquinone flavoprotein (Nduf), a component of complex I. In accord with the mRNA data, Complex I activity was reduced by 50% ($p < 0.05$) by 4 hr treatment with PGE2, from 1.32 ± 0.36 to 0.66 ± 0.08 mOD/min. Cytochrome c oxidase subunit 8 (Cox8c) mRNA was also reduced from a control value of 1.00 to -1.75 ± 0.20 ($p < 0.005$) after PGE2 treatment. Immuno-fluorescence showed that JC-1 aggregates were reduced after 1 or 3 hr treatment with either 1 μM PGE2 or the EP3 agonist, sulprostone, suggesting reduced mitochondrial membrane potential. Subsequent experiments also showed that ATP levels were reduced 16% from 11.18 ± 0.71 nmol to 9.39 ± 0.83 nmol after treatment with sulprostone for only 1 hr. Taken together, these results suggest that increased PGE2 in hypertension may contribute to impaired mitochondrial function and provide yet another link between inflammation and cardiac dysfunction.

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P353

Attenuation of Cardiac Fibrosis, Hypertrophy and Myopathy by AT2R Agonist NP-6A4

PrimaryAuthor.AuthorBlock:**Lakshmi Pulakat**, Univ of Missouri-Columbia, Columbia, MO; Madhavi Gavini, Novopyxis. Inc, Cambridge, MA; Abuzar Mahmood, Anthony Belechia, Ryan Toedebusch, Paige Beauparlant, Vincent DeMarco, Senthil Kumar, Sivakumar Ardhanari, Univ of Missouri-Columbia, Columbia, MO

Adverse cardiac remodeling (hypertrophy, fibrosis and myopathy) underlies cardiac dysfunction and heart failure. Male Zucker obese (ZO:fa/fa) rat that suffers from cardiac dysfunction, adverse remodeling, and heart failure with preserved ejection fraction is a useful model to study the effects of cardioprotective drugs. We reported that NP-6A4 (Novopyxis Inc. Cambridge, MA), a peptide agonist of the Angiotensin II Type 2 Receptor (AT2R), protected mouse cardiomyoblast HL-1 cells and human coronary artery vascular smooth muscle cells (hCAVSMCs) from acute nutrient serum deficiency stress better than β-AR-Blockers, ARBs, and AT2R agonist CGP42112A. This effect was inhibited by AT2R-specific antagonist PD123319, confirming that NP-6A4 acts through AT2R. AT2R is a cardiac and vascular reparative molecule that protects the heart and vasculature from structural damage and fibrosis. No current drugs increase AT2R expression. We report that NP-6A4 treatment (1μM) increased AT2R mRNA (up to 4 fold; $p \leq 0.05$) in hCAVSMCs and human coronary artery endothelial cells (up to 8 fold; $p \leq 0.05$). This effect was inhibited by PD123319 (10 μM). Treatment of 11-week old male ZO rats with heart disease with NP-6A4 (1.8mg/kg/day in saline, delivered once daily subcutaneously; N=7) increased cardiac AT2R expression and mitigated cardiac dysfunction and adverse remodeling. Quantitative RT-PCR and

immunohistochemistry analysis showed that 2 weeks of NP-6A4 treatment increased cardiac AT2R mRNA (up to 9 fold) and protein (1.5-3 fold) compared to controls (N=6) receiving saline ($p<0.01$). NP-6A4 treatment improved cardiac parameters; endocardial circumferential strain ($p\leq0.05$), myocardial performance index (MPI) ($p\leq0.005$), and E/E' ratio ($p\leq0.002$). NP-6A4 treatment also increased cardiac capillary density (118% compared to saline treatedl; $p\leq0.002$), and reduced interstitial fibrosis (77% compared to saline treated; $p\leq0.039$.) At 19-weeks (after 8 weeks of treatment), cardiac tissues from 5 out of 6 control rats exhibited regions of myopathy. Only 2 out 7 NP-6A4 treated rats exhibited similar levels of myopathy in the heart. We propose that NP-6A4 attenuates adverse remodeling and improves cardiac function by increasing AT2R expression in cardiac cells.

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P354

Left Ventricular Mass and Exagerated Hypertensive Blood Pressure Response to Bruce Exercise Stress Test in Diabetics and Nondiabetics

PrimaryAuthor.AuthorBlock:Michael Balderas, Joaquin Cigarroa, UT Health, San Antonio, TX; Daniel Lozano, South Texas Veterans Healthcare System, Audie L Murphy Hosp, San Antonio, TX; Joan Hecht, **Rene A Oliveros**, UT Health, San Antonio, TX

The aim of or study was to see if an exaggerated BP response during the Bruce exercise test could differentiated target organ damage (TOD) in a population of diabetics v/s nondiabetic pts . We studied 105 pts for the performance of a stress test because of chest pain whose systolic BP rose 50mmHg or more at their peak exercise compared to their resting BP. There were 45 diabetics pts (DM) and 60 nondiabetic pts (NDM) pts. All pts were hypertensive on medication but their beta blocker medication was on hold for the performance of the stress test. The echocardiograms were performed using a Phillips IE 33 echo machine and the LV mass was calculated using the Deveraux formula using single plane views.
The was no statistical difference between both groups with respect to age, BMI and systolic and diastolic BP's during rest and exercise. The HR at rest was not different between both groups but the HR at peak exercise was significantly higher for NDM compared to DM ($p < 0.016$). The LVMI was significantly higher in DM compared to NDM pts ($p = 0.04$).
In conclusion, an elevated LVMI which is evidence for hypertensive target organ damage should be suspected in hypertensive pts who have an exaggerated BP response to the exercise Bruce stress test.
The reason for a significantly higher HR at peak exercise for NDM patients compared to DM pts may be related to better physical performance and therefore longer duration of exercise. Other possibilities will be discussed.

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Prevalence of Diastolic Dysfunction With Preserved Left Ventricular Systolic Function in Hypertensive and Type II Diabetic Patients in a Large Community Practice in Greenville, South Carolina

PrimaryAuthor.AuthorBlock:**Naveen Raj Saxena**, Juhi Saxena, Anju Saxena, Vinita Srivastava, Peace Medical Ctr, Simpsonville, SC

Diastolic dysfunction with preserved left ventricle systolic function is a major cause for adverse cardiovascular events in hypertensive and Type II Diabetic patients. Review of medical literature reveals prevalence of diastolic dysfunction in the range of 20 to 60 percent in hypertensive and diabetic patients. The aim of this study is to examine prevalence of diastolic dysfunction with normal systolic function in hypertensive and Type II Diabetic patients in a community practice. This is a retrospective chart review of 3085 hypertensive and 899 type II Diabetic patients. All patients underwent 2D color Doppler studies for the evaluation of diastolic dysfunction using American Society of Echocardiography criteria. E wave velocity, A wave velocity, E to A ratios, and deceleration time were measured. Patients with known systolic heart failure were excluded from the study. The age distribution of the patients in the study ranged from 45 to 85 years with a mean age of 65 years. The sex distribution of the hypertensive patients were 45% male and 55% female. The sex distribution of Type II Diabetic patients were 44% male and 56% female. Given the data, we conclude prevalence of diastolic dysfunction in hypertensive patients is 29% and prevalence of diastolic dysfunction in type II Diabetic patients is 33%. Echocardiography is an excellent tool to risk stratify hypertensive and type II Diabetic patients. Aggressive management of this high-risk group may reduce cardiovascular mortality and morbidity.

RESULTS:

Hypertensive Patients		Total Population	Males	Females
	Hypertensive Patients	3085	1388	1697
	Diastolic Dysfunction	902	405	497
Prevalence of diastolic dysfunction in hypertensive patients is 902/3085 (29%)				
Diabetic Patients		**Total Population**	**Males**	**Females**
	Diabetic Patients	899	395	505
	Diastolic Dysfunction	299	131	168
Prevalence of diastolic dysfunction in Type II Diabetic patients is 299/899 (33%)				

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Mmp-2/timp-2 Imbalance and Increased Collagen Production are Crucial to the Transition From Compensated Cardiac Hypertrophy to Heart Failure in Rats Subjected to Abdominal Aorta Constriction

PrimaryAuthor.AuthorBlock:**Cibele M Prado**, Univ of Sao Paulo, Ribeirao Preto, Brazil; Thayane R Pizzo, Herminio Ometto Univry Ctr, Araras, Brazil; Aline Fernandes, Herminio Ometto Univry Ctr, ARARAS, Brazil; Vanessa A Amorin, Univ of Sao Paulo, Ribeirao Preto, Brazil; Elen Rizzi, Univ of Ribeirao Preto (UNAERP), Ribeirao Preto, Brazil; Flavio V Meirelles, Univ of Sao Paulo, Pirassununga, Brazil; Helio C Salgado, Univ of Sao Paulo, Ribeirao Preto, Brazil

Background. Hypertension causes cardiac hypertrophy, cardiac dysfunction, and heart failure (HF). The role of matrix metalloproteinases (MMPs) in the cardiac remodeling induced by hypertension has been demonstrated. Our objective was to evaluate

whether temporal changes during the transition from compensated cardiac hypertrophy to HF may be dependent of MMP-2 activity in a model of pressure overload cardiac hypertrophy. **Methods.** Male Wistar rats were subjected to abdominal aorta constriction and observed after 30, 60, 90 days post surgery (dps). Systolic and diastolic cardiac functions were analyzed. Picrosirius red staining was used to quantify interstitial collagen in the left ventricle. Western blotting was performed for MMP-2 and tissue inhibitor of matrix metalloproteinase (TIMP)-2. Data were considered significant when $p<0.05$. **Results.** At 90 dps, 70% (28 of 40) presented hypertrophic hearts (HH) and 30% (12 of 40) hypertrophic+dilated hearts (HD). The ejection fraction (EF) and fractional shortening (FS) at 30, 60 and 90 dps in the HH group were not different from sham. In the HD group, a decrease of 45% in the EF (35.46±3.29%) and 41.5% in the FS (21.60±5.34%) was observed compared to sham (62.62±5.82%; 35.28±2.47%, respectively). In relation to diastolic parameters, the mitral E-wave velocity increased 47% (1279±127) and E/E' ratio increased 60% (68.14±16.78) only in the HD group in relation to sham (866±134; 26.99±5.47, respectively). Increased interstitial collagen was observed at 30 (2.08±0.14%), 60 (2.48±0.29%) and 90 dps in the HH (2.83±0.18%) and HD groups (2.82±0.16%) when compared to sham (1.62±0.15%). There is no alteration in the MMP-2 expression at 30 and 60 dps. An increase of 54.4% in the HH group (1.22±0.23) and 51% in the HD (1.19±0.34) was observed in relation to sham (0.79±0.32). TIMP-2 expression increased 82% at 60 dps (1.30±0.17), 62% in the HH group (1.15±0.31) and 51% in the HD group (1.07±0.31) when compared to sham (0.71±1.12). **Conclusion.** The imbalance between MMP-2 and TIMP-2 expression associated with increased collagen content suggests that the deregulation of MMP-2 expression by TIMP-2 could contribute to the transition from compensated cardiac hypertrophy to heart failure.

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Central BP Variability is Increased in Hypertensive-related Target Organ Damage

PrimaryAuthor.AuthorBlock:**Alejandro De La Sierra**, Julia Pareja, Sergi Yun, Eva Acosta, Francesco Aiello, Hosp Mutua Terrassa, Terrassa, Spain; Anna Oliveras, Susana Vazquez, Hosp Del Mar, Barcelona, Spain; Pedro Armario, Pere Blanch, Hosp Moises Broggi, Sant Joan Despi, Spain; Cristina Sierra, Hosp Clinic, Barcelona, Spain; Francesca Calero, Patricia Fernandez LLama, Fundacio Puigvert, Barcelona, Spain

Peripheral BP variability (BPV) has been found to be increased in hypertensives with target organ damage (TOD). It is unknown if central BP variability follows the same pattern. We aimed to evaluate short-term BPV in a group of hypertensive subjects classified as having or not cardiac, renal, or vascular TOD.
A total of 178 hypertensives (33% women; age 57±12) were evaluated. TOD was defined by the presence of left ventricular hypertrophy (LVH) on echocardiography, renal alterations (urinary albumin excretion >30mg/g or eGFR < 60 ml/min/1.73m^2) or aortic stiffness (aortic pulse wave velocity > 10 m/s). BPV was estimated by

24-hour peripheral and central BP monitoring. Parameters evaluated included: night-to-day ratios (NDR), standard deviation (SD) and coefficient of variation (CV) for 24 hours, day and night, weighted standard deviation (WSD) and average real variability (ARV).
Ninety-two patients (51.7%) had TOD, distributed as follows: 66 (37.1%) had LVH, 47 (26.4%) had renal alterations, and 37 (20.8%) increased aortic stiffness. Systolic and diastolic night-to-day ratios and systolic BPV were increased in patients with TOD with respect to those without (table). No differences were observed in diastolic BPV. The increase in systolic BPV was also observed when comparing groups with or without LVH, renal damage or aortic stiffness. Brachial BPV exhibited the same increased variability as central BPV.
We conclude that the presence of hypertensive cardiac, renal or vascular organ damage is associated with increased systolic central BPV and reduced systolic and diastolic nocturnal fall in BP. Central BPV follows the same pattern as observed with peripheral BPV.

NDR (%) and BPV (mmHg)	With TOD (n=92)	Without TOD (n=86)	P value
Systolic NDR	94.2 ± 9.0	91.3 ± 8.7	0.028
Diastolic NDR	88.5 ± 9.2	84.6 ± 9.9	0.008
Systolic Day SD	13.9 ± 4.6	11.0 ± 3.6	<0.001
Systolic Night SD	12.3 ± 4.5	11.1 ± 3.2	0.048
Systolic Day CV	11.1 ± 3.4	9.2 ± 3.1	<0.001
Systolic Night CV	10.8 ± 3.7	9.9 ± 2.6	0.062
Systolic Weighted SD	13.4 ± 4.0	11.0 ± 2.8	<0.001
Systolic ARV	11.2 ± 3.3	9.4 ± 1.9	<0.001

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P359

Achieving Normal Blood Pressure with Cloud-based Monitoring and Management

PrimaryAuthor.AuthorBlock:**Jorge Sanchez**, Oasis Pavilion Rehabilitation, Casa Grande, AZ; Tanzid Shams, Mountain States Medical Group, Johnson City, TN; Clifford Fonner, Virginia Cardiac Services Quality Initiative, Virginia Beach, VA; Nirav Shah, Swedish Neurology Inst, seattle, WA

Introduction
We assessed the feasibility and impact of remote monitoring and medication management on hypertension control in a nursing home.
Hypothesis:
Uncontrolled hypertension is a causal factor for cardiovascular disease. We hypothesized that increased sensitivity to elevated blood pressure (BP) readings via remote monitoring and targeted medication management alerts would lead to higher rates of controlled hypertension.
Methods:
We followed 48 long-term nursing home patients (average age=68.4) over a 10-week period, of whom 21 were identified as uncontrolled hypertension with a reading >140/90 mmHg. BP readings were taken 3 times a week as compared to a norm of 1 measurement per 3 weeks. Blood pressure data was stored in a cloud and analyzed via algorithms. The physician was given a summary of data identifying those patients who met criteria for elevated BP with averaged per week mean systolic and diastolic blood pressure. Furthermore, patients were further stratified via an algorithm where systolic BP over 140 mmHg was reported as a fraction of the week with uncontrolled hypertension. Together these 3 variables guided the physician in making a determination of a decision to escalate pharmacologic therapy.
Results:
After monitoring and interventions there were significant decreases in the patients' average BP (130.6 mmHg vs. 135.6 mmHg, p=0.027). Neurological (headache, vision changes) and

cardiac symptoms (chest pain) were resolved or improved in 83% of patients (5 of 6). By end of the pilot 4.2% (2 of 48) remained hypertensive as compared to the initial 43.8% (21 of 48) found to be hypertensive at start of pilot; 95.8% (46 of 48) remained controlled by end of study. Furthermore, when compared with a similar cohort of patients, hospital admissions were 80% (2 versus 10) fewer in the study population.

Conclusions:

In agreement with our hypothesis, more frequent BP measurements and medication adjustments led to increased rates of hypertension control. We believe that normotension is achievable using cloud-based monitoring, reporting, and treatment. In conclusion, this new method requires serious consideration versus the current standard of care and could help provide improvements in skilled nursing facilities and other care settings.

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Diagnostic Accuracy Provided by Different Approaches to Office Blood Pressure Measurement: How Many Readings Are Enough?

PrimaryAuthor.AuthorBlock:**Ian M Kronish**, Donald Edmondson, Daichi Shimbo, Columbia Univ, New York, NY; Jonathan Shaffer, Univ of Colorado at Denver, Denver, CO; Lawrence Krakoff, Icahn Sch of Med at Mount Sinai, New York, NY; Joseph Schwartz, Columbia Univ, New York, NY

Importance: Guidelines lack consensus about the optimal approach to measuring office blood pressure (BP) when screening for hypertension. **Objective:** To compare the accuracy provided by different office BP measurement approaches that differ based on number of office BP readings within a visit, number of visits, and assessment method. **Design**: Cross-sectional **Setting:** Primary care. **Participants**: 707 employees without prior diagnosis of hypertension or cardiovascular disease and with screening BP <160/105 mmHg. **Measures**: Six standardized BP readings were taken during each of 3 office visits at least 1 week apart, using 2 assessment methods (mercury sphygmomanometer and BpTRU oscillometric device) for a total of 12,645 readings. Confirmatory factor analysis was used to develop a model from which estimates of the probability of correctly classifying an individual's office BP status using differing numbers and types of office BP readings were generated. **Results**: A single systolic BP reading

correctly classified an individual as having BP above or below the cutpoint for elevated office BP (i.e. 140 mmHg) when the reading was <129 mmHg or >155 mmHg, respectively. Averaging three systolic BP readings across two visits correctly classified an individual 95% of the time if the averaged reading was <134 mmHg or >148 mmHg. There was more confidence gained by increasing the number of visits than the number of readings within a visit. There was no clinically significant confidence gained by dropping the first reading versus averaging all readings, nor by measuring with a manual mercury device versus with an automated oscillometric device. **Limitations**: Only evaluated research quality BP readings. Similar probabilities may not apply to BP measured in routine clinical practice. **Conclusions and Relevance**: Averaging BP readings across two or more office visits might best balance increased confidence in BP status with efficiency of BP measurement, though the preferred measurement strategy may vary with the clinical context. Patients with average readings modestly below the 140/90 mmHg cutpoint (e.g., 134-139 mmHg) might be appropriate for referral to out-of-office BP testing as one cannot gain strong confidence that their "true" office BP is below goal.

Disclosures: **I.M. Kronish:** G. Consultant/Advisory Board; Modest; Center for Disease Control. **D. Edmondson:** None. **D. Shimbo:** G. Consultant/Advisory Board; Modest; Center for Disease Control. **J. Shaffer:** None. **L. Krakoff:** None. **J. Schwartz:** G. Consultant/Advisory Board; Modest; Center for Disease Control.

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P361

Prevalence & Predictors of Orthostatic Hypotension at a Tertiary Care Hypertension Clinic With New Diagnostic Thresholds

PrimaryAuthor.AuthorBlock:**Swapnil Hiremath**, Mohammad Ali Faraz, Brendan McCormick, Marcel Ruzicka, Ottawa Hosp, Ottawa, ON, Canada

Background: Orthostatic hypotension (OH), defined as a decrease of blood pressure (BP) of 20/10 mm Hg (systolic/diastolic) on change in posture from supine to standing is seldom assessed in routine practice because of logistical constraints. A recent study reported a sit-to-stand decrease of 15/7 mm Hg as also having good diagnostic yield. We measured the prevalence & risk factors associated with OH with the new threshold of sit-to- stand of either ≥ 15 mm Hg in systolic (SBP) or ≥ 7 mm Hg in diastolic BP (DBP). Methods: We reviewed medical charts of patients being followed at Renal Hypertension Center, a referral centre for difficult to control hypertension. Sitting BP is measured after 5 minutes of resting, as an average of 5 measurements with an automated device. Standing BP is measured three times at one minute intervals and averaged. OH was determined on the basis of the difference in either average SBP or DBP. Demographic characteristics, comorbidities, medication details, laboratory values and BP measurements were extracted. Results: Data from 219 patients was extracted (see table). The overall difference in SBP (sitting - standing)was 0.94 and DBP was 2.1 mm Hg. 190 patients (87%) did not have OH, whereas 29 (13%) had OH using either SBP or DBP thresholds. The difference in SBP and DBP was 17 mm and 6 mm Hg in those with OH, versus 1.6 and 3 mm Hg amongst those without OH respectively. Higher SBP was significantly associated with OH; age, gender, diabetes, number and hypertension drug class were not.

Conclusion: Amongst referred patients to a specialist hypertension clinic, the prevalence of OH using a threshold of 15/7 mm Hg was 13%. The new diagnostic threshold allows for easy assessment of OH.

Categories	Overall	OH	No OH
Number (n/%)	219	29 (13%)	190 (87%)
Age (mean ± sd, years)	61.2 ± 17.7	63.8 ± 17.6	60.8 ± 17.8
Gender, male (N, %)	103 (47%)	15 (52%)	88 (46.3%)
BMI (mean ± sd, kg/m^2)	31.2 ± 15.7	36.7 ± 39.3	30.3 ± 6.5
Ever Smoker (%)	55 (25.7%)	7 (24%)	48 (26.0%)
Comorbidities (N, %)			
Diabetes	73 (34.3%)	11 (37.9%)	62 (33.7%)
Cardiovascular disease	40 (18.9%)	4 (13.8%)	36 (19.7%)
Peripheral vascular disease	22 (10.4%)	4 (13.8%)	18 (9.8%)
Cerebrovascular disease	18 (8.5%)	2 (6.9%)	16 (8.7%)
Resistant hypertension	77 (35.2%)	10 (34.6%)	67 (35.3%)
Blood Pressure Measurements (mean ± standard deviation)			
Sitting SBP (mm Hg)	137.1 ± 22.2	151.3 ± 33.1	134.9 ± 19.2
Standing SBP (mm Hg)	136.2 ± 21.6	133.8 ± 32	136.5 ± 19.6
Sitting DBP (mm Hg)	76.6 ± 15.2	80.3 ± 23.1	76.0 ± 13.6
Standing DBP (mm Hg)	78.7 ± 14.9	74.5 ± 18.6	79.3 ± 14.2
Medications (N, %)			
ACEi or ARBs	157 (71.7%)	24 (82.8%)	133 (70%)
Beta blockers	103 (47%)	14 (48.3%)	89 (46.8%)
Calcium channel blockers	130 (59.4%)	12 (41.4%)	118 (62.1%)
Alpha-blockers	6 (2.7%)	0 (0%)	6 (31.6%)
Loop diuretics	15 (6.8%)	1 (3.5%)	14 (7.4%)
K-sparing diuretics	29 (13.2%)	2 (6.9%)	27 (14.2%)
Thiazide/ thiazide-like diuretic	96 (43.8%)	16 (55.2%)	80 (42.1%)

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P362

What is the Cost of Measuring a Blood Pressure?

PrimaryAuthor.AuthorBlock:**Steven A Yarows**, IHA, Chelsea, MI

Importance: Blood pressure (BP) measurement has transitioned to the oscillometric method in most hospitals in the United States, however out-patient offices mainly use the auscultatory technique. **Objective:** Is the Welch Allen oscillometric Connex Vital Signs Monitor time and cost-saving in out-patient Primary Care Offices compared to the auscultatory technique? **Design:** An analysis to determine the time to measure BP in consecutive office patients. The Welch Allen Connex Vital Signs Monitor (WA) was initially used and subsequently the standard wall-mounted ausculatory monitor (Manual) method. **Setting:** A single Family and Internal Medicine practice. **Participants:** Patients presenting for an office visit. **Main Outcome(s) and Measure(s):** The patient demographics were recorded (birthdate, gender, BMI, and date of measurement) and the time of measurement of each technique was recorded. **Results:** The average time to manually measure BP was 58.6±13.9 seconds, whereas the WA average was 39.8±23.0 seconds, 18.8 seconds faster ($p<0.05$). There was an improvement with experience only with the WA device. The last 1/3 of measurements showed significant improvement ($p<0.05$) in the speed of measurement for WA (29.3 seconds) vs. Manual (57.5 seconds), which did not significantly change between the first 1/3 and last 1/3 of measurements ($p=0.25$). The average MA cost to measure a single BP using the Manual method was $0.35 vs. the WA method was $0.24, or a saving with the WA method of $0.11 per measurement. After the proper oscillometric technique was mastered, the cost of the last 1/3 of measurements for the Manual was virtually the same at $0.34, however the WA method decreased to $0.17, a saving of $0.17 per measurement. **Conclusions and Relevance:** The oscilliometric Welch Allen Connex Vital Signs Monitor saved 17 cents per measurement in our out-patient primary care practice which potentially saves $1,119 per year.

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P363

Dual Monitor Protocol Comparing the Accuracy of the Oscar 2 & Spacelabs 90207 24-hr Ambulatory Blood Pressure Monitors

PrimaryAuthor.AuthorBlock:**V P Lombardi**, Univ of Oregon, Depts of Biology and Human Physiology, Eugene, OR; P C Reichhold, N R Dietz, D L Pate, Univ of Oregon, Depts of Biology & Human Physiology & Inst of Neuroscience, Eugene, OR

BACKGROUND. Our dual monitor protocol enables simultaneous blood pressures (BPs) using two ambulatory monitors (ABPMs) and two observers with a mercury (Hg) column. In normotensives, hypertensives and alcoholics, an *auscultatory* ABPM underestimated diastolic BP (DBP) progressively from supine to seated to standing. Our goal was to test the accuracy and reliability of *oscillometric* ABPMs. **METHODS.** We programmed an Oscar 2 and Spacelabs 90207 to record simultaneous, opposite arm measurements for 24 hr in a hypertensive male (62 yr). Clocks were synchronized and cuffs were switched every 2-3 hr during waking hr. In the lab observers assessed simultaneous same arm BPs using an Hg column and ABPMs recorded simultaneous opposite arm BPs. Lab techniques were repeated in two young (21 yr) male normotensives. **RESULTS.** The monitors' SBP, DPB and mean arterial pressure differed significantly (all $p < 0.001$) for 109 simultaneous 24-hr measures. The Oscar averaged 154/101 ± 12/9 mm Hg (Stage II), while the Spacelabs 146/98 ± 10/8 (Stage I), differing by 8/3 ± 7/9 mmHg. Both machines exhibited troubling run-away inflations during walking and stair climbing as sustained motion artifacts were interpreted as not achieving MAP or peak SBP. The ABPMs common variance for SBP was 63% ($R = 0.79$) and for DBP was 48% ($R = 0.69$), significantly lower than BPs for trained clinicians using an Hg column (SBP 97%, DBP 95%). In the lab, for SBP the Oscar (151 ± 3 mm Hg) was significantly ($P<0.05$) higher than the Spacelabs (143 ± 3 mm Hg) and observers (144 ± 4 mm Hg). For DBP, the Spacelabs (88 ± 3 mm Hg) tended ($P=0.051$) to be lower than the Oscar (90 ± 3 mm Hg) and observers (92 ± 4 mm Hg). **CONCLUSIONS.** Subjects can be classified differently based on the ABPM model used. The Oscar and Spacelabs ABPMs demonstrate greater variability estimating DBP. If results from multiple ABPMs are pooled in meta-analyses, interpretations may be muddled especially if one is overestimating and another underestimating 24-hr pressures. Though our dual monitor protocol is challenging to implement, we hope to conduct studies in a large, diverse group. US and international protocols should require postural and exercise testing to ensure that devices truly can be classified as ambulatory and are more consistently accurate and reliable.

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P364

Altered Circadian Rhythm in Blood Pressure in Obstructive Sleep Apnea

PrimaryAuthor.AuthorBlock:**Saurabh S Thosar**, Matthew P Butler, Oregon Health and Sci Univ, Portland, OR; Kun Hu, Frank AJL Scheer, Brigham and Women's Hosp, Boston, MA; Carolina Smales, Carilion Clinic, Roanoke, VA; Pamela N DeYoung, UC San Diego, San Diego, CA; Mohammad Hussain, Brigham and Women's Hosp, Boston, MA; Miki Morimoto, Steven A Shea, Oregon Health and Sci Univ, Portland, OR

Background: In people with obstructive sleep apnea (OSA), adverse cardiovascular (CV) events occur most commonly during the night, about 6 hours earlier than the morning peak of events observed in the general population. In

healthy individuals, circadian rhythms in many CV risk markers peak in the morning, around the common time of occurrence of adverse CV events. We therefore tested whether circadian rhythms are advanced in those with OSA, which might explain the earlier time of adverse CV events.
Methods: Eleven volunteers (4 healthy controls and 7 OSA; 55 ± 5 years of age) completed a 5-day laboratory protocol with sleep and all other behaviors evenly spaced across the 24 h circadian period. To achieve this, after a baseline night, participants completed 10 consecutive short-day cycles of 2 h 40 min each of wakefulness and sleep opportunity. Blood pressure and heart rate were recorded 10 min after each wake time. Salivary melatonin and cortisol concentration were measured at 1-1.5h intervals during wakefulness. Cosinor analyses were performed to determine the rhythmicity of the all variables. We also compared OSA data with larger groups of historical healthy controls from our laboratory.
Results: The circadian times of peak systolic and diastolic blood pressure were significantly advanced (up to 7 hours) in OSA as compared to healthy controls, $p<0.05$. Circadian peaks in all other measured variables were not different between OSA and controls.
Conclusions: These results suggest that OSA is associated with a change in the timing of endogenous circadian blood pressure rhythms. This may be specific to the cardiovascular system as there was no evidence for altered phase of melatonin or cortisol secretion patterns. The relatively advanced blood pressure rhythms in OSA is similar to the advancement of the time of adverse CV events in OSA and should be further investigated.

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Blood Pressure Evaluation From Ppg Signal Analysis and Artificial Neural Network

PrimaryAuthor.AuthorBlock:Francesco Lamonaca, Dept of Engineering, University of Sannio, Benevento, Italy; **Vitaliano Spagnuolo**, Unit of Hypertension, Azienda Ospedaliera of Cosenza, Cosenza, Italy; Serena De Prisco, Unit of Hypertension (trainee), Azienda Ospedaliera of Cosenza, Cosenza, Italy; Domenico Luca Carnì, Domenico Grimaldi, Dept of Informatics, Modelling, Electronic and System, Univ of Calabria, Rende, (Cosenza), Italy

The analysis of the PPG signal in the time domain for the evaluation of the blood pressure (BP) is proposed. Some features extracted from the PPG signal are used to train an Artificial Neural Network (ANN) to determine the function that fit the target systolic and diastolic BP. The data related to the PPG signals and BP used in the analysis are provided by the Multi-parameter Intelligent Monitoring in Intensive Care (MIMIC II) database. The pre-analysis of the signal to remove inconsistent data is also proposed. A set of 1750 valid pulse is considered. The 80% of the input samples is

used for the training of the network. Instead, the 10% of the input data are used for the validation of the network and 10% for final test of this last. The results show as the error for both the systolic and diastolic BP evaluation is included in the range of ±3 mmHg. Tab.1 shows the results for 20 PPG pulses randomly selected analyzed together with the systolic and diastolic blood pressure furnished by MIMC and evaluated by the trained ANN.
Tab.1 experimental results comparing MIMIC and the ANN results.
Moreover, a suitable hardware to validate the ANN with the sphygmomanometer is designed and realized. This hardware allows clinicians to collect data according to the requirements of the validation procedure. With the sphygmomanometer the systolic and diastolic values are referred to two different PPG pulses. As a consequence, it is proposed a new hardware interface allowing the synchronized acquisition and storage of the PPG signal and clinician voice. For the validation, the clinician: (i) evaluates the BP on both the arms and assesses that no significant differences occur; (ii) plugs the PPG sensor on the finger of one arm; (iii) starts the recording of both the PPG signal and the audio signal; (iv) evaluates the BP on the other arm with sphygmomanometer and says the systolic and diastolic values when detected.
Through suitable post processing algorithm, the Systolic and Diastolic values are associated to the corresponding PPG Pulses. Following this procedure, the dataset to further validate the ANN according the standard is obtained.
Once the ANN is validated it will be implemented on smartphone to have always in the pocket a reliable measurement system for Blood Pressure, oximetry and heart rate.

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P366

Blood Pressure Checks for Diagnosing Hypertension (BP-CHECK)

PrimaryAuthor.AuthorBlock:**Beverly B Green**, Melissa L Anderson, Andrea J Cook, Jerry Campbell, Yoshio N Hall, Clarissa Hsu, Dwayne Joseph, Pedja Klasnja, Kaiser Permanente Washington, Seattle, WA; Karen L Margolis, Health Partners, Minneapolis, MN; Jennifer B McClure, Kaiser Permanente Washington, Seattle, WA; Sean A Munson, Matthew J Thompson, Univ of Washington, Seattle, WA

Importance: The US Preventive Services Task Force recommends screening adults for high blood pressure (BP). If BP is high, out-of-office BPs are recommended before making a new diagnosis of hypertension, preferably using 24 hour ambulatory BP monitoring (ABPM), because over 30% have normal BPs outside of clinic or “White coat” hypertension with risk of cardiovascular events and death similar to those without hypertension. Currently few physicians use and few patients have heard of ABPM, which may be less convenient than clinic, home, or kiosk-based monitoring.
Objective: The BP Check randomized controlled diagnostic study will compare the accuracy and acceptability of clinic, home, and kiosk-based BP monitoring compared to ABPM for diagnosing hypertension. The study aims, design, protocols, materials, and evaluation described below are informed by patient, health care, policy, and expert stakeholders.
Methods: Adults ages 18-85 will be recruited and randomized to routine screening via clinic screening, home BP monitoring over 5 days, or kiosk-based monitoring on 3 separate days. After completion all participants will complete

ABPM. Mean BP assessed via each screening method (clinic, home, and kiosk) will be compared with 24 hour APBM to assess the accuracy of each method. We will also assess the acceptability of each method from the patients' perspective and the impact of the tests longer-term. Finally, a mixed method analysis is planned to explore physician knowledge and beliefs about BP measurement, diagnosing hypertension, and the perceived feasibility of using each of the tested BP strategies in routine clinical practice.
Results: Enrollment began in May of 2017 with a target of randomizing 510 participants.
Conclusion and Public Health Impact: BP-CHECK will inform which hypertension diagnostic methods are most accurate, acceptable to patients, and feasible to implement in primary care. This evidence-base is critical to compare alternate methods of diagnosing hypertension to ABPM

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P367

Masked Hypertension is Not Associated With Orthostatic Hypertension

PrimaryAuthor.AuthorBlock:Joaquin Cigarroa, Michael Balderas, UT Health, San Antonio, TX; Christopher Baez, South Texas Veterans Healthcare System, Audie L Murphy VA Hosp, San Antonio, TX; Benjamin Garza, South Texas Veterans Healthcare System, Audie L Murhy VA Hosp, San Antonio, TX; Joan Hecht, **Rene A Oliveros**, Univ of Texas Health Science, San Antonio, TX; Robert Chilton, UT Health, San Antonio, TX

The aim of this study was to see if there is any association between masked hypertension (MHTN) and orthostatic HTN (OHTN). In addition, we evaluated if MHTN was associated with diabetes (DM). Lastly we compared BP and HR changes between DM and non-diabetics (NDM)pts.
We performed ambulatory BP monitoring (ABPM) in 70 consecutive pts referred to us for evaluation of hypertension. From this number of pts there were 20 pts with OHTN for an incidence of 28%. There were 8 pts with MHTN for an incidence of 11.4%. There were 26 pts with DM and 44 pts were NDM
The studies were performed after 5 minutes of rest. We obtained BP's in the dominant arm. We subsequently performed the 24hs ABPM using Space Lab On Trak 90227 monitors.
Out of 8pts with MHTN, none had OHTN and only 2 were pts with DM. In addition, we compared age, weight, BP sitting and standing as well as HR sitting and standing in our 70 patients that were divided in 2 groups, DM v/s NDM. The following table depicted our results:
In summary, we found a higher sitting diastolic BP in nondiabetic pts. There was no significant difference between both groups in the remaining parameters.
In conclusion, in our small population of pts there was no association between MHTN and OHTN. The association between MHTN and DM was very low. When comparing 8 different parameters depicted in the table the only significant difference between DM and NDM was on the sitting diastolic BP.

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P368

Effects of Empagliflozin on Day-to-day Variability of Home Blood Pressure and Heart Rate in Patients with Type 2 Diabetes

PrimaryAuthor.AuthorBlock:**Tatsuo Misawa**, Yumi Kawagoe, Fujiko Oda, Misao Ogata, Yamagata Prefectual Kahoku Hosp, Yamagata, Japan

Background: The effects of empagliflozin in addition to standard care, on day-to-day variability of self-measured home blood pressure (BP) and heart rate (HR) at home in patients with type 2 diabetes (T2DM) at high cardiovascular risk are not known. **Method**: We followed twenty-three consecutive T2DM patients (mean age: 69 years old, 12 men) to add 10 mg of empagliflozin once daily for three months. Home BP and HR were measured once every morning at home, using an oscillometric device. The variability of BP and HR were defined as the standard deviations (SD) of measurements which were performed on seven consecutive days. **Results**: For home blood pressure, empagliflozin significantly reduced systolic blood pressure (SBP) from 130±11 mmHg at baseline to 126±11 mmHg at the first week(1W) of the administration (P<0.05). SBP achieved the target home BP goal (125±11 mmHg) at the second week(2W) and was maintained during the study (P<0.01). As regards day-to-day variability of SBP, SD decreased from 7.3±3.5 mmHg at baseline to 6.7±2.5 mmHg at 1W (4W: 6.4±2.8 mmHg, 8W: 5.2±2.3 mmHg, P<0.05). In diastolic blood pressure (DBP), there was a significant reduction of SD compared with that at baseline (4.9±1.6 mmHg at baseline, 4W: 4.3±1.6 mmHg, 8W: 3.9±1.8 mmHg, 12W: 4.4±1.6 mmHg, P<0.05); however, there was no change of DPB (71±10 mmHg at baseline, 12W: 71±8 mmHg). Similarly, there was a decreasing trend in SD of HR (3.9±1.0 beats per minute (bpm) at baseline, 4W: 3.3±1.3 bpm, 12W: 3.1±1.3 bpm, P<0.1), although there was no significant change in HR (62±13 bpm at baseline, 12W: 61 ± 12 bpm). **Conclusion**: Empagliflozin tended to reduce the day-to-day variability of self-measured morning home BP and HR.

Table. Baseline characteristics

Diabetic treatment	N	Cardiovascular medication	N
Diet only	4	AIIR	17
Biguanide	13	ACE inhibitor	4
α -Glucosidase inhibitor	4	Ca channel blocker	17
Sulfonylurea	2	β -blocker	9
DPP-4 inhibitor	3	Diuretic	4
Insulin	3	Statin	15

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P369

Ambulatory Blood Pressure Monitoring Profile as a Useful Tool - Assessment of an Adult Population in the Real World

PrimaryAuthor.AuthorBlock:Yasmin Suemy Prado, Livia Freitas Rodrigues, Patricia de Lima Lopes, Ligia de Carvalho Rocha, Maria Andries Lucci, Mariella Pacheco dos Santos, Paulo Emilio Almeida, Estácio de Sá Univ, Rio de Janeiro, Brazil; **Lílian Soares da Costa**, Estácio de Sá Univ e Faculdade de Medicina Souza Marques, Rio de Janeiro, Brazil

Introduction: The pattern of therapeutic and diagnostic control of arterial hypertension (AH) has been better evaluated with the use of Ambulatory Blood Pressure Monitoring (ABPM) in clinical practice. Objective: The purpose of the study was to evaluate the blood pressure

(BP) profile through data obtained in an adult population (18-59 years), and to further determine the differences of BP readings between users (G1) and non-users (G2) antihypertensive drugs. Method: In a cohort of 2222 non-elderly subjects, both genders, mean 43 years (56.8% female), referred for ABPM from an outpatient set-up, we studied 24-hour ABPM patterns. Results: Of 2222 adults, 1324 (59.6%) were off antihypertensive medications (G2) and of the remaining, 751 (33.8%) used one or two drugs and 6.6% used three or more drugs. Analysis of variance was applied for a comparative study of therapeutic control between groups, with a significance level of *p* less than 0.05. From the G2, 898 subjects (67,8%) had 24-hour BP within normal limits (119x71mmHg) and, 426 (32,2%) had an elevated mean 24-hour BP (139x87mmHg). Of the G1, subjects in monotherapy, double or triple or more antihypertensive therapies presented satisfactory control in 68% (118x71mmHg), 72% (117x70mmHg) and 66% (119x71mmHg) respectively, with no statistically significant difference between groups. The mean nocturnal drop expressed in percentage for the SBP and DBP in G2, was 11.9±2.03 and 18.4±7.19 in normotensive subjects, and 11.1±4.05 and 16.8±8.02 in hypertensive subjects, with no significant difference between different gender groups (p=0.6). Irrespective of normal nocturnal drop values in G1, we observed a significant attenuation in the subgroup using three or more drugs (percentage of 10.1±6.02 and 14.8±6.13) compared to those with monotherapy or double therapy (11.0±4 and 16.6±4.08), both with similar pattern in their dipping profile. Conclusion: The percentage diagnosis of AH by ABPM (32%) is similar to the reported papers in the literature. The pattern of nocturnal drop may help the identification of target organ damages and help in determination of therapeutic adjustments, corroborating literature data, which emphasize the use of ABPM study in the diagnostic and therapeutic evaluation.

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P370

Nocturnal Blood Pressure Fall on Ambulatory Monitoring in a Cohort Data of Elderly Subjecsts

PrimaryAuthor.AuthorBlock:Patricia de Lima Lopes, Livia Freitas Rodrigues, Yasmin Suemy Prado, Ligia de Carvalho Rocha, Paulo Emilio Almeida, Maria Andries Lucci, Mariella Pacheco dos Santos, Estácio de Sá Univ, Rio de Janeiro, Brazil; **Lílian Soares da Costa**, Estácio de Sá Univ e Faculdade de Medicina Souza Marques, Rio de Janeiro, Brazil

Introduction: The use of Ambulatory Blood Pressure Monitoring has offered parameters that directly support stratification and independent association of cardiovascular risk (CV), especially in the elderly. Objective: To evaluate systolic (SBP) and diastolic (DBP) patient's blood pressure (BP) on 24-hour, daytime and night time periods, pulse pressure (PP) and systolic and diastolic nocturnal drop in a population of elderly referred to be clinically healthy. Method: In a cohort of 1652 individuals over 60 years of age, both genders, we selected 434 subjects with no prior history of CV disease and off CV medications. Of these, 242 (56%) had normal and 192 (44%) had elevated mean 24-hour BP (G2), being 68 with isolated systolic hypertension (G2A) and 124 with systolic and diastolic hypertension (G2B). Analysis of variance was applied for a comparative study

between groups, assuming a significance level of *p* less than 0.05. Results: Mean 24-hour, daytime and night time SBP in G1 were 118.94 ± 7.2, 122.27 ± 3.0, 110.23 ± 4.1 mmHg in men and 117.05 ± 6.3, 120, 46 ± 2.4 and 107.57 ± 3.4 mmHg in women (p = 0.06). Mean DBP values in the same periods were, respectively, 67.66 ± 6.62, 70.61 ± 5.3 and 60.24 ± 2.4 mmHg in men and 67.94 ± 5.2, 70.70 ± 5, 2 and 60.57 ± 5.1 mmHg in women (p = 0.08). Statistical analysis showed no difference for PP values between genders in different periods evaluated. In G2, there was a significant difference between subgroups A and B for all parameters, with mean 24-hour BP being 137,18x71,85 in G2A and 142,67x86,33 in G2B, p 0.003 SBP and 0.001 DBP, and 24-hour PP 65.32 and 56.33 mmHg, respectively (P 0.001), with no significant difference between genders. Regarding mean systolic and diastolic nocturnal drop, we observed values in G1 10,06 ± 7,07 and 14,37 ± 8,18, in G2A 5,24 ± 10,2 and 9,73 ± 9,8 and in G2B 8,93 ± 6.47 and 13.36 ± 6.5 mmHg, respectively; that is, lower values in G2A compared to G1 and G2B (p 0.001 and p 0.033), with G1 and G2B with no significant difference. CONCLUSIONS: Since the nocturnal drop pattern is the most consistent predictor of CV outcome, we have confirmed in our findings, previous literature data, which shown reduced nocturnal BP falls in the elderly population as a marker of BP elevation itself, after adjusting for age, gender and comorbidities.

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P371

Incremental Changes in Systolic Blood Pressure Post-mild Exercise, in Normotensive Subjects, is Associated With Significant Structural and Functional Cardiovascular Abnormalities

PrimaryAuthor.AuthorBlock:**Susan Tucker**, Arjun Byju, Mahfouz El Shahawy, Cardiovascular Ctr of Sarasota, Sarasota, FL

Background: Abnormal rise in systolic blood pressure (SBP) post-mild exercise (PME) has been associated with early structural and functional cardiovascular abnormalities.
Purpose: To determine if the differential amount of rise in SBP PME, in normotensive subjects, effected the risk of early structural and functional cardiovascular abnormalities.
Methods: 1416 untreated, asymptomatic subjects were screened for early indicators of cardiovascular disease using the Early CVD Risk Score (ECVDRS), also known as Rasmussen Risk Score (RRS), which consists of a panel of 10 tests; large (C1) and small (C2) artery stiffness, resting BP and post mild exercise (PME), CIMT, abdominal aorta and left ventricle ultrasounds, retinal photography, microalbumuria, ECG, and pro-BNP. 996 subjects were normotensive. Of those subjects, 30 subjects had an abnormal rise in SBP PME between 30-40mmHg and 14 had an abnormal rise in SBP PME >40 mmHg. Focus was placed on the three tests recommended for early CVD assessment; C1, C2 and CIMT.
Results: As seen in Figure 1.0, an abnormal rise in SBP PME greater than 40mmHg is associated with a statistically significant increase in risk of early structural and functional cardiovascular abnormalities in untreated, asymptomatic, normotensive subjects opposed to those who only had an increase between 30-40mmHg.
Conclusion: Assessment of SBP PME is an easy, noninvasive, inexpensive test that can be performed by any health care practitioner to

evaluate the risk of CVD in patients. Based on the severity of the increase in SBP PME should warrant physicians on the urgency to further investigate and treat patients to divert the progression of CVD.

Figure 1.0

Increase in SBP PME Between 30-40mmHg		Increase in SBP PME >40mmHg	
Total Number of Subjects	30		14
Number of Males	15		14
Number of Females	15		2
Average Age	60		68
Average BMI	27.3		32
Average Waist	38.0		36
Abnormal C1	0/30 (0%)		3/14 (22%)
Abnormal C2	2/29 (7%)		2/14 (14%)
Abnormal CIMT	5/30 (17%)		6/14 (43%)
Average R/S	3		6

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P372

Validation of a m-Health Platform for Blood Pressure Control and Reduction

PrimaryAuthor.AuthorBlock:**Giovanni Peretto**, Univ Vita-Salute San Raffaele, Milano, Italy; Gaia Del Mauro, Tommaso Bordignon, Eros Colombo, Martina Fabi, Amicomed Inc, San Francisco, CA; Stefano Negri, Vittoria Vergani, Univ Vita-Salute San Raffaele, Milano, Italy; Domenico Cianflone, Amicomed Inc, San Francisco, CA

Background: Self-measured blood pressure (BP) monitoring, has been proposed as a means of improving BP control. Lifestyle modifications are indicated in hypertension at any stage. *AHA's Life Simple 7* provides a straightforward call-to-action. mHealth services (mHs) may provide additional tools to effective BP control. **Aim**: We tested the reproducibility of the initial results of a mHs for remote BP monitoring and lifestyle counseling. The mHs in study is a *cloud*-based BP -recording, -interpretation and -trend evaluation service (BT) coupled with personalized lifestyle-change programs (LC). Initial results showed a significant reduction of BP at the end of the LC program. BT collects BPs, and after consistency-checks, feeds a proprietary CE-certified medical device algorithm to provide actual BP interpretation and BP trend assessment. Through a separate proprietary algorithm, LC provides a 3-month personalized lifestyle modification program for BP reduction, built on the *AHA's Life Simple 7*, after collecting detailed clinical and lifestyle data. BP changes were evaluated comparing BPs recorded at the beginning and at 3 months in both groups. We studied 2601 subjects (50±14 years old) that voluntarily and unsolicited joined LC+BT or BT-only, from June 2016 to April 2017, via App or website. **Results**: During the study period a total 60044 BP values were collected, subjects with discontinuous BP measurements were excluded from evaluation. We hence analyzed the data of 575 LC+BT subjects and 1653 BT-only subjects. We observed a significant BP reduction in LC+BT subjects at the end of the program for both systolic and diastolic BPs: 4.7±1.2 and 3.3±0.8 (mean±SEM, mmHg), $p<0.0001$, respectively. In BT-only subjects the reduction of BP values at 3-months was *2.5 ±0.9 and 1.7±1.0, p<0.001, respectively.* The subjects of the LC+BT group with $\geq$ 3 systolic BPs above normal values in the first 10 days, experienced a greater BP reduction: *7.9±1.4 and 4.2±1.1, p<0.0001, respectively.* **Conclusions**: The data confirm that this mHealth service provides a strong and effective adjunctive tool for BP reduction. These results support the value of the combination of algorithm based remote BP monitoring and a personalized implementation of *AHA's Life Simple 7* recommendations.

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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; AMICOMED.

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P373

A Cohort Study in Elderly Population - Differences Between Older and Very Older Subjects

PrimaryAuthor.AuthorBlock:Livia Freitas Rodrigues, Patricia de Lima Lopes, Yasmin Suemy Prado, Mariella Pacheco dos Santos, Maria Andries Lucci, Ligia de Carvalho Rocha, Paulo Emilio Almeida, Estácio de Sá Univ, Rio de Janeiro, Brazil; **Lílian Soares da Costa**, Estácio de Sá Univ e Faculdade de Medicina Souza Marques, Rio de Janeiro, Brazil

Introduction: It is known that isolated systolic hypertension (ISH) and elevated pulse pressure (PP) play an important role in the development of cerebrovascular disease, congestive heart failure, and coronary heart disease, which are the major causes of cardiovascular morbidity and mortality in the population aged older than 65 years. The systolic blood pressure (BP) has been proven to be a stronger predictor of cardiovascular diseases than diastolic BP and a correlation between mortality and high BP has been extensively proven for those in late life until around the age of 80 years. Objective: To evaluate BP profile on 24-hour, daytime and night time periods, pulse pressure (PP), riser BP pattern and systolic and diastolic nocturnal drop in an elderly population, referred to be clinically healthy with no prior history of CV disease and off CV medications. Methods: We analyzed a cohort of 1300 elderly patients aged over 65 years and compared the ambulatory blood pressure data of elderly individuals (G1) aged 65-79 years (1020 subjects, mean 67.16 years, 64.7% female) and very elderly individuals (G2) aged over 80 years (280subjects, mean 83.89 years, 69.3% female). Results: Differing trends for systolic and diastolic BP between groups resulted in large differences in ambulatory PP, it being significantly greater (p = 0.001) throughout the entire 24-hour in G2, even after correcting for age (24-hour, daytime and night time periods being 55,07 x 66,76mmHg, 55,31 x 67,03mmHg and 54,55 x 65,95mmHg , respectively in G1 and G2). The prevalence of non-dipping was not significantly higher in G2. Largest difference between groups was in the prevalence of the riser BP pattern, asleep systolic BP mean greater than awake SBP mean (18,7% vs. 6,5% in patients G2 and G1, respectively; p 0.001). Conclusions: There is a growing recognition of the importance of the systolic component of BP. About 65% of hypertension in the elderly is ISH, and CVD risk increases with PP, which is not simply a marker for stiff diseased arteries; elevated PP and asleep systolic BP mean was the major basis for the diagnosis and stratification of ISH.

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P374

Smartphone Application for Blood Pressure Monitoring: Whitings vs MobileRapid (Pic S)

PrimaryAuthor.AuthorBlock:**lucia prati**, Asl di Frosinone, paliano, Italy; Valerio Pecchioli, Asl di Frosinone, frosinone, Italy

HBPM has a limits: the poor compliance with the correct method and the compilation of the paper diary. Telemonitoring of arterial pressure - wants to overcome these predetermined limits. The goal of telemonitoring of the pressure is to improve the management of the hypertensive condition. Aim of our study: to evaluate the perception of qualitative parameters for the extensive use of blood pressure telemonitoring such as: Eases, clarity of the display, speed, noise, practicality and reliability, comparing two different oscillometric devices for blood pressure measurement with Bluetooth connectivity to a smartphone app with the ability to send the data press directly to its reference center : MobileRapid (A), Whitings (B).
Materials and Methods: We have enrolled 60 hypertensive patients, 30 M and 30 F with an average age of 40.5± 9.74 y. All patients were asked to carry out the automated blood pressure with the two devices. Each patient was given a questionnaire that investigated the perception of subjective parameters by the patient of a device in comparison to the other (A vs C) and the patient's orientation about the possibility of connecting the pressure measuring device to a specific app to send the data.
Results: 50 out of 60 pts (83.33%)of the sample perceived overall easier to use the device A against 9 out of 60 (15%) C ($p < 0.0001$) 43 out 60 (71.67%) found it clearer ($p < 0.0001$), 31 out of 60 (51.67%) faster ($p = 0.0078$), while for the silent parameter 25 out of 60 (41.67%) of patients preferred C and for 28 out of 60 (46.67%) there was no No difference ($p = 0.0016$). Device A appeared to be more practical in 45 out of 60 (75%) patients compared to 20% for Device C (12 pts out of 60) ($p < 0.0001$), and in 46 out of 60 (76.67%) of cases worldwide, MobileRapid appeared more reliable than Whitings ($p < 0.0001$).
98% of the sample (58 pts) is interested in having a dedicated app.
Conclusions: The MobileRapid device is perceived reliable, faster than Whitings. The good thing is that our sample, albeit not numerically elevated, has shown almost total interest in being 'connected' with your doctor as well as constantly monitoring your pressure values.

Disclosures: **L. prati:** G. Consultant/Advisory Board; Modest; artsana. **V. Pecchioli:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Artsana group.

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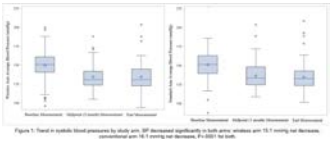
P375

Community Based Wireless Blood Pressure Monitoring Improves Patient Centeredness

PrimaryAuthor.AuthorBlock:**Ryan James Moran**, Anna Narezkina, Kevin Patrick, UCSD, San Diego, CA

Objective: Studies exploring wireless-based systems to monitor patients in underinsured communities are lacking. We evaluated blood pressure (BP) control with wireless vs. conventional home-based BP machines in a pilot study nested within a larger trial in San Diego County focusing on optimizing cardiovascular risk reduction. Methods: Patients with a new diagnosis of hypertension (HTN), or those with BP above 140/90 on a current regimen were enrolled from three federally qualified healthcare center systems in low-income communities. Participants were randomized to conventional automated BP cuff

(CBP) or a wireless cloud-based system (WBP), and followed with a clinical visit at 3, and 6 months. Exit surveys were conducted to evaluate patterns of use. Results: At the time of this analysis complete clinical data are available for 151 participants (71 WBP; 80 CBP). Baseline BPs did not differ between treatment arms (systolic: 149.9 mmHg WBP; 151 mmHg CBP; P=0.72; diastolic: 83.5mmHg WBP; 81.3 CBP; P=0.23). BP decreased significantly in both arms by the end of the pilot (Figure 1). No differences were seen between groups for both net change of BP (P=0.79) or for end mean systolic BP (P=0.96). Of 60 participants in WBP arm who completed the exit survey, those with BP still not at goal (N=20) were more likely to report “Strongly Agree” or “Agree” that the wireless system helped them feel more engaged in their clinical care (P=0.02). Discussion: Both wireless and conventional BP monitoring had a clinically significant impact on BP control among underinsured individuals in this study, and the wireless system appears to improve engagement in care.



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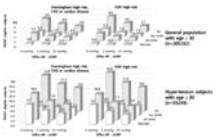
P376

Impact of Selected Differences Between Automated versus Conventional Office Blood Pressure and Adherence on the Prevalence of SPRINT Eligibility in Korean Population

PrimaryAuthor.AuthorBlock:**Jinho Shin**, Hanyang Univ Medical Ctr, Seoul, Korea, Republic of; Sung-Il Choi, Hanmmaeum General Hosp, Chang-Won, Korea, Republic of; Sungha Park, Yonsei Unviersity Medical Ctr, Seoul, Korea, Republic of; Ki Chul Sung, Sungkyunkwan Univ Coll of Med, Seoul, Korea, Republic of; Kwang-il Kim, Seoul Natl Unviersity Bundang Hosp, Bundang, Korea, Republic of; Sang Hyun Ihm, Catholic Univ Coll of Med, Seoul, Korea, Republic of; Wook Bum Pyun, Ewha Womens Univ Sch of Med, Seoul, Korea, Republic of; Soon Kil Kim, Hanyang Univ Medical Ctr, Seoul, Korea, Republic of

Background: For the applicability of Systolic Blood Pressure (BP) Intervention Trial (SPRINT) is controversial due to regional cardiovascular (CV) risk stratification system, AHM adherence, and the automated office BP (AOBP) methodology. **Method**: General population aged 30 or more (n=205282) and 55208 hypertension subject in Korean National Health Insurance Service - National Sample Cohort (NHIS-NSC) 2010 data were analyzed. Three BP criteria 1, 2, and 3 according to the selected difference between office BP and AOBP of 0 mmHg, 5 mmHg, and 10 mmHg, respectively. Also according to the risk groups and adherence status, prevalence of SPRINT eligible subjects were investigated. **Results:** SPRINT eligibility subjects were observed in 6.5[6.4~6.7]% vs 5.6[5.5~5.7]% in the general population 15.9[15.7~16.0]% vs 14.8[14.7~15.0]% in hypertension patients by KSH vs. FRS, respectively ([ ], 95% confidence interval). According to BP criteria 1 to 3, SPRINT eligibilities by KSH were different significantly in

the general population (6.5[6.4~6.7]%, 4.0[3.9~4.1]%, and 2.7[2.6~2.7]%, respectively) and in hypertension patients (15.9[15.7~16.0]%, 11.8[11.7~12.0]%, and 9.9[9.8~10.0]%, respectively). When the SPRINT eligibility was allowed only in PDC >= 300 days per year in hypertension patients, the prevalence according to the BP criteria 1 to 3 were 8.0[7.7~8.2]%, 5.4[4.3~4.7]%, and 4.1[3.9~4.2]%, respectively by KSH and 8.1[7.8~8.3]%, 5.7[5.5~5.9]%, and 4.4[4.3~4.6]%, respectively by FRS. **Conclusion:** SPRINT eligibility can be markedly differed not by the risk stratification systems but by the application of AOBP to conventional office BP and adherence.



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P377

Real World of Ambulatory Arterial Stiffness Index and Central Aortic Pressures of Hypertensive Patients Treated by Their Primary Care Physicians

PrimaryAuthor.AuthorBlock:**Ricardo Cabrera-Sole**, Liliana Urrera Rivera, Caridad Turpin Lucas, Santiago Garcia Ruiz, Univ Hosp of Albacete, Albacete, Spain

BACKGROUND: THE AMBULATORY ARTERIAL RIGIDITY INDEX (aasi) is a good method to measure the vascular risk of patients with hypertension. However, in a few cases this index is taken into account when treating patients at high risk. In this paper, we present our experience on AASI levels in patients with AHT (PHTT) and high cardiovascular risk referred by their primary care physicians (PCP)
OBJECTIVES: to evaluate the AASI in patients with hypertension who received previous treatment and to correlate these values with central arterial pressures.
MATERIALS AND METHODS: We studied 256 PHTAs (100 women and 156 men, (69 + 8 years old) treated by their PCP, who underwent a 24-hr ABPM study, and arterial stiffness was measured by AASI, , as well as the central arterial pressures and the simple or combined drugs that were used at the beginning and the end of the 16-week follow-up after modifying the treatment and evaluating them by telephone at least once a month. The details are described in the following table:

DATA	SBP mmHg	DBP mmHg	AASI	SBPC mmHg	DBPC mmHg	Nº of drugs
BASELINE	152±6	92±4	0.78±0.2	124±3	88±3	3±1.1
FINAL	134±4*	83±3*	0.45±0.2*	101±2*	79±2*	2±1

* Means p values less than 0.05.
CONCLUSIONS: In the real world, PHTTs are not well controlled despite taking more than two drugs, requiring adjustment and closer follow-up to obtain desired results in the control of hypertension and in improving parameters of arterial stiffness measured by AASI as well as central pressures and consequently decrease cardiovascular risk. For follow-up, telephone follow-up may be useful for better control of these patients.

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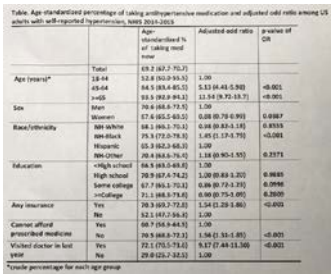
P378

Current Progress of Antihypertensive Medication Use According to Healthy People 2020 Goals Among Adults With Hypertension-the National Health Interview Survey 2014-2015

PrimaryAuthor.AuthorBlock:**Jing Fang**, Carma Ayala, Fleetwood Loustalot, CDC, Atlanta, GA

Background. Hypertension is a major risk factor for heart disease and stroke; yet only half of those with hypertension have it under control. One of the Healthy People 2020 goals is to increase the proportion of adults with hypertension who are taking antihypertensive medications to lower their blood pressure from a baseline of 63.2% to a target of 69.5% by 2020. The objective of this study was to examine progress towards meeting this national objective and to assess disparities mid-decade.
Methods: Adult participants (≥18 years) with self-reported hypertension from the National Health Interview Survey in 2014 and 2015 were included in this study. Current antihypertensive medication use was assessed and age-standardized for analysis. Multivariable logistic regression models were used to determine the odds ratio of antihypertensive medication use, adjusting for age, sex, race/ethnicity, education and health care access status.
Results: Among the 21,050 (26.7%) adults with self-reported hypertension, 69.2% reported current antihypertensive medication use and disparities were noted among subgroups. Adults with estimates of antihypertensive medication use that fell below the Healthy People 2020 goal (69.5%) included women, non-Hispanic whites, Hispanics, and those with access health care barriers (Table).
Conclusion: To control hypertension, most patients require antihypertensive medications to achieve control. Understanding disparities in antihypertensive medication utilization is needed for all population to achieve Healthy People 2020 targets by the end of the decade.



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P379

Patient and Caregiver Experiences With the Diagnosis of Neurogenic Orthostatic Hypotension

PrimaryAuthor.AuthorBlock:**Lawrence Arthur Hewitt**, Lundbeck LLC, Deerfield, IL; Charles H. Adler, Mayo Clinic, Scottsdale, AZ; Daniel O. Claassen, Vanderbilt Univ Medical Ctr, Nashville, TN; Christopher H. Gibbons, Beth Israel Deaconess Medical Ctr, Harvard Medical Sch, Boston, MA; Satish R. Raj, Libin Cardiovascular Inst, Univ of Calgary, Calgary, AB, Canada

Objective: To understand the challenges to diagnosis in patients with neurogenic orthostatic hypotension (nOH) **Background:** nOH is a sustained reduction in blood pressure (BP) with postural change associated with autonomic dysfunction. Despite symptoms of nOH, many patients struggle to find an accurate diagnosis. **Methods:** An online, US-based survey designed by the authors was conducted by Harris Poll. Eligible participants were ≥18 years of age with Parkinson disease, multiple system atrophy, or pure autonomic failure and ≥1 of

the following: orthostatic hypotension (OH), nOH, low BP, OH/nOH symptoms, or were caregivers of eligible participants. **Results:** The survey included 363 patients and 128 caregivers. Groups were separate, where caregivers were not the caregivers to patient responders. Respondents indicated that patients experienced nOH symptoms long term **(Table 1)**. Most patients (69%) and caregivers (59%) reported discussing nOH symptoms with a healthcare provider (HCP) within the first year of symptom onset, but only 36% of patients and 16% of caregivers reported a formal diagnosis of OH or nOH. Of those with a formal diagnosis, the majority of patients (50%) were frustrated by the path to diagnosis and more than 40% of patients and caregivers reported that the patient saw ≥3 HCPs before diagnosis. After diagnosis, most patients (70%) and caregivers (60%) reported improved symptom management. **Conclusions:** This survey reveals that patients and caregivers may find the path to nOH diagnosis challenging and suggests increased awareness among HCPs is needed. Once a diagnosis is made nOH symptoms are better managed.

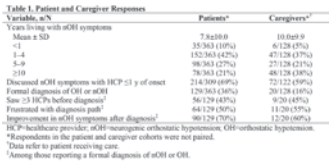

Table 1. Patient and Caregiver Responses

Variable, n/N	Patients*	Caregivers*
Years living with nOH symptoms		
Mean ± SD	7.8±10.0	10.0±9.9
<1	35/363 (10%)	6/128 (5%)
1–4	152/363 (42%)	47/128 (37%)
5–9	98/363 (27%)	27/128 (21%)
≥10	78/363 (21%)	48/128 (38%)
Discussed nOH symptoms with HCP ≤1 y of onset	214/309 (69%)	72/122 (59%)
Formal diagnosis of OH or nOH	129/363 (36%)	20/128 (16%)
Saw ≥3 HCPs before diagnosis†	56/129 (43%)	9/20 (45%)
Frustrated with diagnosis path†	64/129 (50%)	11/20 (55%)
Improvement in nOH symptoms after diagnosis†	90/129 (70%)	12/20 (60%)

HCP=healthcare provider; nOH=neurogenic orthostatic hypotension; OH=orthostatic hypotension.
*Respondents in the patient and caregiver cohorts were not paired.
†Data refer to patient receiving care.
‡Among those reporting a formal diagnosis of nOH or OH.

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P380

Blood Pressure Control Rates When Measured in a Specialty Clinic versus Primary Care Within a Large Integrated Health System

PrimaryAuthor.AuthorBlock:**Sarah J Billups**, Joseph J Saseen, Univ of Colorado Skaggs Sch of Pharmacy and Pharmaceutical Sciences, Aurora, CO; Joseph P Vande Griend, Univ of Colorado Health, Aurora, CO; Lisa M Schilling, Univ of Colorado Sch of Med, Aurora, CO

Background:
Treating patients with hypertension to goal blood pressure (BP) values is a pillar of

cardiovascular risk reduction. Its importance is prominent in quality metrics used in public reporting, accreditation, and value-based reimbursement programs. We hypothesized that BP measurements performed in a specialty (SP) clinic would be less likely to be at goal compared to when measured in a primary care (PC) clinic.

Objective(s):

Identify differences in rates of BP control for patients with hypertension using measurements performed in a SP clinic versus a PC clinic.

Methods:

Clinic-based BP measurement performed in 2016 for adults in Colorado's UCHealth Clinically Integrated Network with a hypertension diagnosis were included. The most recent measurement for each patient was classified as controlled (<140/90 mm Hg) or uncontrolled, and from a PC or SP clinic. Logistic regression was used to calculate the OR and 95% CI for the likelihood of having controlled BP in a SP versus PC clinic, controlling for age and gender. A secondary analysis of patients with measurements done in both clinic types used conditional logistic regression to compare the likelihood of control based upon each patient's most recent SP versus PC measurement.

Results:

Of the 86,632 hypertensive adults with BP measurements in 2016, 51% (43,850/86,632) had their most recent measurement performed in a SP clinic. Control rates for BP were lower in SP versus PC clinics (63% (27,771/43,850) vs 67% (28,843/42,782), OR=0.84 (95% CI 0.81-0.86)). Among patients with measurements performed in both clinics in 2016, control rates for the same patients were lower in SP compared to PC clinics (63% (17,900/28,455) vs. 71% (20,077/28,455), OR=0.64 (0.61-0.66)). This pattern persisted even in patients whose measurements were done within 7 days of each other (63% (1,634/2,593) vs 69% (1,781/2,593), OR=0.70 (0.61-0.80)).

Conclusions:

BP measurements performed in SP clinics are significantly less likely to be controlled compared to those in PC clinics, even between measurements performed on the same patient within a short time. This has significant treatment and quality measurement implications for health systems. Investigation is needed to identify underlying reasons and to resolve discrepancies**.**

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P381

Understanding the Symptoms of Neurogenic Orthostatic Hypotension: Results From a Survey of Patients and Caregivers

PrimaryAuthor.AuthorBlock:**Lawrence Arthur Hewitt**, Lundbeck LLC, Deerfield, IL; Charles H. Adler, Mayo Clinic, Scottsdale, AZ; Daniel O. Claassen, Vanderbilt Univ Medical Ctr, Nashville, TN; Christopher H. Gibbons, Beth Israel Deaconess Medical Ctr, Harvard Medical Sch, Boston, MA; Satish R. Raj, Libin Cardiovascular Inst, Univ of Calgary, Calgary, AB, Canada

Objective: To understand the burden and impact of symptoms of neurogenic orthostatic hypotension (nOH) on patients **Background:** nOH and its symptoms such as dizziness/lightheadedness are common in patients with Parkinson disease (PD) and other forms of autonomic dysfunction. **Methods:** An author-designed, US-based survey was conducted by Harris Poll. Eligible participants were aged ≥18 years with PD, multiple system atrophy (MSA), or pure autonomic failure and

≥1 of the following: orthostatic hypotension, nOH, low BP, OH/nOH symptoms, or were caregivers of eligible patients. **Results**: Most patients (90%) had a diagnosis of PD, and most caregivers (88%) cared for a patient with PD **(Table 1)**. Patients (34%) and caregivers (49%) reported experiencing nOH symptoms before PD or MSA motor symptoms and >40% indicated that nOH symptoms were more troublesome than motor manifestations of PD or MSA. Less than a quarter (22%) of respondents suggested symptoms were most severe in the morning; more (30%) reported a consistent severity throughout the day. Patients (40%) and caregivers (63%) reported trouble managing symptoms during the day. In the past 12 months, a fall due to nOH symptoms was reported by 57% of patients and 80% of caregivers. **Conclusions:** These findings suggest that nOH symptoms may predate the onset of motor symptoms in neurodegenerative conditions linked to alpha-synuclein pathology. Many respondents report nOH symptoms are the same severity through the day. Patients with nOH may have trouble managing symptoms and note an increased risk for falls.

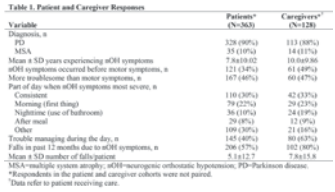

Table 1. Patient and Caregiver Responses

Variable	Patients* (N=363)	Caregivers*† (N=128)
Diagnosis, n		
PD	328 (90%)	113 (88%)
MSA	35 (10%)	14 (11%)
Mean ± SD years experiencing nOH symptoms	7.8±10.02	10.0±9.86
nOH symptoms occurred before motor symptoms, n	121 (34%)	61 (49%)
More troublesome than motor symptoms, n	167 (46%)	60 (47%)
Part of day when nOH symptoms most severe, n		
Consistent	110 (30%)	42 (33%)
Morning (first thing)	79 (22%)	29 (23%)
Nighttime (use of bathroom)	36 (10%)	24 (19%)
After meal	29 (8%)	12 (9%)
Other	109 (30%)	21 (16%)
Trouble managing during the day, n	145 (40%)	80 (63%)
Falls in past 12 months due to nOH symptoms, n	206 (57%)	102 (80%)
Mean ± SD number of falls/patient	5.1±12.7	7.8±15.8

MSA=multiple system atrophy; nOH=neurogenic orthostatic hypotension; PD=Parkinson disease.
*Respondents in the patient and caregiver cohorts were not paired.
†Data refer to patient receiving care.

Disclosures: **L.A. Hewitt:** A. Employment; Significant; Lundbeck. **C.H. Adler:** A. Employment; Significant; Mayo Clinic College of Medicine, Mayo Clinic. B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; National Institutes of Health, Michael J. Fox Foundation for Parkinson's Research. G. Consultant/Advisory Board; Modest; Acadia, Acorda, Adamas, Cynapsus, Jazz, Minerva, Lundbeck, Neurocrine, Sunovion. **D.O. Claassen:** A. Employment; Significant; Vanderbilt University Medical Center. B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; National Institutes of Health, Michael J. Fox Foundation for Parkinson's Research. G. Consultant/Advisory Board; Modest; Lundbeck, Teva Neuroscience, Acadia, AbbVie. H. Other; Modest; Vaccines, AbbVie, CHDI, Auspex/Teva Neuroscience. **C.H. Gibbons:** A. Employment; Significant; Beth Israel Deaconess Medical Center, Harvard Medical School. B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Grifols, Celgene. G. Consultant/Advisory Board; Modest; Lundbeck, Pfizer, Janssen, Astellas. **S.R. Raj:** A. Employment; Significant; University of Calgary. B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; Canadian Institutes of Health Research, Cardiac Arrhythmia Network of Canada (CANet). G. Consultant/Advisory Board; Significant; Lundbeck, GE Healthcare. H. Other; Significant; Medtronic, Bristol Myers Squibb.

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P382

Management and Treatment of Neurogenic Orthostatic Hypotension: Results From a Survey of Patients and Caregivers

PrimaryAuthor.AuthorBlock:**Lawrence Arthur Hewitt**, Lundbeck LLC, Deerfield, IL; Charles H. Adler, Mayo Clinic, Scottsdale, AZ; Daniel O. Claassen, Vanderbilt Univ Medical Ctr, Nashville, TN; Christopher H. Gibbons, Beth Israel Deaconess Medical Ctr, Harvard Medical Sch, Boston, MA; Satish R. Raj, Libin Cardiovascular Inst, Univ of Calgary, Calgary, AB, Canada

Objective: To understand the patient and caregiver experience with the management of neurogenic orthostatic hypotension (nOH)

Background: nOH is a sustained reduction in blood pressure (BP) with postural change associated with autonomic dysfunction. Clinical symptoms may include falls, leading to activity limits and a diminished sense of well-being.

Methods: A US-based survey designed by the authors was conducted by Harris Poll. Eligible participants had Parkinson disease (PD), multiple system atrophy, or pure autonomic failure and ≥1 of the following: orthostatic hypotension (OH), nOH, low BP, OH/nOH symptoms, or were caregivers of eligible patients. Descriptive statistics are reported.

Results: PD was the most frequent underlying diagnosis **(Table 1)**. Healthcare provider (HCP) communication with patients was rated as satisfactory by ≥75% of respondents, yet when nOH symptoms were first discussed with the HCP, only 35% of patients and 31% of caregivers reported the patient received guidance on management. The most frequently recommended interventions are listed in **Table 1**; 25% of patients reported that no interventions were recommended. Medication prescribed to treat nOH was reported by 34% of patients and 45% of caregivers. Among patients treated, 79% of patients and 71% of caregivers said that the patient's symptoms were somewhat to very well managed.

Conclusions: Survey findings suggest the need for increased awareness of nOH and engagement with patients and caregivers regarding symptom management. A variety of interventions were recommended; no single treatment approach was noted. Respondents felt nOH symptoms were at least somewhat well managed with treatment.

Table 1. Patient and Caregiver Responses

Variable, n/N	Patients*	Caregivers*
PD diagnosis	328/363 (90%)	113/128 (88%)
Satisfied with HCP communication with patient	272/363 (75%)	99/128 (77%)
Received guidance on nOH symptom management (upon initial discussion with HCP)	109/309 (35%)	38/122 (31%)
Intervention suggested by HCP		
Avoid quick positional changes	177/363 (49%)	62/128 (48%)
Increase fluid intake	172/363 (47%)	67/128 (52%)
Adjust PD drugs	100/363 (28%)	55/128 (43%)
Increase salt intake	98/363 (27%)	25/128 (20%)
No intervention suggested	92/363 (25%)	13/128 (9%)
Medication ever prescribed	122/363 (34%)	57/128 (45%)
Symptoms managed with treatment/medication†		
Very well	71/272 (26%)	22/112 (20%)
Somewhat well	145/272 (53%)	57/112 (51%)

HCP=healthcare provider; nOH=neurogenic orthostatic hypotension; PD=Parkinson disease.
*Respondents in the patient and caregiver cohorts were not paired.
†Data refer to patient receiving care.
‡Among those receiving treatment or medication.

Disclosures: **L.A. Hewitt:** A. Employment; Significant; Lundbeck. **C.H. Adler:** A. Employment; Significant; Mayo Clinic College of Medicine, Mayo Clinic. B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; National Institutes of Health, Michael J. Fox Foundation for Parkinson's Research. G. Consultant/Advisory Board; Modest; Acadia, Acorda, Adamas, Cynapsus, Jazz, Minerva, Lundbeck, Neurocrine, Sunovion. **D.O. Claassen:** A. Employment; Significant; Vanderbilt University Medical Center. B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; National Institutes of Health, Michael J. Fox Foundation for Parkinson's Research. G. Consultant/Advisory Board; Modest; Lundbeck, Teva Neuroscience, Acadia, AbbVie. H. Other; Modest; Vaccines, AbbVie, CHDI, Auspex/Teva Neuroscience. **C.H. Gibbons:** A. Employment; Significant; Beth Israel Deaconess Medical Center, Harvard Medical School. B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Grifols, Celgene. G. Consultant/Advisory Board; Modest; Lundbeck, Pfizer, Janssen, Astellas. **S.R. Raj:** A. Employment; Significant; University of Calgary. B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; Canadian Institutes of Health Research, Cardiac Arrhythmia Network of Canada (CANet). G. Consultant/Advisory Board; Modest; Lundbeck, GE Healthcare. H. Other; Modest; Medtronic, Bristol Myers Squibb.

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P383

Pharmacist-led Digital Care Improved Community Hypertension Control by Increasing Medication Adherence in China

PrimaryAuthor.AuthorBlock:Wenhao Qu, Sinopharm Group Company Limited, Shanghai, China; Hongwei Wang, Zhifang Yang, Shanghai Univ of Med and Health Science, Shanghai, China; Zhen Wang, **Kai Liu**, CareLinker Co., Ltd., Shanghai, China

Objective: To identify the correlation between medication adherence and community hypertension control when a digital solution was introduced to assist retail pharmacists in a randomized controlled trial (RCT). Methods: Hypertensive patients (≥ 140/90 mm Hg, n=90) taking ARB or CCB anti-hypertensive drugs were recruited and randomized into 3 study groups: 1) usual care; 2) retail pharmacy in-store care which was offered as a walk-in service and provided by pharmacists who were equipped with the CareLinker solution consisting of blue-tooth BP monitor, an App and a cloud database to display history BP data, personalized meal plans and health coaching contents; or 3) home-based tele-medical care by which patients used GSM-embedded BP monitor to auto-send BP data to the pharmacists who then resorted to the CareLinker Decision Support Solution, a cloud-based backend engine, to consult patients over phone on a biweekly basis. This was a 12-month real-world research and the two primary end points were the anti-hypertensive medication adherence measured by Proportion of Days Covered (PDC) and changes of SBP. Results: PDC of the 3 study groups at baseline was 84.0% (group-1, control), 85.4% (group-2, pharmacy in-store intervention) and 87.6% (group-3, home-based tele-medical care), $p > 0.05$ between the 3 groups. At the end of the 12-month study, PDC was reduced to the annual average of 31.9% (group-1), 49.5% (group-2) and 61.2% (group-3), with $p<0.05$ between group-1 and the 2 intervention groups (group-2 and -3). Reduction of SBP was 3.4±2.4 mm Hg (p=0.166, group-1), 11.7±3.2 mm Hg (p=0.001, group-2), and 15.5±2.6 mm Hg ($p=2.26*10^{-6}$, group-3), with p= 0.014 (group-1 vs group-2) and p= 0.002 (group-1 vs group-3). DBP was reduced by 3.2±1.5 mm Hg (p=0.043, group-1), 6.8±2.4 mm Hg (p=0.008, group-2), and 6.8±2.8 mm Hg (p=0.004, group-3). Plotting PDC with BP reduction found a strong positive correlation for SBP: r= 0.692, $p<0.001$, and a weaker correlation for DBP: r= 0.301, P=0.004. Conclusion: Pharmacist-led digital healthcare can improve adherence of anti-hypertensive medication by as much as 84% (group-3 vs group-1), leading to enhanced community BP control.

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Switching Insufficient Hypotensive Monotherapy to ARB and CCB Combination Tablet in Elderly Patients Increases Both Patient and Physician Satisfaction Rates and Contributes Better Patient-physician Concordance

PrimaryAuthor.AuthorBlock:**Yoshiyuki Ikeda**, Kagoshima Univ, Kagoshima, Japan; Takeshi Sasaki, Makurazaki Municipal Hosp, Kagoshima, Japan; So Kuwahata, Tarumizu Chuo Hosp, Tarumizu Municipal Medical Ctr, Kagoshima, Japan; Yuichi Akasaki, Kagoshima Univ, Kagoshima, Japan; Chikuma Hamada, Faculty of Engineering, Tokyo Univ of Science, Tokyo,

Japan; Mitsuru Ohishi, Kagoshima Univ, Kagoshima, Japan

Background: Treatment must take into consideration patient-physician concordance conducted with good communication. Whereas it has been reported that there are discrepancies in awareness between patients and physicians regarding the blood pressure (BP) levels, there are no studies addressing patient and physician satisfaction with hypertension (HT) treatment. Reducing the pill burden and medication costs using combination drug, such as ARB/CCB tablet might improve adherence and concordance. Thus, the aim of this study is to evaluate the satisfaction of both hypertensive elderly patients and their physicians and QOL of patients with angiotensin II type I receptor blocker (ARB) and calcium channel blocker (CCB) combination drug (CD) treatment. Methods and Results: An open-label, multicenter, intervention study was conducted. Patients with insufficient hypotensive effect under the treatment with ARB or CCB monotherapy were enrolled, and medication was switched to CD and taken for 12 weeks. Physicians and patients participated in satisfaction surveys concerning HT treatment and patients also participated in QOL survey. Both home and clinic BP measurements showed a significant decrease after treatment was switched to an CD. Patient satisfaction rates showed an increase for treatment (69.4% to 90.1%), antihypertensive drugs (60.0% to 76.5%), clinic BP (37.0% to 68.8%), and home BP (41.2% to 67.5%). Significant differences were found between patients and physicians in the proportions of satisfaction and dissatisfaction at baseline, and ratios for both patient and physician satisfaction increased at 12 weeks. The QOL survey for patients showed significantly increase in the QOL score for general health ($p = 0.0191$). After CD treatment, satisfaction rates for both patients and physicians increased and improvement in the discrepancy between patient and physician satisfaction was achieved in addition to better BP control. Conclusions: In elderly patients with HT, switching insufficient hypotensive monotherapy to ARB and CCB combination tablet treatment increases both patient and physician satisfaction rates and contributes better patient-physician concordance.

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Switching Insufficient Hypotensive Monotherapy to ARB/CCB Combination Tablet in Elderly Patients Increases Both Patient and Physician Satisfaction Rates and Contributes Better Patient-physician Concordance (Patients Voice study)

PrimaryAuthor.AuthorBlock:**Yoshiyuki Ikeda**, KAGOSHIMA UNIVERSITY, Kagoshima, Japan

Aim: This study was conducted to evaluate the effect of ARB/CCB combination tablet treatment considering patient-physician concordance on the patient and physician satisfaction for the hypertensive treatment. Methods: In an open-label, multicenter study, patients with insufficient hypotensive effect receiving treatment with ARB or CCB monotherapy were enrolled and their medication switched to an ARB/CCB combination tablet taken for 12 weeks. Home and clinic BP rates were evaluated at baseline and at 12 weeks. Both physicians and patients participated in satisfaction surveys concerning the treatment at baseline and at 12 weeks. Results: One hundred twelve patients were screened and 85 patients were enrolled.

Significant differences were found between patient and physician rates of satisfaction and dissatisfaction in most survey items at baseline. Both home and clinic BP rates showed a significant decrease after switching to the ARB/CCB combination tablet. Patient satisfaction increased for the following survey items: treatment, 69.4% (59/85) to 90.1% (73/81); antihypertensive drugs, 60.0% (51/85) to 76.5% (62/81); clinic BP, 37.0% (31/84) to 68.8% (55/80); and home BP, 41.2% (35/85) to 67.5% (54/80). Component ratios of both patient and physician satisfaction showed significant increases in the following survey items: satisfaction with treatment, 18.8% (16/85) to 65.4% (53/81); involvement in treatment, 44.7% (38/85) to 65.4% (53/81); antihypertensive drugs, 18.8% (16/85) to 55.6% (45/81); clinic BP, 10.7% (9/84) to 46.3% (37/80); and home BP, 9.5% (8/84) to 46.3% (37/80). Our results indicate that, together with the effectiveness of the ARC/CCB combination tablet, participation in a satisfaction survey and access to a home BP monitor may have contributed to an increase in patient involvement in their treatment, a more mutual relationship between patients and physicians, and improvement in patient satisfaction. Conclusion: After switching to a ARB/CCB combination tablet treatment, better control of BP was found in patients with insufficient hypotensive effect from ARB or CCB monotherapy. Surveys showed that satisfaction rate for patients and physicians were improved in addition to patient-physician concordance.

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P386

The Paraventricular Nucleus in Control of Blood Pressure and Its Role in Hypertension

PrimaryAuthor.AuthorBlock:**Tyler M Basting**, Eric Lazartigues, Snigdha Murkerjee, Jiaxi Xu, Louisiana Health Sciences Ctr, New Orleans, LA

Rationale and objectives: In conscious mammals the importance of neural control over sympathetic tone in relation to cardiovascular (CV) function is well established. Neuro-CV dysregulation leads to increased sympathetic activity and neurogenic hypertension. The paraventricular nucleus (PVN) of the hypothalamus and both the rostral ventrolateral medulla (RVLM) and nucleus of the tractus solitarius (nTS) in the brainstem are currently viewed as key hubs for BP control and are implicated in producing or relaying the increased sympathetic tone in hypertension. We propose increased activity in the PVN, potentially through an upregulation of the renin angiotensin system, causes an increase in blood pressure. We test this theory by examining how stimulation or lesioning of the excitatory PVN neurons in conscious mice affects blood pressure and sympathetic activity.
Methods: PVN and nearby glutamatergic neurons were unilaterally transduced with channelrhodopsin using an adeno-associated virus (CamKII-ChR2-eYFP-AAV) in wildtype mice. We then measured the effect of acute stimulation of excitatory PVN neurons on resting blood pressure (telemetry) and baroreflex in conscious mice. Additionally, in vGlut-cre mice glutamatergic neurons of the PVN were bilaterally lesioned utilizing a cre-dependent caspase (Dio-Caspase-AAV). We then recorded baseline resting blood pressure and baroreflex in conscious mice before and after DOCA-Salt hypertension. Finally, we measured nor-epinephrine levels as a quantification of sympathetic activity.

Results and conclusions: Unilateral PVN excitation increased blood pressure from baseline by ~10 mmHg. Glutamatergic lesions of the PVN resulted in a blunted rise in BP when animals went through the DOCA-Salt protocol. These experiments demonstrate that the autonomic dysfunction seen in hypertension could be due to changes in the PVN. Overactivation of the PVN in hypertension is probably one of multiple factors that increase sympathetic drive resulting in an increased blood pressure.

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P387

Depletion of Perivascular Macrophages in the Brain Delays Blood Pressure Elevation in Angiotensin II-salt Hypertensive Rats

PrimaryAuthor.AuthorBlock:**Masanobu Yamazato**, Akio Ishida, Yoriko Yamazato, Takuto Nakamura, Yusuke Ohya, Graduate Sch of Med, Univ of the Ryukyus, Nishihara-cho Okinawa, Japan

Objectives: Perivascular macrophages in the cerebrospinal fluid (CSF) are immune surveillance cells in the brain. Increase in angiotensin II (Ang II) in the periphery gradually produces neurogenic hypertension which is associated with brain inflammation. However, little is known on the role of perivascular macrophages in the CSF on neurogenic hypertension. We hypothesized that perivascular macrophages in the CSF have an important role in the development of neurogenic hypertension by relaying and amplifying excess peripheral Ang II signals to brain parenchyma. Methods: Sprague-Dawley rats had surgery to instrument radio telemetric pressure transducers to abdominal aorta. Following a one-week recovery period, the rats had surgery to implant either saline or Ang II filled osmotic minipump subcutaneously and received intracerebroventricular (icv) injection of either control liposome or clodronate liposome. The clodronate liposome is a drug that selectively induces apoptosis to macrophages which have liposome phagocytic activity. After the surgery, all rats were provided high-salt diet (2.0% NaCl). Blood pressure of rats was recorded three times a week for 2 weeks. Different set of rats had hexamethonium challenge test to evaluate sympathetic tone in 7 or 9 day after initiation of the infusions. Results: Rats that received Ang II infusion with control icv (n=7) gradually increased mean arterial pressure (MAP) compared with rats that received saline infusion (n=5) and reached significant increase in 6 days after the initiation of infusions. Rats that received Ang II infusion with clodronate icv (n=6) had delayed increase in MAP and reached significant increase in 10 days and had significantly lower MAP (91±4 mmHg) compared with rats that received Ang II infusion with control icv (111±4 mmHg) in day 8. Peak depressor response to hexamethonium was increased only in rats that received Ang II infusion with control icv when compared with saline infused rats examined in 7 or 9 days after initiation of the infusions. Conclusion: Perivascular macrophages in the CSF may have important role in the development of angiotensin II-salt neurogenic hypertension.

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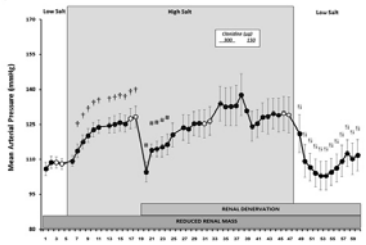
P388

Acute but Not Long-term Blood Pressure Lowering by Renal Denervation in Reduced Renal Mass, Salt Sensitive Hypertension

PrimaryAuthor.AuthorBlock:Ionut Tudorancea, Grigore T. Popa Univ of Med and Pharmacy, Iasi, Romania; Thomas E. Lohmeier, Barbara T. Alexander, Univ of Mississippi Medical Ctr, Jackson, MS; Dragos Pieptu, Dragomir Serban, **Radu Iliescu**, Grigore T. Popa Univ of Med and Pharmacy, Iasi, Romania

Chronic kidney disease is prevalent among patients with resistant hypertension and activation of the sympathetic nervous system is common in both conditions. While renal nerve ablation lowers blood pressure (BP) in some resistant hypertensive patients, the influence of kidney disease on the antihypertensive response is largely unknown. Systemic hemodynamics and sympathetically-mediated low frequency oscillations of systolic BP were determined continuously by telemetry in rats before and after unilateral nephrectomy and surgical excision of the poles of the remaining kidney to reduce renal mass (RRM) ~ 80%. Before RRM, BP was not salt sensitive. However, placing rats with RRM on a high salt diet induced sustained increases in mean arterial pressure (Figure) and cardiac output while suppressing sympathetic activity. After denervation of the remnant kidney, sympathetic activity and heart rate increased, concomitant with reductions in BP and cardiac output. However, these acute responses waned over the 2 weeks of follow up. Subsequent central sympathetic inhibition with clonidine reduced sympathetic activity, heart rate and cardiac output, but not BP as peripheral resistance increased. Finally, when the low salt diet was restored, BP decreased back to normotensive levels. These results show that renal denervation does not chronically lower BP in this model of salt-sensitive hypertension associated with substantial nephron loss, but without active parenchymal disease, thus providing insight into conditions likely to impact the antihypertensive response to renal-specific sympathoinhibition in subjects with advanced kidney disease.

Figure



† p<0.05 vs. Low Salt (days 3-4); # p<0.05 vs. High Salt (days 17-18); § p<0.05 vs. Clonidine washout (days 45-46).

Disclosures:**I. Tudorancea:** None. **T.E. Lohmeier:** None. **B.T. Alexander:** None. **D. Pieptu:** None. **D. Serban:** None. **R. Iliescu:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; "The East European Network of Excellence for Research and Development in Chronic Diseases CHRONEX-RD" 2/1/149 MIS ETC Code 1840 and National Heart, Lung, and Blood Institute Grant HL-51971..

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P389

Post-traumatic Stress-induced Sensitization of Angiotensin II Hypertension is Reversed by Blockade of Angiotensin-converting Enzyme or Tumor Necrosis Factor-alpha

PrimaryAuthor.AuthorBlock:Baojian Xue, Yang Yu, Shun-Guang Wei, Terry G Beltz, Fang Guo, Robert B Felder, **Alan Kim Johnson**, Univ of Iowa, Iowa City, IA

Using an Induction-Delay-Expression experimental paradigm, our previous studies

demonstrated that post-traumatic stress disorder (PTSD) sensitizes angiotensin (ANG) II-elicited hypertension, which was associated with upregulation of central renin-angiotensin system (RAS) components and proinflammatory cytokines (PICs). The present study investigated whether inhibition of angiotensin-converting enzyme (ACE) or tumor necrosis factor-α (TNF-α) prior to PTSD blocks sensitization of ANG II-elicited hypertensive response. The resident-intruder paradigm was used to model PTSD. Each intruder rat (male Sprague-Dawley) was pretreated with ACE inhibitor (captopril, 0.5 mg/Ml) or with TNF-α inhibitor (pentoxifylline, PTX, 100 mg/kg/day) in the drinking water for two weeks and then exposed to a different resident (male Long-Evans) for 2 hours on three days with each session separated by 1 day. Beginning 3 days after the last exposure, the intruder (PTSD) rats and unstressed control rats received a subcutaneous infusion of ANG II (120 ng/kg/min) for 2 weeks. The PTSD rats had a significantly enhanced hypertensive response to the ANG II infusion and an upregulation of mRNA expression of RAS and PIC components and of a microglial marker in the lamina terminalis (LT) when compared to control rats. Both the sensitized hypertensive response and enhanced gene expression were blocked by pre-treatment with either ACE inhibitor or TNF-α antagonist. These results suggest that upregulation of the brain RAS and PICs produced by a severe stress contribute to PTSD-induced sensitization of the hypertensive response to ANG II.

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P390

Combining Olmesartan and Renal Denervation Elicits Strong Blood Pressure Reduction in Association With Sympatho-inhibitory and Aldosterone-reducing Effects in Hypertensive Mice With Chronic Kidney Disease

PrimaryAuthor.AuthorBlock:**Masaaki Nishihara**, Yoshitaka Hirooka, Kyushu Univ, Fukuoka, Japan

Background: Augmented sympathetic nerve activity (SNA) and renin-angiotensin-aldosterone system (RAAS) are involved in the pathogenesis of hypertension (HT) accompanied with chronic kidney disease (CKD). The oxidative stress in the hypothalamus in the brain increases SNA in HT. The renal denervation (RDN) or angiotensin II receptor blocker (olmesartan; OLM) exerts an antihypertensive effect in HT with CKD; however, the precise mechanisms of the combination therapy are not fully elucidated. In the present study, we examined whether combining RDN and OLM reduces SNA through oxidative stress in the hypothalamus and RAAS in hypertensive mice with CKD.
Methods and Results: In 5/6-nephrectomized ICR-mice (Nx, n=19) at 4-weeks after nephrectomy, systolic blood pressure (SBP) was significantly increased (147±3 vs. 107±3mmHg, $p<0.001$), accompanied by increased SNA (urinary norepinephrine (uNE): 504±21 vs. 243±25μg/24hrs, $p<0.001$) and albuminuria (547±80 vs. 108±15mg/g creatinine, $p<0.01$) compared with those in control-mice (n=9). Nx-mice were orally-administered OLM (10mg/kg/day), vehicle, or performed RDN under OLM-administration, and divided into Nx-OLM (n=5), Nx-VEH (n=9), and Nx-OLM/RDN (n=5), respectively. In Nx-OLM and -OLM/RDN at 8-weeks after therapy, SBP were significantly decreased (124±3 and 116±3 vs. 144±2mmHg, n=5-9, $p<0.05$) accompanied with inhibition of both uNE (379±69 and 347±61 vs.

568±24μg/day, n=5-9, p<0.05) and oxidative stress in the hypothalamus (thiobarbituric acid-reactive substances: 0.952±0.06 and 1.012±0.041 vs. 1.222±0.047nmol/mg protein, n=5-9, p<0.05) compared with those in Nx-VEH, without worsening creatinine clearance. In Nx-OLM and -OLM/RDN, the albuminuria was also suppressed compared with that in Nx-VEH (476±56 and 499±82 vs. 1059±165mg/g creatinine, n=5-9, p<0.05). In Nx-OLM/RDN, but not in Nx-OLM, the plasma aldosterone concentration level was significantly decreased compared with that in Nx-VEH (441±35 vs. 675±33pg/ml, n=5-9, p<0.05).
Conclusion: These findings suggest that the OLM/RDN combination therapy have a strong antihypertensive effect associated with inhibiting SNA through reducing oxidative stress in the brain and the plasma aldosterone in hypertensive mice with CKD.

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P391

Local Ionotropic Glutamate Receptors Contribute to the Sympathoexcitatory Effects of Leptin in the Paraventricular Nucleus

PrimaryAuthor.AuthorBlock:Zhigang Shi, **Virginia L Brooks**, Oregon Health & Science Univ, Portland, OR

In male rats, intracerebroventricular infusion of leptin increases sympathetic nerve activity (SNA), and this response can be partially reversed by nanoinjections of the ionotropic glutamate receptor (iGluR) blocker, kynurenate (KYN), and also the melanocortin type 3/4 receptor (MC3/4R) antagonist, SHU9119, into the hypothalamic paraventricular nucleus (PVN). These results suggest that leptin increases SNA in part via activation of PVN iGluR and MC3/4R. More recently, we found that injection of leptin directly into the PVN also dose-dependently increases SNA. However, the role of PVN iGluR or MC3/4R in this response is unknown. Therefore, we determined if PVN KYN (2.7 nmol in 60 nL) or SHU9119 (30 pmol) reverses the increases in lumbar SNA (LSNA), mean arterial pressure (MAP), and heart rate (HR) evoked by bilateral PVN nanoinjections of leptin (30 ng in 60 nL; n=6) or, as a control, of artificial cerebrospinal fluid (aCSF; n=4). After ninety min, PVN leptin increased (all P<0.05) LSNA to 164±10 % of control, MAP from 106±5 to 118±5 mmHg, and HR from 360±12 to 399±16 bpm. Then, PVN injections of KYN decreased (all P<0.05) LSNA to 133±8 % control, MAP to 108±4 mmHg, and HR to 379±16 bpm. On the other hand, neither PVN aCSF injections (LSNA, to 105±3 % control; MAP, from 107±8 to 109±7 mmHg; and HR, from 363±31 to 367±20 bpm) nor subsequent PVN KYN injections (LSNA, to 102±5 % control; MAP, to 108±6 mmHg; and HR, to 373±17 bpm) significantly altered these variables. In a second set of animals, while PVN leptin (n=4) elicited similar increases (all P<0.05) in LSNA (to 158±8 % control), MAP (from 101±6 to 113±4 mmHg), and HR (from 367±19 to 405±11 bpm), subsequent SHU9119 had no effects (LSNA, to 151±7 % control; MAP, to 108±5 mmHg; and HR, to 400±14 bpm). Again, no changes in these variables were observed in response to PVN injections of aCSF (n=4), even when followed by SHU9119 (LSNA, to 114±11 and then 110±13 % control; MAP, from 107±7 to 109±8 and then 106±9 mmHg; and HR, 357±31 to 355±24 and then 354± 25 bpm). In conclusion, activation of local iGluR, but not MC3/4R, contribute to the increases in LSNA, MAP, and HR in response to PVN leptin.

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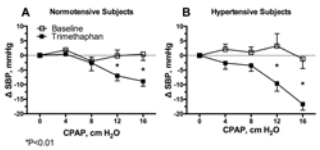
P392

Autonomic Reflexes Mask the Hemodynamic Effects of Continuous Positive Airway Pressure

PrimaryAuthor.AuthorBlock:**Luis E Okamoto**, Alfredo Gamboa, Jorge E Celedonio, Andre Diedrich, Sachin Y Paranjape, Italo Biaggioni, Vanderbilt Univ Medical Ctr, Nashville, TN

Continuous positive airway pressure (CPAP) improves cardiac function and blood pressure (BP) in patients with sleep-breathing disorders by reducing increased sympathetic tone. The direct hemodynamic effects of increased intrathoracic pressure with CPAP, however, are not known and are likely masked by baroreflex buffering. To determine the acute hemodynamic effects of CPAP in the absence of autonomic modulation, we applied 5 levels of CPAP (0, 4, 8, 12 and 16 cm H_2O, each for 2 min) to 7 healthy normotensive (NTN, 33±3 years, BMI 29±3, 4 females) and 12 hypertensive subjects (HTN, 50±2 years, BMI 30±2, 8 females) before and during autonomic withdrawal with the ganglionic blocker trimethaphan. Hemodynamic parameters were measured at the end of each CPAP level. At baseline, CPAP had no effect on systolic BP (SBP), heart rate (HR), cardiac output (CO), stroke volume (SV) or systemic vascular resistance (SVR) in either group. During the autonomic blockade, CPAP significantly decreased SBP in both groups (Figure). The maximal BP drop was twice as much in HTN than NTN (-17±2 vs. -8±2 mmHg at 16 cm H_2O; $P<0.02$) but the final SBP was the same in both groups (85±3 mmHg). In HTN, CO and SV decreased -25±4% and -24±4% at 16 cm H_2O, respectively; whereas in NTN, the decreases were -20±5% and -24±4%. Neither SVR nor HR changed significantly with CPAP in either group. We conclude that, in the absence of autonomic modulation, CPAP acutely decreases SBP significantly, due to decreases in CO and SV likely reflecting a decrease in venous return and increase in venous capacitance. The effect on BP was greater in HTN suggesting that a sympathetically mediated reduction in capacitance may contribute to the hypertension.



Disclosures: **L.E. Okamoto:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHA Mentored Clinical & Population Research Award 14CRP20380211. **A. Gamboa:** None. **J.E. Celedonio:** None. **A. Diedrich:** None. **S.Y. Paranjape:** None. **I. Biaggioni:** None.

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P393

Orthostatic Heart Rate-Blood Pressure Relationship Identifies Neurogenic Orthostatic Hypotension

PrimaryAuthor.AuthorBlock:Alejandro Velasco, Univ of Alabama at Birminghan, Birminghan, AL; **Luis E Okamoto**, Emily M Garland, VANDERBILT UNIVERSITY MEDICAL CENTER, Nashville, TN; Chang Yu, VANDERBILT UNIVERSITY, Nashville, TN; Italo Biaggioni, VANDERBILT UNIVERSITY MEDICAL CENTER, Nashville, TN

Background:
Neurogenic orthostatic hypotension (nOH) is a chronic disabling condition associated with significant morbidity and mortality. Currently, the diagnosis of nOH relies on impaired

autonomic reflexes as determined by testing available only in specialized centers. Our objective was to test the hypothesis that a blunted heart rate (HR) increase in response to a given systolic blood pressure (SBP) fall will correctly diagnose nOH.

Methods:

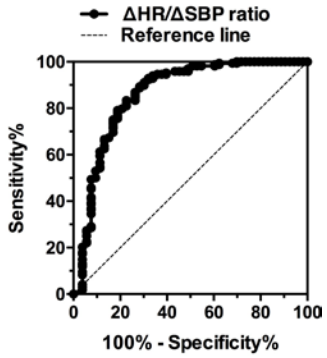
We performed a retrospective study of nOH patients (studied in an inpatient environment off medications that can interfere with autonomic function) and control subjects. nOH was diagnosed on the basis of SBP fall ≥20 mmHg on standing associated with a decrease in SBP≥ 20 mmHg during phase 2 of the Valsalva maneuver and absence of phase 4 SBP overshoot. Controls had any SBP fall on standing but intact autonomic reflexes. Receiver operator characteristic curve (ROC) analysis was performed on the ratio of the changes from supine to 3 min standing in HR and SBP (ΔHR/ΔSBP).

Results:

We studied 171 nOH patients (66±1 years, males 61%, multiple system atrophy 42%, Parkinson disease 14%, pure autonomic failure 32% and undetermined 12%) and 53 controls (51±3 years, males 28%). nOH patients had a greater drop in standing SBP (-63±2 vs. -16±4 mmHg in controls; p<0.01) but a smaller increase in HR (13±1 vs. 18±1 bpm in controls; p<0.01). The ROC analysis at 95% Confidence Interval showed that a ΔHR/ΔSBP ratio <0.445 had a 81% specificity and 79% sensitivity in identifying nOH (AUC=0.86, p<0.01. Figure).

Conclusions:

Our study suggests that a simple ratio of ΔSBP/ΔHR <0.445 during a posture test in clinic can reliably identify patients with nOH.



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P394

Reduced Serum Butyrate Is Associated With Dampened Central Effects Of Butyrate On Blood Pressure In The Shr

PrimaryAuthor.AuthorBlock:**Tao YANG**, Kacy Magee, Rebeca Arocha, Thomas Vickroy, Jasenka Zubcevic, UNIVERSITY OF FLORIDA, Gainesville, FL

Introduction: The link between gut dysbiosis and hypertension is characterized by a significant reduction in butyrate-producing bacteria in the spontaneously hypertensive rat (SHR) compared with its Wistar Kyoto (WKY) control. Butyrate, a major end-product of gut microbiota fermentation, has beneficial effects in multiple dysbiosis-related diseases. Here, we

investigated the link between serum butyrate levels and central effects of butyrate in regulation of blood pressure (BP) in WKY and SHR.
Methods: Serum samples were collected from 12 weeks old male WKY and SHR, and butyrate quantification was performed using LC-MS. Quantitative real time PCR was employed to measure relative expression levels of butyrate sensing receptors (Gpr41, Gpr43) in the hypothalamus of SHR and WKY. Central effects of butyrate on BP were evaluated by acute ICV injection in anaesthetized WKY and SHR.
Results: Significantly lower levels of serum butyrate were observed in the SHR (WKY 1.23±0.14µmol/L vs SHR 0.23±0.033µmol/L, N=4, P=0.0005). Moreover, lower expression of Gpr43 were observed in the hypothalamus of SHR (WKY 0.0002 ± 2.7e-005 vs SHR 6.9e-005 ± 1.9e-005, N=4/3, P=0.0152). ICV administration of butyrate reduced BP in both strains; however, the reduction in arterial BP was greater in the WKY at two time points (400s post-injection: WKY -5.83±0.49mmHg vs SHR -2.62±0.63mmHg, N=4, P=0.0235; 1000s post-injection: WKY -19.7±1.85mmHg vs SHR -13.26±1.1mmHg, N=3, P=0.05).
Conclusion: Reduced serum butyrate is associated with reduced sensitivity to central butyrate. This may contribute to elevation in BP in the SHR.

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P395

A New Technique for Selective Denervation of the Aortic and Carotid Baroreceptors

PrimaryAuthor.AuthorBlock:Jaci A Castania, Pedro L Katayama, Fernanda Brognara, Univ of São Paulo, Ribeirão Preto, Brazil; João P Sabino, Federal Univ of Piauí, Teresina, Brazil; Rubens Fazan Jr., **Helio C Salgado**, Univ of São Paulo, Ribeirão Preto, Brazil

The traditional sinoaortic denervation (SAD) technique in rats includes aortic and carotid baroreceptors denervation, but also leads to concomitant denervation of the carotid chemoreceptors. Here we report a new technique to denervate the carotid and aortic baroreceptors, selectively, preserving the carotid chemoreceptors intact. Wistar rats were subjected to selective aortic and carotid baroreceptor denervation sparing the carotid chemoreceptors intact (BAROS-X), or sham surgery (SHAM). The animals were also implanted with femoral arterial and venous catheter for arterial pressure recording and drug administration. In unanesthetized, freely moving rats, baroreflex or peripheral chemoreflex activation was elicited by intravenous injection of phenylephrine or potassium cyanide (KCN), respectively. Phenylephrine caused a significant hypertensive response in SHAM (Δ = 46 ± 3 mmHg, P<0.001) and BAROS-X (Δ = 38 ± 3 mmHg, P<0.001) combined with a reflex bradycardia in SHAM (Δ = -54 ± 11 bpm, P<0.001) but no change in heart rate in BAROS-X subjects (Δ = -5 ± 2 bpm, ns); confirming the absence of aortic and carotid baroreceptors in BAROS-X rats. KCN elicited significant hypertensive response in SHAM (44 ± 6 mmHg, P<0.001) and BAROS-X (39 ± 5 mmHg, P<0.001) subjects followed by bradycardic response in SHAM (-88 ± 17 bpm, P<0.001) and BAROX-S (-103 ± 23 bpm, P<0.01) rats; confirming that the carotid chemoreceptors were intact in both groups. These data provide support to a new surgical technique that allows working with conscious rats exhibiting selective inactivation of the aortic and carotid

baroreceptors, combined with intact carotid chemoreceptors.

Disclosures:**J.A. Castania:** None. **P.L. Katayama:** None. **F. Brognara:** None. **J.P.J. Sabino:** None. **R. Fazan Jr.:** None. **H.C. Salgado:** None.

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P396

The Stroke Belt: Forged in the Heat of the Buckle? A Hypothesis

PrimaryAuthor.AuthorBlock:**Clarence Grim**, High Blood Pressure Consulting, Stateline, NV

The “stroke belt”(SB) in the SE USA has significantly higher stroke rates in whites (W) and African Americans (AA). HTN is the major driving force. The “Buckle” (B) of the SB, with even higher rates, lies along the costal “low country” plains of the Carolinas. Being born in the B is a powerful exposure that increases risk. Forty percent of all US slaves came thru, slaved and many died in the B of the SB. While reading the scholarly review “Slavery, Disease and Suffering in the Southern Low Country”, by Dr. Peter McCandless, (Cambridge U Press, 2011) I was stuck with the effect of the hot, humid weather with morbidity and mortality in the B. He details three decimating “fevers”. The first two, Malaria and Yellow Fever, are known to have left their mark on the human genome. The third fever, “Malignant Fever” (MF), coined by James Lind in “An Essay on Diseases Incidental to Europeans in Hot Climates” (London, 1768). MF was characterized as a fever of sudden onset resulting in rapid death in a few days. Lind describes MF morality in sailors “wooding” onshore during hot days who died overnight if they failed to return to the “cleaner air” of the ship. Many new arrivals, including military forces, to the SB/B succumbed to MF in days. Especially in the summer. Treatment of MF was removal to an area of “healthier air”/high country which quickly “cured” MF. I suggest this lowered the heat index, improving survival in heat illness/stroke or “Malignant fever”. In addition, an affliction called the “Dry Agues” characterized by muscle spasms, weakness and physical and mental fatigue was a major cause of disability during the summer. I interpret this as the classic features of hypokalemia driven by Na/K losses during heat adaptation and modulated by intakes of Na, K and the RAAS. As death from terminal heat stoke manifests as yellow skin/bleeding diathesis many of these deaths were likely wrongly attributed to Yellow Fever. Recent US military research documents a lower susceptibility to heat injury in both Ws and AAs from the SB/B. As the genes sing the songs of survival, I propose that selective survival, related to heat illnesses, plays a role in today’s greater prevalence of HTN in Ws and AAs in the SB and its B. Study of family trees, their genes and the physiology of heat control systems should be informative.

Disclosures:**C. Grim:** None.

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P397

Living in Ethnic-enclave Neighborhoods May Attenuate the Negative Effect of Acculturation on Blood Pressure in Refugees and Maintain Immigrant Health Effect

PrimaryAuthor.AuthorBlock:**Ahmed Dalmar**, Aurora Res Inst, AHC, Milwaukee, WI; Douglas Pryce, Hennepin County Medical Ctr, Minneapolis, MN; Brian Martinson, Farhiya Faraha, HealthPartners Inst for Education and Res, Minneapolis, MN; Sandra Wewerka,

HealthPartners Inst for Education and Res, Minniapolis, MN; Diana Dubois, WellShare Intl, Minneapolis, MN; Laura Sanka, Wellshare Intl, Minneapolis, MN; Osman Ahmed, East Africa Health Project, Minneapolis, MN; Bjorn Westgard, HealthPartners Inst for Education and Res, Minneapolis, MN

BACKGROUND: Researchers have long hypothesized that acculturation (duration of U.S. residence [DOR]) is associated with increases in blood pressure in immigrant populations. However, little is known about the potential effects of acculturation on hypertension (HTN) in refugees living in ethnic-enclave neighborhoods. We conducted a cross-sectional study of Somali refugees and immigrants (a largely enclaved population) living in Minneapolis-St. Paul, Minnesota. **METHOD:** We analyzed data from 1155 Somali refugees/immigrants who arrived in United States from 1990 to 2015 and are participating in an ongoing study of Somali refugees in the Twin Cities. Using 10 years as a threshold, the study population was divided into two groups based on DOR: short (&lt 10 years) or long (&gt= 10 years). Main outcome was HTN, which was defined using the protocol of international HTN studies. Differences between the two groups were determined by chi-squared test and t-test. Multivariate logistic regression was used to determine association between HTN and DOR. **RESULTS**: Of 1155 subjects, 572 (49.6%) had DOR &gt= 10 years. Mean DOR was 9.8 ± 6.0 years (median of 10), and 58.1% (559 of 1155) were women. The short DOR group was younger (mean age 44.6 ± 18.0 vs 51.4 ± 18.1 years, P&lt 0.001. There was no difference in mean systolic blood pressure (SBP) between groups (117 mmHg vs 119 mmHg, P=0.087) and no correlation between SBP and DOR (linear R^2=0.012). Prevalence of HTN was 29.5% (341 of 1155), with 179 of these 341 (52.5%) having uncontrolled HTN. HTN increased with aging: 4.1% (19 of 457) for age group 18-39, 15.3% (47 of 306) for ages 40-59, and 29.7% (114 of 384) for ages 60**+**. After adjusting for body mass index, marital status, English language (written and spoken), health insurance, income**/**poverty ratio, language spoken at home, employment, education and DOR, age was the only predicator of HTN. Even after matching two groups by age, the distribution of HTN was similar between the two age-matched DOR groups (P=0.1879). **CONCLUSIONS:** DOR (acculturation) was not a predictor of HTN in this highly enclaved refugee/immigrant sample. Further studies are needed to identify which cultural and neighborhood environment factors may operate to blunt the effects of acculturation on blood pressure.

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P398

Screening of Primary Aldosteronism by Clinical Features and Daily Laboratory Tests: Combination of Urine pH, Sex, and Serum K^+

PrimaryAuthor.AuthorBlock:**Tomohisa Yamashita**, Norihito Moniwa, Masayuki Koyama, Kohei Ohno, Dept of Cardiovascular, Renal and Metabolic Med, Sapporo Medical Univ, Sapporo, Japan; Hideaki Yoshida, Div of Cardiology and Nephrology, JR Sapporo Hosp, Sapporo, Japan; Tetsuji Miura, Dept of Cardiovascular, Renal and Metabolic Med, Sapporo Medical Univ, Sapporo, Japan

Introduction: There is a clinical need for an easy-to-use method to determine who should be screened for primary aldosteronism (PA). **Objective:** The aim of our study is to develop

and validate a scoring system from clinical features and daily laboratory tests to select patients who should proceed to endocrinolgic examinations of PA from newly diagnosed hypertensives. **Methods:** A multivariate logistic regression analysis which entered possible PA markers, age < 40 years, female sex, moderate to severe hypertension, hypokalemia, serum Na^+ minus Cl^- ≥ 40 mmol/L, serum uric acid ≤ 237.92 µmol/L (4.0 mg/dL), and urine pH (U-pH) ≥ 7.0 were undertaken in consecutive outpatients newly diagnosed hypertension. The diagnosis of PA was made by plasma aldosterone concentration-to-plasma renin activity ratio (ARR) ≥ 20 and at least one positive result in challenge tests. **Results:** In derivation study, 24 of 130 patients were diagnosed PA. The area under the receiver operating characteristic curve (AUC) for a logistic model incorporating all possible PA markers was 0.73 (95% CI: 0.61-0.85). Removing high U-pH, female sex, and hypokalemia from the full model decreased the AUC by 0.059, 0.035, and 0.011, respectively. We devised the diagnostic score for predicting possible PA, in which one point each was assigned to high U-pH, female sex and hypokalemia, and named it PFK score. The prevalence of PA in patients with 0, 1, 2, and 3 points were 11% (6 of 50), 14% (7 of 50), 42% (8 of 19), and 60% (3 of 5), respectively. In external validation datasets including patients already treated, 23 of 106 patients were diagnosed PA. AUC of PFK score was 0.73 (95% CI: 0.63-0.83), significantly higher than that of hypokalemia alone (0.53, 95% CI: 0.44-0.63, *P*<0.01). **Conclusion:** We successfully developed the easy-to-use diagnostic score for predicting possible PA, PFK score which is superior to hypokalemia alone.

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P399

The Association of Serum Aldosterone with Sex, Age, and Sodium Status

PrimaryAuthor.AuthorBlock:**Ajay D Rao**, Huaqing Zhao, Lewis Katz Sch of Med at Temple Univ, Philadelphia, PA; Ramachandran S Vasan, Boston Univ Sch of Med, Boston, MA; E Victor Adlin, Lewis Katz Sch of Med at Temple Univ, Philadelphia, PA

Primary aldosteronism (PA) is now recognized as the most common cause of secondary hypertension. Although the aldosterone-to-renin ratio is recommended for case detection, screening rates for PA remain low. Based on our prior study in low-renin hypertension, we hypothesized that a statistical adjustment for age, sex, and sodium status would improve the diagnostic accuracy of aldosterone measurements.
Data from the cohort of 3,345 individuals in the sixth examination cycle of the Framingham Offspring Study was analyzed. In addition to the standard study protocol, blood was drawn for later measurement of aldosterone. After exclusions, the final analysis consisted of 1,468 normotensives (652 men and 834 women), aged 29 to 85. To compare levels of continuous variables in two groups, we used independent samples t tests. We regressed natural-log-transformed values of aldosterone on age, sex, and the urine sodium/creatinine ratio. Unimodality of aldosterone (adjusted and unadjusted) was tested utilizing dotplots and the dip test.
The mean aldosterone levels were 10.4 ± 5.89 ng/dL in women and 11.3 ± 6.14 ng/dL in men ($p < 0.005$). Age, sex, and urine sodium/creatinine ratio were each significantly associated with aldosterone ($p < 0.005$). The proportion of variability explained by age, sex,

and urine sodium/creatinine was 0.91%,1.3%, and 8%, respectively. The fully adjusted model $r^2 = 0.0948$. When adjusted for age, sex, and urine sodium/creatinine ratio, the distribution of aldosterone was unimodal (dip test: 2.26 ± 0.17 ng/dL, p = 0.9973); when unadjusted, the distribution of aldosterone was not unimodal (dip test, p ≤ 0.005). The dotplot and Q-Q plot showed a normal distribution of aldosterone after adjustment.
In this large, community-based population, adjustment for age, sex, and sodium status resulted in a more normal distribution of aldosterone. PA has been described as a condition "in which aldosterone production is inappropriately high for sodium status." Our study suggests that appropriate adjustment of the measured serum aldosterone concentration may provide a more accurate approximation of the renin-angiotensin aldosterone system. Studies in populations of PA are needed to test for improved diagnostic accuracy that may result from this adjustment.

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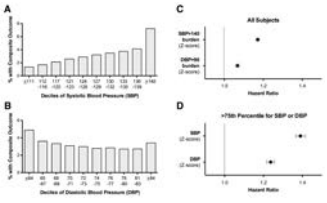
P400

Relative Contributions of Systolic and Diastolic Blood Pressure to Adverse Cardiovascular Outcomes in an Outpatient Cohort of 1.3 Million Adults

PrimaryAuthor.AuthorBlock:**Alexander C Flint**, Kaiser Permanente, Redwood City, CA; Carol Conell, Kaiser Permanente, Oakland, CA; Xiushui Ren, Nader Banki, Sheila L Chan, Vivek A Rao, Ron B Melles, Kaiser Permanente, Redwood City, CA; Deepak L Bhatt, Harvard Univ, Boston, MA

Systolic hypertension is believed to have a greater influence than diastolic hypertension on adverse cardiovascular events. We tested this hypothesis using data from an integrated healthcare system, with 36.8 million measures from 1.3 million adults. Increasing SBP was associated with risk of a composite outcome of MI, ischemic stroke, or hemorrhagic stroke (Fig A). DBP showed a very different relationship with outcomes, with higher risk at the lowest and highest levels of DBP (Fig B). We examined the impact of systolic and diastolic pressures in multivariable survival analysis controlling for age, sex, race, and comorbidities using two different approaches. We first used the burden of systolic (SBP>=140) and diastolic (DBP>=90) hypertension as predictors. Both systolic burden (hazard ratio [HR] 1.17 for z-score, 95% CI 1.16 to 1.18, P<0.001) and diastolic burden (HR 1.07 for z-score, 95% CI 1.06 to 1.08, P<0.001) were associated with outcomes (Fig C). To examine a broader range of pressures from normal to elevated, where both measures are directly correlated with outcome, we used weighted average SBP and DBP from subjects above the 75th percentile for SBP (>133) or DBP (>78). Again, both SBP (HR 1.39 for z-score, 95% CI 1.37 to 1.41, P<0.001) and DBP (HR 1.24 for z-score, 95% CI 1.22 to 1.26, P<0.001) independently predicted outcomes (Fig D). A complicated relationship exists between SBP, DBP, and outcomes; at mid-normal to elevated pressures, both SBP and DBP are independently associated with the risk of adverse cardiovascular events.



Disclosures:**A.C. Flint:** None. **C. Conell:** None. **X. Ren:** None. **N. Banki:** None. **S.L. Chan:** None. **V.A. Rao:** None. **R.B. Melles:** None. **D.L. Bhatt:** None.

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P401

The Progress of Carotid Atherosclerosis is Associated With Incident Stroke and Coronary Heart Disease: The Suita Study

PrimaryAuthor.AuthorBlock:**Yoshihiro Kokubo**, Makoto Watanabe, Aya Higashiyama, Yoko M Nakao, Fumiaki Nakamura, Yoshihiro Miyamoto, Natl Cerebral and Cardiovasc Ctr, Suita, Osaka, Japan

Background and Purpose: We observed that the maximum of intima-media thickness (IMT) more than 1.1 mm in the common carotid artery (CCA), i.e. CIMT-plaque, is the best predictive marker for incident cardiovascular disease (CVD) in the Suita Study. There has been no study of the association between the progress of carotid atherosclerosis and CVD in a general population. We assessed our hypothesis that CIMT-plaque progression could predict the risk of new-onset CVD in a general population.
Methods: We studied 3,722 men and women (mean age 59.8 years without CIMT-plaque or CVD at baseline) who completed a baseline survey and carotid ultrasonography. CIMT-plaque was defined as a maximum IMT in the CCA more than 1.1 mm. During the follow-up periods from April 1994 to March 2005, we observed 632 new CIMT-plaques. After new incident CIMT-plaque or as of April 2005, we conducted a prospective cohort study of the cases until the December 2013 endpoint. We compared the CVD risk by a Cox proportional hazards model after adjusting for age, sex, body mass index, prehypertension, hypertension (grades I and II+III), TC, HDL, antihypertensive drug and/or statin use, diabetes, impaired fasting glucose, chronic kidney disease, smoking, and excessive drinking (more than 4 units/day).
Results: After new incident CIMT-plaques in CCA, we observed 234 incident CVD events (139 strokes and 95 coronary heart disease [CHD]) during 38,243 person-years of follow-up. Age, men, hypertension grades I and II+III, diabetes, and hypercholesterolemia were associated with the progression of CIMT-plaque. After new incident CIMT-plaques, the adjusted hazard ratios (95% confidence intervals) for CVD, stroke, and IHD during follow-up were 1.58 (1.15 to 2.17), 1.54 (1.03 to 2.31), and 1.72 (1.03 to 2.88).
Conclusions: The novel finding of this study is that CIMT-plaque progression could be a good predictor of new-onset CVD in a general population.

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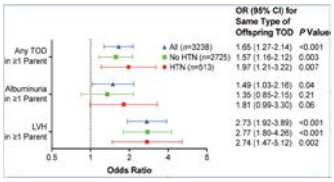
P402

Familial Clustering of Hypertensive Target Organ Damage in the Community

PrimaryAuthor.AuthorBlock:**Teemu J. Niiranen**, Framingham Heart Study, Framingham, MA; Honghuang Lin, Boston Univ Sch of Med, Boston, MA; Martin G. Larson, Ramachandran S. Vasan, Framingham Heart Study, Framingham, MA

Prior studies suggest that hypertensive target organ damage (TOD) is a heritable trait. However, the risk that parental TOD confers on propensity for TOD in their offspring, and how hypertensive TOD clusters in the background of parental versus offspring hypertension status remain unclear. We studied 3238 Framingham

Heart Study participants (mean age 39±8 years, 53% women) with available parental data on TOD. Parents and offspring underwent measurements for echocardiographic left ventricular hypertrophy, microalbuminuria, and conventional risk factors. Prevalence of any TOD (left ventricular hypertrophy or microalbuminuria) in participants with 0 and ≥1 parents with any TOD was 7% (131 of 1860) and 13% (173 of 1378), respectively (*P*<0.001 for difference). As illustrated in the **Figure**, having ≥1 parent with TOD was associated with greater odds of TOD in offspring compared to individuals without parental TOD (multivariable-adjusted odds ratio [OR], 1.65; 95% confidence interval [CI], 1.27-2.14). Similarly, parental left ventricular hypertrophy was associated with offspring left ventricular hypertrophy (OR, 2.73; 95% CI 1.92-3.89) and parental albuminuria was related to offspring albuminuria (OR, 1.49; 95% CI 1.03-2.16). These associations remained robust upon additional adjustment for risk factors and in analyses of subgroups defined according to parental or offspring hypertension status (**Figure**). Overall our data suggest that familial clustering of TOD in the community is independent of blood pressure. Additional studies are warranted to confirm our observations.



Disclosures:**T.J. Niiranen:** None. **H. Lin:** None. **M.G. Larson:** None. **R.S. Vasan:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH.

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P403

Seasonal Variation in the Daily Urinary Sodium Excretion in Outpatients From the Northern Japan

PrimaryAuthor.AuthorBlock:**Tomoko Hashimoto**, Iwate Prefectural Hosp, Morioka, Japan

Although the daily urinary sodium excretion (UNaV) is considered to provide the most reliable estimate of the daily sodium intake, it may be affected by salt loss due to sweating in summer. However, theseasonal variation in the daily UNaV associated with a normal lifestyle is unknown. This study was performed in 348 outpatients from the Morioka region during three seasons: summer(summer 1), winter, and the following summer (summer 2). The daily UNaV (g salt/day) was estimated by the second morning urine method three times during each season. Seasonal variation was defined as a significant trend across the three seasons together with a significant difference between winter and both summers. In women, the daily UNaV was higher in winter (11.8±3.0 g salt/day) than in summer 1 (11.2±2.9g salt/day) or summer 2 (11.0±2.9 g salt/day). In contrast, there was no marked seasonal variation in men. An analysis stratified by age (4 quartiles) identified seasonal variation in the older 2 quartiles of women (aged ≧68 years). In these women, the mean seasonal difference in the daily UNaV was 0.9 g of salt/day for both winter vs. summer 1 and winter vs. summer 2, while it was 0.1-0.8 g of salt/day in the other groups. Seasonal variation in the daily UNaV only occurred in older female patients and was relatively small. This is evidence for restricting salt intake throughout the year and should reassure patients who are anxious about salt loss due to sweating in summer.

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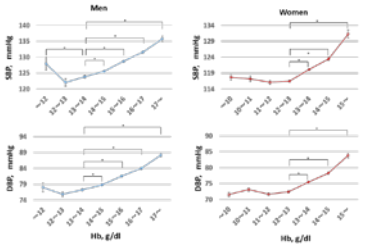
P404

Association of Hemoglobin Level With Blood Pressure in the Japanese General Population

PrimaryAuthor.AuthorBlock:**Yoshiaki Ohyama**, Clinical Investigation and Res Unit, Gunma Univ Hosp, Maebashi, Japan; Keisuke Ichikawa, Dept of Cardiovascular Med, Gunma Univ Graduate Sch of Med, Maebashi, Japan; Kunihiko Imai, Dept of Internal Med, Gunma Chuo Hosp, Maebashi, Japan; Hisako Sumiyoshi, Clinical Investigation and Res Unit, Gunma Univ Hosp, Maebashi, Japan; Yuichiro Saito, Systen integration Ctr, Gunma Univ Hosp, Maebashi, Japan; Tetsuya Nakamura, Clinical Investigation and Res Unit, Gunma Univ Hosp, Maebashi, Japan; Masahiko Kurabayashi, Dept of Cardiovascular Med, Gunma Univ Graduate Sch of Med, Maebashi, Japan

Background; The aim of this study is to evaluate association between hemoglobin (Hb) level and blood pressure in the Japanese general population. **Methods**; The present study is based on annual health check-up program in Gunma, Japan. We studied 21006 participants free of anti-hypertensive medication. Participants were classified into subgroups according to their Hb level using a 1 g/dl interval in each sex, then we compared the systolic and diastolic blood pressure (SBP and DBP) among Hb subgroups. We also used piecewise linear regression models to assess the relationship of Hb level with SBP and DBP adjusted for age, body mass index, fasting blood sugar, current smoking, and total cholesterol, due to the non-linear relationship of Hb with blood pressure. **Results**; . Participants were aged 49 ± 9 years and 37% women. Mean Hb level was 14.3 ± 1.5 (15.2 ± 1.0 in men and 12.9 ± 1.3 in women) g/dl. Higher SBP and DBP were seen with higher Hb level in men with Hb ≧13 g/dl and in women with Hb ≧12 g/dl (Figure). In multiple regression analysis, higher Hb level was associated with higher SBP (B=3.1[95%CI, 2.8 to 3.4] mmHg per 1 g/dl increase in Hb) and DBP (B=2.7 [2.5 to 2.9] mmHg) in men with Hb ≧ 13 g/dl. Similar results were observed in women with Hb ≧ 12g/dl (SBP, B=4.2 [3.7 to 4.8] mmHg; DBP, B=3.4 [3.1 to 3.7] mmHg). **Conclusion**; Hemoglobin levels were positively associated with SBP and DBP in men with Hb ≧ 13 g/dl and women ≧ 12g/dl independent of other risk factors in the Japanese general population.

Figure and Table. Association of hemoglobin and blood pressure



Men	Hb<13 g/dl		Hb≧13 g/dl	
	β (95% CI)	p	β (95% CI)	p
SBP, mmHg	-2.1 (-4.27 to 0.01)	0.06	**3.11 (2.82 to 3.40)**	**<0.001**
DBP, mmHg	-0.33 (-1.78 to 1.13)	0.659	**2.70 (2.50 to 2.90)**	**<0.001**

Women	Hb<12 g/dl		Hb≧12 g/dl	
	β (95% CI)	p	β (95% CI)	p
SBP, mmHg	-0.51 (-1.22 to 0.21)	0.16	**4.21 (3.74 to 4.68)**	**<0.001**
DBP, mmHg	0.0069 (-0.47 to 0.48)	0.98	**3.37 (3.06 to 3.68)**	**<0.001**

Oneway ANOVA with Bonferroni correction was used to compare blood pressure among each Hb subgroups in Figure. Dots represents mean value of Hb and bars represent standard error of Hb.
*p<0.05
B coefficient and 95% confidence intervals (in brackets) were measured using piecewise linear regression model to assess the non-linear relationship of Hb (per 1 g/dl) and blood pressure in Table. The goodness-of-fit tests suggested that the 2-piece simple linear models with Hb cutoff at 13 g/dl in men and 12 g/dl in women give adequate description of the relationships of Hb and blood pressure. Adjust were performed for the following risk factors: age, body mass index, fasting blood sugar, current smoking, and total cholesterol.
Hb indicates hemoglobin; SBP, systolic blood pressure; DBP, diastolic blood pressure

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P406

Sleep Health as an Important Determinant of Elevated Blood Pressure: A Population Based Study

PrimaryAuthor.AuthorBlock:**Sumaira Khalid**, West Virginia Univ, Morgantown, WV

Introduction: One mechanism elucidating the association between lack of sleep and elevated blood pressure includes over-activity of the sympathetic neurotransmitters which in turn raises the levels of adrenaline in the blood. Persistently raised adrenaline levels directly elevate heart rate, systolic blood pressure (SBP) and the diastolic blood pressure (DBP). We aimed at evaluating the association of sleep duration with blood pressure among the adult population in the United States after adjusting for the confounding effects of age, gender, BMI, income poverty ratio (IPR), and race. **Methods:** Our study was based on a population-based cross-sectional study design, comprising of a nationally representative sample of US adults derived from the National Health and Nutrition Examination Survey (NHANES) data cycle 2013-2014 (N=6266). Study population comprised of adults aged 16 years or above who had participated in both interview and medical examination components of the NHANES survey. Measurements on SBP, DBP, and BMI were obtained at the designated clinic by trained personnel, while age, gender, IPR, and race were recorded during the interview by self-report. **Results:** Of 6266 participants, aged between 16 to 80 years with a mean age of 45($\pm$9.2) years, a total of 2302 (34%) reported short sleep duration (<6 hours per day) (Table 1). The outcome variables, SBP and DBP, were significantly different across short and normal sleep duration (p-value <0.05) (Table 1). SBP and DBP were also significantly different among males and females (p-value <0.0001) (Table 2). Both unadjusted and adjusted analyses showed a statistically significant association between sleep duration and DBP [$b_{adjusted}$= -0.42, sd=0.16, 95%CI= -0.74, 0.08, p-value= 0.02]. When stratified by gender, the association was only significant for males [$b_{adjusted}$= -0.50, sd=0.20, 95%CI= -0.90, -0.09, p-value = 0.02] (Table 3). **Conclusion:** Sleep health is a significant determinant of elevated blood pressure in our data even when BMI was held constant. Prospective studies are needed to augment the understanding of this association to seek early public health and behavioral interventions for the prevention of premature cardiovascular morbidity.

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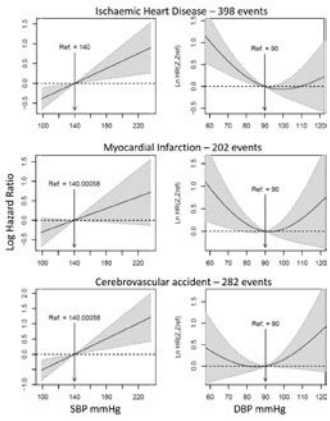
P407

Five-year Longitudinal Bp In Treated Hypertensive Patients Shows Evidence For Diastolic J-curve And Cardiovascular Outcomes

PrimaryAuthor.AuthorBlock:**Li E Tan**, Stefanie Lip, Linsay McCallum, Rhian Touyz, Anna F Dominiczak, Sandosh Padmanabhan, Univ of Glasgow, Glasgow, United Kingdom

Current guidelines recommend a BP target <140/90 mmHg irrespective of cardiovascular (CV) risk level. The SPRINT showed significant benefit of treating SBP to <120 mmHg. Concerns exist regarding the potential increased CV risk from intensive DBP lowering - the diastolic J-curve. We analysed the relationship of longitudinal on-treatment SBP and DBP on CV events. **Methods**: Longitudinal BPs were obtained from clinic records for hypertensive patients attending the Glasgow BP Clinic. AUC BP for the first year (Y1) and 2-5years (Y2-5) were calculated for patients who had >2 BP measurements during these periods. Survival analyses were performed using Cox proportional hazard model adjusted for age, sex, cholesterol, smoking status, BP (time-dependent) and Charlson comorbidity index (time-dependent). **Results:** There were 4813

eligible patients (mean age 54±14 years; female 52%). The average baseline, Y1 and Y2-5 BPs were 167/97±26/12, 153/91±19/9 and 146/87±16/8 mmHg respectively. There were 398, 452 and 455 first admissions with IHD in 15, 25, 35 years (31,563, 39,255 and 40,714 person-years respectively). DBP demonstrated a J-shaped relationship with IHD and MI events but not for CVA (Figure). Compared to DBP80-90 mmHg, DBP<80mmHg was associated with a nearly two-fold increased risk of IHD admissions 1.95[95%C.I. 1.46;2.61, P=7.6E-06]. Similar results were observed for 25- and 35-year follow-up periods. **Conclusions:** Low DBP (<90mmHg) is associated with increased risk of IHD but not CVA. Increasing SBP is linearly associated with increased risk of IHD and CVA. Therefore, intensive SBP reduction measures should recognise potential risks of concomitant DBP reduction.



Disclosures:**L.E. Tan:** None. **S. Lip:** None. **L. McCallum:** None. **R. Touyz:** None. **A.F. Dominiczak:** None. **S. Padmanabhan:** None.

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P408

Waist-to-height Ratio Index For Predicting The Incidence Of Hypertension

PrimaryAuthor.AuthorBlock:**Jung Ran Choi**, Yonsei Univ, Wonju, Korea, Republic of

Background Several anthropometric indices such as body mass index (BMI) and waist circumference (WC) have been examined as indicators of cardiovascular diseases in both adults and children. Waist-to-height ratio (WHtR) has been considered a superior predictor for detecting cardiovascular risk factors than BMI. We investigated the association between WHtR and incident hypertension in prospective study. **Methods** A total of 1,718 subjects aged 39-72 years in a longitudinal study were recruited. Participants were divided into two groups according to development of hypertension during 2005-2008 (baseline) and 2008-2011 (follow-up). Logistic regression models were used to evaluate WHtR as a significant predictor of the hypertension. **Results** During 2.8 years of follow-up, 185 new cases of hypertension (10.8%) were diagnosed with an incidence rate of approximately four percent per year. The WHtR was significantly higher in the subjects who had developed hypertension than in those who had not (0.54±0.05 vs. 0.51±0.05, *p*<0.001). After adjusted for baseline age, gender, smoking status, alcohol intake, regular exercise, total cholesterol (LDL), and systolic blood pressure (SBP), logistic regression analysis indicated participants with the highest quartile of WHtR (WHtR≥0.54) were 4.51 times more likely to have hypertension than those with the lowest quartile (OR 4.51; 95% CI 2.41-8.43; p<.0001). The area under the curve (AUC) for WHtR in identifying hypertension risk was significantly greater than BMI (p=0.0233). **Conclusion** A positive association between WHtR and the incidence of hypertension was found in Korean adults. We assumed the WHtR may be a better predictor of incident hypertension in a community-based prospective study.

Disclosures:**J. Choi:** None.

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P409

Defective Renal Ang III and AT2 Receptor Signaling in Pre-hypertensive Spontaneously Hypertensive Rats (SHR)

PrimaryAuthor.AuthorBlock:**Brandon A. Kemp**, Nancy L. Howell, Shetal H. Padia, Susanna R. Keller, John J. Gildea, Robert M Carey, Univ of Virginia Health System, Charlottesville, VA

The intrarenal renin-angiotensin (RAS) system controls blood pressure and electrolyte balance. Angiotensin II (Ang II), the principal RAS effector peptide, is metabolized by aminopeptidase A to des-apartyl^{1}-Ang II (Ang III). Our previous studies showed that Ang III, not Ang II, is the preferred endogenous agonist for Ang type-2 receptor (AT_2R)-induced natriuresis in normal rats, and that hypertensive 12-week old SHR lack natriuretic responses to Ang III. The present study tested whether Ang III induced natriuresis in 4 week old pre-hypertensive SHR by activating/translocating intrarenal AT_2Rs and internalizing the major renal proximal tubule (RPT) sodium transporter NHE-3. Female 4 week old SHR (N=6) and WKY (N=6) rats were studied after 24 h systemic AT_1R blockade with candesartan. The left kidney received a 30 min renal interstitial (RI) infusion of vehicle followed by cumulative RI infusions of Ang III (3.5, 7.0, 14, and 28 nmol/kg/min; each dose for 30 min). The right kidney received vehicle RI infusions. In 4 week old WKY, RI Ang III increased urine sodium excretion ($U_{Na}V$) dose dependently from control of 0.04 ± 0.01 to 0.08 ± 0.02 (P<0.05), 0.09 ± 0.02 (P<0.01), 0.07 ± 0.01 (P<0.05), and 0.07 ± 0.01 (P<0.05) µmol/min. In 4 week old pre-hypertensive SHR, RI Ang III failed to induce natriuresis at all doses except 28 nmol/kg/min (0.03 ± 0.01 vs. 0.05 ± 0.01 µmol/min; P<0.05). There was no change in $U_{Na}V$ in right control kidneys of WKY or SHR. Also, Ang III had no effect on mean arterial pressure (MAP) in WKY or SHR. However, MAP was slightly higher, but normotensive, in SHR than WKY. Control and Ang III infused kidneys were processed for confocal microscopy from a separate group of rats. In WKY, RI Ang III induced translocation of AT_2Rs from subapical to apical membranes of RPT cells [RPTC] (1767 ± 41 vs. 1313 ± 47 RFU; P<0.001). Simultaneously, Ang III induced retraction of NHE-3 from in SHR, intrarenal Ang III failed to induce AT_2R translocation, NHE-3 retraction, and $pSer^{552}$-NHE-3 upregulation. These results apical to subapical membranes of RPTCs (1703 ± 90 vs. 1388 ± 91 RFU; P<0.05). Consistent with NHE-3 retraction, Ang III increased $pSer^{552}$-NHE-3 (1102 ± 57 vs. 830 ± 66 RFU; P<0.01). In contrast,demonstrate defective Ang III and AT_2R signaling in pre-hypertensive SHR.

Disclosures:**B.A. Kemp:** None. **N.L. Howell:** None. **S.H. Padia:** None. **S.R. Keller:** None. **J.J. Gildea:** None. **R.M. Carey:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH grants.

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P410

Characterization of the Renin-angiotensin System in Induced Pluripotent Stem Cell-derived Human Kidney Organoids

PrimaryAuthor.AuthorBlock:**Ryousuke Satou**, Courtney M Dugas, Dept of Physiology, and Hypertension and Renal Ctr of Excellence, Tulane Univ Sch of Med, New Orleans, LA; Jiao Liu, Dept of Pediatrics, Tulane Univ Sch of Med, New Orleans, LA; Samir El-Dahr, Dept of Pediatrics, Tulane Univ Sch of Med, New

Orleans, LA; Zubaida Saifudeen, Dept of Pediatrics, Tulane Univ Sch of Med, New Orleans, LA

Intrarenal renin-angiotensin system (RAS), including proximal tubular angiotensinogen (AGT), plays crucial roles in the progression of hypertension, kidney injury, and kidney development and has been investigated *in vivo* using animal models. For translational relevance, we sought to further our investigations in human tissue. This study investigated RAS expression and AGT regulation by histone deacetylase 9 (HDAC9), an epigenetic repressor of AGT, in human iPSC-derived kidney organoids. After pre-treatment of human iPSC with CHIR99201, a glycogen synthase kinase inhibitor, and fibroblast growth factor 9, cells were moved to transwell membranes. Cells were harvested on day 0, 5, 12 or 18 to determine mRNA copy numbers of developmental markers and RAS genes by digital PCR. Marker genes of renal structures were induced during the culture with concomitant decrease in progenitor markers including Cited1. Immunostaining revealed that the organoids contain podocytes, proximal tubules expressing AGT and distal tubules. Angiotensin II type 1 receptor (AT1R) levels were higher than other RAS components on day 0 and the expression was downregulated on day 5. AT2R induction peaked on day 5 and reduced until day 18. Renin expression was strongly induced on day 5 and sustained until day 18. Angiotensin-converting enzyme levels were moderately augmented during the culture. AGT levels were elevated on day 12 and remained until day 18 (7.6-fold, ratio to day 0). Conversely, HDAC9 levels decreased by day 18. On day 18, AGT and HDAC9 levels were inversely correlated in a CHIR99201 concentration-dependent manner. Moreover, an HDAC9 inhibitor increased AGT expression (2.25±0.21-fold, ratio to control). These results suggest that all RAS components are expressed and independently regulated during the development of iPSC-derived human kidney organoids. The existence of all RAS components including high renin expression, their regulation, and epigenetic regulation of AGT in the organoids support previous findings in rodent models. Concerted effort, including this study, to overcome technical challenges to generate complete nephrons will provide human kidney organoids to study development, pathophysiological mechanisms, novel drugs and clinical therapies.

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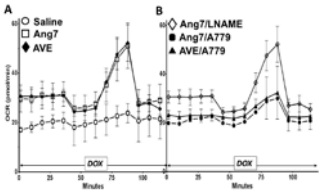
P411

Angiotensin-(1-7) Preserves Mitochondrial Function in Doxorubicin-exposed Renal Epithelial Cells

PrimaryAuthor.AuthorBlock:David Soto-Pantoja, Wake Forest Sch of Med, Winston Salem, NC; Nildris Cruz-Diaz, Wake Forest Sch of Med, Winston-Salem, NC; **Mark C Chappell**, Wake Forest Sch of Med, Winston Salem, NC

We recently identified components of an Angiotensin-(1-7) system (Ang7) within mitochondria (Mito) of the renal cortex, as well as angiotensinogen uptake and trafficking to the Mito and nucleus in proximal tubules. Although activation of the Ang II-AT1 receptor (AT1R) axis is deleterious to the Mito, the functional role of Ang7 is unknown. Thus, we evaluated the effects of Ang7 and its agonist AVE0991 (AVE) to attenuate doxorubicin (DOX)-induced Mito toxicity. NRK-52 renal epithelial cells were exposed to DOX (10 µM, 24 hours)

and either saline, Ang7 (100 nM), AVE (100 nM), the MasR antagonist A779 (10 μM) or LNAME (1 mM). Mito function in NRK cells was evaluated by a Seahorse XF-96 analyzer; the OCR data were expressed as the mean ± SD. Both Ang7 and AVE attenuated the decline in Mito function by DOX exposure (Fig_A); the calculated maximal respiration (MR) rates for Ang7 and AVE treated DOX cells were similar to control cells without DOX [25.5 ± 4.4 and 26.7 ± 7.8 vs. 33.9 ± 11.4 pmol/min; p>0.05]. Preservation of Mito function by Ang7 and AVE in DOX cells was reversed by the A779 antagonist (Fig_B). Ang7 stimulation of the nitric oxide synthase (NOS)-NO pathway is a key signaling event in various cell types; however, the NOS inhibitor LNAME failed to block the Ang7 response in DOX exposed cells [MR: 32.2 ± 7.6 pmol/min; Fig_B]. DOX also increased phospho-ERK1/2 over 10-fold [1.12 ±. 0.27 vs. 0.08 ± 0.03], but Ang7 did not attenuate the MAPK response [1.19 ±. 0.34]. We conclude that Ang7 preserves mitochondrial function following DOX exposure in tubular epithelial cells. Moreover, the protective effect of the Ang7-MasR axis does not apparently reflect the contribution of the NOS-NO or MAPK pathways.



Disclosures:**D. Soto-Pantoja:** None. **N. Cruz-Diaz:** None. **M.C. Chappell:** None.

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Funding Component: Mid-Atlantic Affiliate (Maryland, North Carolina, South Carolina, Virginia & Washington, DC)

P412

Genetically Increased Angiotensin I-converting Enzyme and Peripheral and Renal Vascular Reactivity in Mice

PrimaryAuthor.AuthorBlock:Catherine Chollet, Sandrine Placier, Christos Chatziantoniou, INSERM, Paris, France; Annette Hus-Citharel, CIRB Coll de France, Paris, France; Nathalie Caron, Univ of Namur, Namur, Belgium; Ronan Roussel, **Francois Alhenc-Gelas**, Nadine Bouby, INSERM, Paris, France

Angiotensin 1-converting enzyme (ACE) levels in man are under strong genetic influence. Genetic variation in ACE has been linked to risk for and progression of cardiovascular and renal diseases. Causality has been documented in genetically modified mice but mechanisms underlying causality may remain incompletely documented. To further document the vascular and renal consequences of a moderate genetic increase in ACE we studied mice carrying three copies of the ACE gene (ACE3) and littermate wild type 2-copy animals (WT). We studied peripheral and renal vascular reactivity to angiotensin II and bradykinin, by measuring blood pressure and renal blood flow (RBF) after intravenous administration, and also reactivity of isolated glomerular arterioles, by following intracellular calcium mobilisation. Vasoconstrictor responses to angiotensin II were significantly enhanced in ACE3 compared to WT over the whole range of doses tested (0.25, 0.5, 1 and 2 ng, n=5/6 per group, ANOVA, genotype effect, MAP $p<0.01$, RBF $p<0.05$). The lowest dose of Ang II increased MAP by 5.3±1.3 and 16.7±4.0 mm Hg in WT and ACE3, respectively and decreased RBF by 0.136±0.035 and 0.486 ±0.097 ml/min. ACE gene copy-number had no influence on the response to intravenous norepinephrine (2, 4 and 10 ng) or bradykinin (25, 50 and 100 ng). In isolated glomerular afferent arterioles, maximal calcium

response to angiotensin II (10^{-12} to 10^{-7} mol/L) was increased in ACE3, consistent with the hemodynamic study. Δ $[Ca^{2+}]_i$ max (nmol/l) was 239 ± 22 in ACE3 versus 189 ± 16 in WT (n=9, p<0.01). Duplication of ACE gene also altered the signalling pathways triggered by endothelial activation by bradykinin or carbachol in pre-constricted muscular efferent arterioles. While in WT the NOS-NO pathway was not functional in these arterioles, with lack of NOS mRNAs and lack of effect of L-NAME, in ACE3 NOS3 gene expression was induced and NO mediated the effect of bradykinin or carbachol, which was inhibited by L-NAME. These data document new, unexpected vascular consequences of a genetic increase in ACE synthesis. Enhanced vasoconstrictor effect of angiotensin II, probably due to loss of counter-regulatory mechanisms, may contribute to the risk for cardiovascular and renal diseases linked to genetically high ACE.

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P413

Vascular AT1 Receptors Control Blood Pressure by Augmenting Peripheral Vascular Resistance in Female Mice

PrimaryAuthor.AuthorBlock:Erin Wolf, Edward Diaz, Hooman Azad, Aaron Kupin, **Matthew A Sparks**, Duke Univ Medical Ctr, Durham, NC

Angiotensin (Ang) II is a major mediator of hypertension pathogenesis and end organ damage. Our understanding of how Ang II elicits these effects on a cellular level continues to evolve. However, the majority of these studies utilize male subjects, despite the awareness that males and females differ in mechanisms of BP regulation. Here we investigated the role of vascular smooth muscle cell (VSMC) AT1A receptors to BP control in female mice. Using Loxp technology and Cre transgenes, we created female mice with cell-specific deletion of AT_{1A} receptors in smooth muscle cells (SMKO mice). We found that elimination of these receptors led to a significant (8 mmHg) reduction in baseline BP in female SMKO mice (similar to male SMKO mice) compared with controls (108±2 versus 116±1 mmHg; *P*=0.004) with no effect on modulating sodium sensitivity in female SMKO mice (unlike male SMKO mice in which sodium sensitivity was enhanced). Over a 4-week Ang II infusion, the severity of Ang II-dependent hypertension was minimally affected during the first 2 weeks of Ang II infusion, corresponding to no difference in sodium excretion. However, female SMKO mice had a 35% less BP elevation compared to controls over the final 2 weeks of Ang II infusion (SMKO, 20±2 versus control, 30±3 Change in SBP mmHg; *P*=0.01). The acute vasoconstrictor responses to Ang II in the systemic vasculature were reduced (by approximately 50-75%) in female SMKO mice (0.1 μg/kg- SMKO, 3.6±1.2 mmHg versus control, 7.3±0.7 mmHg; *P*=0.01, 0.3 μg/kg- SMKO, 3.3±1.8 mmHg versus control, 12.1 ± 1.3 mmHg; *P*=0.0008, 1.0 μg/kg- SMKO, 8.5±1.9 mmHg versus control, 19.4±2.0 mmHg; *P*=0.002). In addition, the acute Ang II reduction in renal tissue perfusion was nearly eliminated in female SMKO mice. In contrast, we previously reported that in male SMKO mice have a much larger (50%) reduction in BP during chronic Ang II infusion associated with increased natriuresis, but relatively preserved vasoconstrictor response (25% reduction) during acute Ang II infusions. These findings suggest that in female mice, direct actions of AT_{1A} receptors in VSMCs are essential for regulation of basal BP and Ang II-dependent hypertension. However, this effect

is mediated by reductions in peripheral vascular resistance rather than sodium retention.

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P414

Blockade of Sodium Glucose Cotransporter 2 by Canagliflozin Suppresses High Glucose-induced Angiotensinogen Augmentation in Renal Proximal Tubular Cells

PrimaryAuthor.AuthorBlock:**Ryousuke Satou**, T. Cooper Woods, Kayoko Miyata, Michael W Cypress, Akemi Katsurada, Courtney M Dugas, Daniel Lightell Jr, L. Gabriel Navar, Dept of Physiology and Hypertension and Renal Ctr of Excellence, Tulane Univ Sch of Med, New Orleans, LA

Intrarenal angiotensinogen (AGT) is mainly expressed in proximal tubular cells (PTC). AGT is increased by hyperglycemia (HG) in type 1 and 2 diabetes mellitus, which causes elevated intrarenal angiotensin formation contributing to the development of hypertension and kidney injury. Sodium glucose co-transporter 2 (SGLT2) is abundantly expressed in early PTC and may promote increased intrarenal AGT by increasing intracellular glucose levels. This study tested the effects of canagliflozin (CANA), an SGLT2 inhibitor, on HG-induced AGT elevation in cultured PTC. Mouse PTC were treated with 5, 10 or 25 mM glucose. 0-10 µM CANA was applied one hour before glucose treatment. AGT mRNA and protein levels were measured by digital PCR and western blot analysis. Levels of intracellular reactive oxygen species (ROS) were determined with H_2DCF-DA. Tempol, an antioxidant, was used to test if elevated ROS is involved in HG-induced AGT upregulation. 10 mM glucose increased AGT protein levels at 12 hours (3.06±0.48-fold compared with 5 mM glucose) and treatment with 10 µM CANA attenuated the AGT augmentation (1.68±0.05-fold). AGT protein levels were also increased by 25 mM glucose; but CANA did not suppress the AGT levels caused by this glucose concentration. In PTC treated with 10 mM glucose for 12 hours, the suppressing effect on AGT upregulation was observed with 1 and 10 µM CANA. Lower concentrations of CANA (0.01 and 0.1 µM) did not lower AGT protein levels significantly. Elevated AGT mRNA expression by glucose was also attenuated by CANA. Treatment of PTC with 1 mM pyruvate also increased AGT expression levels, indicating that glycolysis is involved in HG-induced AGT upregulation. After incubation of PTC with 10 mM glucose for 12 hours, intracellular ROS levels were elevated compared to baseline (4.24±0.23-fold) and these were also inhibited by CANA (0.2±0.08-fold). Furthermore, tempol attenuated AGT upregulation in HG-treated PTC. These results indicate that enhanced glucose entry via SGLT2 into PTC elevates intracellular ROS generation by stimulation of glycolysis and consequent AGT augmentation. Thus, SGLT2 inhibition by CANA may limit HG-induced AGT stimulation which suppress intrarenal angiotensin formation and reduce kidney injury in diabetes mellitus.

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ACE N-domain Regulates High-glucose Mediated Interleukin-1 beta Production by Renal Epithelial Cells Independently From Angiotensin II Generation

PrimaryAuthor.AuthorBlock:**Jorge F Giani**, Ellen A. Bernstein, Masahiro Eriguchi, Cedars-Sinai Medical Ctr, Los Angeles, CA; Romer A. Gonzalez-Villalobos, Internal Med Res Unit, Pfizer, Inc, Los Angeles, CA; Kenneth E. Bernstein, Cedars-Sinai Medical Ctr, Los Angeles, CA

Research studies demonstrated that interleukin (IL)-1β contributes to the development of diabetic nephropathy and hypertension. However, the origin and regulation of IL-1β synthesis during diabetic kidney injury are still unknown. Here, we hypothesize that renal epithelial cells produce IL-1β in response to a high glucose stress and that angiotensin converting enzyme (ACE) plays a key role in this process. To study this, we isolated proximal tubular (PT) epithelial cells from wild-type (WT) and mice lacking either the ACE N-domain (NKO) or the C-domain (CKO) catalytic activity. These cells were exposed to normal (5 mM) or high (30 mM) glucose for 24 hours. IL-1β produced by PT cells were assessed by ELISA and RT-PCR. High glucose induced WT PT cells to release significant amounts of IL-1β (from 5±1 to 70±6 pg/ml, $p<0.001$; n=6). When WT PT cells were exposed to a high glucose media in the presence of an ACE inhibitor (lisinopril, 10 mM), IL-1β levels were significantly reduced (from 70±6 to 38±6 pg/ml, $p<0.01$). In contrast, AT1 receptor blockade by losartan did not change the amount of IL-1β produced by WT PT cells. To determine which ACE domain is associated with IL-1β production, NKO and CKO PT cells were exposed to high glucose. Strikingly, NKO PT cells released lower amounts of IL-1β when exposed to high glucose compared to WT (NKO: 15±7 vs. WT: 79±9 pg/ml, $p<0.01$, n=4). No differences were observed between WT and CKO PT cells. Since the ACE N-domain degrades the anti-inflammatory tetrapeptide N-acetyl-Ser-Asp-Lys-Pro (AcSDKP), we tested whether the lower IL-1β production in NKO PT cells was due to an accumulation of AcSDKP. For this, we pre-treated NKO PT cells with a prolyl endopeptidase inhibitor (S17092, 50μM) to prevent the production of AcSDKP. Notably, this treatment increased the IL-1β response to high glucose in NKO PT cells (2.1±0.3-fold increase, $p<0.01$, n=4). Our data indicate that: 1) PT cells can sense and respond to high glucose by secreting IL-1β and 2) the absence of the ACE N-domain blunts the production of IL-1β through a mechanism that involves AcSDKP accumulation. In conclusion, ACE might contribute to the inflammatory response that underlays diabetic nephropathy independently from angiotensin II generation.

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P417

The Elevation of Blood Pressure in Adipocyte Prorenin Receptor Deficient Female Mice is Mediated by a Local AngII Dependent Mechanism

PrimaryAuthor.AuthorBlock:Eva Gatineau, Diane Cohn, Ming Gong, **Frederique Yiannikouris**, Univ of Kentucky, Lexington, KY

Studies of acquired or genetic lipodystrophy are becoming increasingly significant because it may provide new insights to decode mechanisms leading to obesity related hypertension. The objective of the study was to determine the mechanism by which lipodystrophy contributes to hypertension in adipocyte-(pro)renin receptor (PRR) deficient female mice. Control ($PRR^{fl/fl}$) and adipocyte-PRR deficient (PRR^{Adi}) female mice were fed a high fat diet (HF) for 31 weeks (n=7-8/groups) and blood pressure was recorded by radiotelemetry. As predicted, the deletion of adipocyte PRR prevented HF diet-induced obesity, decreased by 85% (8.5 of 10) the fat mass and increased significantly systolic blood pressure (SBP) in female mice. To determine the mechanism by which the deletion of adipocyte PRR led to an increase in blood pressure, female mice were injected with a β-adrenergic antagonist (propranolol), a parasympathetic agonist (atropine), a ganglionic blockade (hexamethonium) and an AT1R antagonist (Losartan, Los). The bradycardic response after propranolol injection, the tachycardic response induced by atropine or the sensitivity of ganglionic blockade to the elevated BP were similar in $PRR^{fl/fl}$ and PRR^{Adi} female mice suggesting that hypertension was not para- or sympathetically mediated. However, the decrease of SBP induced by Los was more pronounced in PRR^{Adi} compared to control female mice (PRR^{Adi}, -14.2± 0.9 mmHg; $PRR^{fl/fl}$, -5.3±1.2 mmHg; P<0.05) whereas the decrease of SBP induced by Los was similar in $PRR^{Adi/Y}$ and $PRR^{fl/Y}$ male mice. Analyze of systemic and local angiotensin peptides revealed a local increase of AngII peptides in the cortex of the kidney of PRR^{Adi} compared to control female mice (PRR^{Adi}, 236 ± 24; $PRR^{fl/fl}$, 119± 16 pg/g; P<0.05). Urinary level of vasopressin was higher in PRR^{Adi} mice compared to control female mice (PRR^{Adi}, 19.4± 2.8; $PRR^{fl/fl}$, 7.3± 0.7 pg/mg of creatinine; P<0.05) and was positively correlated with plasma sPRR levels (P<0.05). Our results demonstrated that, elevation of blood pressure due to adipocyte PPR deficiency is driven by a local AngII dependent mechanism in female mice whereas this elevation might be mediated by the autonomic nervous system in male mice and suggest an important role of PRR in blood pressure control.

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PPARγ Targets both (Pro)Renin Receptor and Site-1 Protease in the Collecting Duct to Mediate Rosiglitazone-induced Fluid Retention

PrimaryAuthor.AuthorBlock:**Tianxin Yang**, Fei Wang, Univ Utah, Salt Lake City, UT; Ziwei Fu, Sun Yat-sen Univ, Guangzhou, China; Chang-Jiang Zou, Kexin Peng, Univ Utah, Salt Lake City, UT; Jiajia Hu, Sun Yat-sen Univ, Guangzhou, China; Guangju Guan, Shandong Univ, Jinan, China; Long Zhao, Univ Utah, Salt Lake City, UT

Collecting duct (CD)-specific deletion of PPARγ attenuates the fluid retention side effect of thiazolidinedione including rosiglitazone (Rosi) but the underlying mechanism largely remains unknown. (Pro)renin receptor (PRR) is a newly described regulator of CD function. Recent results have demonstrated site-1 protease (S1P) but not furin or ADAM19 as the predominant PRR cleavage protease. A histidine-tagged soluble PRR, sPRR-His, stimulates AQP2 expression. Here, we examined involvement of PRR/S1P during Rosi-induced fluid retention. PRR promoter contains two putative PRREs at positions -834~-828 bp (AGGTTA) and -318~-

312 bp (GGTGCA). A 2.0-kb mouse PRR promoter showed a 10-fold increase in luciferase activity in mpkCCD cells exposed to 10 μM Rosi. The Rosi-induced response was first mapped to -421~ -219 bp by the deletion analysis and then to the second PPRE by mutagenesis. Rosi treatment at 80 mg/kg diet in C57/BL mice rapidly induced a 1.9-fold increase in renal PRR protein, a 2.4-fold increase in urinary sPRR, a 2.0-fold increase in urinary renin activity, a 48% reduction of urine volume, and a 4.8-fold increase in renal AQP2 protein, without an effect on expression of ENaC subunits, at 36 h, all of which were diminished thereafter. On day 3, Rosi induced a 9% body weight gain (BWG) and a 15% reduction of Hct, a 20% increase of fluorescein isothiocyanate dextran (FITC-d)-measured plasma volume. In contrast, the indices of Rosi-induced fluid retention along with AQP2 upregulation were all nearly abolished by a PRR antagonist PRO20 and CD PRR deletion. The sensitivity to Rosi-induced volume expansion as assessed by Hct and FITC-d in the null mice was fully restored by a 3-d i.v. infusion of sPRR-His at 30 μg/kg/d. A 2.1-kb mouse S1P promoter also contained two PPRE sites and showed a 3-fold increase in luciferase activity in response to Rosi. Administration of a S1P inhibitor PF-429242 via minipump in C57/BL6 mice attenuated Rosi-induced FITC-d-measured plasma volume (Control 1.28 $\pm$ 0.1 vs. Rosi 1.61 $\pm$ 0.05 vs. Rosi $\pm$ PF429242 1.37 $\pm$ 0.08 ml/kg), BWG, and Hct drop. Overall, PPARγ simultaneously targets PRR and S1P to generate sPRR to increase AQP2 expression to expand plasma volume during Rosi treatment.

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Klotho Ameliorate Medullary Fibrosis and Pressure Natriuresis in Hypertensive Rat Kidneys

PrimaryAuthor.AuthorBlock:**Tsuneo Takenaka**, Hirofumi Kobori, Intl Univ of Health, Tokyo, Japan; Tsutomu Inoue, Takashi Miyazaki, Hiromichi Suzuki, Saitama Medical Univ, Saitama, Japan; Akira Nishiyama, Kagawa Univ, Kagawa, Japan; Naohiro Ishii, Kitasato Univ, Kanagawa, Japan; Matsuhiko Hayashi, Keio Univ, Tokyo, Japan

Recent data indicate that klotho interacts with the renin-angiotensin system (RAS). However, the effects of klotho protein supplementation on hypertensive renal injury have not been examined yet. First, the in vivo experiments were performed using spontaneously hypertensive rats (SHR). To elucidate in vivo observations, the in vitro studies were carried out to examine the interactions between klotho protein and angiotensin type-1 (AT1) receptor with immunoprecipitation and cell culture (HK-2 cell) methods. Uninephrectomized SHRs were treated with exogenous klotho protein or vehicle. Exogenous klotho protein supplementation to uninephrectomized SHRs decreased blood pressure, renal angiotensin II levels, angiotensinogen expression, HIF-1α abundance, and medullary fibronectin with increased renal klotho expression and serum and urine klotho levels. Klotho supplementation also reduced kidney weight, renal phosphorylated Akt and mTOR abundance. Furthermore, klotho supplementation restored renal autoregulation of glomerular filtration rate and renal plasma flow, and improved pressure-induced natriuresis in SHR. Klotho protein bound to AT1 receptors without affecting the bindings of angiotensin II to AT1 receptor. Klotho decreased the presence of AT1 receptors on HK-2 cells, attenuating inositol

triphosphate generation. Angiotensin II elevated angiotensinogen expression in HK-2 cells, and klotho protein suppressed angiotensin II-induced increases in angiotensinogen expression. Of note, although angiotensin II improves glomerular autoregulatory ability especially at lower pressure range, angiotensin II impairs pressure-induced natriuresis. Collectively, the present data demonstrate that klotho binds with the AT1 receptors to suppress angiotensin signal transduction and inactivate the renal RAS. In addition, our results suggest that exogenous klotho supplementation suppresses Akt-mTOR signaling to decline renal hypertrophy and restores renal autoregulatory ability in uninephrectomized SHRs. Finally, the present findings implicate that klotho supplementation inhibits HIF-1α pathway to suppress medullary fibrosis, presumably involved in improvements of pressure-natriuresis and reduction in blood pressure.

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Sexual Difference of Tubular Renin Angiotensin System (RAS) in 2 Kidney 1 Clip Rats

PrimaryAuthor.AuthorBlock:**Ju Young Moon**, Yang Gyun Kim, Chun-Gyoo Ihm, Tae Won Lee, Kyung Hwan Jeong, Yu Ho Lee, Kipyo Kim, Nephrology, Seoul, Korea, Republic of; Il Suk Sohn, Cardiology, Seoul, Korea, Republic of; Seon Hwa Park, Dong Jin Kim, Nephrology, Seoul, Korea, Republic of

Background: Premenopausal female manifests lower blood pressure compared with age-matched male. Intrarenal RAS is thought to be an important role in hypertension and hypertensive renal disease, while there is rare information about sexual difference in intrarenal RAS. This study was performed to evaluate sexual difference of tubular RAS in 2 kidney 1 clip(2K1C) rat model.
Methods: A 2.5-mm clip was inserted into the left renal artery of male and female Spargue-Dawley rats and they were euthanized at 5 week following the operation. Systolic blood pressure(SBP) was measured via the tail-cuff method at 10 day interval. Medullary area for tubular RAS and cardiac tissue was collected for analysis of local RAS expression.
Results: At 30 days after clipping operation, SBP and albuminuria was significantly increased in 2K1C male rats (SBP: male 176.75±5.58 vs. female 106.71±8.68 mmHg, p 0.004, albumin/Cr ratio: male 116.93±33.96 vs. female 56.40±13.94 mg/g, p 0.029). Also, left ventricular hypertrophy was observed only in male rats, however cardiac ACE2 and MasR mRNA did not show sexual difference. The clipped kidney(CK) of 2K1C male rat presented worse glomerulosclerosis and more macrophage infiltration. Renin mRNA was more expressed in female CK, but protein expression was rather decreased in female CK and non-clipped kidney(NCK). 2K1C female rats exhibited highly augmented ACE2 and Mas receptor(MasR) in mRNA and protein, but ACE was augmented in mRNA and reduced in protein. Immunohistochemistry showed that tubular renin and ACE was increased in 2K1C male rats, in contrast ACE2 and MasR were increased in 2K1C female rats. Medullary angiotensin II(AngII) was significantly lower in male CK, but angiotensin 1-7(Ang1-7) was higher in female CK but did not show significance (CK AngII: male 201.88±10.40 vs. female 137.01±19.33 fg/mg p 0.030, CK Ang1-7: male 22.21±4.51 vs. female 57.93±19.59 fg/mg

p 0.190).
Conclusions: Same insult of renal artery stenosis aroused different clinical outcome in 2K1C male and female. Differently activated tubular RAS might influence difference in clinical manifestation. Superiority of nonclassic tubular RAS in female rats could limit the adverse effect of classic RAS under renovascular hypertension.

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The Effects of Dual Angiotensin Receptor and Neprilysin Inhibitor on Organoprotection in Experimental Model of Chronic Heart Failure

PrimaryAuthor.AuthorBlock:**Libor Kopkan**, Lenka Hosková, Vojtech Melenovský, Zuzana Husková, Ludek Cervenka, Inst for Clinical and Exp Med, Prague 4, Czech Republic

The cardiac effects of dual angiotensin receptor and neprilysin inhibitor (LCZ696) as a novel therapeutic approach for inhibition of renin-angiotensin system was assessed in volume overload-induced chronic heart failure (CHF) in hypertensive Ren2 transgenic rats (TGR) with aorto-caval fistula (ACF) and compared with valsartan (VAL). CHF model was induced by ACF in male TGR at 8 weeks of age. After 5 weeks of ACF induction, LCZ696 was administered in standard dosing at 68 mg/kg/day and VAL at 31 mg/kg/day for next 15 weeks to assess the effect on mortality. In second series, 12 weeks after ACF, the animals were anaesthetized and echocardiography was performed using a 7.5 MHz probe (Vivid 7, GE) to determine left ventricular internal dimensions (LVIDd), fractional shortening (FS) and stroke volume (SV). Animals were euthanized to determine heart weight and left ventricular mass (LVm). Untreated ACF group displayed 100 % mortality till 17 weeks after ACF induction. On the other hand, LCZ696-treated ACF TGR exhibited only 12 % mortality at the end of experiment. Valsartan also markedly reduce mortality in these animals to 21 %. In comparison to intact animals, ACF significantly increased LVIDd (10,2 ± 1,1 vs. 7,6 ± 0,4 mm). Only LCZ significantly lowered LVIDd in ACF TGR (8,2 ± 0,7 mm). We also observed significantly altered ventricular function in ACF group compared to intact TGR: FS 37 ± 5 vs. 53 ± 4 % and SV 763 ± 124 vs. 395 ± 36 ml/beat. There was a significant improvement of the left ventricular function in both treated groups, where FS was increased in ACF LCZ (52 ± 4 %) or in ACF VAL (47 ± 3 %) and SV significantly decreased in LCZ (509 ± 62 ml/beat) and ACF VAL (591 ± 78 ml/beat). Compared to untreated ACF group, the LVm was significantly reduced in ACF LCZ (1958 ± 97 vs. 1697 ± 69 mg). We did not observe any adverse effect during the treatments with VAL or LCZ confirming the safety of these drugs in this model. LCZ696 treatment resulted in significant reverse left ventricular remodeling. However, we did not observe any additional effects of treatment on cardiac function with LCZ696 compared to VAL in short term follow-up. Prolonged treatment period is advised to verify possible cardioprotective benefits of LCZ696 in this model.

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Two-kidney One Clip (2k1c) Hypertension Reduces Complexity of Heart Rate Variability in Mice

PrimaryAuthor.AuthorBlock:Fernanda L Rodrigues, Luiz E Silva, Carlos A Silva, Univ of San Paulo, Ribeirao Preto, Brazil; Fernando S Carneiro, Rita C Tostes, Univ of San Paulo, Ribeirão Preto, Brazil; Rubens Fazan Jr, **Helio C Salgado**, Univ of San Paulo, Ribeirao Preto, Brazil

Introduction: Hypertension is the most common chronic cardiovascular disease, being multifactorial in origin and an important cause of morbidity and mortality worldwide. Complex behaviors of heart rate series have been widely recognized and the loss of complexity in heart rate variability (HRV) has been shown to predict adverse cardiovascular outcomes. We hypothesized that two-kidney one clip (2K1C) hypertension reduces the HRV complexity in mice. **Methods and results:** C57BL/6 mice were anesthetized with isoflurane and submitted to 2K1C hypertension by placing a silver clip (0.12 mm) around left renal artery. After 4 weeks, mice were implanted with subcutaneous electrocardiogram (ECG) electrodes and allowed to recover for 48 h. On the day of the experiment, the ECG was recorded for 30 minutes in conscious, unrestrained mice. At the end of the recording, arterial pressure (AP) was directly measured in each mouse under isoflurane anesthesia. RR interval time series were generated and the complexity of HRV was determined using detrending fluctuation analysis (DFA) and multiscale entropy (MSE). Mean AP was higher in 2K1C mice (133±2 vs 93±4 mmHg) while the HR was similar between groups. DFA scaling exponents were calculated in short (5 to 15), mid (30 to 200) and long (200 to 1500) window sizes, but only the long-term exponent was different between groups (1.27±0.09 vs 0.89±0.08 in 2K1C and sham mice, respectively). MSE was calculated up to scale 20 and averaged in short (1 to 5) and long (6 to 20) time scales. In both short (0.75±0.16 vs 1.25±0.11) and long (0.76±0.17 vs 1.22±0.09) ranges, entropy is lower in hypertensive mice. **Conclusions:** The complexity of HRV dynamics was found lower in renovascular hypertensive mice. Both sympathetic and vagal control of the heart seems to be involved in this process, as predictability (MSE) and fractality (DFA) is affected in various temporal scales. Nevertheless, the greatest entropy difference between groups is found at scale 6, which is closely related to respiration.

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Chronic Doxycycline Administration Inhibits Angiotensin Converting Enzyme Activity and Exerts Antioxidants Effects in Renovascular Hypertensive Rats

PrimaryAuthor.AuthorBlock:**Elen Rizzi Sanchez**, Giselle F Bonacio, Univ of Ribeirao Preto, Ribeirao Preto, Brazil; Danielle A Guimaraes, Gustavo Oliveira Paula, Jefferson Henrich Amaral, Univ of Sao Paulo, Ribeirao Preto, Brazil; Victoria Becker M Fernandez, Univ of Ribeirao Preto, Ribeirao Preto, Brazil; Jose

Eduardo Tanus-Santos, Univ of Sao Paulo, Ribeirao Preto, Brazil

Doxycycline (Dox), an established matrix metalloproteinase (MMP) inhibitor, and angiotensin-converting enzyme inhibitors (ACEi) present beneficial cardiovascular effects which could be consequence of their antioxidants properties. Furthermore, some evidences have shown other biochemical similarities between Dox and ACEi suggesting that Dox could also inhibit ACE and ACEi directly interact with MMPs and inhibit these proteases. Supporting this idea, Dox is able to chelate divalent metals, such as calcium and zinc, which are essential for the ACE activity. In this regard, we hypothesized that Dox inhibits ACE activity in hypertensive rats which leads to a reduction in ROS production and decrease hypertension. Sham-operated or 2K1C hypertensive rats were treated with Dox (30 mg/Kg/day) or water for 4 weeks. Systolic blood pressure (SBP) was monitored weekly. Dox treatment reduced SBP in 2K1C rats from 200±12 mmHg to 158±8 mmHg ($P<0.05$). In addition, Dox treatment attenuated reactive oxygen species (ROS) levels in hypertensive animals measured by lucigenin chemiluminescense (in RLU/mg of aorta: from 711±1.0 to 377±93; $P<0.05$). ACE activity in aorta was increased in untreated 2K1C rats (22±0.8 nmols/min/g) when compared with the Sham group (12±1.0 nmols/min/g; $P<0.05$) and Dox treatment was able to reduce ACE activity in 2K1C rats (15±2.0 nmols/min/g; $P<0.05$). To evaluate whether Dox inhibits ACE activity *in vitro*, aortic tissues from 2K1C rats were incubated with Dox or Captopril (a known ACEi). Dox *in vitro* did not affect ACE activity while captopril inhibited 80% of its activity. To verify whether Dox could inhibit ACE activity *in vivo*, an acute assay was performed with different doses of angiotensin I (in µg/Kg: 0.03, 0.3 and 3), after the single administration of Dox or saline (i.p.). Angiotensin I infusion increased mean arterial pressure dose-dependently and Dox pretreatment did not attenuated these increases significantly ($P>0.05$) as Captopril.Taken together, these results show that chronic treatment with Dox inhibits ACE activity in aorta of 2K1C rats, which contribute to ROS reduction and SBP attenuation. However, the mechanism by which Dox inhibits ACE activity needs further investigation.

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P424

Assessment of the RAAS-status Using Triple-A-Testing: Combining Molecular Profiling of Hypertension With Advanced Screening for Primary Aldosteronism in a Single Blood Test

PrimaryAuthor.AuthorBlock:Oliver Domenig, Cornelia Schwager, Dunja van Oyen, Martin Leitner, Claudia Ziegelmayer, Christian Fabsich, **Marko Poglitsch**, Attoquant Diagnostics, Vienna, Austria

Triple-A testing is a novel mass spectrometry based approach providing a comprehensive biochemical evaluation of the circulating renin-angiotensin-system (RAS) on the basis of equilibrium angiotensin levels and circulating aldosterone levels. In contrast to previous technologies involving complex sampling procedures, RAS-Equilibrium-Analysis combines the robustness and accuracy of LC-MS/MS based quantification with the versatility of serum sampling to generate a highly accurate readout containing multiple layers of information regarding the biochemical features of the circulating RAAS. Equilibrium Angiotensin

I (Ang I), Angiotensin II (Ang II) and Aldosterone were simultaneously quantified in 500µl of standard collected serum samples from healthy volunteers or hypertensive patients receiving different anti-hypertensive first-line therapies. Stable-isotope labeled internal standards were used to control for analyte recovery. Following analyte extraction, samples were subjected to UPLC-MS/MS analysis and diagnostic ratios were calculated. ACE inhibitor therapy resulted in a significant reduction of the Ang II-to-Ang I-Ratio in equilibrium analysis, which was accompanied by an up-regulation of renin, as expected. Surprisingly, PRA showed a high correlation with the sum of equilibrium Ang I and Ang II, which was independent of ACE inhibitor treatment. While the ARR was strongly suppressed in the presence of ACE inhibitor treatment, the Aldosterone-to-Angiotensin II-Ratio (AA2-Ratio) was not affected, suggesting superior applicability in screening for primary aldosteronism (PA). Triple-A testing is a mass spectrometry based multiplex assay combining Ang I, Ang II and Aldosterone to diagnostic values that draw a comprehensive picture of a patient's "RAAS Status" . While the sum of Ang I and Ang II serves as a strong PRA surrogate marker, ACE activity and ACE inhibitor therapy efficacy can be monitored using the Ang II/Ang I-Ratio. On top, the AA2-Ratio serves as an advanced diagnostic marker for PA that might pave the way for patient screening without the need of withdrawing anti-hypertensive therapies.

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Ras Blockade Alone or Combined With Inhibition of Soluble Epoxide Hydrolase: Effects on the Course of Congestive Heart Failure in Ren-2 Transgenic Hypertensive Rats With Aorto-caval Fistula

PrimaryAuthor.AuthorBlock:**Petr Kala**, Lenka Sedlakova, Zdenka Vanourkova, Libor Kopkan, Ctr for Experimental Med, Inst for Clinical and Experimental Med, Prague, Czech Republic; Janusz Sadowski, Elzbieta Kompanowska-Jezierska, Dept of Renal and Body Fluid Physiology, Mossakowski Medical Res Ctr, Warsaw, Poland; Sung Hee Hwang, Bruce Hammock, Dept of Entomology and Nematology, and UC Davis Comprehensive Cancer Ctr, Univ of California, Davis, CA; Ludek Cervenka, Ctr for Experimental Med, Inst for Clinical and Experimental Med, Prague, Czech Republic

Rationale and Objective: We recently showed that increasing epoxyeicosatrienoic acids (EETs) in kidney by blocking soluble epoxide hydrolase (sEH), an enzyme responsible for EETs degradation, markedly attenuated the development of renal dysfunction and progression of aorto-caval (ACF)-induced congestive heart failure (CHF) in Ren-2 transgenic hypertensive rats (TGR). Therefore, in this study we examined if additional inhibition of sEH to RAS blockade could further improve the course of ACF-induced CHF in TGR. **Methods:** The treatment regimens were started from one week after creation of ACF and the follow-up period was 60 weeks. RAS blockade was achieved by administration of angiotensin-converting enzyme inhibitor (ACEi, trandolapril, 3 mg/L in drinking water) and sEH was blocked

using a sEH inhibitor (sEHi, *c*-AUCB, 3 mg/L in drinking water). The following experimental groups were investigated: 1) Sham-operated TGR; 2) Untreated ACF TGR; 3) ACF TGR + ACEi; 4) ACF TGR + ACEi + sEHi (n = 36 in each ACF group). In separate groups renal hemodynamics and excretory function were evaluated two weeks post-ACF, just before the onset the decompensated phase of CHF. **Results:** After 29 weeks post-ACF, no animal survived. ACEi treatment greatly improved the survival rate (87%) at the end of study. Surprisingly, combined treatment with ACEi and sEHi worsened the rate (53%, $p < 0.05$). After 2 weeks post-ACF, untreated TGR group showed lower mean arterial pressure (MAP) (124 ± 3 vs. 146 ± 4 mmHg, $p < 0.05$), renal blood flow (7.6 ± 0.3 vs. 10.5 ± 0.3 $mL.min^{-1}.g^{-1}$, $p < 0.05$) and absolute sodium excretion (0.18 ± 0.06 vs. 1.09 ± 0.19 $\mu mol.min^{-1}.g^{-1}$, $p<0.05$) than sham-operated TGR group, respectively. The treatment with ACEi alone or combination treatment with sEHi did not prevent the changes in renal hemodynamics and sodium excretion. **Conclusion:** We found that addition of sEHi to ACEi treatment did not provide better protection against CHF progression and the survival rate, indeed, decreased significantly. Thus, increasing bioavailability of tissue EETs in individuals with pharmacologically-induced suppression of the RAS is not a promising approach to further attenuate renal dysfunction and progression of CHF.

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P426

Suppression of Intrarenal Ras With a 20-hydroxyeicosatetraenoic Acid Antagonist Attenuates Ang II-dependent Malignant Hypertension and Reverses Established Ang-II-dependent Hypertension in Cyp1a1-ren-2 Transgenic Rats

PrimaryAuthor.AuthorBlock:**Lenka Sedlakova**, Libor Kopkan, Zuzana Huskova, Sona Kikerlova, Věra Certikova-Chabova, Ctr for Experimental Med, Inst for Clinical and Experimental Med, Prague, Czech Republic; John Falck, Dept od Biochemistry, Univ of Texas Southwestern Medical Ctr, Dallas, TX; Janusz Sadowski, Dept of Renal and Body Fluid Physiology, Mossakowski Medical Res Ctr, Warsaw, Poland; Ludek Cervenka, Ctr for Experimental Med, Inst for Clinical and Experimental Med, Prague, Czech Republic

Rationale and Objective: Interplay of 20-HETE and ANG II might be a factor in pathophysiology of many forms of experimental hypertension. We hypothesized that intrarenal 20-HETE potentiates prohypertensive actions of ANG II in Cyp1a1-Ren-2 transgenic rats (TGR), a model of ANG II-dependent malignant hypertension. Therefore, we evaluated the antihypertensive effectiveness of a new, orally active 20-HETE antagonist (SOLA) in this model. **Methods:** Treatment with SOLA (10 $mg.kg^{-1}.day^{-1}$ in drinking water) was started either simultaneously with induction of hypertension (early treatment) by indol-3-carbinol or 10 days later, during established hypertension (late treatment). Systolic blood pressure (SBP, measured by radiotelemetry) was monitored continuously and indices of renal and cardiac injury, and kidney 20-HETE and ANG II levels were determined at the end of experiments. **Results:** In TGR with induced hypertension, early SOLA treatment reduced SBP elevation (to 161±3 vs. 199±3 mmHg in induced TGR,

p<0.001), reduced albuminuria (16±2 vs. 35±3 mg/24 h, p<0.002), glomerulosclerosis index (0.12±0.01 vs. 0.32±0.02, p<0.001) and cardiac hypertrophy (left ventricle weight (mg)/tibial length ratio: 16.9±0.4 vs 20.6±0.5, p<0.02). TGR with induced hypertension exhibited elevated intrarenal 20-HETE levels (15.2±0.03 vs. 9.2±0.02 µg/g in noninduced rats, p<0.01); however, the elevation was not altered by SOLA treatment. Hypertensive TGR showed also augmented kidney ANG II levels (405±30 vs. 51±2 fmol/g in noninduced rats, p<0.001). SOLA treatment significantly lowered kidney ANG II (95±19). Remarkably, in TGR with established hypertension, late SOLA treatment also decreased SBP (from 187±4 to 158±4 mmHg, p<0.002) and exhibited cardio- and renoprotective effects in addition to a marked suppression of kidney ANG II levels (72±12 fmol/g). **Conclusion:** In hypertensive TGR the new orally active 20-HETE antagonist attenuated the development and largely reversed the established ANG II-dependent experimental malignant hypertension, likely via suppression of intrarenal ANG II levels. This suggests that intrarenal RAS activation by 20-HETE is important in pathophysiology of this hypertension form

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Inhibition of the Regulator of G Protein Signaling 14, a Novel Anti-Hypertensive Mechanism

PrimaryAuthor.AuthorBlock:**Jie Zhang**, John J Guers, Marko Oydanich, Dorothy E Vatner, Stephen F Vatner, Rutgers Univ- New Jersey, Newark, NJ

The Regulator of G Protein Signaling 14 (RGS14) knockout (KO) mouse is unique in that it not only extends longevity, but also enhances several aspects of healthful aging. The RGS14 KO mouse, compared with wild type (WT), is protected against obesity and diabetes, and has increased exercise capacity, similar to levels achieved with chronic exercise training, all potentially important anti-hypertensive mechanisms. The goal of this investigation was to test more directly that the RGS14 KO mouse is protected against hypertension. Accordingly, we chronically infused angiotensin II (1.44 mg/kg/day) by implanted osmotic pump for 14 days. Stroke volume was measured by cardiac echocardiography. Cardiac output was calculated as the product of stroke volume and heart rate. Heart rates were not different in WT and RGS14 KO prior to angiotensin II infusion or after infusion (425±11 beats/min in WT vs. 420±13 beats/min in KO). Prior to angiotensin II baseline values for mean arterial pressure were similar in WT (84±3 mmHg) and RGS14 KO (79±2 mmHg) and baseline values for systemic vascular resistance, calculated as mean arterial pressure/cardiac output, were also similar in WT (3.7±0.1 mmHg/mL/min) and RGS14 KO (2.2±0.2 mmHg/mL/min) before angiotensin II infusion. As expected, the angiotensin infusion for 14 days increased mean arterial pressure by 69±8 % and systemic vascular resistance by 43±9 % in WT. Surprisingly, angiotensin II failed to increase either mean arterial blood pressure or systemic vascular resistance significantly in RGS14 KO mice. Thus the increases in arterial pressure and vasoconstriction, pathognomonic of angiotensin II, were completely blocked in the RGS14 KO mouse, implicating this mechanism as a potential novel therapeutic modality for treating hypertension.

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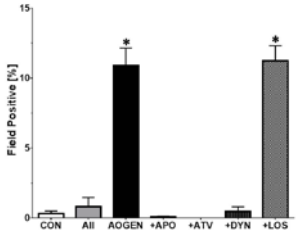
Internalization of Angiotensinogen is Coupled to Oxidative Stress in Human Retinal Epithelial Cells

PrimaryAuthor.AuthorBlock:Victor M Pulgar, Nildris Cruz-Diaz, Brian M Westwood, **Mark C Chappell**, WAKE FOREST SCHOOL MEDICINE, Winston Salem, NC

We previously reported that isolated proximal tubules internalize angiotensinogen (Aogen); labeled Aogen accumulated in the nuclear and mitochondrial fractions and was associated with increased oxidative stress. Aogen internalization in non-renal epithelial cell types is unknown, thus, we assessed Aogen uptake in human retinal ARP-19 cells. Cells, maintained in serum-free media to remove extracellular sources of Aogen and renin were exposed to 125I-Aogen at 37°C and washed in HCl-glycine to dissociate any membrane-bound label. Subcellular fractionation revealed that the majority of the labeled Aogen localized to the nucleus with a lower accumulation in the mitochondrial and cytosolic fractions [42.0 ± 0.2% vs. 12.0 ± 0.2% and 5.2 ± 0.2%, respectively, $p<0.01$; n=3]. The rate of Aogen internalization in the retinal cells was 244 ± 15 fmol/hr/mg [n=3]; however, the cellular uptake of 125I-labeled Ang II, Ang I or Ang 7 was not evident [< 1 fmol/hr/mg]. Cells were then exposed to a low concentration of Aogen [200 pM] for 60 mins and oxidative stress assessed. As shown in the figure, Aogen elicited a marked increase in oxidative stress that was abolished by Apocynin (APO). Atorvastatin (ATV) also blocked the Aogen response, but did not attenuate Aogen uptake. Ang II (AII, 200 pM) had no effect and the AT1R antagonist losartan (LOS, 10 μM) failed to block Aogen uptake or its response. Finally, the dynamin inhibitor dynasore (DYN) blocked internalization and abolished the Aogen response. We conclude that human retinal cells internalize Aogen through a pathway distinct from the Ang II-AT1R axis to elicit an acute increase in oxidative stress.

*[*P<0.01 vs. control(CON); n=4-5 all groups]*



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P430

Soluble Epoxide Hydrolase Inhibition Augments Ras Blockade Renoprotection in 5/6 Nephrectomized Ren-2 Transgenic Hypertensive Rats With Chronic Kidney Disease

PrimaryAuthor.AuthorBlock:**Věra Certikova-Chabova**, Petr Kujal, Petra Skaroupkova, Zdenka Vanourkova, Libor Kopkan, Zuzana Huskova, Lenka Sedlakova, Ctr for Experimental Med, Inst for Clinical and Experimental Med, Prague, Czech Republic; Janusz Sadowski, Elzbieta Kompanowska-Jezierska, Dept of Renal and Body Fluid Physiology, Mossakowski Medical Res Ctr, Warsaw, Poland; Sung Hee Hwang, Bruce Hammock, Dept of Entomology and UCD

Cancer Ctr, Univ of California, California, CA; John Imig, Dept of Pharmacology and Toxicology, Medical Coll of Wisconsin, Prague, WI; Ludek Cervenka, Ctr for Experimental Med, Inst for Clinical and Experimental Med, Prague, Czech Republic

Rationale and Objective: We showed recently that increasing kidney tissue epoxyeicosatrienoic acids (EETs) by blocking soluble epoxide hydrolase (sEH) and thereby blocking EETs degradation to inactive dihydroxyeicosatrienoic acids (DHETEs) substantially attenuated the progression of chronic kidney disease (CKD) in Ren-2 transgenic hypertensive rats (TGR) subjected to 5/6 renal mass reduction (5/6 NX). In this study we examined if in this model addition of sEH inhibition to the complex (dual) renin-angiotensin system (RAS) blockade would bring additional renoprotective effects in already established CKD. **Methods:** TGR aged 9 weeks underwent 5/6 NX and then were left untreated for 6 weeks to develop CKD. Then dual RAS blockade : ACE inhibition (trandolapril) + angiotensin AT_1 receptor blockade (losartan) was instituted, alone or combined with sEH inhibition (c-AUCB, 3 mg/l in drinking water). During the 60 weeks' follow-up period albuminuria and urinary creatinine excretion was repeatedly determined. The following experimental groups were investigated: ; 1) sham-operated TGR; 2) Untreated 5/6 NX TGR; 3) 5/6 NX TGR + RAS blockade ; 4) 5/6 NX TGR + RAS + sEH blockade. Sham-operated transgene-negative normotensive Hannover-Sprague Dawley (HanSD) rats served as basic controls. In separate groups renal glomerular and tubulointerstitial injury was assessed, and effects of two weeks' treatments on systolic blood pressure (SBP, measured by telemetry) and on kidney ANG II, ANG 1-7, EETs and DHETEs levels were determined. **Results:** All untreated TGR died by week 14 after 5/6 NX. RAS blockade increased the final survival rate to 23%, normalized SBP (116 ± 3 vs. 198 ± 3 mmHg, $p<0.0015$), reduced albuminuria (46 ± 5 vs. 102 ± 12 mg/24 h, $p<0.001$) and intrarenal ANG II (27 ± 8 vs. 189 ± 14 fmol/g $p<0.0015$) and did not alter kidney EETs/DHETEs ratio. After addition of sEH blockade kidney EETs/DHETEs ratio increased to 2.89 ± 0.42 ($p<0.001$ vs. 5/6 NX TGR treated with RAS blockade), the final survival rate increased to 42% and indices of renal glomerular and tubulointerstial injury improved. **Conclusion:** Addition of sEH blockade to the RAS blockade brings additional renoprotective effects on the CKD progression in 5/6 NX TGR, even when applied in the advanced phase of the disease.

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P431

Renal Artery Stenosis Caused by a Bcr-Abl Tyrosine Kinase Inhibitor: Blood Pressure Response to Revascularization

PrimaryAuthor.AuthorBlock:**Swapnil Hiremath**, Syed Obaid Amin, Marcel Ruzicka, Kevin Burns, Ottawa Hosp, Ottawa, ON, Canada

Introduction: Bcr-Abl tyrosine kinase inhibitors (TKI) are first line agents for management of chronic myelogenous leukemia (CML). Amongst the second generation TKIs, which have less resistance and improved side effect profile, nilotinib and ponatinib have also been reported to be associated with vascular adverse events (VAEs), including systemic and peripheral

arterial stenosis. We report on a patient with CML treated with ponatinib, who developed bilateral renal artery stenoses, difficult to control hypertension, and responded to revascularization.
Case: A 55 year old man, with CML due to a Bcr-Abl truncating mutation diagnosed in 2007, was treated with ponatinib 45 mg daily starting in 2011, which induced a complete remission. He subsequently developed hypertension. Hypertension appeared angiotensin II-dependent as BP normalized on combination of candesartan 32 mg once daily and hydrochlorothiazide 25 mg once daily. This treatment was however associated with an increase in serum creatinine from 1.4 to 2.6 mg/dL. Consistent with our clinical suspicion, a computed tomography angiogram revealed bilateral renal artery stenosis. He eventually required bilateral renal artery angioplasty and stenting as the BP could not be controlled without renin-angiotensin system blockers despite using up 5 classes of BP lowering drugs. Furthermore, follow up imaging of renal arteries showed progressive renal artery stenosis bilaterally. He underwent angioplasty and stenting in the left renal artery and angioplasty of both the branches of the right main renal artery, which could not be stented because of the early branching. After 6 months, BP is within target with 8 mg candesartan, amlodipine 10 mg and bisoprolol 2.5 mg daily, and the renal function is stable (creatinine 1.1 mg/dL). Overall, in this case, renal artery angioplasty and stenting stabilized renal function and improved control of hypertension.
Discussion: Second generation TKIs are associated with VAEs, including renal artery stenosis. Renal artery angioplasty and stenting stabilized renal function and improved control of hypertension in this case. Future research should clarify the mechanisms of VAEs with TKIs, natural history, and long term response to revascularization in this setting.

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The Effect of Carbonyl Stress on Renal Injury Induced by Renal Congestion

PrimaryAuthor.AuthorBlock:**Yusuke Ohsaki**, Chika Takahashi, Tohoku Univ, Sendai, Japan; Takefumi Mori, Tohoku Medical and Pharmaceutical Univ, Sendai, Japan; Sadayoshi Ito, Tohoku Univ, Sendai, Japan

Background; Due to the functional linkage between cardiac function and renal function, a decline in renal function exacerbates cardiac function and a decline in cardiac function exacerbates renal function. Renal congestion is reported to contribute to the decline of renal function in heart failure patients. Regarding the mechanism of kidney failure caused by congestion, although it is suggested that ischemia is involved due to an increase in venous pressure and a decrease in blood flow, it has not been sufficiently elucidated. Since overexpression of glyoxylose-1 (GLO1), a methylglyoxal (MG) metabolizing enzyme, which is a type of carbonyl stress, has been reported to attenuate impairment due to renal ischemia reperfusion (I/R). In this study, we aimed to investigate the effect of carbonyl stress on kidney damage due to renal congestion using GLO1 overexpressing rats.
Methods; Male Wistar wild type (WT) rat and GLO1 transgenic (GLO1) rat were used for experiment. Left kidney was congested by placing silver clip on left renal vein under anesthesia. Two weeks after surgery, renal cortical and outer medullary mRNA markers of fibrosis [transforming growth factor-β (TGF-β)

and fibronectin (FN)], tubular injury [osteopontin (OPN) and kidney injury molecule 1 (KIM-1)] and inflammations [monocyte chemoattractant protein-1 (MCP-1) and tumor necrosis factor-α (TNF-α)] were measured. Results; Relative mRNA expression of cortical TGF-β (contralateral kidney; 1.00 ± 0.09, congested kidney; 2.44 ± 0.33, respectively), FN (1.00 ± 0.11, 2.71 ± 0.46), OPN (1.00 ± 0.18, 6.41 ± 1.47), KIM-1 (1.00 ± 0.59, 21.20 ± 6.31), MCP-1 (1.00 ± 0.24, 2.93 ± 0.56) and TNF-α (1.00 ± 0.14, 1.62 ± 0.18) were significantly increased in congested kidney compared to contralateral kidney. Relative mRNA expression of cortical TGF-β (GLO1tg; 1.69 ± 0.20, WT; 2.44 ± 0.33, respectively), FN (1.53 ± 0.33, 2.71 ± 0.46), TNF-α (1.00 ± 0.14, 1.62 ± 0.18) in GLO1tg rat were significantly decreased compared to WT rat. No significant change was observed between renal medullary area of WT rat and GLO1tg rat. Conclusion; Renal congestion induces renal fibrosis, tubular injury and inflammation, and carbonyl stress which could contribute to the pathophysiological mechanism on renal injury in cardiac failure.

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P433

Impact of Renal Function on Outcomes After Percutaneous Transluminal Renal Angioplasty in Hypertensive Patients With Renal Artery Stenosis

PrimaryAuthor.AuthorBlock:**Yoshio Iwashima**, Natl Cerebral and Cardiovascular Ctr, Suita City Osaka, Japan; Tetsuya Fukuda, Natl Cerebral and Cardiovascular Ctr, Suita City, Osaka, Japan; Takeshi Horio, Kawasaki Hosp, Kawasaki Medical Sch, Okayama city, Okayama, Japan; Shin-ichiro Hayashi, Hiroshi Kusunoki, Masatsugu Kishida, Natl Cerebral and Cardiovascular Ctr, Suita City Osaka, Japan; Kei Kamide, Osaka Univ Graduate Sch of Med, Suita City Osaka, Japan; Yuhei Kawano, Teikyo Univ Fukuoka, Oomuta, Fukuoka, Japan; Fumiki Yoshihara, Natl Cerebral and Cardiovascular Ctr, Suita City Osaka, Japan

Objective-Atherosclerotic renal artery stenosis (ARAS) is associated with secondary hypertension. Renal dysfunction is often present in ARAS patients; however, evidence for the impact of pretreatment renal function on outcomes after percutaneous transluminal renal angioplasty (PTA) is limited.**Methods-**A total of 139 hypertensive patients with ARAS (mean age, 69.5±8.9 years, 112 male) who underwent renal PTA were included. Renal function was evaluated based on estimated glomerular filtration rate (eGFR) and albuminuria/proteinuria, and classified into three categories according to eGFR (≥45, 30-44, <30 ml/min/1.73m^2) and albuminuria/proteinuria [normal-to-mild: albumin/creatinine ratio (ACR) <3.0, protein/creatinine ratio (PCR) <15; moderate: ACR 3.0-30.0, PCR 15-50; severe: ACR >30.0, PCR >50 mg/mmol]. The primary end point of this study was first occurrence of the composite of cardiovascular and renal events including all-cause death, myocardial infarction, stroke, adverse aortic events, or end-stage renal failure requiring regular hemodialysis.**Results-**During a median follow up of 5.4 years, 36.0% (50 of 139) of patients developed the primary composite end point including cardiovascular and renal outcomes. In multivariate Cox regression analysis, eGFR <30 (hazard ratio [HR] 3.47, p <0.01) as well as severe albuminuria/proteinuria (HR 2.63, p <0.05) was an independent predictor of worse outcome. In the subgroup without events within one year after PTA (n=117), the outcome differed among

the three renal functional categories at one year based on eGFR (log-rank χ^2=16.28, p <0.001) as well as on albuminuria/proteinuria (log-rank χ^2=8.30, p <0.05). At one year, 24 of 117 patients (20.1%) showed a ≥20% decrease in eGFR, and their outcome was worse than that of those with a ≥20% increase (n=23) (HR 3.50, p <0.05). Multiple logistic regression analysis indicated that pretreatment moderate-to-severe albuminuria/proteinuria was an independent predictor of a ≥20% eGFR decrease (odds ratio 2.82, p <0.05).**Conclusion-** Impaired renal function, and in particular, a poor response of eGFR to PTA, is associated with worse outcome. Therapeutic effectiveness of renal angioplasty seems to be limited in patients with albuminuria/proteinuria.

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P434

Role of Dusp5 in Hypertension Induced Chronic Renal Disease

PrimaryAuthor.AuthorBlock:Xiaochen He, Chao Zhang, Sydney Murphy, Paige N Mims, **Fan Fan**, Univ of Mississippi Medical Ctr, Jackson, MS

We recently identified 4 single nucleotide polymorphisms in Dusp5 in FHH as compared with Brown-Norway (BN) rats, two of which alter CpG sites and another one causes G155R mutation. We then created a Dusp5 knockout (KO) rat in the FHH.1^{BN} genetic background, in which a small region in Chr. 1 of BN rats containing 15 genes, including Dusp5 into the FHH rats. We found that knockout of Dusp5 enhances the myogenic response and autoregulation of cerebral circulation. In the present study, we evaluated whether Dusp5 also plays a role in the regulation renal function. The expression of Dusp5 was lower in FHH. 1^{BN}. Dusp5 KO rats in comparison to the FHH.1^{BN} rats. The levels of p-PKC and p-ERK1/2 were elevated in Dusp5 KO vs. FHH.1^{BN} rats. The blood pressure and urinary protein excretion was similar in FHH. 1^{BN}. Dusp5 KO (n = 43) and FHH.1^{BN} rats (n = 30) when they were at 12-week of age (114 ± 2 vs. 115 ± 1 mmHg and 32 ± 2 vs. 29 ± 3 mg/day). The autoregulation of RBF was similar in FHH.1^{BN} (n=22) and Dusp5 KO (n = 9) rats in response to elevation of MAP from 100 to 140 mmHg, but was impaired in FHH rats (n=21) that increased by 37.3 ± 2.3% when they were at 12-week of age. In response to an elevation in pressure from 60 to 120 mm Hg, the renal afferent arterioles constricted by 29 ± 2% and 11 ± 1%, respectively in Dusp5 KO (n = 6) in comparison to FHH.1^{BN} rats (n = 17). Blood pressure was similar in Dusp5 KO (n = 11) vs. FHH.1^{BN} rats (n = 14) after 3-week DOCA-salt treatment (169 ± 5 vs. 177 ± 5 mmHg). However, proteinuria was significantly reduced in Dusp5 KO rats (258 ± 22 mg/day, n = 12) in comparison to FHH.1^{BN} rats (338 ± 30 mg/day, n = 12). The renal injury induced by DOCA-salt was markedly reduced in Dusp5 KO vs. FHH.1^{BN} rats as the glomerular injury scores were 2.49 ± 0.03 vs. 2.54 ± 0.01; the areas of renal fibrosis were 5.5 ± 0.5% vs. 8.3 ± 0.3%; the areas of renal protein casts were 0.5 ± 0.2% vs. 2.4 ± 1.0% and renal arteriolar media-to-lumen ratios were 0.64 ± 0.03 vs. 0.81 ± 0.04, respectively. These results indicate that KO of Dusp5 enhances renal vascular function to protect against DOCA-salt hypertension induced chronic renal injury via activation of PKC/ERK signaling pathway. In conclusion, an activating mutation in Dusp5 may contribute to the impaired myogenic response and promote the development of chronic renal injury in FHH rats.

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P435

Differential Responses of Cerebral Cortical and Renal Cortical Microvessels to Perfusion Pressure and Angiotensin II: Effect of Angiotensin II or DOCA/salt Hypertension

PrimaryAuthor.AuthorBlock:**Lingli Li**, Georgetown Univ, Washington DC, DC; En Yin Lai, Georgetown Univ, Washington DC, Zhejiang Univ, Hangzhou, China; William J. Welch, Christopher S. Wilcox, Georgetown Univ, Washington DC, DC

Background: The brain and kidney autoregulate their blood flow well yet both suffer from hypertensive damage. We found that a pressor infusion of angiotensin II (Ang II) reduced renal blood flow yet did not change cerebral blood flow. Therefore, we tested the hypothesis that their myogenic and Ang II responses differed. **Methods**: Cerebral cortical microvessels (cerebral) and renal afferent arterioles (afferent) were isolated and perfused from mice after 4 weeks of hypertension from Ang II infusion /high salt/uninephrectomy (Ang II hypertension) or DOCA/high salt/uninephrectomy (DOCA/salt hypertension) or normotensive controls without Ang II or DOCA (n=4-6 per group). **Results**: Normal cerebral and afferents had similar myogenic responses (Δ diameter: cerebral -21±3 versus afferent-19±2%, NS), but bath addition of Ang II or norepinephrine contracted afferents strongly (Ang II: -48±5%, P<0.001, NE: -95±2%, P<0.001), yet cerebrals were entirely unresponsive. Myogenic responses in Ang II hypertension were reduced selectively by 40% in cerebral microvessels compared to controls (-13±3 versus -21±3%, P<0.001) yet maintained in afferents (-17±3 versus -19±2%, NS). However, myogenic responses in DOCA/salt hypertension were maintained in both groups. Contractions to Ang II in cerebral microvessels were increased in Ang II hypertension (-5±2 versus 0±1%, P<0.01) and increased in DOCA/salt hypertension (-18±8 versus -2±2%, P<0.01). In contrast, contractions to Ang II in afferent arterioles were reduced 50% in Ang II hypertension (-23±5 versus -48±5%, P<0.001) and reduced 25% in DOCA/salt hypertension (-38±6 versus -50±10%, P=0.05). C**onclusions:** The kidney is well protected from hypertension and excessive Ang II vasoconstriction. However, the breakdown of myogenic responses in the cerebral microvessels during Ang II hypertension and the enhanced Ang II responses in the cerebral microvessels during Ang II and DOCA/salt hypertension make the brain especially vulnerable to hypertensive ischemia or damage.

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Intrarenal Ghrelin Receptor Inhibition Prevents Sodium Retention and Reduces Blood Pressure in Angiotensin II-induced Hypertension

PrimaryAuthor.AuthorBlock:**Brandon A Kemp**, Nancy L. Howell, Shetal H. Padia, Univ of Virginia, Charlottesville, VA

The renal ghrelin receptor (GR) is localized to collecting duct (CD) cells where it increases $E_{Na}C$-dependent sodium (Na^+) reabsorption. We have previously shown that in uninephrectomized Sprague-Dawley (SD) rats, systemic Ang II infusion induces hypertension after 1 day (129.2 ± 5 vs. 158.2 ± 2.6 mmHg; P<0.01), and this increase is sustained over a 6 day period (191.2 ± 4.0 mmHg; P<0.001). The increase in BP is attenuated with concomitant intrarenal (IR) GR siRNA infusion (F=28.9; P<0.001), but the mechanism is unknown. In this study, we tested whether the reduction in Ang II-induced hypertension in the presence of IR GR siRNA is the result of the prevention of antinatriuresis. Uninephrectomized SD rats (N=33) received a subcutaneous osmotic pump for chronic systemic delivery of Ang II (200 ng/kg/min) or 5% dextrose in water (D_5W). Rats received IR bolus infusion of D_5W, GR siRNA (15 µg x 3 over 6 days), or scrambled siRNA (SCR siRNA, 15 µg x 3 over 6 days). Systemic Ang II + SCR siRNA reduced 24 h $U_{Na}V$ from 0.90 ± 0.04 to 0.34 ± 0.08 µmol/min (P<0.001) and increased cumulative Na^+ balance from 0 to 0.54 mEq Na^+ (P<0.01) on day 1, with no significant differences for either group for the remainder of the study. IR GR siRNA infusion prevented Ang II-mediated Na^+ retention (0.86 ± 0.06 vs. 0.87 ± 0.06 µmol/min) and induced a decrease in cumulative Na^+ balance from 0 to -0.37 ± 0.08 mEq on day 1 with no significant differences for either group for the remainder of the study. At 24 h of Ang II + SCR siRNA infusion, IR GR expression was increased in whole cell kidney (0.55 ± 0.03 vs. 0.91 ± 0.08 DU; P<0.05) and major CD Na^+ transporter $E_{Na}C$ translocated from intracellular sites to apical plasma membranes to mediate Na^+ reabsorption (0.34 ± 0.05 vs. 0.51 ± 0.02 DU; P<0.05). In rats receiving IR GR siRNA infusion, however, Ang II failed to recruit $E_{Na}C$ to the apical plasma membranes of CD cells. There was no change in total whole cell $E_{Na}C$ for all conditions. These data demonstrate that chronic inhibition of IR GR activity significantly reduces $E_{Na}C$-dependent Na^+ retention, resulting in a negative cumulative Na^+ balance, thereby preventing Ang II-induced hypertension in rats. Thus, renal GRs represent a novel therapeutic target for the treatment of hypertension and other Na^+-retaining states.

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The Extracellular Domain of Na^+/K^+ATPase Serves as a Receptor for Cyclic GMP in Mediating Natriuresis

PrimaryAuthor.AuthorBlock:Brandon A. Kemp, Nancy L. Howell, Susanna R. Keller, John J. Gildea, Josh D. Hinkle, Jeffrey Shabanowitz, Donald F. Hunt, **Robert M Carey**, Univ of Virginia Health System, Charlottesville, VA

Previous studies from our laboratory have shown that extracellular renal interstitial (RI) cyclic guanosine 3’5’-monophosphate (cGMP) inhibits renal proximal tubule (RPT) sodium reabsorption and induces natriuesis via activation of Src family kinase *in vivo*. The cellular mechanisms by which extracellular cGMP regulates this process are unknown. Previous immunofluorescence competitive binding studies in isolated RPTs from normal rat kidneys demonstrated that cGMP and ouabain (OUA) compete for binding to Na^+/K^+-ATPase

(NKA). We hypothesized that cGMP binds to the extracellular domain of the α1-subunit of NKA on basolateral membranes of RPT cells thereby inhibiting Na^+ transport. In the present study, we cross-linked cGMP to isolated RPTs from normal rat kidneys (N=6). We incubated RPT cells with 4-N_3-PET-8-Biotin-11-cGMP (azido cGMP) or 8-N_3-6-Biotin-10-cAMP (azido cAMP; both at 2 μM) in the presence or absence of UV light to induce cross linking; azido cAMP served as a negative control. Immunoprecipitation was then performed using strepavidin beads followed by Western blot analysis probing for NKA. RPTs with cross linked azido cGMP exhibited a strong signal for NKA that was significantly weaker for non-cross linked azido cGMP (33.4 ± 6%; P<0.0001) and cross linked (45.8 ± 8%; P<0.001) or non-cross linked (35.2 ± 6%; P<0.001) azido cAMP samples. We further demonstrated that in the presence of OUA (10 μM) NKA was reduced in cross linked azido cGMP RPTs, providing confirmation for previous immunofluorescence competition studies. Azido cGMP cross linked samples (N=3) were subsequently separated in a Tris-HCl gel and stained with Coomassie Blue. The protein band corresponding to NKA was excised and processed for mass spectrometry. NKA was identified as the second most ubiquitous protein in that band with 50 unique peptides for NKA represented covering 47% of all the amino acids in NKA. Together, these data demonstrate that cGMP competes with OUA for binding on NKA and that NKA serves as a receptor for cGMP thereby mediating natriuresis.

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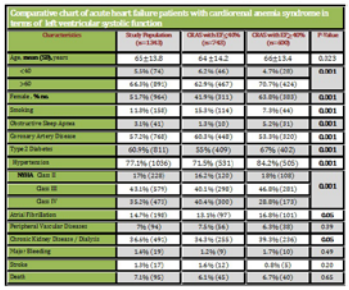
P438

Cardio-Renal Anemia Syndrome in acute heart failure patients: An Observational data from "Gulf-CARE" Heart Failure Registry

PrimaryAuthor.AuthorBlock:Mohammed Al Jarallah, **Rajesh Rajan Sr.**, Sabah Al Ahmad Cardiac Ctr , Al-Amiri Hosp, Kuwait City, Kuwait; Ahmed R. Al-Saber, Kuwait Technical Coll (K-Tech), Kuwait City, Kuwait; Raja Dashti, Bassam Bulbanat, Sabah Al Ahmad Cardiac Ctr , Al-Amiri Hosp, Kuwait City, Kuwait; Mustafa Ridha, Adan Hosp, Kuwait City, Kuwait; Khadhim Sulaiman, Prashanth Panduranga, Royal Hosp, Oman, Oman; Alawi Alshekh-Ali, Inst of Cardiac Sciences, Sheikh Khalifa Medical City, Abu Dhabi, United Arab Emirates; Khalid Al Habib, King Fahad Cardiac Ctr, Riyadh, Saudi Arabia; Jassim Al Suwaidi, Qatar Cardiovascular Res Ctr, Doha, Qatar; Wael Al Mahmeed, Clevland Clinic, Abu Dhabi, United Arab Emirates; Hussam Al Faleh, King Fahad Cardiac Ctr, Riyadh, Saudi Arabia; Abdelfatah Elasfar, King Salman Heart Ctr, Riyadh, Saudi Arabia; Ahmed Al Motarreb, Faculty of Med, Sana'a Univ, Sana'a, Yemen; Nooshin Bazargani, Dubai Hosp, Dubai, United Arab Emirates; Nidal Asaad, Qatar Cardiovascular Res Ctr, Doha, Qatar; Haitham Amin, Mohammed Bin Khalifa Cardiac Ctr, Bahrain, Bahrain

Aims:
Cardio renal anemia syndrome is a condition (CRAS) where heart failure, anemia and renal failure co-exist. The purpose of this article is to measure and analyze the role of CRAS in acute heart failure in terms of left ventricular (LV) systolic function.
Subjects & Methods:
Gulf aCute heArt failuRe rEgistry (Gulf-CARE)

study analyzed 5005 consecutive patients admitted with AHF to 47 hospitals in middle-eastern Gulf countries between 14 February and 14 November 2012. Out of which we analyzed the data of patients with CRAS and divided into two groups. The first group G1 that consists of CRAS patient with ejection fraction (EF) less than 40%, where the second group G2 that consists of CRAS patient with ejection fraction (EF) more than 40%. Chi-square test of independence was utilized for G1 and G2. **Results:** Out of total study population of 5005 patients, 26.8 % (1343) were identified as CRAS patients. Anemia was observed in 54.5% (2728/5005) and chronic kidney disease (CKD) in 45.1% (2257/5005) patients. G1 had 743 patients, and G2 had 600 patients. It was overserved that in G1, around 40.4% (300) were in NYHA Class IV, where G2 has only 28.8 % (173) with a *p* value=0.001. Cardiogenic shock, Intubation, and major bleeding were reported almost same in both groups. In-hospital stroke was seen more in G1 1.6% (12) when compared to GII 0.8% (5) without any statistical significance (*p*=0.20). Out of total CRAS patients, 36.6% (491/1343) had dialysis. Mortality rates were almost similar in both groups 6.1% (45) in GI and 6.7% (40) in GII. **Conclusions:** In the setting of acute heart failure in CRAS patients LV function has no significant role in the incidence of in-hospital stroke, major bleeding and death.



Comparative chart of acute heart failure patients with cardiorenal anemia syndrome in terms of left ventricular systolic function

Characteristics	Study Population (n=1343)	CRAS with EF≤40% (n=743)	CRAS with EF>40% (n=600)	P Value
Age, mean (SD), years	65±13.8	64±14.2	66±13.4	0.023
<40	5.5% (74)	8.2% (66)	4.7% (28)	0.001
>40	94.5% (991)	92.9% (467)	71.7% (424)	
Female, % no.	53.7% (964)	41.9% (311)	63.8% (332)	0.001
Smoking	13.8% (188)	15.8% (114)	7.3% (44)	0.001
Obstructive Sleep Apnea	3.1% (41)	1.8% (10)	5.2% (31)	0.001
Coronary Artery Disease	57.2% (768)	60.3% (448)	53.3% (320)	0.001
Type 2 Diabetes	60.9% (811)	55% (409)	67% (402)	0.001
Hypertension	77.1% (1036)	71.5% (531)	84.2% (505)	0.001
NYHA Class II	17% (228)	16.2% (120)	18% (108)	0.001
Class III	43.1% (579)	40.1% (298)	46.8% (281)	
Class IV	35.2% (473)	40.4% (300)	28.8% (173)	
Atrial Fibrillation	14.7% (198)	13.1% (97)	16.8% (101)	0.05
Peripheral Vascular Diseases	7% (94)	7.5% (56)	6.3% (38)	0.39
Chronic Kidney Disease / Dialysis	36.6% (491)	34.3% (255)	39.3% (236)	0.05
Major Bleeding	1.4% (19)	1.2% (9)	1.7% (10)	0.49
Stroke	1.3% (17)	1.6% (12)	0.8% (5)	0.20
Death	7.1% (95)	6.1% (45)	6.7% (40)	0.65

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Fibromuscular Dysplasia Of The Iliac And Renal Arteries: A Rare Case Report And Literature Review

PrimaryAuthor.AuthorBlock:**Luyun Fan**, Wenjun Ma, Huimin Zhang, Jun Cai, State Key Lab of Cardiovascular Disease, Natl Ctr for Cardiovascular Diseases, Fuwai Hosp, Chinese Acad of Medical Sciences and Peking Union Medical Coll, Beijing, China

Background: Iliac arterial fibromuscular dysplasia (FMD) is an orphan type of FMD, a non-atherosclerotic non-inflammatory arteriopathy predominantly among women aging from 20 to 60. The demographic, clinical features and therapeutic algorithm of iliac arterial FMD have not been precisely described. **Patient concerns:** A 31-year-old Chinese male was referred for 3-month-ago onset hypertension, low serum potassium, and small-sized right kidney with normal renal artery under ultrasound examination. Spirolactone was poorly effective in this patient.

Diagnosis: Contrast-enhanced computed tomographic angiography (CTA) and three-dimensional reconstruction of the whole aorta discovered an aneurysm from right common iliac artery (CIA) to the internal iliac artery, consistent with left CIA dissection and a remarkable right renal artery aneurysm before a stenosis, which was then confirmed through digital subtraction angiography. **Intervention:** Percutaneous transluminal angioplasty (PTA) of right renal artery was operated and a stent was deployed in left CIA. **Outcome:** This patient was normotensive, asymptomatic and free from recurrence without any antihypertensive agents at an 8-month follow-up. **Conclusions:** A total of 111 iliac arterial FMD cases (female, 84.7%; median age, 52±12.8 years) have been reported. Asymptomatic condition (49.5%) and claudication (40.5%) consist the majority of clinical presentations. Bruit (64.9%) and pulse deficits (39.4%) are the most popular signs. External iliac artery involved is approximate threefold of common and internal iliac arteries, usually accompanied with renal artery (71.2%, presenting hypertension) or carotid artery involvement (50.5%). A system screening among iliac or renal arterial FMD patients is therefore suggested with CTA from neck to pelvis and MRA in head. Dissection accounts for 15.3% cases, usually presenting ischemia symptoms with an inclination to young males. Conservative medication is efficient among 61.3% patients and PTA was operated in 15 patients with satisfactory outcomes in 86.7%. Stenting is an optimal recommendation of iliac artery FMD with progressive dissection. PTA is the first choice of short-duration renovascular hypertension due to FMD especially in young patient.

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P440

Renin-angiotensin Signal Develops Arterial Senescence and Atherosclerosis via Modulation of Mitochondrial Dynamics

PrimaryAuthor.AuthorBlock:**Yoshiyuki Ikeda**, Masaaki Iwabayashi, Yuichi Sasaki, Yuichi Akasaki, Koji Higuchi, Masaaki Miyata, Mitsuru Ohishi, Kagoshima Univ, Kagoshima, Japan

Introduction: Mitochondria are dynamic organelles that undergo fusion and fission. It is reported that renin-angiotensin signal (RAS) inhibition retards arterial senescence and development of arteriosclerosis. This study aims to clarify whether mitochondrial dynamics is involved in the effects of RAS-inhibition on arterial senescence and atherosclerosis. Methods and Results: Vascular smooth muscle cells (VSMC) were used in in vitro experiments. Administration of Angiotensin II to VSMCs increased p53 and p21 protein expressions and senescence associated beta galactosidase (SA-beta Gal) positive cells, indicating that angiotensin II induces cellular senescence. Administration of oxidized low-density lipoprotein (ox-LDL) to VSMC also facilitated cellular senescence through RAS stimulation. Dynamin-related protein 1 (Drp1), which mediates mitochondrial fission, was activated with phosphorylation at serine 616 and mitochondrial fission assessed by electron microscopy increased in VSMC with either angiotensin II or ox-LDL administration. Administration of pharmacological Drp1 inhibitor, mdivi1, to VSMC with either angiotensin II or ox-LDL stimulation decreased mitochondrial fission, increased fused mitochondria and retarded cellular senescence. These results suggest that mitochondrial fission is crucial to develop stress induced cellular senescence. We also conducted in vivo

experiments using C57BL6, apolipoprotein E knockout (ApoE KO) and double KO mice (DKO) of angiotensin II type Ia receptor KO and ApoE KO. Ser616p-Drp1 and mitochondrial fission increased in the artery of ApoE KO compared to age-matched C57BL6. The degree of arterial senescence and atherosclerosis are greater in ApoE KO than in C57BL6 assessed by SA-beta Gal and oil red o staining and immunoblot of p53 and p21. ATP production was lower and reactive oxygen species (ROS) production was higher in ApoE KO. DKO showed lower degrees of arterial senescence, ROS generation and atherosclerosis compared to AopE KO. Expression of arterial Ser616 p-Drp1 was lower and the number of fused mitochondria and ATP production were higher in DKO than ApoE KO. Conclusion: Mitochondrial fusion plays a crucial role to retard arterial senescence and atherosclerosis by RAS-inhibition.

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P441

Accelerated Age-dependent Cardiovascular and Cognitive Decline in Dahl-S Rats is Associated with Elevated Levels of an Endogenous Na/K-ATPase Inhibitor

PrimaryAuthor.AuthorBlock:**Olga V Fedorova**, Yulia N Grigorova, Jeffrey M Long, Rebecca L McPherson, Ondrej Juhasz, Wen Wei, Valentina Zernetkina, Natalia Petrashevskaya, Kenneth W Fishbein, Richard G. Spencer, Peter R Rapp, Edward G Lakatta, Natl Inst on Aging, NIH, Baltimore, MD

Age-associated central arterial stiffening contributes to both cerebral arterial fibrosis and to cognitive impairment. Accelerated aging, accompanied by a gradual increase in blood pressure (BP) and aortic remodeling, occurs in Dahl-S rats (DSS) vs. normotensive Sprague-Dawley rats (S-D) counterparts even in the absence of a high salt intake. A novel pro-hypertensive factor marinobufagenin (MBG) is implicated in DSS hypertension. Here we determined whether an increase in MBG is also implicated in age-associated arterial remodeling in DSS.

Methods. Life span was measured in 60 S-D and 78 DSS. BP, pulse wave velocity (PWV), behavioral water maze test, ANGII, MBG and aortic collagen were assessed in 3 and 9-mo S-D and DSS on a normal 0.5% NaCl intake.

Results. Median life span in DSS is reduced by 50% vs. S-D (12±1 vs. 24±2 mo, $p<0.01$). At 3-mo DSS had higher SBP, PWV, ANGII, MBG, aortic and large cerebral arterial wall remodeling vs. 3-mo S-D (Table). Between 3 and 9-mo DSS, but not S-D, exhibited further increase in SBP, PWV, MBG and aortic collagen deposition. In a redundant place-cue version of the water maze test, 3-mo DSS demonstrated numerically impaired spatial hippocampal memory vs. 3-mo S-D, and by 9-mo, performance in DSS suggested the development of motor impairments, thus precluding an uncontaminated assessment of their spatial memory.

Conclusions. In DSS high MBG occurred concurrently with fibrosis of aorta and large cerebral arteries and numerically impaired spatial memory. With advancing age of DSS further increase in BP, aortic stiffness and spatial learning/motor deficit occurred in context with an increase in MBG, which suggested an implication of MBG in these declines.

Table

	S-D; n=8 per group		DSS; n=8 per group	
Age (months)	3	9	3	9
Body weight (BW) (g)	509 ± 12	715 ± 22 *	373 ± 4 *	465 ± 7 * † *
SBP (mmHg)	128 ± 2	129 ± 4	143 ± 2 *	181 ± 6 * † *
PWV (m/s)	2.4 ± 0.1	3.3 ± 0.1	4.7 ± 0.8 *	6.5 ± 0.5 * † *
Plasma ANGII (pg/ml)	19.8 ± 1.6	19.5 ± 1.6	43.8 ± 1.4 *	41.6 ± 3.5 * † *
Urine MBG (pmol/24 hr/kg BW)	38 ± 4	42 ± 2	62 ± 4 *	103 ± 13 * † *
Aortic wall collagen (%)	8.0 ± 1.2	9.6 ± 0.7	14.9 ± 3.0 *	20.8 ± 1.7 * † *
Aortic wall thickness (μm)	102 ± 5	115 ± 5	122 ± 3 *	153 ± 6 * † *
Cerebral arterial wall collagen (%)	3.58 ± 0.57	-	5.86 ± 0.61 #	-

Means±SE. *P<0.05 vs. 3-mo S-D; †P<0.05 vs. S-D 9-mo; #P<0.05 vs. 3-mo DSS, by 1-way ANOVA followed by Newman-Keuls test; #P<0.05 by t-test.

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Estradiol Treatment Attenuates the Ovariectomy-induced Increase in Blood Pressure in the Middle Aged Dahl Salt Sensitive Rat as Long as Treatment is Initiated Immediately After Ovariectomy

PrimaryAuthor.AuthorBlock:**Hong Ji**, Crystal A West, Xie Wu, Amrita Pai, Aline Souza, Parnika Kadam, David A West Jr, Michelle J Zhu, Kathryn Sandberg, Georgetown Univ Sch of Med, Washington, DC

Introduction: Several studies have shown that ovariectomy increases blood pressure and that 17beta-estradiol (**E_2**) treatment can attenuate this effect; however, the majority of these studies were conducted in young animals in which E_2 replacement was initiated soon after ovariectomy. Since most women who experience ovarian hormone loss are middle aged rather than young, this study investigated the effect of E_2 replacement in middle aged Dahl salt-sensitive (**DS**) rats. In addition, the time at which E_2 replacement was initiated was examined. **Methods:** DS rats were ovariectomized (**OVX**) at 4.5 months (**mo**) (**DS-OVX^Y**) and 7mo (**DS-OVX^O**). At 7mo, half of OVX DS rats in each group were treated with E_2 for 4 weeks (**DS-OVX^Y+E_2, DS-OVX^O+E_2**). Mean arterial pressure (**MAP**) was measured by telemetry in all treatment groups from 7-8mo. Body weights were measured throughout the experiment and plasma angiotensin II (**AngII**) was determined at time of sacrifice (8mo) by RAS fingerprint. **Results:** E_2 treatment at 7mo had no effect on MAP in the DS rats OVX at early age [MAP (mmHg) at 8mo: DS-OVX^Y, 180±5.3 vs. DS-OVX^Y+E_2, 179±4.6, ns; n=4/group]. In contrast, one month of E_2 treatment prevented the ovariectomy-induced increase in MAP when OVX at late age [MAP (mmHg): DS-OVX^O, 191±1.6 vs. DS-OVX^O+E_2, 177±2.9, $p<0.0001$ (Two-way ANOVA); n=4/group]. E_2 reduced the body weight (**BW**) by 26g in the DS-OVX^Y group and prevented the ovariectomy-induced gain in BW by 39g in the DS-OVX^O rats [BW(g): DS-OVX^Y, 349±9 vs. DS-OVX^Y+E_2 323±7, $p<0.05$; DS-OVX^O, 334±7 vs. DS-OVX^O+E_2, 295±6, $p<0.01$; n=7-9/group]. E_2 also reduced plasma AngII in both the DS-OVX^Y and DS-OVX^O groups to similar extents [AngII (pg/ml): DS-OVX^Y, 112±19 vs. DS-OVX^Y+E_2, 68.0±7.8, $p<0.05$; DS-OVX^O, 113±11 vs. DS-OVX^O+E_2, 63.9±6.1, $p<0.05$; n=7-9/group]. **Conclusions:** These findings suggest E_2 replacement can attenuate the increase in MAP in middle age as a result of ovarian hormone loss. This study also supports the timing hypothesis that suggests E_2 treatment loses its protective blood pressure lowering effects if there is a significant time delay between ovarian hormone loss and E_2 replacement. These findings have implications for women who are ovarian hormone deficient and are considering E_2 replacement therapy.

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P443

Multiple Chronic Conditions in Older Adults: Implications for Clinical Trials & Guidelines in Hypertension

PrimaryAuthor.AuthorBlock:**Brent M Egan**, Susan E Sutherland, Valinda Rutledge, Robert A. Davis, Care Coordination Inst, Greenville, SC; Peter L Tilkemeier, Univ of South Carolina Sch of Med - Greenville, Greenvile, SC; Angelo Sinopoli, Care Coordination Inst, Greenville, SC

Background: Multiple chronic conditions ([M]CCs), including hypertension (HTN) and clinical CVD, increase sharply with age and account for most U.S. healthcare costs. In 2001, the Institute of Medicine recommended integrated clinical guidelines for MCC. The dearth of integrated guidelines reflects limited inclusion of complex patients in clinical trials and continued focus on individual diseases. Methods: To explore implications of MCC for clinical trials and HTN guidelines in older adults, hierarchical clustering was used to segregate beneficiaries in one large Medicare Shared Savings Program into clusters with similar groups of MCC. Clusters were named for the most prevalent CC and described by number of CCs, prevalent HTN, CVD, behavioral health diagnoses and paid claims. Results: The 50,627 beneficiaries (mean 72 yrs) segregated into 12 clusters; 36,533 (72.2%) had HTN. A total of 33,262 beneficiaries (65.7%) segregated into 6 complex clusters (CHF, CKD, Diabetes, Cancer, COPD, Vascular) with a high prevalence of CVD; 27,324 (82.1%) had HTN. The CHF and CKD clusters had the highest mean number of CCs (9.8, 7.5, respectively), HTN prevalence (94.3%, 91.9%), and yearly costs ($37,700, $26,700/beneficiary). Diabetes, cancer, COPD and vascular disease clusters also had a large burden of CCs (5.9, 5.8, 5.1, 5.4) and HTN (88.3%, 73.6%, 70.7%, 83.9%) with annual healthcare costs from $19,500 (cancer) to $12,900 (COPD); more than 1/3 of patients in the CHF, CKD, diabetes and vascular clusters had a behavioral health diagnosis, most often depression. Of 17,365 (34.3%) beneficiaries in less complex clusters, 9,209 (54%) had HTN, 90+% were candidates for primary CVD prevention, less than 10% had behavioral health diagnoses, and costs were lower. Conclusions: HTN impacts ~82% of older adults with a higher burden of MCC, and ~75% (27,324/36,533) of Medicare beneficiaries with HTN have a large burden of MCCs. Behavioral health diagnosis, associated with adverse outcomes and costs, are common with MCCs. Clinical care, outcomes and costs for older adults with HTN and MCCs could improve with more representative inclusion in clinical trials and translation through integrated clinical guidelines developed by multi-specialty/disciplinary teams.

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Surveillance, Control, and Prevention Systems and Community Engagement Process of Hypertension in Singburi, Thailand

PrimaryAuthor.AuthorBlock:**Vichai Tiienthavorn**, Univ of Phayao, Muang Phayao, Thailand

Background Recently,the patients of non-communicable diseases are increasing in Thailand; especially hypertension. However,finding high blood pressure early,

treating and keeping it in the normal range can reduce the risk of developing complications such as Stemi and Stroke. Thailand has 10 million hypertension patients in 2013. Hence, Thai Government has policy to reduce the NCDs such as hypertension.

Methods This study was to convert disease oriented to health promotion approach to primary health care, using " VICHAI's 7 Color Balls Model", which was used for primary screening of hypertension. The target population aged 15-65 which covering more than 90% of Singburi population. The screening result was classified by types and levels of severity of hypertension (blood pressure).The 7 colors are referred to normal <120/80 mmHg, white, risk 120/80-139/89 mmHg, light green, risk medication <139/89 mmHg, dark green, mild 140/90-159/99 mmHg, yellow, moderate 160/100-179/109 mmHg, orange , severe >180/110 mmHg, red, and patients with complication (black). The normal and risk group measured BP every 3 months, while the patient group did every month.

Results The control and prevention systems were developed to follow up patients using investigation, health education to encourage strictly medication and their behavioral change with best practice of 3Es(Eating,Exercise,Emotion) and 3Rs (Reducing tobacco,alcohol, obesity). Screening Hypertension was coverage 97.41% (2014-2015),normal group (white) increased from 72.55% to 74.94%, Significantly severe patients (red) were decreased to moderate (orange) from 0.11% to 0.07% and the complicated patient with STEMI decreased of 17.14% Furthermore, treatment cost decreased 3.9 million baht.

Conclusions This project is expanding in all provinces of Thailand as it is now one of the priority government policy. And it is the first demonstration of knowledge transfer to community engagement by student, which is the sustainable education in primary health care.

Main Message

Finally, outcome of this study not only reduce the patient and mortality rate but also increase the quality of life, could apply in different areas and propose to be the national policy, effectively for a long term operation.

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P445

Sympathetic and Renal Mechanisms of Age-related Hypertension

PrimaryAuthor.AuthorBlock:**Richard D Wainford**, Alissa A Frame, Boston Univ, Boston, MA

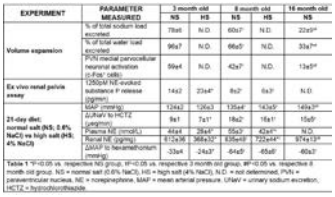
Hypothesis Sympathoexcitation and sodium retention contribute to age-related hypertension (HTN).

Methods Three, 8, and 16 month old male SD rats underwent an IV volume expansion (VE; 5% BW) and MAP, HR, urine output and paraventricular nucleus (PVN) neuronal activation (c-Fos expression) were assessed. In separate groups of rats fed a 21 day normal (NS; 0.6% NaCl) or high salt (HS; 4% NaCl) diet, measures of 1) ex vivo afferent renal nerve (ARN) activity (norepinephrine (NE)-evoked substance P release) or 2) MAP, HR, NCC activity (ΔUNaV to IV HCTZ, 2mg/kg) and sympathetic tone (plasma and renal NE content, ΔMAP to IV hexamethonium) were assessed. Separate groups of rats underwent bilateral renal denervation (RDNX) or chronic NCC antagonism (SC HCTZ, 4mg/kg/d, 14d) and MAP was assessed (N=4/gp).

Results Acute VE-evoked natriuresis, diuresis, and PVN parvocellular neuronal activation were

impaired in aged rats. Aged rats exhibited reduced ARN activity on a NS diet and failed to increase ARN activity during a HS diet. In aged rats, basal MAP, NCC activity and sympathetic tone were increased and HS-evoked suppression of NCC activity and sympathetic tone was impaired. Chronic NCC antagonism and RDNX attenuated age-related HTN (MAP [mmHg] 16mo 149±3 vs RDNX 139±1 vs SC HCTZ 136±5, P<0.05).

Conclusion Our data suggest that the ARN, which have been implicated in the regulation of sympathetic outflow and renal sodium excretion, are impaired in age-related HTN. We speculate that an age-related decrease in ARN activity promotes sympathoexcitation, perhaps in part via reduced activation of PVN sympathoinhibitory parvocellular neurons, resulting in NCC-mediated sodium retention and HTN.



EXPERIMENT	PARAMETER MEASURED	3 month old		8 month old		16 month old
		NS	HS	NS	HS	NS
Volume expansion	% of total sodium load excreted	78±6	N.D.	60±7	N.D.	22±9*
	% of total water load excreted	96±7	N.D.	66±5*	N.D.	33±7**
	PVN medial parvocellular neuronal activation (c-Fos+ cells)	59±4	N.D.	42±7	N.D.	13±5**
Ex vivo renal pelvis assay	1250pM NE-evoked substance P release (pg/min)	14±2	23±4*	8±2	6±3	N.D.
21-day diet: normal salt (NS; 0.6% NaCl) vs high salt (HS; 4% NaCl)	MAP (mmHg)	124±2	126±3	135±4*	143±5*	149±3**
	ΔURNV to HCTZ (μg/min)	9±1	7±1*	18±2*	16±1*	15±5*
	Plasma NE (nmol/L)	44±4	28±4*	65±3*	42±4**	N.D.
	Renal NE (pg/mg)	612±36	368±32*	836±59*	722±45*	974±73**
	ΔMAP to hexamethonium (mmHg)	-33±4	-24±3*	-64±5*	-65±6*	-60±3*

Table 1 *P<0.05 vs. respective NS group, #P<0.05 vs. respective 3 month old group, ##P<0.05 vs. respective 8 month old group. NS = normal salt (0.6% NaCl), HS = high salt (4% NaCl), N.D. = not determined, PVN = paraventricular nucleus, NE = norepinephrine, MAP = mean arterial pressure, UNaV = urinary sodium excretion, HCTZ = hydrochlorothiazide.

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P446

Sestrin2 Suppresses Age-related Hypertrophy by Inhibiting mTORC1 Signaling Pathway

PrimaryAuthor.AuthorBlock:Nanhu Quan, Courtney Cates, Thomas Rousselle, **Ji Li**, Univ of Mississippi Med Ctr, Jackson, MS

Introduction: The mechanistic target of rapamycin complex 1 (mTORC1) plays a critical role in the regulation of cell growth and energy state. A novel stress-inducible protein, Sestrin2 was recognized as a sensor for mTORC1 pathway.

Hypothesis: The cardiac mTORC1 activation modulated by Sestrin2 is impaired in aging that sensitizes heart to hypertrophy.

Methods: C57BL/6J young WT (4-6 months) and aged WT mice (24-26 months), and young Sestrin2 knockout mice (4-6 months) were subjected to transverse aortic constriction (TAC) for pressure overload. The *ex vivo* working heart perfusion was used for measuring substrate metabolism.

Results: The protein levels of cardiac Sestrin2 were decreased with aging. There are no phenotypic differences in young WT, aged WT and Sesn2 KO mice under normal physiology, while aged WT and Sesn2 KO *versus* young WT mice exhibit bigger hearts after 4 weeks of TAC surgery. The echocardiography showed an impaired cardiac function of aged WT and Sesn2 KO hearts by pressure overload. The pressure overload-induced phosphorylation of mTOR and mTORC1 downstream effectors 4E-BP1 and p70S6K were augmented in aged WT and Sesn2 KO *versus* young WT hearts. The swollen mitochondria with severely disrupted cristae and higher levels of redox markers $pShc^{66}$ and 4-hydroxynonenal were observed in aged WT and Sesn2 KO *versus* young WT hearts by pressure overload. The rate of glucose oxidation and fatty acid oxidation were impaired in the aged WT and Sesn2 KO *versus* young WT hearts by pressure overload. Intriguingly, pressure overload induced an interaction between Sestrin2 and GATOR2, a complex of unknown function that positively regulates mTORC1. Moreover, the binding affinity between Sestrin2 and GATOR2 is impaired in the aged WT hearts ($p<0.05$ *vs.* young WT). Furthermore, Adeno-associated virus 9 (AAV9)-Sestrin2 were delivered into the aged WT and Sesn2 KO hearts

via a coronary delivery approach that rescued the protein levels of Sestrin2, attenuated mTORC1 activation and increased the tolerance of both aged WT and Sesn2 KO hearts to pressure overload.
Conclusions: Cardiac Sestrin2 is a sensor for mTORC1 pathway in response to pressure overload. Sestrin2 deficiency in aging could be a reason for an increased sensitivity to hypertrophy in the elderly.

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P447

Hesperidin, Ingredient of Citrus Fruits Juice, Attenuates Cognitive Impairment Induced by Ischemic Brain Damage

PrimaryAuthor.AuthorBlock:**Moe Kawakami**, Jun Iwanami, Kana Tsukuda, Akinori Higaki, Li-Juan Min, Masaki Mogi, Masatsugu Horiuchi, Ehime Univ Graduate Sch of Med, Tohon Ehime, Japan

Objectives: We reported that drinking *Citrus iyo* juice (CI) inhibited more effectively vascular remodeling in inflammation-induced vascular injury mouse model than *Citrus unshiu* juice (CU) (PLoS One. 2015). We aim to explore the possibility that citrus fruits juice drinking could attenuate cognitive decline in transient cerebral ischemia model, focusing on the effects of flavanone, hesperidin, which is more abundantly contained in CI compared with CU and has antioxidant activity. **Methods:** C57BL/6 mice were administrated 10% CI or CU in drinking water or 100 mg/kg/day hesperidin orally by gavage. Two weeks after administration, brain ischemia was induced by bilateral common carotid artery occlusion (BCCAO) for 18 minutes. Cognitive function was determined by Y-maze task 2 weeks after BCCAO (the number of alternations/total arm entry). Cerebral blood flow (CBF) was measured by a laser speckle flowmetry. Cerebral superoxide anion production 24 hours after BCCAO was measured by dihydroethidium staining and depicted as arbitrary unit of intensity vs control. mRNA levels were measured by quantitative real-time RT-PCR
Results: Cognitive function was impaired with the increase in superoxide anion production after BCCAO (71% (16 of 23) vs 55% (14 of 27) in Y maze). The cognitive impairment was more effectively attenuated by the administration of CI with the significant increase in CBF than CU (CI, 66% (13 of 23); CU, 61% (17 of 27)). Interestingly, we observed that the treatment with hesperidin significantly prevented cognitive decline (67% (13 of 21)) after BCCAO with the increase in CBF. Administration of CI, CU or hesperidin did not influence systolic blood pressure, body and brain weight. Superoxide anion production was attenuated by CI or hesperidin, not by CU (BCCAO, 4.0; CI, 1.2; hesperidin, 1.1; CU, 3.0). The increases in mRNA levels of NADPH oxidase subunit such as p22 and p47, and TNFα in the cortex of the brain after BCCAO seemed to be prevented by hesperidin. AT1, AT2, and Mas receptor mRNA levels in the brain cortex did not differ among these groups. **Conclusion:** Taken together, these results suggest that the intake of hesperidin in CI should prevent cognitive decline after brain ischemia at least in part due to reduction of oxidative stress and an increase in CBF.

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None. **A. Higaki:** None. **L. Min:** None. **M. Mogi:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; Daiichi-Sankyo Pharmaceutical Co., Ltd, Nippon Boehringer Ingelheim Co., Ltd., Novartis Pharma K. K. **M. Horiuchi:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; Daiichi-Sankyo Pharmaceutical Co., Ltd, Nippon Boehringer Ingelheim Co., Ltd., Novartis Pharma K. K..

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P448

Interaction Between Ischemic Brain Injury and Amyloid-β Deposition in Cognitive Decline; Possible Cognitive Protection by AT_2 Receptor Activation

PrimaryAuthor.AuthorBlock:**Li-Juan Min**, Masaki Mogi, Kana Tsukuda, Hui-Yu Bai, Bao-Shuai Shan, Akinori Higaki, Jun Iwanami, Masatsugu Horiuchi, Ehime Univ Sch of Med, Tohon, Ehime, Japan

Objectives: Cerebrovascular damage could breakdown amyloid-β (Aβ) clearance and accelerate Aβ deposition. We examined the interaction between ischemic brain damage and Aβ deposition in cognitive function, focusing on the roles of angiotensin II type 2 (AT_2) receptor in vascular smooth muscle cells (VSMC).
Methods: Male wild-type mice (WT) or the mice with VSMC-specific AT_2 receptor overexpression ($smAT_2$) were used. Mice were subjected to ICV injection of Aβ1-40. Ischemic brain injury was induced by bilateral common carotid artery occlusion (BCCAO) 24 hours after Aβ1-40 injection. Three weeks after Aβ1-40 injection, cognitive function was evaluated by the Morris water maze test. Brain samples obtained 8 days after Aβ1-40 injection were used to study the related signals.
Results: ICV injection of Aβ1-40 in WT showed impaired cognitive function (arriving time to platform at day 5: control, 26.53±4.46 sec; Aβ, 65.35±7.44 sec), whereas BCCAO alone did not decline significantly cognitive function. In contrast, BCCAO following Aβ1-40 injection exhibited more marked cognitive impairment (84.27±8.00 sec) compared to Aβ injection alone with the increase in expressions of NADPH oxidase subunits such as p22phox and p67phox in the hippocampus of mice. Aβ1-40 injection with BCCAO tended to increase the mRNA levels of inflammatory cytokines such as MCP-1 and TNFα. BCCAO significantly enhanced the expression of Aβ clearance factor, RAGE (receptor for advanced glycation end product). Aβ1-40 injection did not increase the neuron pyknosis in the hippocampus, whereas the number of neuron pyknosis was increased significantly with BCCAO (control, 6.33±0.88/field; Aβ with BCCAO, 46.33±4.10/field). On the other hand, $smAT_2$ did not show cognitive impairment, the changes of the expression for NADPH oxidase subunits and inflammatory cytokines, and neuron pyknosis, which were induced by BCCAO with/without Aβ1-40 injection in WT.
Conclusion: Ischemic brain injury could enhance Aβ-induced cognitive impairment with possible involvement of enhanced oxidative stress, neuron degeneration, and breakdown of RAGE-mediated Aβ clearance. AT_2 receptor activation in VSMC could play inhibitory roles in the cognitive decline induced by ischemic brain damage and Aβ deposition.

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K. K.. **K. Tsukuda:** None. **H. Bai:** None. **B. Shan:** None. **A. Higaki:** None. **J. Iwanami:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; Daiichi-Sankyo Pharmaceutical Co., Ltd., Nippon Boehringer Ingelheim Co., Ltd. **M. Horiuchi:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; Daiichi-Sankyo Pharmaceutical Co., Ltd., Nippon Boehringer Ingelheim Co., Ltd., Novartis Pharma K. K..

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P450

AT2 Receptor Stimulation Reduces Ischemic Brain Damage Through AT2 Receptor-interacting Protein Signal

PrimaryAuthor.AuthorBlock:**Jun Iwanami**, Masaki Mogi, Xiao-Li Wang, Kana Tsukuda, Akinori Higaki, Masanori Kukida, Hirotomo Nakaoka, Toshifumi Yamauchi, Hui-Yu Bai, Bao-Shuai Shan, Li-Juan Min, Masatsugu Horiuchi, Ehime Univ Graduate Sch of Med, Tohon Ehime, Japan

Objectives: We reported the preventive effects of angiotensin II type 2 (AT2) receptor stimulation on ischemic brain damage. Moreover, we have cloned ATIP (AT2 receptor interacting protein) as a protein interacting specifically with the C-terminal tail of the AT2 receptor, and suggest that ATIP should play key roles in diverse mechanisms of AT2 receptor actions. However, the effect of ATIP on ischemic brain damage is unclear. Therefore, we investigated the effects of the ATIP and compound 21 (C21), a selective non-peptide AT2 receptor agonist, on focal cerebral ischemia. **Method:** Ten-week-old male ATIP-transgenic (ATIP-Tg) and littermate (WT) mice were subjected to middle cerebral artery occlusion (MCAO) with silicon-coated micro-filament. C21 (10 μg/kg/day) was administered 2 weeks before MCAO. Twenty-four hours after MCAO, ischemic volume was determined and depicted as mm^3. Expression of methyl methanesulfonate sensitive 2 (MMS2) mRNA as a neuroprotective factor was determined by real-time RT-PCR. Cerebral blood flow (CBF) before and after MCA occlusion were measured by laser speckle flowmetry. Collateral circulation was evaluated by the perfusion of India ink. **Results:** Systolic blood pressure did not differ among all groups. There was no significant difference in ischemic size without C21 treatment between two strains (WT, 80 mm^3; ATIP-Tg, 75 mm^3). Treatment with C21 significantly decreased ischemic size in both strains (WT, 66 mm^3; ATIP-Tg, 50 mm^3). Interestingly, this protective effect of C21 was more marked in ATIP-Tg compared with WT mice (WT, 16% (66 of 80); ATIP-Tg, 27% (50 of 75) reduction, respectively). MMS2 expression increased in ipsilateral hemisphere of ATIP-Tg mice compared with contralateral hemisphere. There were no significant differences in CBF of core region of ischemic area, among all groups. However, the reduction of CBF in penumbra region after MCA occlusion was attenuated in ATIP-Tg mice with C21 administration. Treatment with C21 tended to increase the cerebral collateral number before MCA occlusion in ATIP-Tg mice not in WT. **Conclusions:** These results suggested that ATIP could enhance the cerebral protective effects of AT_2 receptor stimulation at least in part due to the increase of CBF and MMS2 expression after ischemia.

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Ingelheim Co., Ltd. **M. Mogi:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; Daiichi-Sankyo Pharmaceutical Co., Ltd, Nippon Boehringer Ingelheim Co., Ltd., Novartis Pharma K. K.. **X. Wang:** None. **K. Tsukuda:** None. **A. Higaki:** None. **M. Kukida:** None. **H. Nakaoka:** None. **T. Yamauchi:** None. **H. Bai:** None. **B. Shan:** None. **L. Min:** None. **M. Horiuchi:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; Daiichi-Sankyo Pharmaceutical Co., Ltd, Nippon Boehringer Ingelheim Co., Ltd., Novartis Pharma K. K..

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P451

Cerebrovasuclular Events in Patients With Primary Aldosteronism: Results of the PA Sendai Study Registry

PrimaryAuthor.AuthorBlock:**Masataka Kudo**, Ryo Morimoto, Div of Nephrology, Endocrinology and Vascular Med, Tohoku Univ Graduate Sch of Med, Sendai, Japan; Syuji Mugikura, Dept of Diagnostic Radiology, Tohoku Univ Graduate Sch of Med, Sendai, Japan; Yuta Tezuka, Kei Omata, Mashiro Nezu, Yasuhiro Igarashi, Div of Nephrology, Endocrinology and Vascular Med, Tohoku Univ Graduate Sch of Med, Sendai, Japan; Kazumasa Seiji, Kei Takase, Dept of Diagnostic Radiology, Tohoku Univ Graduate Sch of Med, Sendai, Japan; Sadayoshi Ito, Div of Nephrology, Endocrinology and Vascular Med, Tohoku Univ Graduate Sch of Med, Sendai, Japan; Fumitoshi Satoh, Div of Clinical Hypertension, Endocrinology & Metabolism, Tohoku Univ Graduate Sch of Med, Sendai, Japan

Background: Hyperaldosteronism is associated with the risk of cardio-and cerebrovascular events (CVE) that is independent of blood pressure levels and Primary aldosteronism (PA) display a higher risk of CVE compared with essential hypertension (EH). **Objective:** Our objective was to elucidate the prevalence of symptomatic stroke in PA patients and to characterize their clinical features. **Design and Setting:** This was a retrospective cross-sectional single center study (PA Sendai Study registry) between 2007 and 2016 in Japan. **Patient:** 807 patients with PA were registered and analyzed. The all patients were performed adrenal venous sampling(AVS) to make the diagnosis of PA subtypes. **Results**: Of 807 patients (male/female:395/412, age:53.3±11.8), 357 patients (44.2%) with alsosterone producing adenoma underwent adrenalectomy. The other patients were treated by medical therapy mostly administrating MR antagonists. Around the same time performed AVS, 698 patients were also performed MRI brain scans. Unruptured cerebral aneurysms were detected in about 4%, and Silent Brain Infarction were detected mainly around subcortical and basal ganglia region in about 30%. 27 patients (3.35%) suffered symptomatic stroke before the confirmed diagnosis of PA. The ratio of elderly and male was significantly high in stroke patients with PA. These patients had a higher rate of cerebral hemorrhage (thalamus or putaminal hemorrhage) rather than cerebellar infarction (63% vs 37%, $p< 0.05$). 457/807 patients (56.65%) were follow-up continually (period of observation: mean 1907 days) in our medical center. During this follow-up period, 6 patients died (malignant disease: 1, advanced heart failure: 2, cerebellar infarction: 1, unknown: 1). Eventually, the only 6 patients had new onset of cerebrovascular events (cerebral infarction: 4, cerebellar hemorrhage: 2). Over all, the prevalence of new-onset CVE was 1.8% in the patients treated for PA subtypes specific. **Conclusion**: This study suggests that the

aldosterone excess in untreated PA patients particularly damages cerebral small vessels around subcortical and basal ganglia region. By extension, PA subtypes specific treatment and early diagnosis are useful in preventing CVE.

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P452

Evaluation of Pathological Basis of a Shrsp-based Congenic Strain Characterized by Early Stroke Occurrence

PrimaryAuthor.AuthorBlock:**Hiroki Ohara**, Davis L Ngarashi, Toru Nabika, Shimane Univ, Izumo, Japan

Objectives: The stroke-prone spontaneously hypertensive rat (SHRSP) is a well-known model for essential hypertension and cerebral stroke. In the previous studies, we have identified a major blood pressure (BP) QTL on chromosome (chr) 1 of SHRSP and have explored gene(s) on the BP QTL responsible for hypertensive phenotypes of this strain through physiological analyses using reciprocal congenic strains between SHRSP and a normotensive control (Wistar-Kyoto rat, WKY). In a recent observation, we unexpectedly found that a SHRSP-based congenic strain, harboring a 0.3-Mbp fragment of the chr1 QTL, has developed stroke earlier than SHRSP. Here, we investigated pathological basis of this congenic strain and attempted to identify gene(s) related to early stroke occurrence.
Methods: SHRSP and a SHRSP-based congenic strain 〔SHRSP.WKY-(*D1Smu13*)/Izm, abbreviated as SPwch1.71〕 were used in this study. The rats were housed 12-hour light phase-controlled environment with freely accessible SP diet (Funabashi Farm) and drinking water until at least one suggestive sign of stroke (diminished motor activity, paralytic gait, and so on) was found. MRI by MRmini SA 1508 (DS Pharma Biomedical) was employed to confirm stroke occurrence. BP measurement was performed by the tail-cuff method. Cerebral cortex, brainstem, kidney, adrenal gland and heart obtained from 14 weeks old rats (n=5) were snap-frozen for RNA extraction. Quantitative RT-PCR (qRT-PCR) was performed about 15 of candidate genes located on the chr1 QTL covered by SPwch1.71. Nonsynonymous SNPs were scanned based on the whole-genome sequence of SHRSP and WKY.
Results: SPwch1.71 showed significantly high stroke susceptibility compared with SHRSP 〔stroke-free rate until 22 weeks old; SPwch1.71: 0% (0/7), SHRSP: 62.5% (5/8), $p<0.001$〕. Systolic BP at 12 weeks old of SPwch1.71 was significantly higher than that of SHRSP (222±4 vs. 206±5, $p<0.01$, n=5 for each). qRT-PCR identified genes showing modest (<1.5-fold change), but statistically significant different ($p<0.05$), in the expression in each tissue examined. Nonsynonymous variations were found in *Pde2a*, *Inppl1* and *Folr1*. Pathological significance of expression/sequence variations in the genes remain unclear.

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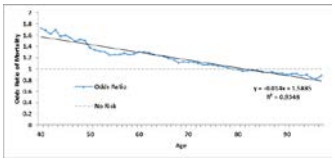
P453

Hypertension Mortality Risk May be Fake News for Nonagenarians

PrimaryAuthor.AuthorBlock:**Raya Elfadel Kheirbek**, George Washington Univ Sch of Med and Health Sciences, washington DC, DC; Farrokh Alemi, George Mason Univ Dept of Health Admin and Policy, Fairfax, VA; Ross D. Fletcher, Georgetown Univ Sch of Med, washington DC, DC

BACKGROUND: Little is known regarding BP control and mortality risk in subjects greater than 90 years of age. **OBJECTIVE:** This paper assesses the association between systolic blood pressure and mortality risk for nonagenarians. **METHODS:** Data from the Veterans Administration Informatics and Computing Infrastructure were used to analyze the survival of 193,651 nonagenarians in 130 Veteran Administration medical centers. Following clinical guidelines, for each day, we selected the lowest Systolic reading of the day exceeding 90 mmHg and defined sustained pressure as the average of two consecutive readings at least one month apart. Kaplan Meier curves and Cox regression was used to analyze survival. **RESULTS:** Odds of mortality from hypertension declined with age. When patients were over 90 years old, the odds of mortality for hypertensives was below 1 to 1, suggesting a protective effect. Patients whose sustained systolic pressures exceeded 140 mmHg survived longer than patients whose highest sustained pressure was between 90 mmHg to 140 mmHg ($p<.0001$). Furthermore, strict control of hypertension of 90 year old patients (SBP 120) was associated with lower days of survival than hypertensives, raising questions about value of strict control of hypertension among 90 year olds. **Conclusions:** For nonagenarians, mortality from hypertension may be a lower concern than mortality from other causes. Randomized clinical trials are needed to examine the impact of control of hypertension of patient over 90 year olds.



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P454

Does Intensive Pulse Pressure control results in lower rates of cardiovascular events? An Analysis of Systolic BP Intervention Trial (SPRINT) database

PrimaryAuthor.AuthorBlock:**Quan Tran**, Pinnacle Health, Harrisburg, PA

Introduction
A wide pulse pressure (PP) is a marker of increased artery stiffness and high cardiovascular risk. A high PP may reflect already diseased arterial walls, with several adverse cardiac implications of potential prognostic value. Previous studies show that arterial stiffness is itself an independent predictor of the risk of all-cause mortality, cardiovascular mortality, coronary events and stroke in hypertensive subjects. No guidelines exist regarding tight pulse pressure in chronic hypertension population.
Method
For the purpose of our study, we examined SPRINT data collected between November 2010 and August 2015. The role of PP and DBP was studied as a qualitative parameter with separation according to the 4 quartiles defined in the studied population. We then combined

the results of both PP and DBP in conjunction with preexistence intensive vs. control arm for further analysis.
Results
Our secondary analysis of SPRINT data first confirmed NEJM article that intensive SBP control reduced rates of heart failure (HF), CVD death, Death from any cause, and SPRINT primary outcome or death. There was a significant difference in primary outcome in each PP quartile. Higher PP quartiles were more likely to have higher rate of MI, stroke, HF, CVD death, and SPRINT primary outcome or death. There also a significant increase in SPRINT primary outcome and Heart Failure when DBP <70 in the intensive treatment arm. Finally, SPRINT data has confirmed that participants in PP_1 and PP_2 with DBP > 70 have significant lower rate of SPRINT primary outcome and death compared to other group regardless of treatment arm.
Conclusion
In the SPRINT database of 9347 participants, we found that higher PP conferred an increased risk for multiple adverse cardiovascular event. Our study is among the largest US studies of PP and adds further support to the prognostic utility of PP. In addition, the SPRINT database offers a contemporary analysis of the relationship between PP and DBP, which is important given that nature pathophysiology of blood pressure. Current study also strengthen the possibility that DBP <70 mm Hg was associated with a higher rate of cardiac events.

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P455

Obesity Types (Body Mass Index vs. Waist Circumference) Affect on Development of Major Cardiovascular Risks: Data From Korean National Health Insurance Service - National Sample Cohort (NHIS-NSC)

PrimaryAuthor.AuthorBlock:**Eun Joo Cho**, Catholic Univ of Korea, Seoul, Korea, Republic of; Sungha Park, Yeonsei Univ of Korea, Seoul, Korea, Republic of; Hae Young Lee, Seoul Natl Univ of Korea, Seoul, Korea, Republic of; Ki Chul Sung, Sungkyunkwan Univ of Korea, Seoul, Korea, Republic of; Wook Bum Pyun, Ewha Womans Univ of Korea, Seoul, Korea, Republic of; Jinho Shin, Hanyang Univ of Korea, Seoul, Korea, Republic of; Myeong-Chan Cho, Chungbuk Natl Univ of Korea, Cheongju, Korea, Republic of

Background: Population based study demonstrated that obesity (defined as increased body mass index, BMI) is important predictor for developing hypertension and diabetes mellitus (DM), which have been well known major cardiovascular (CV) risk factors. Increased waist circumference (WC) representative of central obesity is focused as main factor for developing CV disease and its major risks. This study was to evaluate obesity as a predictor for new onset of hypertension and DM within 1-year in previously normotensives or normoglycemic subjects.
Methods: The Korean National Health Insurance Service - National Sample Cohort (NHIS-NSC) established data about BMI and WC with medical, social and familial histories in 2009 and have been followed up for 1 years. Among total 349257 subjects with age more than 20 year-old, 95124 (normotensive group: 75.2% of population were 25-55 year-old, 54121(56.9%) male) of normotensives for evaluate new hypertension and 120501 (normoglycemic group: 83.6% of population were 25-65 year-old, 67183 (55.8%) male) normoglycemic subjects for evaluate new DM were analyzed. **Results:** During 1-year follow-up period, 3773 (3.97%) new hypertension in

normotensive group and 1594 (1.32%) new DM in normoglycemic group were developed. Binary logistic regression analysis revealed that BMI was the predictor (Exp(B)=1.18, Sig<0.001) for new onset hypertension with age (Exp(B)=1.31, Sig<0.001), sex (Exp(B)=0.664, Sig<0.001), dyslipidemia and family history of hypertension. For new onset DM, WC (Exp(B)=1.04, Sig<0.001), age (Exp(B)=1.26, Sig<0.001), sex Exp(B)=0.664, Sig<0.001) and family history of DM (Exp(B)=1.76, Sig<0.001) were the independent predictors. **Conclusion:** Increase of BMI is independent predictor for new onset hypertension and increase of WC is independent predictor for new onset DM within 1-year. Obesity type might affects development of different major CV risks and hence, obesity itself is the important risk factor for CV disease.

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Examining Framingham Coronary Heart Disease 10-year Risk Reduction in Individuals Treated With Lorcaserin, a Selective $5HT_{2C}$ Receptor Agonist for Weight Management

PrimaryAuthor.AuthorBlock:**Russell Knoth**, Xuan Li, Debanjana Chatterjee, Eisai, Inc., Woodcliff Lake, NJ; Ken Fujioka, Scripps Health, San Diego, CA

Introduction. Obesity contributes significantly to cardiovascular disease (CVD) risk and weight management is associated with CVD risk reduction. Lorcaserin, a selective $5HT_{2C}$ receptor agonist, is indicated as an adjunct to a reduced-calorie diet and increase physical activity for weight management in adults with obesity (BMI≥30) and overweight (BMI 27-29.9 and ≥1 weight-related comorbidity). This analysis used the Framingham 10-year CVD risk to examine the impact of lorcaserin treatment on CVD risk and risk reduction. **Method.** Pooled data from two randomized, double-blind studies (BLOOM & BLOSSOM) investigating the efficacy of lorcaserin vs. placebo among non-diabetic patients with overweight or obesity, were used in this analysis. Framingham CVD risk was calculated for those whose lipid values were available at baseline and at one year. A Framingham CVD risk of <5% was classified as low risk. Patients were stratified by cohort, lorcaserin (LOR) vs. placebo (PBO) and response at week 12 (≥5% weight loss vs. < 5%) for calculating the 10-year CVD risk at one year. Logistic regression was used to adjust for baseline risk and determine if proportional differences in risk reduction were significant. **Results.** A total of 5,658 patients were evaluated, 51% treated with LOR. Among the LOR-treated patients, 43% achieved ≥5% weight loss at week 12 (compared to 19% treated with PBO). At one year, more patients in the LOR cohort had reduced CVD risk (from high to low risk, 21% vs. 15%, respectively). The regression analysis showed LOR-treated patients were 34% more likely to have low CVD risk (p<0.001). Similarly, for patients who lost ≥5% baseline body weight at 12 weeks, more patients in the LOR cohort had reduced CVD risk (from high to low risk, 26% vs. 16% respectively) at one year. The regression showed LOR-treated patients were 23% more likely to have low CVD risk (p<0.05). **Conclusion.** The analysis suggests that patients treated with LOR are more likely to have low CVD risk at one year than those treated with PBO. The effect is magnified if patients have ≥5% weight loss at 12 weeks.

Disclosures: **R. Knoth:** A. Employment; Significant; Eisai, Inc. **X. Li:** A. Employment; Significant; Eisai, Inc. **D. Chatterjee:** A.

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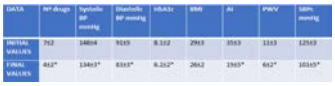
P457

Hypertensive, Type II Diabetic and Obese Patients, Are Better Controlled and Reduce the Number of Drugs When Empagliflozin is Added to Standard Treatment Improving Also Arterial Stiffness and Central Blood Pressures

PrimaryAuthor.AuthorBlock:**Ricardo Cabrera-Sole**, Caridad Turpin Lucas, Liliana Urrera Rivera, Santiago Garcia Ruiz, Manuel Aguilera, Univ General Hosp of Albacete, Albacete, Spain

INTRODUCTION: It is known the difficulty of adequate control of blood pressure (BP) with a single drug, being necessary in most use 2 or more antihypertensive drugs. If they are also obese and diabetic, require even more drugs so we present our experience of adding empagliflozin to the treatment of patients with these characteristics. OBJECTIVES:To assess the benefits of metabolic ,BP and arterial stiffness parameters introducing empagliflozin in obese ,hypertensive and diabetics patients (PHTAD). MATERIAL and METHODS: we studied 32 PHTAD receiving at least 3 drugs (ACE inhibitors, calcium channel blockers, hydrochlorothiazide) and two or more anti diabetic drugs (metformin, glimepirina, sitagliptin, insulin and Statins) for controlling their pathologies). We checked number of drug, BP, glicosylated hemoglobin (A1cHb), BMI and arterial stiffness measured by augmentation index (AI) and pulse wave velocity (PWV) and central systolic blood pressure (SBPc). Patients were followed for 32 weeks, adding to the beginning of the study empagliflozina .Every two months we checked them . The beginning and the end results were compared and are set out in the following table.



DATA	Nº drugs	Systolic BP mmHg	Diastolic BP mmHg	HbA1c	BMI	AI	PWV	SBPc mmHg
INITIAL VALUES	7±2	148±4	91±5	8.1±2	29±3	35±3	11±3	125±3
FINAL VALUES	4±2*	134±3*	83±3*	6.2±2*	26±2	19±5*	6±2*	101±5*

*means p value less than 0.05
CONCLUSIONS: According to our data, the introduction of empagliflozin to the treatment of PHTAD significantly improves metabolic parameters and helps to reduce the number of antihypertensive and antidiabetic drugs they are taking, improving the arterial stiffness indexes and the central pressure values, obtaining a clear improvement in the control of their cardiovascular risk . So it should be thought about this group of drugs when it comes to medicating PHTAD.

Disclosures:**R. Cabrera-Sole:** None. **C. Turpin Lucas:** None. **L. Urrera Rivera:** None. **S. Garcia Ruiz:** None. **M. Aguilera:** None.

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P458

Assessment of Cardiovascular Risk Reduction in Adults With Metabolic Syndrome or Type 2 Diabetes Treated With Lorcaserin: A Pooled Cohort Analysis

PrimaryAuthor.AuthorBlock:**Debanjana Chatterjee**, Xuan Li, Elena Nikonova, Eisai, Inc., Woodcliff Lake, NJ; Michael Miller, Univ of Maryland, Sch of Med, Baltimore, MD

Introduction. Obesity is a risk factor for cardiovascular disease (CVD) and even modest weight loss can contribute to its reduction. Lorcaserin, a selective $5HT_{2C}$ receptor agonist, is indicated as an adjunct to a reduced-calorie diet and increased physical activity in adults with obesity (BMI≥30 kg/m^2) or overweight (BMI 27-29.9 kg/m^2 and at least 1 weight-related comorbidity). Using the 2013 ACC/AHA 10-year

CVD risk assessment pooled cohort equation, we examined risk change in patients with metabolic syndrome or type 2 diabetes (T2DM) undergoing treatment with lorcaserin or placebo for weight loss. **Methods.** We identified patients with metabolic syndrome or T2DM in three randomized, double-blind studies (BLOOM, BLOSSOM, BLOOM-DM) examining the efficacy and safety of lorcaserin 10 mg bid (LOR) vs. placebo (PBO) at one year of treatment. Only patients with lipid values at baseline and one year were included. Once selected, patients were further stratified by weight loss at week 12 of treatment (≥5% vs. <5%). A CVD risk score >5% was classified as high risk, and a 0.5% change in risk over one year was defined as clinically relevant. CVD risk reduction by cohorts, overall and by gender, was compared. **Results.** Overall, 43% LOR (n=449/1056) and 20% PBO (n=207/1041) patients had ≥5% weight reduction at week 12 ($p<0.05$). Baseline CVD risk was 5.43% and 5.14% in the LOR and PBO cohorts, respectively. At one year, PBO-corrected change in CVD risk compared to LOR treatment was -0.21% (95% CI 0.02, 0.04, p=0.03; LOR -0.55% vs -0.3% PBO) overall and -0.56% (95% CI 0.12, 0.99, p=0.012; LOR -1.08% vs PBO -0.41%) in men. CVD risk in women was <5% at baseline, with no significant change seen at one year. For 12-week responders, both LOR and PBO had a clinically relevant change in CVD risk overall (-0.81% and -0.79%) and for men (-1.71% vs -1.02%). For patients with <5% weight loss, only men in the LOR cohort showed clinically relevant CVD risk changes (-0.57% vs -0.26%, respectively). **Conclusion.** The analysis suggests that patients with overweight or obesity and metabolic syndrome/T2DM at risk for CVD, and treated with LOR, experienced a significant CVD risk reduction at one year vs PBO, most notably among men. LOR can effectively reduce weight, which is associated with improvements in CVD risk.

Disclosures: **D. Chatterjee:** A. Employment; Significant; Eisai, Inc. **X. Li:** A. Employment; Significant; Eisai, Inc. **E. Nikonova:** A. Employment; Significant; Eisai, Inc.. **M. Miller:** None.

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P459

The BBSome Mediate the Sorting of the Serotonin 5-HT2C Receptor to the Plasma Membrane in POMC Neurons

PrimaryAuthor.AuthorBlock:**Deng-Fu Guo**, Charles Searby, QiHong Zhang, Darryl Nishimura, Val Sheffield, Kamal Rahmouni, Univ of Iowa, Iowa City, IA

The BBSome, a multiplex of 8 Bardet-Biedl Syndrome (BBS) proteins including BBS1, has emerged as an important regulator of energy homeostasis and cardiovascular function. Disrupting the BBSome, through deletion of its units, globally, in the nervous system, hypothalamus or selective neurons such as the proopiomelanocortin (POMC) neurons causes obesity associated with sympathetic overdrive and hypertension. However, the mechanisms underlying these phenotypes evoked by BBSome disruption remain ill defined. Serotonin 5-HT2C receptor (5-HT2CR) present in POMC neurons plays an important role in the regulation of energy homeostasis. We hypothesized that the BBSome contribute to the regulation of 5-HT2CR signaling. Given the importance of the BSBome for the trafficking of proteins to cilia, we first assessed whether 5-HT2CR localize the cilium in mouse MICD cells transfected with GFP-tagged 5-HT2CR. We found 5-HT2CR present in the plasma membrane, but no evidence for ciliary localization of this receptor. To study the relevance of the BBSome for the trafficking of 5-

HT2CR, we deleted the *Bbs1* gene in IMCD cells using CRISP-Cas9. We found that the 5-HT2CR failed to traffic to the plasma membrane in IMCD cells lacking the *Bbs1* gene. Moreover, the increase in intracellular calcium and membrane potential evoked by serotonin were significantly reduced in cells lacking the *Bbs1* gene. Interestingly, double immunostaining revealed that most of 5-HT2CR was stuck in the late endosome co-localizing with CD63, a marker of this organelle. Next, we analyzed the localization of the 5-HT2CR in the hypothalamic arcuate nucleus of mice lacking the *Bbs1* gene selectively in POMC neurons ($Bbs1^{POMC}$). In control mice, 5-HT2CR was predominantly present in the plasma membrane of POMC neurons and in some non-POMC cells. In $Bbs1^{POMC}$ mice, 5-HT2CR was altered selectively in POMC neurons appearing stalled in the cytoplasm. Finally, i.p. administration of lorcaserin, a 5-HT2C specific agonist, caused a significant decrease in body weight and food intake in control mice, but not in $Bbs1^{POMC}$ mice. These findings demonstrate that the BBSome underlie the trafficking of 5-HT2CR in POMC neurons with important implications for the regulation of energy homeostasis and development of obesity.

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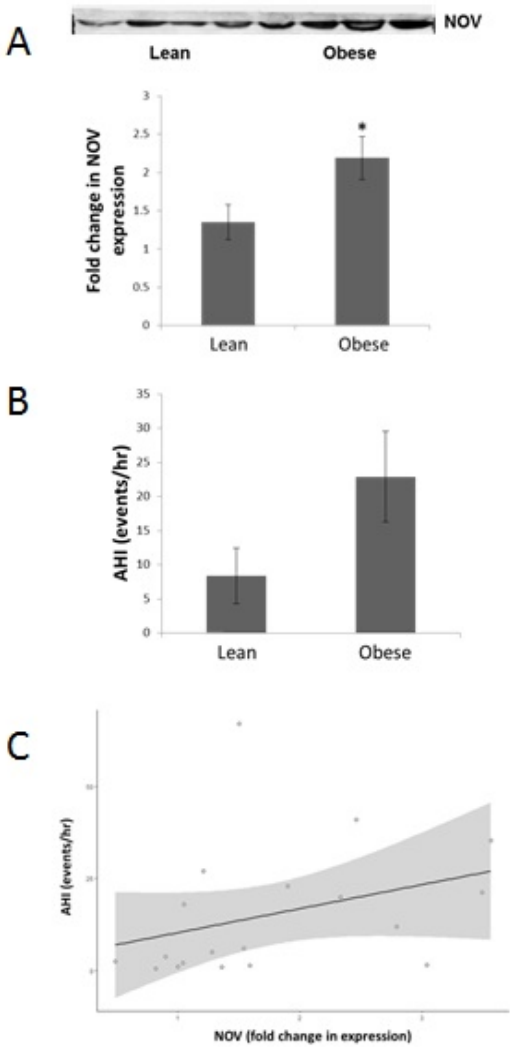
P461

The Association of NOV/CCN3 With Obstructive Sleep Apnea in Severe Morbid Obesity: Preliminary Evidence of a Novel Biomarker in OSA

PrimaryAuthor.AuthorBlock:**Jeremy A. Weingarten**, NewYork-Presbyterian Brooklyn Methodist Hosp, Brooklyn, NY; Lars Bellner, New York Medical Coll, Valhalla, NY; Stephen J. Peterson, Moe Zaw, Puja Chadha, Hemal H. Mehta, NewYork-Presbyterian Brooklyn Methodist Hosp, Brooklyn, NY; Nader G. Abraham, New York Medical Coll, Valhalla, NY

Background: Obstructive sleep apnea (OSA) is strongly associated with cardiovascular and metabolic abnormalities, although the mechanism driving this association is not well understood. NOV/CCN3, a multifunctional extracellular matrix protein, may play a mechanistic and/or prognostic role in these associations. We hypothesized that patients with OSA will have increased levels of NOV, and that NOV can serve as a biomarker in patients to predict OSA as well as metabolic and cardiac risk. **Materials and Methods:** 10 morbidly obese subjects presenting to the sleep laboratory for clinical evaluation of possible OSA and 10 healthy lean controls underwent overnight polysomnography and clinical evaluation. Blood samples were analyzed for NOV levels, adiponectin, and IL-6. **Results:** OSA was found in 9 obese subjects and 3 lean subjects. NOV levels were significantly higher in the obese vs lean group (2.2±0.3 vs 1.4±0.2 fold change, $p<0.03$) while apnea-hypopnea index (AHI) was similarly higher in the obese vs lean group (22.9±6.7 vs 8.4±4.1 events/hr, $p<0.03$). NOV and AHI were positively correlated ($\rho=0.49$, $p=0.033$). IL-6 was elevated while adiponectin was reduced in obese vs lean. **Conclusions:** NOV levels and AHI, a measure of OSA severity, were higher in obese subjects vs lean controls and were correlated with each other. NOV may be a marker of OSA severity and a biomarker for cardiovascular and metabolic disease in OSA patients.



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P462

MiRNA and Biomarkers of Metabolic Syndrome: Correlating Biomarkersfor Early Detection of Metabolic Syndrome in Obese Females in West Virginia

PrimaryAuthor.AuthorBlock:**Hari Vishal Lakhani**, Dana Sharma, Athar Nawab, Komal Sodhi, Marshall Univ Res Corp., Huntington, WV

Metabolic syndrome, a multifactorial disease, causes complications like cardiovascular disease and diabetes mellitus. As metabolic syndrome develops, altered levels of cytokines and miRNAs are measured in the circulation. We constructed a panel detecting abnormal levels of cytokines and miRNAs in patients at risk for metabolic syndrome. Participants included 54 patients from a Family Medicine Clinic at Marshall University School of Medicine and were grouped based on BMI and metabolic syndrome diagnosis: Control, Obese, Metabolic Syndrome (MetS). Serum levels of leptin, adiponectin, leptin: adiponectin ratio, IL-6, six microRNAs (320a, 197-3p, 23-3p, 221-3p, 27a-3p, and 130a-3p), were measured. Among the three groups, leptin, and leptin: adiponectin ratio, and IL-6 levels were highest in MetS, and levels in Obese were greater than Control ($p>0.05$). Adiponectin levels were lower in Obese compared to Control, but lowest in MetS ($p<0.05$). MiRNA levels were lowest MetS, and levels in Obese were lower than Control ($p>0.05$). Our results support the clinical application of biomarkers to diagnose early stage metabolic syndrome, in this population. This would enable attenuation of disease progression before onset of irreversible complications. Since West Virginians are high-risk for developing metabolic syndrome, our biomarker panel could reduce the disease burden on our population.

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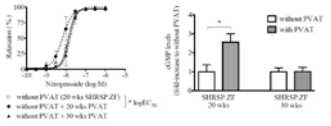
Deterioration of Vasomotor Regulation of Perivascular Adipose Tissue at Later Stage of Metabolic Syndrome

PrimaryAuthor.AuthorBlock:**Satomi Kagota**, Kana Maruyama, Shiori Koyanagi, Saki Iwata, Kazumasa Shinozuka, Mukogawa Women's Univ, Nishinomiya, Japan

Obesity is a critical risk factor for metabolic syndrome, which increases development of cardiovascular complications. Perivascular adipose tissue (PVAT) is known to modulate vascular tone. We reported that PVAT increases vasodilation via enhancing endothelial nitric oxide production in mesenteric arteries of SHRSP.Z-*Lepr*^{fa}/IzmDmcr rats (SHRSP.ZF) with metabolic syndrome (at 20 weeks of age; wks). However, the enhancing effects disappear at later stage (30 wks). We therefore investigated mechanisms underlying deterioration of the compensatory effects of PVAT in metabolic syndrome. Mesenteric arteries were isolated from male 20 and 30-week-old SHRSP.ZF, and vasodilation in response to nitroprusside was examined using bath bioassay techniques. Coexistence of PVAT from SHRSP.ZF at 20 wks increased vasodilation in arteries without PVAT from SHRSP.ZF at 20 wks, but the effects of PVAT were not substituted by replacement with PVAT from rats at 30 wks. Cyclic GMP levels in response to nitroprusside in arteries were increased by the presence of PVAT at 20 wks, but it was not altered by the presence of PVAT at 30 wks. In the presence or absence of vaspin, visfatin omentin, and apelin, vasodilation in response to nitroprusside was unchanged in SHRSP.ZF arteries at 20 wks. These results indicated that the production/release of diffusible vasodilator(s) from PVAT decreases with ageing. Vaspin, visfatin omentin, and apelin could be excluded from potential candidates of vasodilator(s). The deterioration of PVAT's compensatory effects under the conditions of impaired vasodilations may induce cardiovascular complications at later stages of metabolic syndrome.



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P464

Sodium Glucose Cotransporter 2 Inhibition by Canagliflozin Attenuates Intrarenal Angiotensinogen Augmentation in Type 2 Diabetes Mellitus

PrimaryAuthor.AuthorBlock:**T Cooper Woods**, Ryousule Satou, Kayoko Miyata, Akemi Katsurada, Courtney M Dugas, Daniel Lightell, Jr., L Gabriel Navar, Tulane Univ Sch of Med, New Orleans, LA

Type 2 diabetes mellitus (T2DM) is a complex disease where hyperglycemia occurs as a result of the development of insulin resistance. T2DM leads to complications, including renal and cardiovascular injury. Renal proximal tubular angiotensinogen (AGT) is stimulated by hyperglycemia via elevated oxidative stress, and is associated with activation of the intrarenal renin-angiotensin system which contributes to development of high blood pressure and diabetic nephropathy in diabetes mellitus. Sodium glucose co-transporter 2 (SGLT2) is mainly expressed in early renal proximal tubules

and is responsible for most of the glucose reabsorption by the tubules. This study tested the effects of canagliflozin (CANA), an SGLT2 inhibitor, on intrarenal AGT regulation in T2DM mice. Male New Zealand Obese mice were fed a regular fat diet (RFD, 4% fat) or a high fat diet (HFD, 40% fat) to induce diabetes. When the mice fed with HFD exhibited >350 mg/dl blood glucose levels (week 0), both RFD and HFD fed mice were treated with 10 mg/kg/day CANA or vehicle for 6 weeks by daily oral gavage. Systolic blood pressure (SBP) levels were measured by a tail cuff system and 24-hour urine samples were collected using metabolic cages. Intrarenal AGT mRNA and protein levels were determined by digital PCR and western blot analysis. CANA treatment decreased blood glucose levels in HFD mice, which remained suppressed for duration of the study. SBP in HFD groups were higher than in the RFD and RFD+CANA groups (134.7±3.6, 118.7±10.8 and 108.3±7.6 mmHg) at week 6. The elevated SBP was normalized by CANA (110.0±6.0 mmHg). The HFD group exhibited greater renal cortical AGT mRNA levels than the RFD group (3.7±0.4 vs. 7.4±1.0 copies/reaction). Likewise, intrarenal AGT mRNA expression was lower in CANA treated group (4.5±0.8 copies/reaction). Western blot analysis also showed lower AGT protein levels in CANA treated group (1.02±0.08-fold compared with RFD) than in HFD group (1.49±0.08-fold). In the HFD group, CANA treatment also suppressed elevated urinary 8-isoprostane levels, a marker of renal oxidative stress. These results demonstrate that CANA attenuates intrarenal AGT augmentation that occurs in T2DM, which may mitigate the development of diabetic nephropathy and progression of high blood pressure.

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P465

Arterial Hypertension and Orthostatic Hypotension in Diabetic Patients

PrimaryAuthor.AuthorBlock:**Ludovit Gaspar**, Comenius Univ, Bratislava, Slovakia; Slavomira Filipova, Slovak Health Univ and Natl Inst of Cardiovascular Diseases, Bratislava, Slovakia; Matej Bendzala, Iveta Gasparova, Comenius Univ, Bratislava, Slovakia

Introduction: Diabetes mellitus is often associated not only with arterial hypertension. but also with orthostatic hypotension, which is one of the most clinical apparent forms of cardiovascular autonomic neuropathy. **Objective:** To retrospectively assess the association of the orthostatic hypotension (OH) with macrovascular and microvascular complications of diabetes mellitus and to determine its effect on mortality.**Design and Methods:** We retrospectively analysed 187 patients with diabetes mellitus (60 patients with type 1 and 127 patients with diabetes type 2). Patients were divided into groups according to presence or absence of OH and type of diabetes. Association of OH with macrovascular and microvascular complications was evaluated and the effect of OH on 10-year all-cause mortality was also assessed.**Results:** OH was present in 31.7 % (19 of 60) of patients with DM type 1 and 32.3 % (41 of 127) of patients with DM type 2. We found strong positive association between OH and arterial hypertension grade 3 ESH/ESC in DM1 42.1 % (8 of 19) and DM2 48.8 % (20 of 41). OH was

positively associated with the prevalence of myocardial infarction in DM1 (OR=10.667) and with prevalence of stroke in DM2 (OR=3.335). There was also a strong association of OH and the prevalence of peripheral artery obliterative disease in both DM1 (OR=14.18) and DM2 (OR=3.263). Patients with both types of DM and OH had significantly higher prevalence of nephropathy (DM1 OR=8.680, DM2 OR=3.237), retinopathy (DM1 OR=8.095, DM2 OR=4.078) and peripheral neuropathy (DM1 OR=17.143, DM2 OR=7.506). Overall 10-year mortality rate was significantly higher in diabetic patients with OH, in DM1 group 31.6 % (6 of 19) and in DM2 group 31.7 % (13 of 41).**Conclusion:** Presence of orthostatic hypotension in diabetics is associated with higher prevalence of macrovascular and microvascular complications of diabetes mellitus and also with higher 10-year mortality.

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P466

Circadian Rhythm Disruptions in a Novel Diabetic *db/db-mPer2^{Luc}* Mouse Model

PrimaryAuthor.AuthorBlock:Tianfei Hou, Univ Kentucky, Dept. of Pharmacology and Nutritional Sciences, Lexington, KY; Wen Su, **Ming C Gong**, Univ Kentucky, Dept. of Physiology, Lexington, KY; Zhenheng Guo, Univ Kentucky, Dept. of Pharmacology and Nutritional Sciences, Lexington, KY

Db/db mouse, which lacks functional leptin receptor, is an extensively used model of obesity and type 2 diabetes. We and others have demonstrated that *db/db* mouse has disruptions in circadian rhythms of behavior, physiology and some clock genes. However, systemic investigations of the alterations in clock gene oscillations in multiple systems with high time resolution in this model are impeded by the impractical demand for large number of animals. To overcome this limitation, we cross bred the *db/db* mouse with *mPer2^{Luc}* mouse in which the clock gene *Period2* is fused with a luciferase reporter thus allow real-time monitoring of the clock gene *Per2* oscillations. The generated *db/db-mPer2^{Luc}* mice had the typical diabetic mellitus including obesity, hyperglycemia, hyperinsulinemia, glucose intolerance and insulin resistance. In addition, the *db/db-mPer2^{Luc}* mice also exhibited disruptions in circadian rhythms in behavior (locomotor activity), physiology (blood pressure) and metabolism (respiratory exchange ratio and energy expenditure). Using the LumiCycle system, we monitored in real-time of the *Per2* oscillations in both the SCN central clock and multiple peripheral tissues *ex vivo*. The results showed no difference in the phase of the central SCN *Per2* oscillation. However, the peripheral tissues that related to metabolism, such as liver and white adipose clocks, displayed 3.28±0.86 and 4.64±1.06 hours of phase advance respectively. Aorta, mesentery artery and kidney, organs play important role in blood pressure homeostasis, showed 0.99±0.37, and 2.12±0.4, and 2.21±0.5 hours phase advance respectively. Interestingly, no difference was observed in the lung and adrenal gland. We then investigated the *Per2* oscillation *in vivo* by using the IVIS imaging system. Consistent with the *ex vivo* results, the liver *Per2* oscillation were phase advanced in vivo. Our findings demonstrated that clock gene *Per2* oscillations were disrupted in multiple peripheral tissues but not in central SCN. Moreover, the extent of phase advance in peripheral tissue varies largely. Our results suggest dyssynchrony of the clock oscillations among various peripheral systems likely

contribute to the multiple disruptions in physiology and metabolism in diabetic *db/db* mice.

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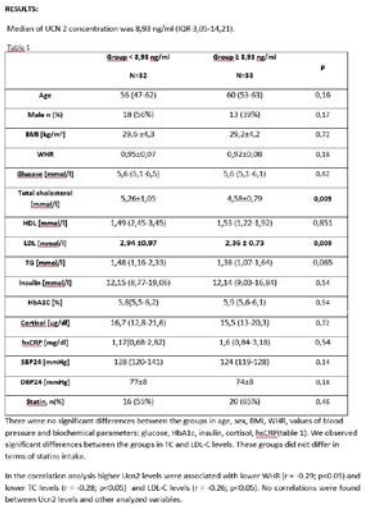
P467

Urocortin 2 Levels and Metabolic Profile in Patients With Primary Hypertension

PrimaryAuthor.AuthorBlock:**Jolanta Walczewska**, Olga Siga, Anna Dzieża-Grudnik, Univ Hosp JUMC, Dept of Internal Diseases and Gerontology, Cracow, Poland; Paweł Wołkow, Agnieszka Borys, Univ Hosp JUMC, Ctr for Medical Genomics OMICRON, Cracow, Poland; Barbara Wizner, Barbara Gryglewska, Tomasz Grodzicki, Univ Hosp JUMC, Dept of Internal Diseases and Gerontology, Cracow, Poland

OBJECTIVE:
Urocortin 2 (UCN 2) has powerful hemodynamic and neurohormonal actions.
Some recent studies suggest that ucn2 may also decrease the steroid production.
The aim of this study was to assess the relationship between the serum concentration of UCN 2 and metabolic parameters in patients with primary hypertension.
METHODS:
We examined 65 participants with essential hypertension treated with at least 3 antihypertensive medications. The evaluation included anthropometric measurements (BMI, WHR) and ambulatory blood pressure monitoring (ABPM). Moreover we evaluated metabolic parameters in blood serum: glucose, total cholesterol , low-density cholesterol (LDL-C), high density cholesterol (HDL-C), triglycerides (TG), HbA1c, insulin, cortisol, hsCRP. For the analysis of UCN 2 serum level we used ELISA Kit.
We divided the surveyed subjects into two groups according to the urocortin's median value. The comparative analyses between the groups were based on the student's T-test, U Mann-Whitney and Chi^2 . To examine the relationships between selected parameters we used Pearson and Spearman correlations.



RESULTS:

Median of UCN 2 concentration was 8,93 ng/ml (IQR 3,05-14,21).

Table 1

	Group < 8,93 ng/ml N=32	Group ≥ 8,93 ng/ml N=33	p
Age	55 (47-62)	60 (53-63)	0,16
Male n (%)	18 (56%)	13 (39%)	0,17
BMI [kg/m²]	29,6 ±4,3	29,2±4,2	0,72
WHR	0,95±0,07	0,92±0,08	0,18
Glucose [mmol/l]	5,6 (5,1-6,5)	5,6 (5,1-6,1)	0,62
Total cholesterol [mmol/l]	5,26±1,05	4,58±0,79	**0,005**
HDL [mmol/l]	1,49 (1,45-3,45)	1,53 (1,22-1,92)	0,851
LDL [mmol/l]	**2,94 ±0,97**	**2,36 ± 0,73**	**0,009**
TG [mmol/l]	1,48 (1,16-2,33)	1,38 (1,07-1,64)	0,085
Insulin [mmol/l]	12,15 (8,77-19,01)	12,14 (9,03-16,84)	0,54
HbA1C [%]	5,8(5,5-6,2)	5,9 (5,6-6,1)	0,54
Cortisol [ug/dl]	16,7 (12,8-21,6)	15,5 (13-20,3)	0,72
hsCRP [mg/dl]	1,17(0,68-2,92)	1,6 (0,84-3,18)	0,54
SBP24 [mmHg]	128 (120-141)	124 (119-128)	0,14
DBP24 [mmHg]	77±8	74±8	0,18
Statin, n(%)	16 (55%)	20 (65%)	0,46

There were no significant differences between the groups in age, sex, BMI, WHR, values of blood pressure and biochemical parameters: glucose, HbA1c, insulin, cortisol, hsCRP(table 1). We observed significant differences between the groups in TC and LDL-C levels. These groups did not differ in terms of statins intake.

In the correlation analysis higher Ucn2 levels were associated with lower WHR ($r = -0.29$; $p<0.05$) and lower TC levels ($r = -0.28$; $p<0.05$) and LDL-C levels ($r = -0.26$; $p<0.05$). No correlations were found between Ucn2 levels and other analyzed variables.

CONCLUSION:
Subjects with higher level of UCN 2 have lower concentration of total cholesterol and LDL-C, however changes in UCN 2 concentration were not associated with others metabolic parameters.
Ucn2 may play a protective role in cardiovascular morbidity through reduction of cholesterol synthesis and development of visceral obesity in patients with arterial hypertension.

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P468

Stimulation of Diuresis and Salt Excretion by Renomedullary Infusion of a Dual Inhibitor of Fatty Acid Amide Hydrolase (FAAH) and Monoacylglycerol Lipase (MAGL)

PrimaryAuthor.AuthorBlock:**Joseph K. Ritter**, Ashfaq Ahmad, Zdravka Daneva, Sara Dempsey, Guangbi Li, Ningjun Li, Aron Lichtman, Justin Poklis, Pin-Lan Li, Virginia Commonwealth Univ, Richmond, VA

The endocannabinoid, anandamide (N-arachidonoyl ethanolamide, AEA), is proposed to have a critical role in blood pressure regulation in the renal medulla. Infusion of AEA into the renal medulla of anesthetized C57BL6/J mice stimulated diuresis and sodium and potassium excretion without affecting blood pressure or glomerular filtration. AEA may act by direct stimulation of its cognate receptors or by either its hydrolysis and conversion to prostaglandins or a cyclooxygenase-2 (COX-2) dependent metabolite. The finding that intramedullary infusion of isopropyl dodecyl fluorophosphate (IDFP) produced the same profile of effects as AEA suggests that the hydrolysis of AEA to release arachidonic acid is not necessary for its efficacy and supports that endogenous endocannabinoids contribute to control of basal tone of renal excretory function. Blockade of the urine formation- and salt excretion- stimulating effects of intramedullary AEA and IDFP by a COX-2 (celecoxib) but not COX-1 (SC-560) inhibitor is consistent with elevated AEA in the renal medulla enhancing formation for a COX-2 metabolite of AEA that mediates the observed increases in diuresis and salt excretion. The effect of IDFP on urine formation rate was blocked in $FAAH^{-/-}$ mice, but unexpectedly IDFP intramedullary infusion lowered mean arterial pressure in FAAH KO mice, which may be explained by release into the blood of another endocannabinoid metabolized by MAGL or other serine hydrolase. It is concluded that the diuretic effect of intramedullary IDFP is mediated by inhibition of FAAH leading to elevation of endogenous AEA and a COX-2 dependent metabolite of AEA.

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CRISPR-Cas9 Gene Editing Yields Genetic Regulation of Obesity and Metabolism in Female LH-derived Rats

PrimaryAuthor.AuthorBlock:**Karen C Clark**, Justin L Grobe, Anne E Kwitek, Univ of Iowa-Carver Coll of Med, Iowa City, IA

Despite strong evidence that the Metabolic Syndrome (MetS) and its defining features (central obesity, dyslipidemia, hypertension, hyperglycemia) are highly heritable, the genetic etiology is complex and relatively few causative genes are known. For this reason, we employ the Lyon Hypertensive (LH) rat, a well-characterized genetic model of MetS. Previously, we identified a novel gene (*RGD1562963*) on LH rat chromosome 17 (RNO17) that affects components of MetS, using a combination of genetic mapping and gene network analyses. In this study, frameshift mutations (by CRISPR-Cas9) in exon 2 of *RGD1562963* is shown to change resting metabolic rate (RMR) and body composition in LH-derived female rats, measured at 8 and 11 weeks of age, despite no differences in either total body weight or food consumption. *RGD1562963*$^{-/-}$ (*RGD*$^{-/-}$) females have significantly more body fat mass than their wild-type control at 8 weeks of age, measured by nuclear magnetic resonance (NMR) as a percentage of total body weight ($^{\#}$ 8 weeks: WT = 5.08±0.099, n=5; *RGD*$^{-/-}$ = 6.52±0.260, n=8; *p<.001), and this difference increases by 11 weeks of age (WT = 5.39±0.140, n=5; *RGD*$^{-/-}$ = 7.81±0.293, n=8, *p<.0001). RMR measurements at 8 weeks showed that both genotypes had identical resting energy expenditure (WT: 0.721±0.033 kcal/hr, n=5; *RGD*$^{-/-}$: 0.721±0.021 kcal/hr, n=6) and body weights (WT = 160.5g±6.884, n=5; *RGD*$^{-/-}$ = 160.7g±4.246, n=8). Though no body weight differences appear by 11 weeks (WT: 196.5g±7.226, n=5; *RGD*$^{-/-}$: 196.1g±3.765, n=8), the wild-type females have an unexpected decrease in RMR, while the RMR of *RGD*$^{-/-}$ females remains high (WT: 0.654±0.005 kcal/hr, n=4; *RGD*$^{-/-}$: 0.759±0.014 kcal/hr, n=7, *p<.01). Our studies suggest *RGD1562963* is an obesity susceptibility gene. Furthermore, inhibition of this gene at the whole body level is tolerated, and unexpectedly induced increased resting metabolic rate despite increased adiposity, possibly as a protective mechanism. The continued study of this rat model of Metabolic Syndrome has the potential to functionally validate an uncharacterized regulatory gene, and provide novel targets for pharmacological intervention in the treatment of obesity. $^{\#}$ all measures are presented as mean±SEM, * RM 2-WAY ANOVA, Sidak's correction

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P470

Cardiac Autonomic Neuropathy Develops As A Result Of Mild Hypercaloric Intake In Absence Of Signs Of Diabetes: Modulation By Anti-diabetic Drugs.

PrimaryAuthor.AuthorBlock:Ahmed El-Yazbi, Ola Al-Assi, Rana Ghali, Abdullah Kaplan, Nahed Mougharbil, Ali H. Eid, **Fouad A. Zouein**, AUBMC, Beirut, Lebanon

Cardiac autonomic neuropathy (CAN) represents a major cause of morbidity and mortality in diabetes. It is usually seen early in the course of diabetes as an impaired heart rate variability (HRV) and baroreflex sensitivity (BRS), and represents an independent risk predictor of cardiac mortality. CAN development is linked to hyperglycemia; however, current understanding extends cardiovascular risk to pre-diabetic patients with slight glycemic changes. As well, recent evidence suggests that anti-diabetic drugs (metformin and pioglitazone) reduced the risk of cardiovascular complications in pre-diabetic patients. Here, we assessed whether CAN develops independent of hyperglycemia and whether metformin or pioglitazone modify this process. Rats were fed a hypercaloric (HC) diet (4.035 KCal/g vs. 3 KCal/g for control rats) composed of: weight (calories) 18.06 % fat (38.68%), 15.8% protein (15.66%), and 46.13% carbohydrates (45.73%). Stable fasting hyperglycemia developed by 16 weeks of feeding. However, at 12 weeks of feeding, there was no elevation in body weight, fasting or random blood glucose, and no difference in oral glucose tolerance, yet an increase in adipose inflammatory cytokines was observed (4- and 40- fold increase in IL-1β and TGF-β expression). No change in systolic blood pressure was observed over the course of feeding. At 12 weeks, carotid and jugular access were established. Mean arterial pressure (MAP) and heart rate (HR) were recorded, and BRS was assessed using Oxford method. HC-fed rats had a higher pressor response to increasing i.v. doses of phenylephrine vs. control rats. BRS sensitivity was blunted (slope of the ΔMAP vs. ΔHR line, -1.018 ± 0.1217 vs. -0.3379 ± 0.04135) indicating reduced parasympathetic feedback. A 2-week treatment with pioglitazone (2.5 mg/Kg) or metformin (100 mg/Kg) normalized the adipose cytokine profile, yet only pioglitazone improved BRS (-0.7463 ± 0.05775). Parasympathetic dysfunction in HC fed rats was further demonstrated by a decreased high frequency power upon frequency domain analysis of HRV data (3098 ± 233 vs. 89 ± 88 μs^2). To our knowledge, this is the first report that CAN occurs prior to any glycemic alterations with a potential role for adipose inflammation and modification by antidiabetic drugs.

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P471

Increased Salt Intake Reduces Diet-induced Thermogenesis in Healthy Men

PrimaryAuthor.AuthorBlock:**Nicola Wilck**, Anja Mähler, Lars Klug, Samuel Klamer, Lajos Marko, Nadine Haase, Jochen Steiniger, Andras Balogh, Michael Boschmann, ECRC, Berlin, Germany; Dominik N. Müller, ECRC and Max Delbrück Ctr for Molecular Med, Berlin, Germany

Objective: High salt intake is a potential risk factor for obesity independent of energy intake, though underlying mechanisms remain unclear. Excessive body fat accumulation is caused by a positive energy balance over an extended period of time. Food intake causes a postprandial increase in energy expenditure. This postprandial effect represents approximately 10% of the total energy ingested over 24h. A decrease in diet-induced

thermogenesis (DIT) contributes to a positive energy balance and weight gain over time. The present study investigated the effect of a 14-day high salt intake on DIT in healthy men. **Methods:** We enrolled eight healthy men (34 ± 7 years, BMI 24 ± 2 kg/m^2) in an exploratory, longitudinal study (Clincaltrials.gov identifier: NCT02509962). Probands received 6 g salt daily using sodium chloride tablets (Slow Sodium tablets, HK Pharma Ltd.) over 14 days on top of their habitual diet. Measurements were taken at baseline (V1), on day 3 (V2) and day 14 (V3) during and on day 9 (V4) after the salt challenge, respectively. At these time points, nocturnal ambulatory blood pressure monitoring, bioelectrical impedance analysis (BIA), food intake from 3-day food records, and resting and postprandial energy expenditure were obtained. Energy expenditure was measured by indirect calorimetry in a respiratory chamber after a 12h overnight fast and a standardized 440 kcal, high-protein meal. **Results:** Nocturnal systolic blood pressure increased after salt challenge (V1: 105.8 ± 8.5, V3: 111.2 ± 9.1 mmHg, *P* = 0.02). There was a trend towards higher energy intakes (V1: 2296 ± 752 kcal, V3: 2866 ± 831 kcal, *P* = 0.1). However, DIT was significantly decreased at all time points during and after salt challenge compared to baseline (V1: 17.3 ± 2.7%; V2: 11.3 ± 2.3%, *P* = 0.01; V3: 10.7 ± 3.6 %, *P* = 0.03; V4: 14.4 ± 2.5%, *P* = 0.04; all vs. V1). After 14 days of high salt, subjects expended 30 kcal less for metabolic processes after meal challenge. Assuming a total energy intake of 2300 kcal/d, this accounts for a 150 kcal positive energy balance. **Conclusion:** A moderate short-term increase of salt intake decreased the thermic effect of a high-protein meal. This could contribute to the observed weight gain in populations consuming a Western diet high in salt.

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Prorenin Independently Causes Hypertension And Renal and Cardiac Fibrosis In Cyp1a1-Prorenin Transgenic Rats

PrimaryAuthor.AuthorBlock:**Yufeng Huang**, Guangyu Zhou, Jie Wu, Chunyan Gu, Alfred Cheung, UNIVERSITY UTAH, Salt Lake City, UT

Plasma prorenin is commonly elevated in diabetic patients and appears to predict the development of diabetic nephropathy. However, the pathological role of prorenin is unclear. In this study, a transgenic, inducible, hepatic prorenin-overexpressing rat model was generated and the effect of prorenin in organ injury was examined. Four groups of rats (Cyp1a1 prorenin transgenic male and female rats and nontransgenic littermates) were assigned to receive a diet containing 0.3% of the transgene inducer indole-3-carbinol (I3C) for 4 weeks. Plasma prorenin concentration rose from 23±6 to 208±44 (µg/ml) and MAP increased from 77±5 to 138±17 (mmHg), whereas renal prorenin/renin protein expression was unchanged, in transgenic rats fed with I3C diet. The intact prorenin, not renin, in plasma and urine samples was further observed by western blot analysis. Importantly, transgenic rats with high levels of prorenin developed albuminuria, glomerular and tubulointerstitial fibrosis associated with increased expression of TGFß1, PAI-1, collagen and fibronectin. These rats also exhibited cardiac hypertrophy determined by echocardiography, with elevated ratio of heart

weight to body weight. Cardiac collagen in interstitial and perivascular area was prominent, accompanied by the increases in mRNA contents of ANP, BNP, ß-MHC, TGFß1 and PAI-1 in the heart tissue. Furthermore, renal protein levels of phosphor-NF-kB-p65 and MCP-1, NAPDH oxidase and MDA, phospho-ß-catenin and phospho-Akt were dramatically increased in prorenin overexpressed rats. These results indicate that prorenin, without being converted to renin, causes arterial hypertension, renal and cardiac fibrosis independently via the induction of inflammation, oxidative stress and the ß-catenin and Akt-mediated signals.

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P473

Group IV Cytosolic Phospholipase A2α is Critical for Norepinephrine-Induced Hypertension

PrimaryAuthor.AuthorBlock:**Ajeeth K Pingili**, Chi Yong Song, Ji Soo Shin, Univ Of Tennessee Health Science Ctr, Memphis, TN; Joseph V Bonventre, Harvard Medical Sch, Harvard Inst of Med, Boston, MA; Kafait U Malik, Univ Of Tennessee Health Science Ctr, Memphis, TN

Previously we reported that angiotensin (Ang) II-induced hypertension and associated cardiovascular and renal dysfunction are mediated by cytosolic phospholipase $A_2\alpha$ ($cPLA_2\alpha$) activation, the release of arachidonic acid (AA), and production of eicosanoids predominantly with pro-hypertensive effects (*Hypertension. 2015; 65: 784-792; 2016; 29: 258-265*). We have also shown that norepinephrine (NE) by activating $cPLA_2$ releases AA, and production of prostanoids in vascular smooth muscle cells (*J Biol Chem. 1996; 217:30149-30157; J. Pharmacol. Exp. Ther. 1993; 266: 1113–1124*). This study was conducted to determine the contribution of $cPLA_2\alpha$ in NE-induced hypertension. Eight weeks old male wild-type ($cPLA_2\alpha^{+/+}$) and $cPLA_2\alpha$ gene disrupted ($cPLA_2\alpha^{-/-}$) mice were infused with NE (10 mg/kg/day, s.c.) or its vehicle using mini-osmotic pumps for 2 weeks, and the systolic blood pressure (SBP) was measured by tail-cuff. Infusion of NE increased the SBP in $cPLA_2\alpha^{+/+}$ mice (148±3 vs. 118±3 mmHg, $P<0.05$, n=4-5); but not in $cPLA_2\alpha^{-/-}$ mice (122±5 mmHg, n=5). The NE-induced increase in SBP was minimized by treatment with AA metabolism inhibitor, 5,8,11,14-eicosatetraynoic acid (ETYA) (25 mg/kg, i.p., every 3rd day) in $cPLA_2\alpha^{+/+}$ mice (125±5 vs. 148±3 mmHg, $P<0.05$, n=4-5). Prostaglandin (PG) E2-EP1 and EP3 receptor activation that increase blood pressure have been implicated in Ang II-induced hypertension. In our study antagonists of the EP3 receptor (L-798106) (10 mg/kg, i.p. every 3rd day) decreased the NE-induced increase in SBP (130±5 vs. 148±3 mmHg, $P<0.05$, n=5/group). These data suggest that $cPLA_2\alpha$ contributes to NE-induced increase in SBP via $cPLA_2\alpha$ activation, the release of AA and generation of eicosanoids, most likely PGE2 that exerts pro-hypertensive effects by stimulating EP3 receptors. Therefore, the development of agents that selectively inhibit the $cPLA_2\alpha$ activity or block EP3 receptors could be useful in treating hypertension and its pathogenesis.

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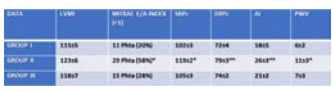
P474

Comparison of the Effect of Different Calcium Channel Antagonists on Arterial Stiffness and Central Pressures in Moderate Hypertensive Patients Receiving Enalapril as First Line Treatment

PrimaryAuthor.AuthorBlock:**Ricardo Cabrera-Sole**, Caridad Turpin Lucas, Liliana Rivera Urrera, Santiago Garcia Ruiz, Manuel Aguilera Saldaña, Univ General Hosp of Albacete, Albacete, Spain

INTRODUCTION:ACE inhibitors reduce blood pressure (BP)satisfactorily, with enalapril (ENL) being one of the most used in daily clinical practice. However, little is known about its effects on arterial stiffness (AS) and central BP when associated with calcium-antagonists (CCA). So we studied three CCA associated with ACE inhibitors. OBJECTIVES: To evaluate the effects of three CCA: amlodipine (AML), nifedipine (NFD)and barnidipine (BND) associated with ENL, on AS, central systolic and diastolic BP: (SBPc, DBPc),diastolic heart function in moderate hypertensive patients (Phta) . MATERIAL AND METHODS: We studied 156 Phta males (68 ± 6 years old) who were treated by their primary care physician with ENL and AML,NFD OR BND. They were grouped into three groups: Group I: 53 Phta receiving ENL +AML, Group II: 50 Phta receiving ENL+ NFD and 53 Phta treated with ENL + BND to maintain BP under 140/90 mmHg. In all, an echocardiogram was performed to measure the Left ventricular mass index (LVMI), the E / A mitral flow index . We measured with 24-hour ABPM , AS by augmentation index (AI)and pulse wave velocity (PWV), and, central BP. The results were compared and presented in Tables I:



* =p value ≤0.05

CONCLUSIONS: according to our data, it is preferable to use AML as a CCA,because it not only reduces BP better but also the central BP, improving AS in small and large vessels. The BND would be the other choice, since it does not present significant differences with respect to the first group. NFD , in addition to, not demonstrating superiority, also does not reduce DBPc in these Phta. Therefore, we consider that the presented data have sufficient clinical relevance to choose CCA associated with an ACE inhibitor.

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What's in a Letter: N vs. J Determines Responses to Ang II/DOCA and DPP4 Gene Deletion

PrimaryAuthor.AuthorBlock:Jianzhong An, Alex Meuth, Shawn Bender, Annayya Aroor, **Ravi Nistala**, Univ Missouri Columbia, Columbia, MO

Activation of the renin-angiotensin-aldosterone system (RAAS) drives blood pressure (BP) responses and kidney injury in humans and rodent models. Previously, we reported activation of dipeptidyl peptidase 4 (DPP4) by RAAS which may play an important role in BP responses and kidney injury. Classically, either angiotensin II (Ang II) or deoxycorticosterone (DOCA)-salt administration to mice, raises their BP over a period of hours to days. Recently, the two have been combined to elicit more robust kidney injury and proteinuria. Although there

are not many head to head comparison studies, it is known through historical association that different strains of mice have different baseline BPs and vary in their response to vasoconstrictor agents for BP peaks as well as associated kidney injury. In this regard, the C57Bl/6J and C57Bl/6N mice are genetically very similar although they differ with respect to their responses to circadian rhythm on salt sensitivity to BP. Therefore, we hypothesized that J versus N strain will have differential BP responses to Ang II/DOCA and kidney injury susceptibility. C57Bl/6 J and N mice (*DPP4*$^{+/+}$ and *DPP4*$^{-/-}$) were subjected to Ang II/DOCA salt infusion for 2 weeks (Ang II 1000ng/kg/min via miniosmotic pumps, DOCA 50mg pellet, 0.9% saline in drinking water) and compared to mice receiving saline only (osmotic pumps). BP was measured by Millar catheter. Kidney injury was quantified via albuminuria and histology. While the Ang II/DOCA treated J strain had mean BP (MBP) of 135mmHg, the Ang II/DOCA treated N strain had MBP of 125mmHg. There was no effect of *DPP4* gene deletion on BP in either strain. The J strain treated with Ang II/DOCA salt had higher albuminuria when compared to the N strain (180ug/ml vs. 60ug/ml). *DPP4*$^{-/-}$ mice on the J strain receiving Ang II/DOCA had a relative improvement in albuminuria (120ug/ml vs. 180ug/ml). Surprisingly, the *DPP4*$^{-/-}$ mice on the N strain had higher albuminuria when compared to the *DPP4*$^{+/+}$ mice receiving Ang II/DOCA (143ug/ml vs. 60ug/ml). Taken together, our results suggest that mice strain plays an important role in BP and kidney injury responses to Ang II/DOCA and *DPP4* gene deletion. These results highlight the importance of selecting patient populations carefully to maximize the benefit of DPP4 inhibitors.

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8-Aminoguanosine Exerts Diuretic and Natriuretic Activity via Conversion to 8-Aminoguanine

PrimaryAuthor.AuthorBlock:**Edwin K Jackson**, Zaichuan Mi, Univ of Pittsburgh Sch Med, Pittsburgh, PA

We previously reported that 8-aminoguanosine and 8-aminoguanine are potent and efficacious K^+-sparing diuretics/natriuretics that may represent a new class of antihypertensive drugs. Moreover, because these compounds are endogenous, they may have physiological roles. It is possible that the diuretic/natriuretic activity of 8-aminoguanosine is mediated mostly via conversion to 8-aminoguanine. To test this concept, we conducted 3 protocols in anesthetized rats. The 1st protocol demonstrated that at 85 to 115 min post intravenous administration, both 8-aminoguanosine and 8-aminoguanine (33.5 μmol/kg) significantly increased urine volume [ml/min: 8-aminoguanosine from 0.3 ± 0.1 to 0.9 ± 0.1 (mean ± SEM; n=7); 8-aminoguanine from 0.3 ± 0.1 to 1.5 ± 0.2 (n=8)] and sodium excretion (μmol/min: 8-aminoguanosine from 12 ± 6 to 109 ± 21; 8-aminoguanine from 18 ± 8 to 216 ± 31). The 2nd protocol showed that intrarenal artery infusions of 8-aminoguanosine (from 0.1 to 1 μmol/kg/min) did not affect urine volume or sodium excretion in either the ipsilateral or contralateral kidney. In contrast, intrarenal artery infusions of 8-aminoguanine

significantly increased ipsilateral (but not contralateral) urine volume [at 1 µmol/kg/min from 0.2 ± 0.02 to 0.7 ± 0.1 (n=17)] and sodium excretion (from 24 ± 4 to 216 ± 31). In a 3rd protocol we administered 8-aminoguanosine and 8-aminoguanine intravenously (33.5 µmol/kg) and measured renal interstitial (medulla) levels of 8-aminoguanosine and 8-aminoguanine using microdialysis combined with ultraperformance liquid chromatography-tandem mass spectrometry. Intravenous administration of 8-aminoguanosine and 8-aminoguanine similarly increased renal interstitial levels of 8-aminoguanine [ng/ml; 8-aminoguanosine from 4 ± 1 to 1025 ± 393 (n=6), and 8-aminoguanine from 2 ± 1 to 1069 ± 407 (n=6)]. Neither 8-aminoguanosine nor 8-aminoguanine affected renal interstitial levels of 8-aminoguanosine. Together these data clearly show that the renal effects of 8-aminoguanosine are not direct, but require conversion in the systemic circulation to 8-aminoguanine. If 8-aminoguanosine is physiologically important it should be viewed as a "pro-hormone." As a pharmacological agent, it is best described as a "pro-drug."

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P477

Effect of Zamicastat on Blood Pressure and Heart Rate - Comparison Between Acute and Repeated Dosing in the SHR

PrimaryAuthor.AuthorBlock:Bruno Igreja, Nuno Pires, Paul Moser, **Patricio Soares-da-Silva**, Bial, Trofa, Portugal

The sympathetic nervous system (SNS) can alter blood pressure by modulation of cardiac output, peripheral vascular resistance and renal function. One strategy for the modulation of sympathetic nerve function is to reduce the biosynthesis of norepinephrine (NE) via inhibition of dopamine β-hydroxylase (DβH), the enzyme that catalyses the conversion of dopamine to NE in sympathetic nerves. Zamicastat is a new peripheral DβH inhibitor that decreases NE levels in sympathetically innervated tissues and slows down the drive of SNS. In order to compare the effect of zamicastat on blood pressure and heart rate after acute and repeated dosing in spontaneously hypertensive rats (SHR), male adult SHR were implanted with telemetry devices (TA11PA-C40, DSI). Zamicastat (30 mg/kg p.o.) was given either as a single dose (n=8) or once a day for five days (n=6). On day 1, the maximal effect (E_{max}) of zamicastat on systolic blood pressure (SBP) was attained 15h after dosing. E_{max} was similar in the acute and repeated dosing groups: -21.1±3.9 and -22.0±8.2 mmHg, respectively. In the acute treatment group, a gradual return of the SBP to the high baseline levels was observed over time. On the other hand, in the repeat dosing group the E_{max} were consistent across the five administrations -22.0±8.2, -25.2±7.9, -22.0±7.5, -20.0±6.0 and -16.1±2.8 mmHg, respectively day 1 to 5. On day 5, after reaching E_{max} a gradual return of SBP to baseline levels was also observed. The effect of zamicastat on diastolic blood pressure was similar with an E_{max} of -18.4±3.5 mmHg in the acute dosing group and -18.8±8.7, -19.2±7.2, -18.2±6.6, -19.6±6.6 and -15.7±4.7 mmHg over the five days of administration in the repeated dosing group. Zamicastat did not affect heart rate under each dosing regimen. In conclusion, there is no tachyphylaxis or sensitization to the cardiovascular effects of zamicastat in this model and blood pressure returns to basal

levels after zamicastat treatment discontinuation.

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Barriers to Health Care Access and Hypertension Treatment Among Us Adults With Self-reported Hypertension

PrimaryAuthor.AuthorBlock:**Jing Fang**, Guijing Wang, Carma Ayala, Sal Lucido, Fleetwood Loustatol, CDC, Atlanta, GA

Background: Hypertension is a major risk factor for heart disease and stroke. Previous studies have shown that access to health care affects hypertension management. The objective of this study was to examine the association between different measures of barriers to health care access and hypertension treatment among US adults with self-reported hypertension. **Methods:** Participants from the National Health Interview Survey in 2014 and 2015, who reported a diagnosis of hypertension were included in the study. Six indicators of barriers to health care access were assessed: 1) no place to see a doctor when needed, 2) cannot afford prescribed medicine, 3) did not see doctor in past 12 months, 4) delayed care due to cost, 5) did not get care due to cost and 6) no health insurance. We compared age-standardized percentages of antihypertension medication use among those with/without barriers to health care access and the odds ratios of using medication after adjusting for age, sex, race/ethnicity, and education. **Results:** Among 21,050 participants with self-reported hypertension (26.7%), 83.2% reported antihypertensive medication use. Antihypertensive medication use was significantly lower among those with barriers to health care access compared to those without (Table). Logistic regression models showed that those with barriers to health care access were significantly less likely to report medication use than those without (Table). **Conclusion**: Although antihypertensive medication use is common among those with hypertension, this study found lower levels of reported antihypertensive medication use among those with perceived barriers to health care access.

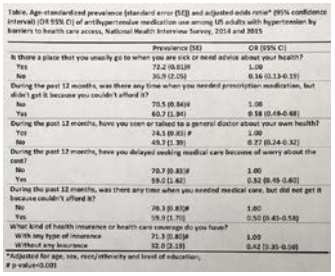

Table. Age-standardized prevalence (standard error (SE)) and adjusted odds ratio* (95% confidence interval) (OR 95% CI) of antihypertensive medication use among US adults with hypertension by barriers to health care access, National Health Interview Survey, 2014 and 2015

	Prevalence (SE)	OR (95% CI)
Is there a place that you usually go to when you are sick or need advice about your health?		
Yes	72.2 (0.61)#	1.00
No	35.9 (2.05)	0.16 (0.13-0.19)
During the past 12 months, was there any time when you needed prescription medication, but didn't get it because you couldn't afford it?		
No	70.5 (0.84)#	1.00
Yes	60.7 (1.84)	0.58 (0.49-0.68)
During the past 12 months, have you seen or talked to a general doctor about your own health?		
Yes	74.1 (0.83) #	1.00
No	49.7 (1.39)	0.27 (0.24-0.32)
During the past 12 months, have you delayed seeking medical care because of worry about the cost?		
No	70.7 (0.83)#	1.00
Yes	59.0 (1.62)	0.52 (0.45-0.60)
During the past 12 months, was there any time when you needed medical care, but did not get it because couldn't afford it?		
No	70.3 (0.83)#	1.00
Yes	59.9 (1.75)	0.50 (0.43-0.58)
What kind of health insurance or health care coverage do you have?		
With any type of insurance	71.3 (0.80)#	1.00
Without any insurance	32.0 (2.19)	0.42 (0.35-0.50)

*Adjusted for age, sex, race/ethnicity and level of education;
p-value<0.001

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P479

Stability of Ambulatory Blood Pressure Patterns Over Time in Children and Adolescents

PrimaryAuthor.AuthorBlock:**Coral D Hanevold**, Univ of Washington Sch of Med, Seattle, WA; Yosuke Miyashita, Univ of Pittsburgh Sch of Med, Pittsburgh, PA; Joseph T. Flynn, Univ of Washington Sch of Med, Seattle, WA

Little is known about the stability of ambulatory blood pressure (ABP) patterns in children and adolescents undergoing evaluation for high blood pressure (HBP). It is possible that children with initially normal ABP may progress to hypertension (HT) or pre-hypertension (PHT), or that those with initial PHT or HT may be normal on repeat. Our objective was to assess stability of ABP patterns over time in children with HBP.
We analyzed changes in ABP classification in patients undergoing a minimum of 2 ABP monitoring (ABPM) studies at least 0.5 years (yrs) apart. Exclusion criteria included known secondary HT, therapy with antihypertensive medication and inadequate recordings. ABPM were interpreted according to the 2014 AHA guidelines using BP thresholds of 95th% for sex and height for children ≤17 yrs. For those ≥18 yrs awake and sleep thresholds were 140/85 and 120/70, respectively. Dipping was considered normal if ≥ 10%, blunted if <10% and reversed if < 0%. For those with > 2 ABPM the difference between the 1st and last were analyzed.
Two hundred ABPM on 100 patients (76 males; median age 14.6 yrs at 1st ABPM) were analyzed. Median interval between ABPM was 1.5 yrs. ABP classification was stable in 53% (53/100). As shown in the table 50% (9/18) of those with normal ABPM showed progression to PHT or HT on follow up. PreHT progressed in 31% (8/26) and improved in 38% (10/26). HT improved in 43% (20/46). Dipping designation was stable in 70% (70/100); but blunted dipping normalized in 48% (10/21).
In our population ABP patterns were not stable over time, supporting the need for follow up studies even with normal initial ABPM. If confirmed in larger studies, these findings support greater use of repeat ABPM.

		ABPM#2				
		Normal	PreHT	HT	Total	% Progressed
ABPM#1	Normal	9	4	5	18	50 (9/18)
	PreHT	10	18	8	26	31 (8/26)
	HT	3	17	26	46	N/A
	Total	22	39	39	100	

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Effects Of Long And Intermediate Acting Dihydropyridine Calcium Channel Blockers In Hypertension: A Systematic Review And Aeta-analysis Of 18 Prospective, Actively Controlled, Randomized Clinical Trials

PrimaryAuthor.AuthorBlock:**Sandip Chaugai**, Vanderbilt Univ, Nashville, TN; Hisatomi Arima, Fukoka Univ, Fukoka, Japan; Lhamo Yangchen Sherpa, Univ Of Oslo, Oslo, Norway; Amir Sepehry, Univ of British Columbia, Vancouver, BC, Canada

Background: Dihydropyridine calcium channel blockers are a heterogeneous group of antihypertensive drugs. Long acting dihydropyridine agent Amlodipine is widely used for mono and combination therapy for hypertension in clinical practice, while intermediate acting dihydropyridine agents such as Felodipine, Nifedipine-GITS etc. have shown inconsistent results in randomized clinical trials (RCTs).
Methods and results: A meta-analysis of 18 prospective, actively controlled RCTs enrolling a total of 80,069 hypertensive patients followed for a mean of 51.7 months was performed. Amlodipine therapy was associated with 24% higher risk of heart failure (RR: 1.24, [95%CI: 1.04 to 1.48], p= 0.019) but 16% lower risk of stroke (RR: 0.84, [95%CI: 0.73 to 0.95], p=0.009) without statistically significant effect on acute myocardial infarction (AMI) (RR: 0.90, [95%CI: 0.78 to 1.03], p=0.128) or cardiovascular event (CVE) (RR: 0.99, [95%CI: 0.93 to 1.06], p=0.814). Intermediate acting dihydropyridine calcium channel blocker therapy was associated with 24% higher risk of heart failure (RR: 1.24, [95%CI: 1.07 to 1.44], p=0.005), 27% higher risk

of AMI (RR: 1.27, [95%CI: 1.04 to 1.55], p=0.019), 14% higher risk of CVE (RR: 1.14, [95%CI: 1.01 to 1.29], p=0.034) without statistically significant effect against stroke (RR: 1.03, [95%CI: 0.87 to 1.21], p=0.580). Amlodipine showed higher risk of heart failure (RR: 1.20, [95%CI: 1.00 to 1.43], p=0.045) and lower risk of stroke (RR; 0.85, [95%CI: 0.75 to 0.96], p= 0.007) compared to Renin-angiotensin-system (RAS) blockers. Intermediate acting dihydropyridine calcium channel blocker showed higher risk of heart failure (RR: 1.31, [95%CI: 1.09 to 1.57], p=0.004) and AMI (RR: 1.46, [95%CI: 1.00 to 2.14], p=0.050) compared to RAS blockers. Multivariate meta-regression suggested that the effect size may differ between intermediate and long acting agents for stroke (B: -0.27, [95%CI: -0.50 to -0.05], p=0.012) and AMI (B: -0.42, [95%CI: -0.69 to -0.16], p=0.002) after adjustment for baseline presence of diabetes. **Conclusions:** This study suggests that Amlodipine offers greater protection against major complications of hypertension compared to intermediate acting dihydropyridine calcium channel blockers.

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P481

Key Findings From Multilevel Stakeholder Input On Awareness, Gaps And Needs Related To The Burden Of Hypertension

PrimaryAuthor.AuthorBlock:**Lakshmi Venkitachalam**, UMKC SCHOOL OF MEDICINE, Kansas City, MO; Tru-Kechia Smith, American Heart Association, Kansas City, KS

Background: Hypertension (HTN) continues to pose a considerable challenge to the health system. Suboptimal rates of screening, awareness, treatment and control, reported at national and local levels, reflect an urgent need for a coordinated and collective approach to action. **Methods:** On May 20, 2017 (World HTN day), we convened a one-day HTN summit in response to an expressed need from stakeholders across the health system (healthcare, public health, community organizations) in the Bi-State Greater Kansas City Area. To help formulate an action plan, we administered a pre-and post-survey to document stakeholder engagement in HTN programs, their awareness of existing key initiates and resources, and perceived needs to address HTN. **Results:** Overall, 24 individuals from 16 organizations registered for the event and completed a pre-event survey - healthcare system (46%), Public health or Government organizations (17%), Community organizations (29%), Academic entity (8.3). Thirteen (54%) attendees completed the post-event survey - 29% delivered healthcare services, 57% conducted community activities, 21% were patients or caregivers. Only 32% of the attendees reported a current program on blood pressure. Less than 10% of participants reported satisfaction with the availability of regularly scheduled learning opportunities or tools for healthcare professionals or patients to improve HTN. Only 21% of participants reported monitoring of performance on metrics related to high blood pressure programs. All attendees expressed interest in continued meetings, patient engagement resources, and an in-depth exploration of the underlying factors contributing to the burden of uncontrolled HTN. Findings also reflected a significant need to improve the awareness of resources like the Missouri Million Hearts initiative and Target BP™. **Conclusion:** In spite of temporal advancements in cardiovascular medicine, there exist significant gaps in the awareness and use

of resources and tools to support HTN management among stakeholders in the health system. Planned strategies include a dedicated working group to proactively identify and support coordinated, multi-stakeholder efforts to reduce the HTN burden and improve cardiovascular health in our communities.

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Different Hemodynamic Phenotypes Of Isolated Systolic Hypertension In Adolescents

PrimaryAuthor.AuthorBlock:**Lukasz Obrycki**, Anna Niemirska, Dept of Nephrology, Kidney Transplantation and Hypertension, Children`s Memorial Health Inst, Warsaw, Poland; Jedrzej Sarnecki, Dept of Radiology, Children`s Memorial Health Inst, Warsaw, Poland; Zbigniew Kulaga, Dept of Public Health, Children`s Memorial Health Inst, Warsaw, Poland; Mieczyslaw Litwin, Dept of Nephrology, Kidney Transplantation and Hypertension, Children`s Memorial Health Inst, Warsaw, Poland

Objective
Isolated systolic hypertension (ISH) is a dominant form of primary hypertension (PH) in adolescents. Some of them present with normal central systolic blood pressure (cSBP), a phenomenon called spurious hypertension (sHT). The study was aimed to describe hemodynamics of PH in relation to cSBP, central pulse pressure (cPP) and target organ damage (TOD) in adolescents referred because of PH.
Patients and methods:
In 267 children (59 girls; 14.9 ±2.6 years) referred with arterial hypertension, in whom secondary hypertension was excluded, 24 hour ABPM, left ventricular mass index (LVMi), carotid intima-media thickness (cIMT), pulse wave velocity (PWV), cSBP, cPP, cardiac index (CI) and stroke volume (SV) was assessed. 64 age and sex matched normotensive control children were control group.
Results
145 subjects had white coat hypertension (WCH) including 24 with ambulatory prehypertension (ambpreHT). Of 122 hypertensive pts, 39 had ambulatory hypertension (ambHT) and 83 severe ambulatory hypertension (severeHT). Normal cSBP was found in all WCH subject and 23 with ambpreHT. 39 of 122 (32%) hypertensive pts had sHT - 47.4% in those with ambHT and 26.5% with severeHT ($p=0.0001$). cIMT, LVMi, PWV, cSBP and cPP increased across blood pressure strata from normotension, through sHT to PH with elevated cSBP (all $p<0.05$). LVMi and cIMT correlated with cSBP ($r = 0.220$; $p = 0.0007$; $r = 0.14$; $p = 0.04$, respectively) and cPP ($r = 0.274$; $p = 0.0001$; $r=0.202$; $p=0.002$, respectively). 36 pts with left ventricular hypertrophy (LVH) had greater cPP (52 ±10 mmHg) in comparison with subjects without LVH (47 ±8 mmHg; $p = 0.027$). Regression analysis revealed cPP as the only predictor of LVMi ($r^2 = 0.09$, $\beta = 0.143$, $p = 0.03$). ROC area for predictors of LVH revealed similar area under curve for cSBP (0.585), cPP (0.618) and 24h systolic ABPM (0.612). Patients with sHT had greater amplification of pulse pressure than normotensive ones. CI and SV was lowest in normotensive controls, intermediate in sHT patients and highest in patients with elevated cSBP ($p<0.05$).
Conclusions
sHT present with intermediate hemodynamic phenotype between normotension and sustained PH. cSBP and cPP differentiates patients with severeHT and TOD from patients with WCH, ambpreHT and ambHT without TOD

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P483

Healing a Broken Heart Using a Closed-loop, Allostatic, Neurotechnology: A Case Study in a Patient Suffering From Takotsubo Syndrome

PrimaryAuthor.AuthorBlock:Charles H Tegeler, Catherine L Tegeler, **Hossam A Shaltout**, Wake Forest Univ, Winston Salem, NC

Background: Stress cardiomyopathy or Takotsubo syndrome (TS) is an acute, reversible disorder of the heart characterized by left ventricular dysfunction, usually triggered by a stressful event. Excessive sympathetic excitation and shift in symapthovagal balance are proposed as mechanisms. High-resolution, relational, resonance-based, electroencephalic mirroring (HIRREM®) is a noninvasive, closed-loop, allostatic, neurotechnology using software algorithms to identify specific brain frequencies, translating them in real time into audible tones, to support self-optimization of brain rhythms and improve autonomic balance. Objective: To evaluate benefits of HIRREM on autonomic function and symptom reduction in a 55 year old female enrolled in an IRB-approved open label feasibility study, after TS diagnosis. Results: The participant received 13 HIRREM sessions over 11 days (9 in office days). Data were collected before, and 21 days after HIRREM completion. Baseline brain patterns had prominent right dominance at temporal lobes (sympathetic pattern), which balanced by the end of the sessions. Cardiovascular autonomic balance also shifted away from sympathetic towards parasympathetic. This was seen as reduced LF/HF ratio (from 1.89 to 0.63), increased heart rate variability (rMSSD from 27 to 40.8 ms), and baroreflex sensitivity (from 11.8 to 24.4 ms/mmHg). Blood pressure dropped from 132/90 to 121/88 with no change in heart rate despite discontinuation of her ACE inhibitor medication due to her BP being “too low.” HIRREM use was also associated with clinically meaningful improvements in multiple symptom inventories including insomnia (ISI) from 15 to 6, depression (CES-D) from 16 to 2, anxiety (GAD-7) from 18 to 2, and perceived stress scale (PSS) from 30 to 14. The patient reported resolution of a feeling of heaviness and discomfort in the chest after starting HIRREM sessions. Conclusion: These data provide the first report of potential cardiovascular benefits of a non-pharmacological therapy to patients suffering from broken heart syndrome.

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Improved Heart Rate Variability, and Symptoms of Insomnia and Stress, With Use of a Closed-loop, Allostatic Neurotechnology in Law Enforcement Officers

PrimaryAuthor.AuthorBlock:Catherine L Tegeler, **Hossam A Shaltout**, Lindsay J Howard, Krystal D Schmidt, Emillee I Garlin, Charles H Tegeler, Wake Forest Sch Univ, Winston Salem, NC

Background: Law enforcement officers have decreased life expectancy, attributed to work-related exposure to traumatic stress and circadian disruption. Autonomic dysregulation is

reported with traumatic stress, and chronic insomnia. Closed-loop therapies with real time monitoring for modulation of biological function offer a precision-guided, patient-centric strategy for brain-based therapies. High-resolution, relational, resonance-based, electroencephalic mirroring (HIRREM®) is a noninvasive, closed-loop, allostatic, acoustic stimulation neurotechnology using software generated algorithms to identify specific brain frequencies, and translate them in real time into audible tones, to support self-optimization of brain rhythms. Objective: To evaluate benefits for autonomic function, and symptoms of insomnia and stress, in a relatively healthy cohort of law enforcement officers, who enrolled in an IRB-approved, open label feasibility study evaluating HIRREM for diverse neuropsychological disorders. Measures done before and after HIRREM included symptom inventories for insomnia (ISI), depression (CES-D), traumatic stress (PCL-C), anxiety (GAD-7), and perceived stress (PSS). Ten minute recordings of heart rate and blood pressure allowed analysis of baroreflex sensitivity (BRS) and heart rate variability (HRV). Results: 7 participants (1 female), mean (SD) age 47 (4.5), received 10.7 (2.6) HIRREM sessions over 11.3 (4.6) days (7.3 in office days). Data were collected before, and 22.6 (1.8) days after HIRREM completion. Use of HIRREM was associated with significantly increased HRV measured as rMSSD [from 25.3 (7.0) to 43.1 (13.0) ms, p=0.02]. BRS measured by high frequency alpha index improved [from 11.3 (8.0) to 20.1 (11.0) ms/mmHg, p=0.03]. All symptom inventories improved significantly ($p<0.05$), even with the small cohort. There were no adverse events or drop outs. Conclusion: These pilot data provide the first report of significant autonomic cardiovascular benefits, and associated symptom improvements, with use of a closed-loop, allostatic therapy for a cohort of sworn law enforcement officers. Further studies are warranted to test the efficacy of this technology in a larger law enforcement cohort

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P485

Use of a Noninvasive, Closed-loop, Allostatic, Neurotechnology Reduced Blood Pressure and Improved Heart Rate Variability in a Pre-hypertensive Cohort

PrimaryAuthor.AuthorBlock:**Hossam A Shaltout**, Catherine L Tegeler, Charles H Tegeler, Wake Forest Univ, Winston Salem, NC

Background: Prehypertension increases risk for hypertension and cardiovascular disease, but effective interventions have not been defined. Disturbed central control of cardiovascular regulation due to trauma, stress, anxiety or other causes can lead to prehypertension and impaired heart rate variability (HRV). High-resolution, relational, resonance-based, electroencephalic mirroring (HIRREM®) is a noninvasive, closed-loop, allostatic, acoustic stimulation neurotechnology using software algorithms to identify specific brain frequencies, and translate them in real time into audible tones, to support self-optimization of brain rhythms. Objective: To evaluate the benefits of this nontraditional therapy on BP and autonomic function, subjects with untreated systolic BP of 120 to 139 or diastolic BP of 80 to 90 mmHg at baseline, who had enrolled in an IRB-approved open label feasibility study evaluating HIRREM for diverse neuropsychological disorders.Results: 66 participants (40 female), mean (SD) age 43.3 (16.5), received 16 (5.7) HIRREM sessions over

22.4 (19.2) days, (9.5 (4.2) days with sessions). Data were collected before, and 14.4 (16.6) days after HIRREM completion. Use of HIRREM was associated with significantly reduced systolic (from 127.5 (8.0) to 122.9 (14.0) mmHg, p=0.011), and diastolic (from 82.0 (8.0) to 78.0 (9.0) mmHg, p= 0.014) arterial pressure, with no change in heart rate. HRV measured as SDNN increased (from 42.0 (17.0) to 50.0 (28.0) ms, p=0.002). Baroreflex sensitivity measured by sequence method improved (from 13.2 (8.0) to 17.2 (12.0) ms/mmHg, p=0.0001), with a trend for reduced sympathovagal tone measured by LF/HF ratio (from 2.5 (2.2) to 2.0 (1.8), p=0.068). There were no adverse events.
Conclusion: These data provide the first report of significant cardiovascular benefits of a closed-loop allostatic therapy for prehypertension. Blood pressure reduction and HRV improvement may prevent progression to more serious cardiovascular symptoms and diseases. Further studies are indicated to investigate the mechanism of the benefits associated with this promising intervention.

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P486

Effects of Isometric Leg Training on Ambulatory Blood Pressure and Morning Blood Pressure Surge in Young Normotensive Men and Women

PrimaryAuthor.AuthorBlock:**Anthony W Baross**, Univ of Northampton, Northampton, United Kingdom; Kevin J Milne, Cheri L McGowan, Univ of Windsor, Windsor, ON, Canada; Ian L Swaine, Univ of Greenwich, Northampton, United Kingdom

Introduction
Cardiovascular disease (CVD) is a major cause of death globally with hypertension reported to be a leading modifiable risk factor. Ambulatory blood pressure (BP), in particular diurnal BP variability, is considered to be associated with CVD risk. In addition, the morning BP surge (MBPS) is thought to be associated with increased stroke risk and to be a destabilizing factor for atherosclerotic plaque. Isometric resistance training (IRT) is an effective method of lowering BP and has been recommended by the American Heart Association as an alternative treatment for reducing BP. To date, few studies have investigated the effects of IRT on ambulatory BP and particularly the morning surge in BP. Therefore, the purpose of this study was to determine whether (i) IRT causes reductions in ambulatory BP and the MBPS, in young normotensives and (ii) there are any sex differences in these changes.
Methods
Ambulatory BP was measured prior to, and after, 10 weeks of bilateral leg IRT using an isokinetic dynamometer (4 x 2 minute contractions at 20% MVC, with 2 minute rest periods on 3 days per week). Twenty normotensive individuals (10 men, age=21 ± 4 years; 10 women, age=23 ± 5 years) were recruited. A two-way repeated measures ANOVA was used to assess the within and between groups ambulatory (mean 24-h, daytime, night time and diurnal variation) BP and MBPS. MBPS was calculated as: mean systolic BP 2 hours after waking minus the lowest sleeping 1 hour mean systolic BP.
Results
There were significant reductions in 24-h ambulatory (4 ± 2 mmHg, p=0.0001; 4 ± 2 mmHg, p=0.0001) systolic BP in both men and women following IRT. This comprised significant reductions in day time (5 ± 5 mmHg, p=0.019; 5 ± 4 mmHg, p=0.002) but not night time (1 ± 5 mmHg, p=0.75; 1 ± 3 mmHg, p=0.3) systolic BP. Additionally, there were significant reductions

in the MBPS (6 ± 8 mmHg, p=0.044; 6 ± 7 mmHg, p=0.019). There were no significant differences between men and women in these changes (p>0.05).

Conclusion

These results support previous research showing that IRT is effective in lowering ambulatory BP. Furthermore, the significant reductions in the MBPS offer the potential for clinically meaningful CVD and stroke risk reduction, provided these effects can be demonstrated in those who are at risk.

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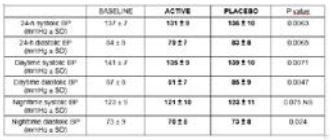
P488

Antihypertensive Effects of Lactotripeptides on Unattended Office and Abulatory Blood Pressure. A Randomized, Double-blind, Placebo-controlled Study

PrimaryAuthor.AuthorBlock:**Giuseppe Crippa**, Hypertension Unit, Guglielmo da Saliceto Hosp, Piacenza, Italy; Filippo Rossi, Food Sciences and Nutrition Inst, Catholic Univ, Piacenza, Italy; Elena Bravi, Manuela Fontana, Dorjan Zabzuni, Hypertension Unit, Guglielmo da Saliceto Hosp, Piacenza, Italy

Milk fermentation by Lactobacillus helveticus produces some tripeptides (VPP and IVP) with sustained ACE-inhibitor activity that have shown to lower blood pressure (BP) in experimental animals and in humans. Grana Padano DOP, an Italian cheese, has shown a potent in vitro ACE-inhibitory effect due to its high concentration of such tripeptides. Present data refer to a randomized, double-blind, placebo controlled, study in which 30 mild-moderate hypertensive patients received a dietary integration with Grana Padano DOP. At base-line all patients had BP not on target (>140 and/or 90 mmHg) after their usual treatment and received a dietary integration with Grana Padano (1 ounce per day) and placebo, in cross over fashion.BP was evaluated at baseline and at the end of the active and placebo treatment (2 months each) by Office and unattended Office BP (using the BpTRU, average of 5 consecutive BP readings) and by ambulatory BP monitoring (ABPM). Results: dietary integration with Grana Padano resulted in a significant decreased in systolic and diastolic office and unattended office BP (-6 mmHg for systolic and - 5 mmHg for diastolic BP). ABPM confirmed a sustained antihypertensive effect of such integration (see table).



	BASELINE	ACTIVE	PLACEBO	P value
24-h systolic BP (mmHg ± SD)	137 ± 7	131 ± 9	136 ± 10	0.0063
24-h diastolic BP (mmHg ± SD)	84 ± 9	79 ± 7	83 ± 8	0.0065
Daytime systolic BP (mmHg ± SD)	141 ± 7	135 ± 9	139 ± 10	0.0071
Daytime diastolic BP (mmHg ± SD)	87 ± 9	81 ± 7	85 ± 9	0.0047
Nighttime systolic BP (mmHg ± SD)	123 ± 9	121 ± 10	122 ± 11	0.075 NS
Nighttime diastolic BP (mmHg ± SD)	73 ± 9	70 ± 8	73 ± 8	0.024

In conclusion, the diaily integration with 1 ounce of Grana Padano DOP cheese caused a significant decrease in systolic and diastolic BP pressure in hypertensive patients. No changes in BMI, total and HDL cholesterol, triglycerides, glucose, serum sodium and potassium levels were observed. The statistically significant drop in BP may be considered also clinically significant since the majority (67%) of the patients reached normal BP levels at the end of active treatment.

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P489

Effect of Continuous Intake of Hesperidin on Blood Pressure in 2-kidney, 1-clip Renovasucular Hypertensive Rats

PrimaryAuthor.AuthorBlock:Ayuna Yamaoka, Yukiko Segawa, Saki Maruyama, Natsumi Saito, Kobe Women's Univ, Kobe, Japan; Hiroko Hashimoto, Osaka Seikei Junior Coll, Osaka, Japan; Tomoko Osera, **Nobutaka Kurihara**, Kobe Women's Univ, Kobe, Japan

Objective: Hesperidin (HES) is a flavonoid which is contained in citrus fruit peel. It has physiological effects on blood vessels such as strengthening capillary vessels. Thus, it is known to be one of the effective ingredients of herbal medicine. Some studies have shown that the intake of HES decreases blood pressure (BP) in spontaneously hypertensive rats. The antihypertensive effect of HES is suggested to be due to vasodilation by nitric oxide (NO). However, its mechanism has not been clarified in detail. In this study, we observed whether HES intake decreases BP in 2-kidney, 1-clip renovasucular hypertensive rats (2K1C) and evaluated endothelial NO synthase (eNOS) mRNA to investigate its role in the mechanism. Methods: Male Sprague-Dawley rats (6 weeks old) were treated with sham operation (SHAM) or clipping the left renal artery (2K1C). After surgery, the rats started receiving continuously a control diet (C) or a diet containing 0.1% (w/w) HES for 6 weeks. The systolic BP (SBP) was measured by a tail-cuff method every week. At the end of the protocol, mean arterial blood pressure (MAP) was measured in each rat under anesthesia. Then, the aortas were removed for extracting mRNA. eNOS mRNA expression was evaluated using real-time RT-PCR. Results: At the end of the protocol, SBP in 2K1C-C was significantly higher than in SHAM-C (170±6 vs 117±6 mmHg, $p<0.001$). On the other hand, 2K1C-HES was lower in SBP (141±4 mmHg) than 2K1C-C ($p<0.01$). There were no significant differences between SHAM-HES (122±7 mmHg) and SHAM-C. MAP at the end of the protocol were similar to in SBP. ANOVA revealed mRNA expression of eNOS was significantly higher in 2K1C than in SHAM ($p<0.05$), and showed no significant difference between C and HES, nor a significant interaction. Conclusion: Continuous intake of HES may suppress BP increase in 2K1C. The role of eNOS mRNA expression may not be involved in the mechanism.

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P490

Dream-Global Study

PrimaryAuthor.AuthorBlock:**Sheldon W Tobe**, Sunnybrook Health Sciences Ctr, Toronto, ON, Canada

Introduction. The DreamGlobal study incorporates mobile health innovations in technology with SMS text messaging and task shifting of blood pressure measurement. Methods. In this pragmatic RCT study

participants with uncontrolled hypertension on or off of medication, received culturally competent text messages over a one year period. All participants lived on First Nations reserves in remote and rural parts of Canada. Initial blood pressure screening was with an automated oscillometric device. Community Health Workers and nurses were trained to measure blood pressure with an approved oscillometric device which was Bluetooth enabled. Once participants were registered online, they received their blood pressure results on their own mobile phone as a text message and guidelines based text messages. Their primary health care provider also received the results as a fax. They were randomized to receive health behaviour change messages alone or active messages including a recommendation to see their health care provider if their blood pressure was above target. The baseline blood pressure was the mean of all readings in the first two months and the final blood pressure, the mean of the last two months. The main outcomes were change in blood pressure between the two groups (active or passive text messages) and the proportion achieving control. Results. One hundred twenty-seven subjects had hypertension. Blood pressure in the first two months of measurement was 143/85 mmHg over all. The blood pressure in the final two months of the study will be reported as will the change in blood pressure in both the active and passive text message groups and the proportion achieving blood pressure control in each group. Health literacy regarding the guideline's based text messages was measured to determine if there was a difference between scores for the passive messages (all participants) and the active messages (active group only). Discussion. Achieving guidelines based management of hypertension is more challenging in remote, resource poor environments. Treatment focused SMS messages may improve hypertension management and allow the provision of guidelines based care even in rural and remote areas with health care resource challenges.

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Characterizing Agreement in Level of Inter-extremity Blood-Pressure Readings of Adults in the Emergency Department (CALIBRATE Study)

PrimaryAuthor.AuthorBlock:**Zain A. Bhutta**, Isma Qureshi, Mohammad Shujauddin, Sarah Thomas, Maarij Masood, Landric B. Dsouza, Navid Iqbal, Furqan B. Irfan, Sameer A. Pathan, Stephen H. Thomas, Hamad Medical Corp, Doha, Qatar

Introduction

Inter-arm blood-pressure difference (IBPD) has been studied previously in multiple settings, but few reports are available from the Emergency department (ED) setting, where BP varies significantly due to acute medical conditions or stress from various factors. CALIBRATE aims to study the inter-arm blood pressure differences in the patients presenting to the ED in Qatar and to assess the IBPD distribution in this population.

Methods

In sitting position, two consecutive BP

measurements were obtained from the right and left arm for each participant using calibrated automated machines and appropriate cuff sizes. Considering the demographic mix of the population presenting to the ED, a 1:1 of male to female and 2:1 for GCC (Gulf Cooperation Council) to non-GCC recruitment strategy was predefined. The data were recorded using predefined data fields including patient demographics, past medical, social and family history. The continuous variables were reported as mean (SD) or median (IQR) based on the distribution of data. The data was analyzed using Stata MP 14.0 (College Station, Texas).

Results

A total of 1800 patients were prospectively recruited from the ED. The mean age was 34 (10) years. The absolute systolic blood pressure (ΔSBP) difference between the right and left arm was same for the first (ΔSBP1) and the second reading (ΔSBP2), as 6 mmHg (3-10). The absolute average of ΔSBP1 and ΔSBP2 was 7 mmHg (4-10). The difference in SBP of less than 20 mmHg for IBP was seen in 95th percentile of the population with single reading, whereas, with the average of two individual readings it was observed in 97th percentile. No meaningful association could be detected between the significant IBPD and the study variables such as age, demographics, regions of interest and risk factors. Although, patients with diagnosed hypertension met the pre-defined criterion for significance, this difference was not clinically significant. There was no significant difference between IBPD noted for the Asia-pacific or Arab population.

Conclusion

In population presenting to the ED, the IBPD of at least 20 mmHg reached at 95th percentile validating the known significant difference. The utility of SBP difference can be improved further by taking the average of two individual readings.

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Lower Body Mass Index is Associated With Arterial Stiffening and Severity of Orthostatic Hypotension in Middle-aged and Older Patients With Abdominal Obesity

PrimaryAuthor.AuthorBlock:**Yusuke Kobayashi**, Saiseikai Yokohamashi Nanbu Hosp, Yokohama, Japan; Tetsuya Fujikawa, Yokohama Natl Univ, Yokohama, Japan; Keisuke Yatsu, Yokohama City Univ, Yokohama, Japan; Tamio Iwamoto, Saiseikai Yokohamashi Nanbu Hosp, Yokohama, Japan; Nobuhito Hirawa, Yokohama City Univ, Yokohama, Japan; Hideo Kobayashi, The Kobayashi Medical Clinic, Yokohama, Japan; Satoshi Umemura, Yokohama Rosai Hosp, Yokohama, Japan; Kouichi Tamura, Yokohama City Univ, Yokohama, Japan

Background Recently, patients with both sarcopenia and abdominal obesity are known to exhibit subclinical atherosclerosis and high mortality risk. Sarcopenic obesity is considered to be latent in those without increase in body mass index (BMI) despite having abdominal obesity. We aimed to investigate whether arterial stiffening and severity of orthostatic hypotension, which is the risk of cardiovascular disease, is associated with lower BMI in patients with abdominal obesity. **Methods** We studied middle-aged and older patients with normal to high BMI (BMI ≥ 18.5) and being treated with life-style related diseases. The patients were divided into 2 groups (with or without abdominal obesity) according to waist

circumference (WC) (abdominal obesity: men; WC ≥ 85 cm, women; WC ≥ 90 cm). We measured cardio-vascular ankle index (CAVI) for an index of arterial stiffness. We assessed sit-to-stand test for measuring orthostatic systolic blood pressure (SBP) change. **Results** One thousand and forty-six patients were included in the study. Mean age of the patients was 68.0 ± 10.1 years (42.5% were men), hypertension dyslipidemia and diabetes mellitus was observed in 83.3%, 74.3% and 20.2% of the patients respectively. There was a significant negative correlation with BMI and CAVI in patients with abdominal obesity (r=-0.309, p<0.001, n=434), whereas no significant association was observed in patients without abdominal obesity (p=N.S., n=612). There was a significant positive correlation with BMI and orthostatic SBP change in patients with abdominal obesity (r=0.213, p<0.001) and in patients without abdominal obesity (r=0.117, P=0.004). Multivariate regression analysis revealed that BMI was an independent determinant of CAVI and orthostatic SBP change in patients with abdominal obesity (p<0.001, p<0.001, respectively), and was an independent determinant of orthostatic SBP change in patients without abdominal obesity (p=0.004). **Conclusion** In patients with abdominal obesity, lower BMI was an independent determinant of arterial stiffening and severity of orthostatic hypotension. Further investigation by evaluating body composition is necessary and now ongoing to assess the risk of lower BMI in patients with abdominal obesity.

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Multiple-ascending-dose Study of IW-1973, a Soluble Guanylate Cyclase Stimulator

PrimaryAuthor.AuthorBlock:**John Hanrahan**, James Wakefield, Phebe Wilson, Marina Mihova, Jennifer Chickering, Ironwood Pharmaceuticals, Cambridge, MA; Dennis Ruff, ICON Early Phase Services, San Antonio, TX; Michael Hall, Todd Milne, Mark Currie, Albert Profy, Ironwood Pharmaceuticals, Cambridge, MA

Background: Soluble guanylate cyclase (sGC), an enzyme that catalyzes the formation of cyclic guanosine monophosphate (cGMP) in response to nitric oxide (NO) binding, is a key mediator of local blood flow, inflammation, and fibrosis. IW-1973 is an orally available sGC stimulator that enhances NOsGC-cGMP signaling and reduces blood pressure (BP) in animal models of hypertension, both alone and in combination with other antihypertensive agents.
Methods: A Phase 1b placebo-controlled, randomized, multiple-ascending-dose study was conducted to assess safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (BP, heart rate, platelet function, and plasma biomarkers) of IW-1973 in healthy subjects. Four successive cohorts of 11 subjects each (8 active and 3 placebo) were enrolled. Subjects received a starting dose once daily (QD) for 14 days followed by an up-titrated QD dose for 7 days.
Results: IW-1973 doses ranging from 15 to 40 mg were tolerated. There were no serious adverse events (AEs), severe AEs, or discontinuations due to AEs. Among the 32 subjects who received IW-1973, the most common AEs (which occurred mainly at doses ≥30 mg) were headache (15 subjects), dizziness/postural dizziness (6) and orthostatic hypotension/BP decreased (4). AEs tended to resolve with continued dosing. The AE profile of

higher doses was not clearly improved by up-titration from a lower starting dose. PK was dose proportional, both for C_{max} and AUC, with a T_{max} of 2-4 hours and an effective half-life of 24-37 hours. After 14 days of treatment, least squares mean change from baseline in 24-h ambulatory systolic BP (±SE) was -0.85±1.32 (placebo), -7.29±1.62 (15 mg), -3.27±1.61 (20 mg), -6.75±1.62 (30 mg), and -5.23±1.61 mmHg (≤40 mg, ≤0.5 mg/kg). After 21 days, the change from baseline was -4.81±1.19 (placebo), -8.21±1.46 (15 to 30 mg), -6.29±1.45 (20 to 40 mg), -9.05±1.56 (30 to 40 mg), and -6.58±1.45 mmHg (≤40 to ≥40 mg). IW-1973 produced a dose-related increase in plasma cGMP indicating target engagement. There was no clear effect of IW-1973 administration on platelet function as assessed by the PFA-100® system.

Conclusion: Further clinical investigation of IW-1973 is ongoing or planned in multiple indications, including resistant hypertension.

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P494

Long-term Outcomes of a Cluster-randomized Trial Testing the Effects Blood Pressure Telemonitoring and Pharmacist Management

PrimaryAuthor.AuthorBlock:**Karen L Margolis**, Stephen E Asche, Anna R Bergdall, Steven P Dehmer, Healthpartners Inst, Minneapolis, MN; Beverly B Green, Kaiser Permanente Washington Health Res Inst, Seattle, WA; JoAnn M Sperl-Hillen, Patrick J O'Connor, Rachel A Nyboer, Pamala A Pawloski, Michael V Maciosek, Nicole K Trower, Healthpartners Inst, Minneapolis, MN

Background/Aims: Hypertension is a common condition and leading cause of cardiovascular disease. We previously reported results of a cluster-randomized trial evaluating a home blood pressure (BP) telemonitoring and pharmacist management intervention, with significant reductions in BP favoring the intervention arm over 18 months. This analysis examined the durability of the intervention effect on BP through 54 months of follow-up and compared BP measurements performed in the research clinic and in routine clinical care.
Methods: The Hyperlink trial randomized 16 primary care clinics having 450 study-enrolled patients with uncontrolled hypertension to either Telemonitoring Intervention (TI) or usual care (UC) study arms. BP was measured as the mean of 3 measurements obtained at each research clinic visit. General linear mixed models utilizing a direct likelihood-based ignorable approach for missing data were used to examine change from baseline to 54 months in systolic and diastolic BP (SBP and DBP).
Results: Research clinic BP measurements were obtained from 326 (72%) study patients at the 54 month follow-up visit. Routine clinical care BP measurements were obtained from 444 (99%) of study patients from 7025 visits during the follow-up period. For TI patients, based on research clinic measurements baseline SBP was 148.2 mm Hg and 54 month follow-up was 131.2 mm Hg (-17.0 mm Hg, p<.001). For UC patients, baseline SBP was 147.7 mm Hg and 54 month follow-up was 131.7 mm Hg (-16.0 mm Hg, p<.001). The differential reduction by study arm in SBP from baseline to 54 months was -1.0 mm Hg (95% CI: -5.4 to 3.4, p=0.63). For TI patients, baseline DBP was 84.4 mm Hg and 54 month follow-up was 77.8 (-6.6 mm Hg, p<.001). For UC patients, baseline DBP was 85.1 mm Hg and 54 month follow-up was 79.1 mm Hg (-6.0 mm Hg, p<.001). The differential reduction by study arm in DBP from baseline to 54 months was -0.6 mm Hg (95% CI: -3.5 to 2.4, p=0.67). SBP and DBP results from routine clinical measurements closely approximated the pattern of results from research clinic measurements.
Conclusion: Significant BP reductions in the TI arm relative to UC were no longer seen at 54 month follow-up. To maintain intervention benefits over a longer period of time additional intervention is needed.

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Effects of *Nitrosomonas Eutropha* D23 Topical Spray on Blood Pressure: Results From a Randomized, Double-blind, Vehicle Controlled, Dose-ranging Study in Normotensive Adults

PrimaryAuthor.AuthorBlock:Peter Hino, Stephens and Associates; Dermatology Ctr of Dallas, Dallas, TX; Yan Liu, Lilly Jiang, Stephens and Associates, Richardson, TX; **Lawrence Weiss**, Spiros Jamas, AOBIome, Cambridge, MA; Joel M Neutel, Orange County Res Ctr, Tustin, CA

Objective: To evaluate the safety and blood pressure effects in normotensive individuals of *Nitrosomonas eutropha* bacteria applied as a topical spray. **Methods:** After initial screening, 36 healthy, normotensive adults participating in a facial acne vulgaris treatment trial were randomized to placebo or one of three concentrations (2, 4, or 8 $x10^9$ cells/ml) of a pure culture of ammonia oxidizing bacteria strain *N. eutropha* D23. Treatment occurred through the application of a topical spray to the face, which delivered ~0.6ml twice daily for 14 days. A standardized safety assessment (including physical exam and 12-lead EKG) and an evaluation of systolic and diastolic blood pressure occurred on Days 1 (pre-treatment), 3, 7, 10, 15 and on Day 28 following a 14 day wash-out. **Results:** A total of 9 subjects in each treatment group were randomized and completed the study (8 men and 28 women). Pre-treatment blood pressure was similar across treatment groups (115-119/75-79 mmHg). A dose-dependent reduction in systolic and diastolic blood pressure was emergent by treatment day 3. At day 15, individuals treated with the 8 $x10^9$ cells/ml concentration experienced a reduction in systolic blood pressure of 6.1 mmHg from baseline (SD=5.9; p=0.014) and diastolic blood pressure was reduced by 3.56 mmHg from baseline (SD=4.49; p=0.041). By comparison, in the placebo group, the treatment phase systolic and diastolic blood pressure changes from baseline were -0.34 (SD=7.80; p=0.94) and -1.19 (SD=4.57; p=0.486) respectively. Blood pressure reductions observed at Day 15 with *N. eutropha* were generally maintained at Day 28. All 3 concentrations of *N. eutropha* and placebo (vehicle alone) were well tolerated and no safety issues were identified. **Conclusions:** Topical treatment with the ammonia oxidizing bacteria *N. eutropha D23* produced a dose-

dependent reduction in systolic and diastolic blood pressure that was statistically significant in the 8 x10^9 cells/ml treatment group. This effect is consistent with the proposed mechanism-of-action of *N. eutropha* treatment: modification of NO/NO_2 levels on the skin. These findings support further development of *N. eutropha* for treatment of acne vulgaris and evaluation of blood pressure lowering effects in hypertensive individuals.

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P497

Re-analysis of the Systolic Blood Pressure Intervention Trial (SPRINT): The Renal Consequences of Intensive versus Standard Blood Pressure Lowering

PrimaryAuthor.AuthorBlock:**Leora Branfield Day**, David M Naimark, Univ of Toronto, Toronto, ON, Canada

Background: BP management guidelines suggest that persons with CKD should be treated to a SBP ≤ 140 mmHg. SPRINT compared this target to intensive SBP lowering (≤ 120 mmHg) in persons with and without CKD and found a reduced rate of CV events and all-cause mortality (ACM). However intensive therapy was associated with an increased risk of AKI. We extrapolated the results of SPRINT over a lifetime horizon to determine whether in the long-term, the benefit in terms of the primary outcome would be less economically attractive when the risks of more frequent AKI and subsequent CKD progression were considered. **Methods:** We re-configured the CKD Simulator, a Markov model of CKD progression, AKI events, fatal and non-fatal CV events, and ESRD. We recalibrated the model to be representative of the SPRINT cohort and compared intensive vs. standard blood pressure control among 10 million simulated persons with and without CKD over their lifetimes. Marginal treatment costs were calculated and hazard ratios for AKI, CV events and ACM observed in SPRINT were applied to the monthly probabilities of these events in the intensive SBP arm. **Results:** Lifetime average, discounted, costs per person associated with intensive vs. standard SBP lowering were predicted to be $35,811 and $30,584, respectively. Quality-adjusted, discounted average lifespans were 196.05 and 190.47 months, respectively. The cost of each quality-adjusted life-year gained by adopting intensive over standard BP lowering would be $11,220, significantly below the accepted cost-effectiveness threshold of $50,000. Intensive SBP control would reduce the lifetime incidence of at least one CV event by 5.5%, but increase the incidence of at least one AKI episode and ESRD by 1.7% and 0.7%, respectively. These differences were associated with average lifetime cost savings per person of $459 for CV events, but losses of $161 and $2,889 for AKI and ESRD. **Discussion:** Intensive SBP management would be cost-effective and associated with a significant lifetime reduction in CV events. However, there would be an

increase in the lifetime risk of AKI and ESRD, contributing to 58% of the total increase in cost of intensive relative to usual SBP control. Intensive SBP lowering should be adopted judiciously in persons at high risk of ESRD.

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P498

Redox Regulation of Vascular Smooth Muscle cGMP Signaling and Blood Pressure by Cytochrome B5 Reductase 3

PrimaryAuthor.AuthorBlock:**Adam C Straub**, Mizanur Rahaman, Anh Nguyen, Megan Miller, Scott Hahn, Univ of Pittsburgh, Pittsburgh, PA; Emil Martin, Univ of Texas at Houston, Houston, TX

Oxidized soluble guanylate cyclase (sGC) heme iron (Fe^{3+}) is desensitized to nitric oxide (NO) and attenuates cGMP production needed for downstream activation of PKG-dependent signaling and vasodilation. While reactive oxygen species drive oxidation of sGC heme iron, the basic mechanism(s) governing sGC heme iron recycling to its NO-sensitive, reduced state (Fe^{2+}), are unknown. Here we report cytochrome *b5* reductase 3 (Cyb5R3), also known as methemoglobin reductase, as a novel sGC heme iron reductase and regulator of cGMP production in vascular smooth muscle cells (VSMCs). Oxidant challenge studies demonstrate that VSMCs have an intrinsic ability to reduce oxidized sGC heme iron and form protein-protein complexes between Cyb5R3 and oxidized sGC. Genetic knockdown and pharmacological inhibition in VSMCs reveal Cyb5R3 expression and activity is critical for NO-stimulated cGMP production and vasodilation. Mechanistically, Cyb5R3 directly reduces oxidized sGC for NO sensitization assessed by biochemical, cellular, and *ex vivo* assays. Furthermore, generation of a smooth muscle specific Cyb5R3 knockout shows increased blood pressure and impaired endothelial dependent vasodilation. Together, these findings uncover new insights into NO-sGC-cGMP signaling and reveal Cyb5R3 as the first identified physiological sGC heme iron reductase in VSMC. The co-expression of Cyb5R3 and sGC in multiple cell types may unveil a fundamental mechanistic partnership that is critical in numerous physiological and pathophysiological processes.

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P499

Protein Kinase Cα Deletion Causes Hypotension Due to Decreased Vascular Contractility

PrimaryAuthor.AuthorBlock:**Brandi M Wynne**, Emory Univ Sch of Med, Atlanta, GA; Cameron G McCarthy, Theodora Szasz, Augusta Univ, Augusta, GA; Janet D Klein, Emory Univ, Atlanta, GA; R. Clinton Webb, Augusta Univ, Augusta, GA; Robert S Hoover, Emory Univ Sch of Med, Atlanta, GA

Protein kinase Cα (PKCα) regulates multiple cell signaling pathways, including those that impact blood pressure. PKCα activation increases vascular smooth muscle contractility, yet

reduces cardiac contractility. PKCα has also been shown to modulate nephron ion transport. We have shown that PKCα deletion leads to hypotension, with compensatory increases in sodium retention. Here, we hypothesized that PKCα deficiency reduces vascular contractility, leading to decreased mean arterial pressure (MAP). MAP, measured by telemetry, was decreased in PKC KO (≈12 mmHg) compared to PKC control (PKC CTL) mice. Aorta and mesenteric arteries were isolated, and concentration response curves (CRCs) to phenylephrine (Phe), acetylcholine (ACh) or sodium nitroprusside (SNP) were performed in the presence of vehicle or the following inhibitors: L-NAME or indomethacin (NOS, COX inhibitor, *resp.*). CRCs to KCL were performed to assess receptor-independent vascular responses. In aorta, we observed a striking reduction in KCl-mediated contraction (5.8±0.3mN *vs.* 10.4±1.1mN control, **p<0.01). PKC KO aorta and mesenteric arteries had decreased contractile responses to Phe, as compared to control (aorta, 12.7±0.5mN R_{max} *vs.* 16.3±0.5mN R_{max}, and mesenteric 9.9±0.3mN R_{max} *vs.* 11.8±0.6mN R_{max}; n=4, **p<0.01), revealing a role for reduced vascular contractility. Endothelium-mediated relaxation responses to ACh were also increased in PKC KO mice, as compared to control (59.3±6.8% R_{max} *vs.* 45.4±3.2% R_{max}, n=4, *p<0.05). Interestingly, NOS inhibition increased contractility in mesenteric arteries from PKC KO mice (8.55±2.65mN R_{max} *vs.* 6.95±0.39mN R_{max} control, n=4, ***p<0.001). However, PKC KO aorta had an enhanced response to COX inhibition (12.2±0.7mN R_{max} *vs.* 10.1±0.6mN R_{max} control, n=4, *p<0.05) suggesting that PKCα may be negatively regulating NOS in mesenteric arteries, and COX-mediated prostaglandin production in the aorta. No differences were observed in the relaxation responses to SNP. These data suggest that global deletion of PKCα results in hypotension due to decreased vascular contractility, and loss of PKCα-mediated inhibition of endothelial relaxing factors. Thus, systemic targeting of PKCα may be beneficial for the reduction of MAP.

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P500

Activation of the Heterodimeric Erythropoietin /β-Common Receptor Impairs Acetylcholine Mediated Vasodilation in Mouse Mesenteric Arterioles

PrimaryAuthor.AuthorBlock:Cody R Kilar, YanPeng Diao, Larysa Sautina, Univ of Florida, Gainesville, FL; Sivakumar Sekharan, Shahar Keinan, Cloud Pharmaceuticals, Research Triangle Park, NC; Kirk P Conrad, Univ of Florida, Gainesville, FL; Mark S Segal, Dept of Veterans Affairs Medical Ctr, North Florida/South Georgia Veterans Health System, Gainesville, FL; **Rajesh Mohandas**, Univ of Florida, Gainesville, FL

Erythropoietin (EPO) increases systemic vascular resistance and blood pressure. However, endothelial cells cultured in the presence of EPO demonstrate increased production of the potent vasodilator, nitric oxide (NO). The mechanism by which EPO causes vasoconstriction despite stimulating NO production may be dependent on its ability to differentially activate the two receptor complexes, the homodimeric EPO ($EPOR_2$) and the heterodimeric EPOR/β-common receptor (βCR). **Objective:**The purpose of this study was to investigate the contribution of the $EPOR_2$ and βCR receptor to the vasoactive properties of EPO. **Methods:** First order, mesenteric arteries isolated from 16-week old male C57BL/6 mice

were cannulated and perfused using a pressure arteriography system. To determine the contribution of each receptor complex, arteries were incubated with EPO stimulating peptide (ESP) which binds and activates only the heterodimeric EPOR/βCR complex or EPO which activates both receptors, 20 min prior to evaluation of vasoconstrictor (phenylephrine and potassium chloride), endothelium-dependent (acetylcholine, bradykinin, A23187) and -independent (sodium nitroprusside) vasodilator responses. Additionally, we studied the effect of a novel βCR inhibitory peptide (βIP) which was developed in silico and validated by demonstrating that it selectively inhibits binding of ligands to the βCR. **Results:** Acetylcholine induced vasodilation was impaired in arteries pretreated with EPO or ESP by 100% and 60%, respectively. EPO and ESP did not affect endothelium-dependent vasodilation by Bradykinin or A23187, endothelium-independent vasodilation by sodium nitroprusside, or vasoconstriction by phenylephrine and KCl. The βIP prevented the impairment of acetylcholine-induced vasodilation by EPO and ESP. **Conclusion:** Together, our findings suggest that activation of the heterodimeric EPOR/βCR leads to selective impairment of ACh-mediated vasodilator response in mouse mesenteric resistance arteries. Thus the βCR might have a role in mediating hypertensive effects of EPO. Therapeutic inhibition of the βCR might prevent vascular complications of EPO without affecting erythropoiesis.

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Editing of Myosin Phosphatase Gene as a Novel Approach for Vasodilator Sensitization and Lowering of Blood Pressure

PrimaryAuthor.AuthorBlock:Mariam Meddeb, Jeanine Ursitti, John Reho, **Steven A Fisher**, Univ of Maryland Baltimore, Baltimore, MD

Myosin Phosphatase (MP) is the primary effector of vascular smooth muscle (VSM) relaxation and a key end target of signaling pathways that regulate vessel tone. Regulated splicing of alternative Exon24 (E24) of Myosin Phosphatase Regulatory/ Targeting subunit (MYPT1) sets vasodilator sensitivity. Skipping E24 codes for a Mypt1 isoform that contains a C-terminal leucine zipper (LZ) motif required for cGK1α binding and NO/cGMP activation of MP resulting in vasodilation. Inclusion of 31 nt E24 shifts the reading frame coding for a Mypt1 isoform with a distinct C-terminus (LZ-) that is unresponsive to NO/cGMP. We are using two editing approaches to test the function of Mypt1 E24 splice variants in the control of BP in vivo. First, LoxP sites were inserted in introns flanking E24, crossed with smMHCCre^{ER}, and treated with Tamoxifen to achieve smooth muscle-specific cKO of E24 (SMcKO E24), thereby converting Mypt1 to the LZ+ isoform. E24 cKO mice had mean BP that was 15$\pm$3 mmHg lower than control (n=3-5; $p<0.05$). Mesenteric arteries from these mice were significantly more sensitive to DEA/NO mediated relaxation (EC_{50}: 2.1+0.5 nM vs 18.2+5.6 µM; n=5-6, $p<0.05$). We now are developing CRISPR/CAS9 editing of Mypt1 for translation into humans with hypertension. Guide(g)RNAs targeting E24 were designed using Benchling.com and selected for further

study based on predicted efficacy, specificity (>10%,>60%) and cross-species conservation. Plasmids were generated by sub-cloning of oligonucleotides into the parent pX601 plasmid for the purpose of co-expression of gRNA and saCas9. These plasmids were transfected into HEK293 cells singly and in combinations and Mypt1 gene editing assayed by PCR, Surveyor nuclease assays and sequencing of genomic DNA. Single gRNAs yielded deletions of 1-3 nt. Combinations yielded deletions of 104-334 nt that removed >80% of E24 with an efficiency of editing that varied from 10% (gRNAs 6+9 and 5+9) to 40% (gRNAs 6+11 and 5+11). We have now generated AAVgE24 and are testing their efficiency of editing of VSM in vivo. These studies support that AAV mediated CRISPR/Cas9 editing of Mypt1 E24 could be a novel strategy for vasodilator sensitization and effective lowering of blood pressure in humans.

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Vascular Tissue Proteomics Enables Detection of Cell Type-specific Changes in Target Expression

PrimaryAuthor.AuthorBlock:Ruth M Mackenzie, Karine Pinel, Emma J Carrick, Katrin Nather, Holger Husi, Delyth Graham, William Mullen, Stuart A Nicklin, **Christian Delles**, Univ of Glasgow, Glasgow, United Kingdom

Introduction

Identification of robust targets from proteomic studies in cardiovascular tissue may prove challenging. Using an angiotensin II (Ang II)-infusion mouse model, we performed a proteomics study in isolated thoracic aortas. Changes in proteins related to cardiovascular pathophysiology were identified and candidate targets selected for validation via traditional techniques.

Methods and Results

C57 black/6 mice were infused with Ang II (24 µg/kg/hour) via osmotic minipump for 6 weeks. Elastic Van Gieson staining demonstrated significantly increased medial area in aortas from Ang II-infused mice compared to water-infused control mice (P<0.005), indicating Ang II-induced remodelling.

Nanoscale liquid chromatography coupled to tandem mass spectrometry (nano LC-MS/MS) revealed a 1.28 fold increase in galectin-3 (LGALS3) expression in vessels from Ang II-infused mice as compared to controls. LGALS3, a β-galactoside binding lectin, is a well known marker of cardiovascular disease reported to play a role in Ang II-induced cardiac remodelling.

Immunohistochemical (IHC) staining showed increased LGALS3 expression throughout the vessel wall and particularly in the endothelial layer (quantification using Image J Fiji software: 110.4 ± 1.37 vs 120.5 ± 2.6 arbitrary units; P=0.005) in Ang II-infused mice compared to controls.

Human primary endothelial cells (ECs) were isolated from saphenous veins of patients undergoing coronary artery bypass graft surgery and translational studies performed. *LGALS3*/LGALS3 expression was detected at both mRNA and protein level by qRT-PCR and immunoblotting respectively. Acute stimulation

of ECs with Ang II (200nM for 24 hours) failed to upregulate *LGALS3*/LGALS3 expression suggesting that the increased endothelial expression observed *in vivo* is due to chronic infusion of Ang II.

Conclusions

We have successfully validated the Ang II-induced increase in LGALS3 identified via vascular tissue proteomics. Despite the use of homogenised whole aortic tissue, nano LC-MS/MS proved sensitive enough to detect elevated expression of a candidate protein that is predominantly expressed in the endothelium. Tissue proteomics can detect changes in expression specific to a single cell type.

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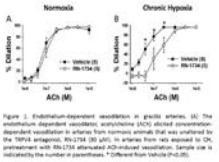
Role of TRPV4 and Endothelial BK Channels in Endothelial-dependent Dilation Following Chronic Hypoxia

PrimaryAuthor.AuthorBlock:**Jay S Naik**, Benjimen R Walker, Univ New Mexico Sch of Med, Albuquerque, NM

Following chronic hypoxia (CH) the systemic vasculature exhibits blunted vasoconstriction due to endothelial cell-dependent hyperpolarization (EDH). Previous data demonstrate that after CH, EDH-mediated dilation switches from a reliance on SK_{ca} and IK_{ca} channels to activation of endothelial BK_{ca} channel (eBK). The mechanism by which endothelial cell stimulation activates eBK channels following CH is not known. We hypothesized that following CH, EDH-dependent dilation involves a transient receptor potential V4 (TRPV4)-dependent activation of eBK channels. ACh induced concentration-dependent dilation in pressurized gracilis arteries from normoxic and CH rats. TRPV4 blockade only attenuated ACh-induced vasodilation in arteries from CH rats (Fig. 1). ACh elicited an increase in endothelial TRPV4-dependent Ca^{2+} events in arteries from both groups. Direct activation of TRPV4 elicited dilation in arteries from normoxic and CH rats (97 ± 2 % and 95 ± 2 % for normoxic and CH, respectively). In arteries from normoxic rats, inhibition of SK_{ca} and IK_{ca} abolished dilation to TRPV4 activation (6 ± 5 %), whereas, luminal iberiotoxin had no effect (89 ± 7 %). In CH rats, only administration of all three K_{ca} channel inhibitors abolished dilation to TRPV4 activation (3 ± 10%). Disruption of endothelial caveolae with methyl-β-cyclodextrin significantly decreased ACh-induced dilation in arteries from both groups. Using a proximity ligation assay, we observed co-localization between caveolin-1, TRPV4, and eBK channels in the endothelium of gracilis arteries. In conclusion, CH results in functional coupling between muscarinic receptors, TRPV4 and K_{ca} channels in gracilis arteries.



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P504

Resistin Mediates Sex-dependent Effects of Perivascular Adipose Tissue on Vascular Function in the SHRSP

PrimaryAuthor.AuthorBlock:Sarah McNeilly, Heather Y Small, Adam Sheikh, Sheon Mary, Karine Pinel, **Christian Delles**, Univ of Glasgow, Glasgow, United Kingdom

Background. Premenopausal women are relatively protected against hypertension compared to males. Estrogen levels have been identified as a potential underlying cause, but the pathophysiological mechanisms remain incompletely understood. We hypothesised that sex-dependent effects of perivascular adipose tissuePVAT mediate altered vascular function in hypertension.
Methods. The effect of PVAT was investigated on resistance vessels of 16 week old male and female stroke-prone spontaneously hypertensive rats (SHRSP).
Results. Wire myography was used on 3rd-order mesenteric vessels (maximum contraction: male +PVAT 113.3±1.1 vs. female +PVAT 91.4±11.36 %). Noradrenaline mediated vasoconstriction was increased in SHRSP males compared to females. K_{ATP} channel-mediated vasorelaxation by cromakalim was impaired in males compared to females (maximum relaxation: male +PVAT 46.9±3.9 % vs. female +PVAT 97.3±2.7 %) A cross-over study assessing function of male PVAT on female vessels and vice versa confirmed the reduced K_{ATP} mediated vasorelaxation induced by male PVAT (maximum relaxation: female +$PVAT_{female}$ 90.6±1.4 % vs. female +$PVAT_{male}$ 65.8±3.5 %). An adipokine array with subsequent western blot validation identified resistin as a potential modifier of vascular reactivity. Resistin was increased by approximately 2-fold in SHRSP male PVAT. Male and female vessels pretreated with resistin (40ng/ml) showed no difference in response to noradrenaline. However, vasorelaxation in response to cromakalim was significantly impaired in resistin treated female vessels, similar to levels observed in male vessels (maximum relaxation: female +PVAT 97.3±0.9 % vs. female +PVAT +resistin 36.8 ±2.3 %).
Conclusion. We identified a novel role for resistin in sex-dependent PVAT mediated vascular function in hypertension through a K_{ATP} channel mediated mechanism.

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Longitudinal Associations Among Flow-mediated Vasodilatation, Preclinical Vascular/renal Damage and Serum Cholesterol Levels in Treated Hypertension

PrimaryAuthor.AuthorBlock:**Hirofumi Tomiyama**, Akira Yamashina, FMDJ Study Group, Tokyo Medical Univ, Tokyo, Japan

Objective: The endothelial dysfunction is thought to be an early precursor of vascular/renal damages. The present study was conducted to examine whether endothelial dysfunction, as assessed by measurement of flow-mediated vasodilatation (FMD), is longitudinally associated with the progression of vascular/renal damage, and also to identify factors that can perpetuate endothelial dysfunction in treated hypertension.
Methods and Results: A 3-year multicenter prospective observational study was conducted in 674 Japanese patients with hypertension receiving antihypertensive medication, in whom the carotid intima-media thickness/plaque (CIMT), brachial-ankle pulse wave velocity (baPWV), estimated glomerular filtration rate (eGFR), urinary albumin/creatinine excretion ratio (UACR) and FMD were measured thrice at 1.5-year intervals. A lower value of the FMD at the study baseline was associated with a higher

rate of increase of the baPWV during the study period (The change of baPWV during the study period was -25 cm/sec in subjects with the highest quartile range of FMD vs. +18 cm/sec in subjects with the lowest quartile range of FMD, $p<0.05$). However, no association of the FMD at the study baseline was observed with any of the other markers of vascular/renal damage examined. Mixed model linear regression analysis revealed a significant inverse relationship of the FMD at each measurement point with the baPWV at each measurement point (beta estimate = -10.97, $P < 0.01$); furthermore, the serum level of low density lipoprotein cholesterol (LDLC) at the study baseline was also inversely associated with the longitudinal changes of the FMD values (estimate = -0.39, $P < 0.01$).
Conclusion: Our results suggest that an "endothelial dysfunction-arterial stiffening continuum" may exist in treated hypertension, and endothelial dysfunction, as measured by FMD, may be more closely associated with the arterial stiffness than with other parameters of preclinical renal/vascular damage. Furthermore, inadequate control of LDLC may also contribute to the progression of endothelial dysfunction.

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Deoxycorticosterone Acetate-salt Promotes Endothelial-mesenchymal Transition in Human Glomerular Endothelial Cells

PrimaryAuthor.AuthorBlock:**Zaiming Luo**, Div of Nephrology and Hypertension, and Hypertension Ctr, Georgetown Univ, Washington, DC; William J. Welch, Christopher S. Wilcox, Div of Nephrology and Hypertension, and Hypertension Ctr, Georgetown Univ, Washington, DC

Endothelial cells (ECs) lose their endothelial specification and gain mesenchymal cell features during endothelial-mesenchymal transition (EndMT). Post-developmental EndMT disrupts EC homeostasis, leading to vascular dysfunction. We found that afferent arterioles from deoxycorticosterone acetate (DOCA)-salt treated mice had > 5 fold upregulation of mRNAs for preproendothelin-1, p47phox ,NOX2 and TGF-β accompanied by microvascular dysfunction. Since these may cause EndMT, we investigated the mechanism in human glomerular endothelial cells (HGECs) treated for 7-21 days with high salt and DOCA. Endothelin-1 (ET-1) in the medium was increased 2.8±0.2 fold by day 7 while the cells gained multiple mesenchymal markers with increased mRNA for alpha-smooth muscle actin (1.78±0.19 and 2.96±0.32 fold, $P<0.05$ and 0.01, n=3) and transgelin (1.96±0.14 and 2.91±0.28 fold, $p<0.05$ and 0.01, n=3) on day 7 and 21, respectively, and markedly downregulated mRNA for endothelial markers with decreased vascular endothelial cadherin (1.99± 0.27 and 2.12±0.24 fold, $P<0.05$ and 0.005, n=3) and platelet endothelial cell adhesion molecule 1 (1.78±0.26 and 1.94±0.23 fold, $P<0.05$ and 0.005, n=3). There were parallel changes in protein expression. Dihydroethidium and MitSox fluorescence probes were used to determine intracellular and mitochondria ROS. The fluorescent intensities were increased by 1.89±0.27 and 1.62±0.22 fold ($P<0.01$, N=6) respectively in the cells treated for 7 days with DOCA-salt accompanied by increased expression of TGF-β and phosphorylated-extracellular signal-regulated kinases (P-ERK

1/2). In conclusion, human glomerular endothelial cells treated with high salt and DOCA for 1-3 weeks have increased cellular and mitochondrial ROS, ET-1, TGF-β and P-ERK that could account for adverse changes of endothelial-mesenchymal transition and associated microvascular dysfunction.

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Atherosclerosis Progression in Chronic Kidney Disease

PrimaryAuthor.AuthorBlock:Hernan A. Perez, Blossom DMO, Cordoba, Argentina; Laura Aballay, CONICET, Cordoba, Argentina; Luis J. Armando, Blossom DMO, Cordoba, Argentina; J. David Spence, Robarts Res Inst, Western Univ, London, ON, Canada; **Nestor H Garcia**, CONICET, Cordoba, Argentina

Cardiovascular risk is very high in CRF, but the underlying mechanisms are not well understood. Traditional cardiovascular risk factors (RF) do not explain the increased risk, and observational studies have observed paradoxical or absent associations between classical RF and mortality in ESRD. CRF studies found that statin therapy does not reduce CV events; these may be the results of "resistant atherosclerosis" observed in these patients. We investigated if carotid total plaque area (TPA) is increased at progressively lower creatinine clearance and whether or not TPA progression is increased in CRF patients not on dialysis. Methods: The Blossom DMO Argentina ethics committee approved the study and informed consent was obtained from each participant. We performed a cohort study in 201 patients with Normal Renal Function (NRF), Stage 2 and 3 CRF. Clinical, laboratory tests and TPA were determined at time 0 and after 1 year. TPA was measured using carotid ultrasonography. Renal function (eGFR) was determined by the MDRD equation. The Study population was divided into quartiles of eGFR. Results: 1st Quartile, (51±1yo, eGFR 89±2 ml/min) had a blood pressure (BP) of 136±2/81±1 mmHg, BMI 31±1, Total Chol (tChol) 196±6 mg/dl, HbA1c 6.7±0.4% and had the lowest Chol 192±5 mg/dl, HbA1c 6.2±0.1% and TPA 47±6mm^2; 3rd Quartile, (59±1yo, eGFR 63±1 ml/min) BP 133±2/82±1, tChol 192±5 mg/dl, HbA1c 6.2±0.1% and TPA 47±6mm^2; 4th Quartile (60±2yo, eGFR 52±1 ml/min) BP 140±3/84±1, tChol 209±5 mg/dl, HbA1c 6.2±0.1% and TPA 76±11mm^2. After one year, the 4th Quartile had the most progression of TPA ($p < 0.005$); it was not influenced by age, hypertension, smoking, dyslipidemia or diabetic status. Conclusions: In CRF, TPA increases as renal function decreases; its progression is not associated with traditional risk factors. Other mechanisms are responsible for the observed excess of cardiovascular disease in CKD. Determination of TPA should be used to measure effects of antiatherosclerotic therapy to decrease the enormous cardiovascular event rate observed in this population.

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Inhibition of Big-conductance Calcium-activated Potassium Channels in Vascular Myocytes is a Key Novel Mechanism for Tacrolimus-induced Hypertension

PrimaryAuthor.AuthorBlock:**Yun-Min Zheng**, Yong-Xiao Wang, Albany Medical Coll, Albany, NY

Background and Purpose: Tacrolimus (TAC, also called FK506), a common immunosuppressive drug, causes hypertension. In this study, we tested a novel hypothesis that TAC inhibits big-conductance Ca^{2+}-activated K^+ (BK) channels in artery smooth muscle cells (SMCs), leading to hypertension. **Experimental Approach:** Noninvasive and invasive blood pressure measurements were used to determine hypertension; organ bath technique and live videomicroscopy to assess vasoconstriction; fluorescence imaging and confocal microscope to detect whole-cell and local intracellular Ca^{2+} concentration; patch-clamp techniques to record channel activity; Western blot analysis and real-time RT-PCR to evaluate channel expression; and gene knockout mice to determine its functions. **Key Results:** Intraperitoneal injection of TAC once a day for a week causes hypertension with a peak at 4 weeks. Application of norepinephrine (NE) induces a larger vasoconstriction in arteries and increase in $[Ca^{2+}]_i$ in artery SMCs from mice treated with TAC for 4 weeks. Whole-cell BK channel activity is significantly decreased in SMCs from TAC-treated mice, whereas Ca^{2+} spark activity is increased. The voltage sensitivity, Ca^{2+} sensitivity and open time of single BK channels are all decreased in TAC-administrated mouse cells. BK channel β1, but not α, subunit protein and mRNA expression levels are significantly decreased in arteries from TAC-treated mice. Chronic administration of TAC fails to further increase blood pressure in BK channel β1 knockout mice with hypertension. **Conclusion and Implications:** Our findings provide novel evidence that TAC inhibits BK channel β1 subunit expression and functions in artery SMCs, thereby leading to enhanced vasoconstriction and hypertension.

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P509

Identification of Radial Vascular Wall Abnormalities by Very-high Frequency Ultrasound in Patients With Fibromuscular Dysplasia: The Fuchsia Study

PrimaryAuthor.AuthorBlock:**Rosa Maria Bruno**, Univ of Pisa, Pisa, Italy; Nicole Di Lascio, Inst of Clinical Physiology - CNR, Pisa, Italy; Daniela Guarino, Saverio Vitali, Piercarlo Rossi, Davide Caramella, Stefano Taddei, Lorenzo Ghiadoni, Univ of Pisa, Pisa, Italy; Francesco Faita, Inst of Clinical Physiology - CNR, Pisa, Italy

Aim: This case-control study is aimed at identifying radial vascular wall abnormalities in

patients with fibromuscular dysplasia (FMD). **Methods:** High-frequency ultrasound scans of the radial arteries and of FMD patients and healthy controls were obtained by Vevo MD (70 MHz probe, FUJIFILM, VisualSonics, Toronto, Canada). Radial wall showed in all individuals two echogenic interfaces: the 1st (lumen-media) and the 2nd (media-adventitia). Intima-media (IMT), adventitia (AT), and global thickness (IMAT) were measured and wall cross-sectional area (WCSA) calculated. The disarray level of the two echogenic interfaces was assessed calculating the root mean square error (RMSE) between 20 gray-level profiles crossing the two interfaces and the profile obtained averaging them. For each echogenic interface, the RMSE was normalized for the maximum value of the corresponding mean profile (RMSE/mean). **Results:** 11 treated hypertensive female FMD patients and 8 healthy control women (C) were enrolled (age 52±5 vs 45±13 years, p=0.51; BMI 26±3 vs 23±3, p=0.12; mean BP 97±7 vs 85±10, p=0.01). Radial internal diameter was similar (1.938±0.432 vs 1.701±0.532 mm, p=0.21). IMT (0.166±0.037 vs 0.128±0.022 mm, p=0.03), AT (0.114±0.029 vs 0.083±0.019 mm, p=0.008) and IMAT (0.281±0.042 vs 0.211±0.027 mm, p=0.003) were higher in FMD. Wall/lumen ratio was similar and WCSA increased in FMD, calculated with either IMT or IMAT. The maximum values of 1st (121±43 vs 157±22, p=0.09) and 2nd interface 109 ±44 vs133±18, p=0.09) tended to be lower, whereas RMSE/mean was higher in FMD (1st 1.31±0.24 vs 0.83±0.32, p=0.006; 2nd 1.37±0.38 vs 0.94±0.32, p=0.03). The difference was attenuated for the 1st but not for the 2nd interface when considering age and mean BP as covariates (p=0.054 and p=0.016 respectively). **Conclusions:** Wall ultrastructure of radial arteries of hypertensive FMD patients showed eutrophic remodeling. Furthermore, a peculiar "blurred" pattern occurred, characterized of loss of echogenicity and inhomogeneity of the two echogenic layers, independent of age and mean BP.

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P510

Nox4 Deficiency Leads to Hypertension and Vascular Damage With Enhanced Effects in Ang II-dependent Hypertension

PrimaryAuthor.AuthorBlock:Silvia Lacchini, ICB - Univ of Sao Paulo, Sao Paulo, Brazil; **Augusto C Montezano**, Rheure A Lopes, Adam P Harvey, Francisco J Rios, ICAMS-Univ of Glasgow, Glasgow, United Kingdom; Katrin Schroder, Ralf P Brandes, Goethe - Univ Frankfurt, Frankfurt, Germany; Rhian M Touyz, ICAMS-Univ of Glasgow, Glasgow, United Kingdom

We previously showed that Nox1 and Nox2 are not involved in chronic Ang II-dependent hypertension, which recapitulates human hypertension. Here we questioned the role of Nox4 by studying transgenic mice expressing human renin (LinA3) crossed with Nox4-/- mice. Four groups were used: wildtype (WT), LinA3, Nox4 KO (Nox4), and LinA3/Nox4 KO (LinA3/Nox4). Blood pressure was measured by tail cuff. Aorta was collected to assess wall thickness, collagen and glycosaminoglycans (GAGs) deposition, and TNFα expression. Mesenteric arteries were used to access vascular function by myography. Blood pressure was increased in LinA3, Nox4 and LinA3/Nox4

mice vs WT ($p<0.05$). All three experimental groups exhibited vascular remodeling with evidence of increased fibrosis. Although LinA3 had increased aortic wall thickness (+31%), there was no significant change in collagen (10.3±3 vs. 8.5±2% in WT) and GAGs (6.6±3 vs 2.8±2% in WT) deposition ($p<0.05$). Nox4 mice, which presented a similar increase in wall thickness to LinA3 (+31%), had significant increase in collagen (20.6±6%) and GAGs (22.3±4%) in aorta ($p<0.05$). In LinA3/Nox4 mice, collagen (24.6±7%) and GAGs (37.1±10%) deposition were increased vs LinA3. TNFα was increased in LinA3 (130.4±6 a.u.) and LinA3/Nox4 mice (129±5 a.u.) vs WT (116.8±9 a.u.) ($p<0.05$). Mesenteric arteries from LinA3, Nox4 and LinA3/Nox4 mice, exhibit increased Phenylephrine-induced vasoconstriction vs WT (Emax: WT 6.79±0.29 vs LinA3 9.37±0.51; Nox4 9.87±1.59; LinA3/Nox4 9.12±1.63, $p<0.05$). Endothelium-dependent vasodilation was not reduced in Nox4 but impaired in LinA3 and LinA3/Nox4 (Emax: WT 86.48±0.01 vs LinA3 59.70±0.03; LinA3/Nox4 33.57±0.26, $p<0.05$). In conclusion, Nox4 deficiency was associated with increased blood pressure, vascular dysfunction and fibrosis, effects that were variably enhanced in LinA3/Nox4 mice. We also observed that the fibrosis in vessels from Nox4 mice was not associated with inflammation. These results suggest that Nox4 may be cardiovascular protective, which when downregulated leads to blood pressure elevation and vascular injury, processes that may be amplified by Ang II-dependent hypertension.

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Targeting Il-1β Protects From Aortic Aneurysms Induced by Disrupted Tgfβ Signaling in Smooth Muscle Cells

PrimaryAuthor.AuthorBlock:**Daniela Carnevale**, Sapienza Univ/IRCCS Neuromed, Pozzilli, Italy; Raimondo Carnevale, IRCCS Neuromed, Pozzilli, Italy; Francesco Da Ros, Univ of Padova, Padova, Italy; Roberta Iacobucci, Manuel Casaburo, Giuseppe Cifelli, Lorenzo Carnevale, Marialuisa Perrotta, IRCCS Neuromed, Pozzilli, Italy; Giorgio Bressan, Univ of Padova, Padova, Italy; Giuseppe Lembo, Sapienza Univ/IRCCS Neuromed, Pozzilli, Italy

Aortic aneurysms represent a life-threatening condition because of the current lack of effective treatments. Aneurysm formation is typically associated with extracellular matrix remodeling and persistent inflammation. Although the molecular mechanisms underlying aortic pathology remain largely unclear, TGFβ signaling is unquestionably implied and its downstream target Smad4 showed protective functions for maintenance of aortic walls' integrity. Using mice with smooth muscle cells (SMCs) specific deletion of *Smad4* in the adult (*Smad4*-SMC^{iko}), developing spontaneous aneurysms (Ascending Aorta Diameter: *Smad4*-SMC^{iko} 2.15±0.03; *Smad4*-SMC^{wt} 1.7±0.03;***$p<0.001$), we investigated the molecular mechanisms activated by dysregulation of TGFβ signaling. Structural disarrangement of ascending aorta in *Smad4*-SMC^{iko} mice was clearly appreciated early after Smad4 deletion as discrete breaks of elastic lamellae (breaks/section: *Smad4*-SMC^{iko} 2.05±0.5; *Smad4*-SMC^{wt} 0.83±0.4;***$p<0.001$). Interestingly, the islands of damage in the aorta of *Smad4*-SMC^{iko} were enriched of immune infiltrate, mainly monocytes/macrophages, as indicated by FACS and immunofluorescence. We then analyzed several pathways

downstream to Smad4 inhibition, finding a selective activation of NF-kB/IL-1β in SMCs. To test the relevance of this pathway in the formation of aneurysms, we deleted *Smad4* in SMCs of mice with *Il1r1 null* background (*Smad4*-SMC^{iko};*Il1r1*$^{-/-}$). Ultrasonographic analyses revealed that ablation of IL1 receptor1 protected *Smad4*-SMC^{iko} mice from the progression of pathology and improved their overall survival. In the end, to test the translational potential of our findings, we neutralized IL-1β signaling with the clinically relevant murine version of the FDA-approved clinical drug canakinumab. During a time course of 16 weeks, while a weekly administration of control immunoglobulins did not change aneurysm progression in *Smad4*-SMC^{iko} mice, treatment with anti-IL-1β antibody significantly hampered aneurysm formation in the aorta (*Smad4*-SMC^{iko} +anti- IL-1β 1.85±0.02; *Smad4*-SMC^{iko} +anti-IgG 2.09±0.03; ***p< 0.001) These findings identify a mechanistic target for controlling aneurysms progression induced by disrupted TGFβ signaling.

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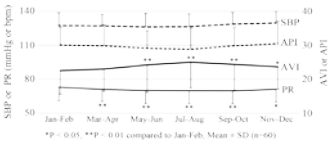
Seasonal Fluctuations of Novel Arterial Stiffness Indexs in Hypertensive Patients

PrimaryAuthor.AuthorBlock:**Toshihiro Kita**, Kazuo Kitamura, Univ of Miyazaki, Miyazaki, Japan

Seasonal fluctuation of blood pressure (BP) is well known and possible relationship is reported between increases of BP and increases of cardiovascular accidents (CVA) in winter. Parameters of arterial stiffness may have seasonal fluctuation but available data are highly limited. Novel arterial stiffness index, Arterial Velocity pulse Index (AVI) and Arterial Pressure volume Index (API), can obtain through usual maneuver of BP measurement, and thus repeated measurement is easy in regular examination in outpatient office. Here, we analyzed seasonal fluctuation of AVI and API in hypertensive patients for 2.5 years (30 months), where the parameters were measured on every visits. Objects are 60 hypertensive patients under stable treatment and continuously visited our outpatient office for 2.5 years. BP, pulse rate (PR), AVI and API was measured using AVE-1500 (Pasesa, Shisei datum, Tokyo, Japan) in sitting position. Six periods were made like January to February, March to April and so on. Every measurements (at least 12 to over 30 per person) were sorted using the six period and averages of the parameters were used for analysis. Obvious seasonal fluctuations in PR (p<0.001) and AVI (p<0.001) and marginal fluctuations in SBP (p=0.055) and API (p=0.067) were confirmed by repeated ANOVA (Figure). SBP, API and PR were decreased in summer as expected, but, unexpectedly, AVI was increased in summer. Coefficient variations were SBP 5.1%, PR 4.8%, AVI 12.5% and API 10.6%. AVI is affected by reflected wave and closely correlated with augmentation index, and high AVI increases cardiac afterload. Decrease of BP and increase of AVI may relate to another CVA risk in summer like ischemic stroke.



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Age-related Differences in the Annual Changes of the Ankle-brachial Pressure Index: Underlying Mechanisms

PrimaryAuthor.AuthorBlock:Shogen Fujii, Kazuki Shiina, Taishiro Chikamori, Akira yamashina, Chisa Matsumoto, **Shunsuke Komatsu**, Hirofumi Tomiyama, Tokyo Medical Univ, Tokyo, Japan

Objectives: The present prospective observational study was conducted to examine the age-related annual changes of the ankle-brachial pressure index (ABI) and their association with the longitudinal changes of the brachial-ankle pulse wave velocity (baPWV), a marker of arterial stiffness, and the radial augmentation index (rAI), a marker of the pressure wave reflection in middle-aged Japanese men.
Methods and Results: In 4264 men (42 ± 9 years old) of a Japanese construction company, the ABI, baPWV and rAI were measured annually over a 9-year observation period. During the study period, ABI (from 1.10 ± 0.07 to 1.13 ± 0.07), baPWV (from 1295 ± 194 to 1344 ± 217 cm/sec) and rAI (from 69 ± 13 to 72 ± 13 %) were increased significantly ($p<0.01$). Mixed model linear regression analysis of the repeated-measures data revealed that the annual increase of the ABI was lower in men aged over 50 years of age (n = 1237: 0.28 ± 0.06) than in those aged under 50 years of age (n = 3027: 0.50 ± 0.04) ($p < 0.01$). Furthermore, while increased baPWV (estimate = 0.017, $p < 0.05$) and increased rAI (estimate 0.254, $p < 0.05$) were significantly related to the annual increase of the ABI in men aged <50 years ($p < 0.01$), no such association was observed in men aged $\geq$50 years.
Conclusion: In middle-aged Japanese men, the ABI increases with age until the age of 50 years, and increased arterial stiffness and increased pressure wave reflection may contribute to this annual increase. In men aged $\geq$50 years, the annual increase of the ABI was attenuated, which could be related, at least in part, to the attenuation of the increase of the pressure wave reflection and also attenuation of the effect of increased arterial stiffness on the hydrostatic pressure in the arteries with age. These findings may suggest the usefulness of ABI measurement as a screening tool for peripheral arterial disease in men over 50 years old.

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Vascular Stiffness is Not Changed by Dipeptidyl Peptidase 4 Inhibitor

PrimaryAuthor.AuthorBlock:**Jose Fernando Vilela-Martin**, Luciana N Cosenso-Martin, Luiz T Giollo-Jr, Medical Sch S Jose Rio Preto, Sao Jose Rio Preto, Brazil

Introduction: The action of dipeptidyl peptidase-4 (DPP-4) inhibitors on blood pressure (BP) and arterial stiffness is controversial. This study aimed to investigate the effects of DPP-4 inhibitor (vildagliptin) on the BP and arterial stiffness. Methods: Fifty patients over 35 years of age with diabetes and hypertension without cardiovascular disease were randomized to vildagliptin (n=25) or glibenclamide (n=25) in a prospective, open,

drug-controlled study. Both groups used metformin and renin angiotensin system blockers. BP was evaluated by digital sphygmomanometer and by 24-hour ambulatory BP monitoring with the Mobil-O-Graph PWA® device, which analyzes parameters of arterial stiffness (central systolic BP, pulse wave velocity (PWV) and augmentation index (AIx75). Laboratory evaluation (glycemia and glycated hemoglobin), and BP and arterial stiffness measurements were performed before and after 12 weeks of treatment. Results: Glycated hemoglobin reduced non-significantly with treatment in both groups. 24-hour systolic BP decreased significantly in the vildagliptin (123.8±12.5 vs 119.1 ± 1.7 mmHg, P=0.03) and glibenclamide group (125.1 ± 12.2 vs 117.3 ± 8.0 mmHg, P=0.002), but without difference between the groups. There were no changes in central systolic BP and PWV in the vildagliptin group before and after treament. However, in the glibenclamide group, central systolic BP decreased (116.4±12.19 vs 109.0±7.5, mmHg P=0.003), as well as PWV (8.5±1.3 vs 8.1±1.1 m/s, P=0.003), but with no difference between groups (P=0.27 and 0.32, respectively). There was also no difference for 24 hour AIx75 for both drugs. Pearson's correlation did not demonstrate a correlation of glycemic control with PWV and AIx75. However, office systolic BP and central pulse pressure showed correlation with PWV in the vildagliptin group (P=0.008 and 0.002, respectively) and glibenclamide (P<0.001 and 0.001, respectively). Conclusions: Vildagliptin does not alter BP and arterial stiffness in diabetic and hypertensive patients. The observed improvement in 24-hour BP is due the antihypertensive treatment, as it was demonstrated in the 2 groups. Unlike blood glucose, BP correlates to arterial stiffness.

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Arterial Stiffness is a Mediator of Uric Acid-related Development of Hypertension

PrimaryAuthor.AuthorBlock:**Hirofumi Tomiyama**, Kazuki Shiina, Chisa Matsumoto, Taishiro Chikamori, Akira Yamashina, Shogen Fujii, Tokyo Medical Univ, Tokyo, Japan

Objectives: Uric acid (UA) is a risk for the development of hypertension, but the underlying mechanisms of this development has not been fully clarified. On the other hand, arterial stiffness is also noted as a risk for the development of hypertension. Then, the present prospective observational study examined whether UA is associated with the development of hypertension mediated by the increase of arterial stiffness.
Methods and Results: In 3274 middle-aged Japanese men (aged 42 ± 9 years old) without hypertension at the study baseline, the brachial-ankle pulse wave velocity (baPWV), radial augmentation index (rAI), systolic and diastolic blood pressure (SBP/DBP) and serum UA level were measured annually over a 9-year period. During this study period, 474 subjects (14.5%) developed hypertension. Binary logistic regression analysis demonstrated that serum UA levels has a significant odds ratio for the development of hypertension even after the adjustment [1.3 (1.1-1.5), p<0.01]. Multivariate linear regression analysis also demonstrated that serum UA levels at the baseline of study

period had a significant association with delta changes of SBP (beta=0.10 p<0.01), DBP (beta = 0.13, p<0.01) and baPWV (beta=1.12 p<0.01), but not that of rAI, during the study period. Mixed model linear regression analysis in repeated measurement data revealed the following: 1) At each annual measurement, higher serum UA levels were associated with a higher annual increase of the SBP (estimate=0.63 p<0.01), DBP (estimate=0.63 p<0.01) and baPWV (estimate=6.70 p<0.01). Mediation analysis demonstrated that baPWV had a significant indirect effect on the association of UA at the baseline with the delta change of SBP (indirect effect = 0.03 p<0.01). Conclusion: UA is a risk for the development of hypertension. In there, UA may elevate blood pressure via increase in arterial stiffness, at least in parts.

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Dialysis Vintage Longer Than Sixty Months Contributes to Increased Arterial Stiffness and Impaired Diastolic Function in Patients with End-stage Renal Disease

PrimaryAuthor.AuthorBlock:Valentin Calvez, Marco Palladino, Giulio Montefusco, Vincenzo Cesario, Claudia Fofi, Giuseppino M Ciavarella, Massimo Volpe, **Carmine Savoia**, Sapienza Univ Rome, Rome, Italy

Hemodialysis (HD) may induce vascular stiffness through several mechanisms. We sought to determine the role of dialysis vintage (DV) on the development of cardiovascular alterations. We studied 14 patients in chronic HD and 24 newly diagnosed never treated hypertensive patients and 16 normotensive controls.
The patients in HD were divided in two groups according to DV: <60-months (DV<60,n=7) or >60-months (DV>60,n=7). After HD session, when dry weight was reached, we evaluated peripheral blood pressure (pBP), the parameters derived by tonometric analysis of the pulse waveform (central blood pressure-cBP-, Subendocardial Viability Ratio-SEVR-, carotid-femoral pulse wave velocity-cf-PWV-) and those derived from echocardiography: ejection fraction (EF-for systolic function) and E/e' (for diastolic function), and the ultrafiltration volume (UV). Calcium/phosphate (Ca/P) levels, serum albumin, and Kt/V were evaluated retrospectively on repeated measurements over the past 5 years.
All the groups were similar for sex and BMI, both DV<60 and DV>60 were older than hypertensives and controls (58.33±3.71 and 59.83±7.98 vs 44.14±1.28 and vs 40.63±2.05 years, respectively, P<0.05). Both DV<60 and DV>60 presented similar levels of Ca/P, serum albumin, Kt/V and UV. pBP was increased and similar to hypertensives in DV>60 vs DV<60 (systolic-pBP: 154.2±4.5 vs 132.5±5.18 mmHg, P<0.01 and diastolic-pBP: 90.4±49 vs 78.5±3.3 mmHg, P<0.01). Likewise cBP was increased and similar to hypertensive patients in DV>60 vs DV<60 (systolic-cBP: 140.8±8.4 vs 111.2±3.36 mmHg, P<0.001 and diastolic-cBP: 88.2±3.73 vs 72.33±7.78 mmHg, respectively, P<0.05). cf-PWV was similar in normotensives, hypertensives and DV<60, and increased only in DV>60 vs DV<60 (9.6±1.4 vs 7.13±1.4 m/s, p<0.05). SEVR and EF were preserved and similar in all the groups. E/e' was significantly increased only in the groups in HD, however it was higher in DV>60 vs DV<60 (9.16±1.14 vs

6.96±0.72, P<0.01).
In conclusion, only patients with DV>60 presented increased aortic stiffness. This was associated to higher BP and diastolic dysfunction. Hence, chronic HD, particularly after 60 months, may play a putative role in developing cardiovascular alterations in patients with end-stage renal disease.

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Abnormal Pressure Wave Reflection Accelerates the Development of Hypertension via the Increase of Arterial Stiffness

PrimaryAuthor.AuthorBlock:**Hirofumi Tomiyama**, Chisa Matsumoto, Kazuki Shiina, Taishiro Chikamori, Akira yamashina, Shogen Fujii, Tokyo Medical Univ, Tokyo, Japan

Objectives: Arterial stiffness (macrovascular damage) and peripheral vascular damage (microvascular damage) affect pressure wave reflection, and several prospective studies have demonstrated that not only arterial stiffness but also abnormal pressure wave reflection are risks for the development of hypertension. However, the association between arterial stiffness and pressure wave reflection in the development of hypertension has not been fully clarified. The present study was conducted to examine whether the abnormal pressure wave reflection accelerates the development of hypertension via the increase of arterial stiffness.
Methods: In 3102 middle-aged healthy Japanese men without hypertension at baseline, systolic/diastolic blood pressures, brachial-ankle pulse wave velocity (baPWV), and radial augmentation index (rAI) were annually measured during a 9-year study period.
Results: In multivariate linear regression analysis and in mixed model linear regression analysis, baPWV was not longitudinally associated with rAI. Linear regression analysis demonstrated that the higher rAI at the baseline was associated with the larger longitudinal increase of baPWV (beta = 0.17, p<0.01). At the end of study period, 404 subjects were developed to hypertension. Binary logistic regression analysis demonstrated that baPWV (per SD increase = 1.43; 1.25 – 1.62, p<0.01) and rAI (per SD increase = 1.56; 1.33 – 1.82, p<0.01) at the baseline had significant odds ratio for the prediction of the development of hypertension. The prevalence rate of the development of hypertension during the study period was higher in subjects with higher baPWV and higher rAI at the baseline (220 in 939 subjects: 23 %) than that in other 3 groups classified by the status of baPWV and rAI at the baseline (e.g. 52 in 942 subjects with low baPWV combined with low rAI: 6%, p<0.01).
Conclusion: The abnormal pressure wave reflection, which may be derived from both arterial stiffness and peripheral vascular damages, may be an accelerator for the development of hypertension via the increase of arterial stiffness. In there, the combination of macro- and micro-vascular damages may accelerate the development of hypertension.

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P519

Arterial Stiffness is Associated With Lower Performance on the Cognitive Tests at Different Domains in Hypertensive Patients

PrimaryAuthor.AuthorBlock:Henrique Muela, Valeria Costa-Hong, Michel F Machado, Natalia C Moraes, Claudia M Memória, Monica S Yassuda, Edson B Shu, Ayrton R Massaro, Ricardo Nitrini, Alfredo Mansur, **Luiz Bortolotto**, Heart Inst (InCor) of HCFMUSP, São Paulo, Brazil

Background: Cognitive impairment and elevated arterial stiffness are described in patients with arterial hypertension(AH), but its correlations are not well studied. **Objectives:** To study the cognitive function at different domains and arterial properties in patients with AH stage 1 to 3 compared to normotensives and to evaluate the correlations between these variables. **Methods:** We evaluated 221 subjects, 71 normotensives (52±14yrs,47%male,65%white) and 150 patients with stage 1-3 AH (52±12yrs,45%male,70%white) under treatment. The global cognitive function was assessed by Mini Mental State Examination(MMSE) and Montreal Cognitive Assessment(MoCA). There was done a validated comprehensive battery of neuropsychological tests(NPE) assessed the following main cognitive areas: memory, language, visuospatial ability, executive function, attention. Pulse wave velocity(PWV) was measured by Complior® device. Carotid properties were assessed by radiofrequency ultrasound(WTS®). Central arterial pressure and augmentation index (AIx) were obtained using applanation tonometry(Sphygmocor®). **Results:** Mean BP of the normotensive group (122.1±8/76.7±7mmHg) was significantly lower than hypertensive patients (135.2±13/83.3±10 and 149.9±29/91.5±16mmHg). Severe HTN group had worse performance in cognitive evaluation either by MMSE (26.8±2.1 vs 27.4±2.1 vs. 28.0±2.0, p=0.004) or MoCA test (23.4±3.7 vs. 24.9±2.8 vs. 25.5±3.2, p<0.001). On the neuropsychological tests hypertensive patients had worse performance mainly in visuoperceptual and visuospatial capacities and executive function. On the multivariate regression analysis, the following independent associations were observed: Aix-language, executive function, visuospatial and attention; cSBP-MoCA; IMT-memory and attention; PWV-memory, executive function, visuospatial and attention. Higher PWV group had more cognitive dysfunction. **Conclusions:** Cognitive impairment at different domains was more frequent in patients with different stages of AH. Arterial functional and structural properties were diversely associated with cognitive performance at different domains

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Low Plasma Levels of Vitamin D are Not Associated With Carotid Artery Stiffness in Hypertension

PrimaryAuthor.AuthorBlock:**Cristiana Catena**, Gianluca Colussi, Gabriele Brosolo, Nicole Bertin, Marileda Novello, Alessandro Frangipane, Andrea Duratti, Leonardo A Sechi, Internal Med, Udine, Italy

Objectives. Recent studies suggest an association between vitamin D deficiency and prevalence of cardiovascular disease in the general population. However, the evidence of a relationship between vitamin D status and vascular functional changes in hypertension is currently controversial. We aimed to investigate if the presence of low serum vitamin D levels is associated with an increased arterial stiffness in essential hypertension. Design and method. In 151 essential hypertensive patients (53±13 y, 71 males) we measured serum 25(OH)-vitamin D levels and performed a carotid ultrasound examination to determine variables of carotid stiffness, such as distensibility, compliance, Young elastic modulus and beta-stiffness. Furthermore, in a subgroup of 86 subjects we evaluated the augmentation index and the pulse wave velocity. The patients were subdivided into two groups according to the 25(OH)-vitamin D levels, lower or higher than 30 nmol/L. Results. A plasma vitamin D level lower than 30 nmol/L was found in 83 (55%) of 151 patients. Patients with low vitamin D levels were significantly older than those with a normal value of serum vitamin D. No significant differences in carotid artery distensibility, compliance, Young elastic modulus, and beta-stiffness were observed between patients with plasma vitamin D below or above 30 nmol/L. Also, the augmentation index and the pulse wave velocity were comparable in hypertensive patients with lower or higher plasma vitamin D levels. Conclusions. This study does not support the hypothesis of an association between vitamin D deficiency and functional vascular changes in hypertensive patients.

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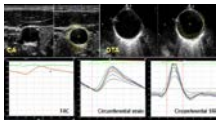
Relationship Between Carotid Arterial Elastic Properties and Central Aortic Hemodynamics in Patients with Stroke

PrimaryAuthor.AuthorBlock:**Sung-ai Kim**, Sang-Ho Jo, Kyung-Ha Park, Hyun-Sook Kim, Hallym Sacred Heart Hosp, An-yang, Korea, Republic of

Objective: Patients with ischemic stroke have more thickened, dilated and stiffer aorta and carotid arteries than patients without. However, there was few data about the relations between the carotid and central aortic elastic properties in those patients. This study aims to investigate the association between carotid artery parameters and central aortic hemodynamics in patients with ischemic stroke. **Methods:** A total of 101 consecutive patients with ischemic stroke who underwent clinically indicated transesophageal echocardiography (TEE) were performed carotid ultrasonography to assess vascular elastic properties. Using velocity-vector imaging (VVI), instantaneous vessel area (cm^2) and deformation as fractional area change (FAC, %) and circumferential strain (%) were obtained in both cross-sectional image of common carotid artery (CA) and descending thoracic aorta (DTA) during TEE. **Results:** In correlation analysis, elastic properties of DTA including FAC, circumferential strain and vessel area were significantly correlated with the corresponding values of CA, respectively (all $p<0.01$). Among the vascular parameters, FAC of CA showed independent association with the circumferential strain of DTA adjusting for age, gender, blood pressure, heart rate, intima-media thickness, pulse wave velocity and vessel area (ß=0.14, p=0.002, 95% CI 0.05-0.24) in linear regression analysis. **Conclusions:** Non-invasively measured elastic properties of CA using VVI showed significant associations with

those of DTA and provide valuable information for better understandings of central aortic hemodynamics in patients with ischemic stroke.



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Endothelial Cell-specific Knockout of Epithelial Sodium Channel Prevents Aortic Stiffness, Cardiac Stiffness and Diastolic Dysfunction in Response to a Western Diet in Female Mice

PrimaryAuthor.AuthorBlock:**Annayya Aroor**, Guanghong Jia, Michael Hill, Javad Habibi, Camila Manrique, Guido Lastra, Brady J Barron, Sadie A Allen, Dongqing Chen, Yan Yang, Zhe Sun, Gerald Meininger, Luis Martinez-Lemus, Adam Whaley-Connell, Vincent G DeMarco, Univ of Missouri, Columbia, MO; Frederic Jaisser, Ctr de Recherche des Cordeliers, Paris, France; James R Sowers, Univ of Missouri, Columbia, MO

A western diet (WD), high in fructose and fat, is often accompanied by insulin resistance and cardiovascular disease characterized by endothelial cell (EC) dysfunction, increased arterial and cardiac stiffness, and diastolic dysfunction. Although premenopausal non-obese women are protected against cardiovascular disease, arterial stiffness and diastolic dysfunction, in obese women these abnormalities are more pronounced than in men. We have recently developed a clinically relevant, WD fed, murine model that exhibits increased aortic stiffness associated with vascular and cardiac dysfunction. In this model, female mice have high plasma aldosterone levels and increased mineralocorticoid receptor expression (MR) in both the vasculature and heart. One of the mechanisms by which MR activation promotes endothelial stiffness is through increased expression and activation of epithelial sodium channel (ENaC) in ECs (EnNaC). We reported increased aortic EC stiffness associated with increased expression of EnNaC in WD fed mice and suppression of aortic stiffness and improved diastolic function by treatment with a low dose of an MR antagonist or the ENaC inhibitor, amiloride. In this study, we tested the hypothesis that specific deletion of EnNaC, decreases aortic EC stiffness and improves vascular relaxation and diastolic function in WD fed female mice. To produce cell specific deletion of the EnNaC gene,"floxed" EnNaC mice were serially crossed with Tie 2-Cre transgene mice. This resulted in marked suppression of EnNaC expression in ECs. Female KO mice and littermate controls were fed a WD with high in fat (46%) and fructose (17.5%) for 12 weeks. Compared to mice fed a control diet (CD), aortic EC stiffness, measured ex vivo by atomic force microscopy (AFM) was significantly increased in WD fed mice and this was prevented in EnNaC KO mice fed WD. Decreased EC stiffness was associated with improved endothelial-dependent aortic relaxation in response to acetylcholine. Moreover, deletion of EnNaC also prevented WD induced impairment of diastolic function. Taken together, these findings support the notion that a WD promotes ECMR mediated activation of EnNaC and associated aortic stiffness, cardiac stiffness and diastolic dysfunction.

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Time and Concentration Dependent Vascular Changes of Cancer Therapy Everolimus in Mesenteric Resistance Arteries

PrimaryAuthor.AuthorBlock:**Patricia Martinez Quinones**, Alexander Warner, Nicole Klee, Camilla F Wenceslau, Cameron G McCarthy, Clinton Webb, Medical Coll of Georgia at Augusta Univ, Augusta, GA

The leading cause of death in cancer survivors is cardiovascular disease, in part due to cancer therapy-induced cardiotoxicity. The short- and long-term cardiovascular side effects of a newer class of targeted chemotherapy, mTOR inhibitors, have not been well established as they have been with anthracyclines, anti-VEGF therapy and tyrosine kinase inhibitors. mTOR inhibitor Everolimus, used as an agent in transplant immunosuppression, is now indicated for the treatment of metastatic breast, colon, renal cell and pancreatic cancer. Retrospective clinical data indicate the possibility of heart failure and hypertension as cardiotoxic effects of Everolimus, although data are inconclusive and no vascular function studies are available. We studied the effects of Everolimus on the contractility and relaxation of mesenteric resistance arteries (MRA) of male Wistar rats (12-15 weeks old) at different drug incubation time frames (1, 12, and 24 hours) on a tension wire myograph. We hypothesized that the longer exposure to Everolimus (i.e. longer incubation time) leads to enhanced contractility to phenylephrine (PE) and impaired relaxation to acetylcholine (ACh). Two concentrations of Everolimus were evaluated, 0.1 µM and 0.1 nM. MRA exposed to Everolimus 0.1 µM showed a decreased contractile response to PE at 1 hour incubation ($LogEC_{50}$, Ctrl: -5.627±0.07 vs. Drug: -5.390±0.10, *p=0.03) and at 12 hours incubation ($LogEC_{50}$, Ctrl: -5.295±0.16 vs. Drug: -5.645±0.08, *p=0.04), while no difference was observed at 24 hours (p>0.05). MRA exposed to Everolimus 0.1 nM for 24 hours showed enhanced contractility to PE ($LogEC_{50}$, Ctrl: -5.295±0.16 vs. Drug=-5.740±0.07, *p=0.005) while no difference at 1 hour or 12 hour incubation times (p>0.05). No differences were observed in the endothelium-dependent relaxation response to ACh for either drug concentration or any of the incubation times. Our data suggest that the effects of Everolimus on resistance arteries from normotensive rats are a time and concentration-dependent: short-term incubation (i.e., < 12hours) increases sensitivity of MRA to adrenergic stimuli. However, long-term treatment with this drug may lead to vascular dysfunction, which could

be associated with cardiotoxicity observed in cancer survivals.

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Vasoprotective Effect of Hesperidin in the Onset of Arterial Hypertension Induced by L-NAME

PrimaryAuthor.AuthorBlock:**Fernanda Priviero**, Augusta Univ, Augusta, GA; Tiago Tomazini Goncalves, Carolina Martins Lazaro, Jackeline Mezencio, Natiele Araujo, Mario A Claudino, Patricia Carvalho, Univ Sao Francisco, Braganca Paulista, Brazil; R Clinton Webb, Augusta Univ, Augusta, GA

Hesperidin has been used to manage venous disease and has been shown to have vasodilator, antiinflammatory and antioxidant properties. The aim of this work was to evaluate the efects of acute treatment with hesperidin (HD) on the vascular reactivity of rats in the onset of arterial hypertension induced by L-NAME. Male Wistar rats were divided into 3 groups as follow: 1- Control (CTL); 2- L-NAME (LN); 3- L-NAME + Hesperidin (LNHD). LN was given by gavage at the dose of 75mg/Kg, concomitantly with HD at the dose of 0.5 mmol/Kg, 24 hours previously to the sacrifice. After sacrifice, the blood was collected and the aorta removed and mounted in a wire myograph to assess concentration response curves to acetylcholine (ACh) and sodium nitroprusside (SNP). ACh elicited concentration dependent relaxation in aorta of CTL rats, reaching a maximum relaxation of 89 ± 3%, which was significantly reduced in LN group (37 ± 7%). Treatment with HD partially prevented this decrease, reaching the maximum relaxation of 72 ± 2%. On the other hand, SNP induced relaxation was more potent on the aorta of LN (pEC_{50} 9.67 ± 0.11) when compared to CTL (pEC_{50} 8.51 ± 0.13) and LNHD rats (pEC_{50} 8.90 ± 0.09). The oxidative status was not changed by any treatment since SOD activity was 11 ± 1, 9 ± 2 and 12 ± 0.5 U/mL, for CTL, LN and LNHD, respectively and lipid peroxidation measured by TBARS assay showed a MDA concentration of 29 ± 8, 35 ± 7 and 23 ± 3 μM for CTL, LN and LNHD, respectively. Altogether, these data suggest that that Hesperidin prevents the impairment of endothelium-dependent vascular relaxation, through a mechanism not related to its antioxidant property.

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Mtor Inhibitor Everolimus Decreases Aortic Sensitivity To Phenylephrine In Normotensive Rats

PrimaryAuthor.AuthorBlock:**Alex Warner**, Patricia Martinez Quinones, Camilla Wencesalu, Cameron McCarthy, Clinton Webb, Medical Coll of Georgia at Augusta Univ, Augusta, GA

While targeted chemotherapeutics have improved cancer clinical outcomes, many of these agents have the drawback of cardiovascular complications. It has been established that the mTOR complexes have a pivotal role in many types of cancer, promoting cell growth, proliferation, and survival. Inhibitors of this pathway such as first generation Everolimus, and second generation PP242, have inhibited cancer cell proliferation and cancer progression. When compared with other target chemotherapeutics such as VEGF and tyrosine kinase inhibitors, first generation mTOR inhibitors have demonstrated decreased incidence of cardiovascular complications, however the specific vascular effects of mTOR inhibitors have not been investigated. Therefore, Everolimus, an inhibitor of mTORC1, and PP242, an inhibitor of mTORC1 and mTORC2, were evaluated for their effects on vascular reactivity of rat aorta. Previous studies have demonstrated that mTORC2 displayed cardioprotective effects. Therefore, we hypothesize that Everolimus, which does not inhibit mTORC2, will have a decreased contractile response to Phenylephrine (PE) and increased Acetylcholine (Ach)-induced relaxation. On the contrary, since PP242 inhibits mTORC2, we hypothesize that PP242 will increase contractile response to PE, and will decrease ACh-induced relaxation. Concentration response curves to PE (1nM - 30μM), and Ach (1nM - 30μM) were conducted in isolated aortic rings from male normotensive Wistar rats (12-15 weeks old) on a pin myograph *ex vivo* after 1 hour of incubation with each drug. Aortic rings treated with 31 μM of Everolimus showed decreased sensitivity to PE ($LogEC_{50}$= -7.322 ±0.078 vs. treated: -6.578 ± 0.1116 $p<0.05$). No differences were observed in the contractile responses to PE for Everolimus (0.1 nM) or PP242 (245 nM) ($p>0.05$). Similarly, no differences were observed in the endothelium-dependent relaxation response to Ach for all three treatments. These results suggest that Everolimus may be a good therapeutic choice for cancer patients also suffering from cardiovascular dysfunction.

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MMP-2 Activation By S-glutathiolation Decreases Calponin-1 During Vascular Remodeling In Hypertension

PrimaryAuthor.AuthorBlock:Juliana Parente, Marcela Mello, Univ of Sao Paulo, Ribeirão Preto, Brazil; Brandon Chan, Richard Schulz, Univ of Alberta, Edmonton, AB, Canada; **Michele Castro**, Univ of Sao Paulo, Ribeirão Preto, Brazil

Aim: Antioxidant tempol may decrease MMP-2 activation by S-glutathiolation as it reduces oxidative stress, and then prevents calponin-1 cleavage and aortic remodeling in hypertension. **Methods:** Male Wistar rats were submitted to the two kidney-one clip (2K-1C) model of hypertension or sham surgery and were treated with tempol (18 mg/kg/day) or water by gavage for one week. Systolic blood pressure (SBP) was accessed by tail-cuff plethysmography. Aortas were used to perform *in situ* zymography, immunofluorescence for dihydroethidium and calponin-1, immunohistochemistry for

nitrotyrosine, co-immunoprecipitation for glutathione followed by Western blot for MMP-2 and histomorphology. Two-way ANOVA followed by Bonferroni test was done (Ethics Committee approval number: 0015/2017). **Results:** SBP increased in 2K-1C rats compared to Sham group (156±2.9 vs. 128.6±4.1, $p<0.05$) and tempol did not reduce it (147.3±4.7, $p>0.05$). Morphological analysis showed increased that 2K-1C aortas had increased media to lumen ratio (M/L) (8.6±0.7 vs. 6.5±0.2) and cross-sectional area (CSA) ($1.4x10^6±1.5x10^5$ vs. $9.7x10^5±4.2x10^4$) ($p<0.05$ vs. Sham), but tempol did not decrease them (M/L: 7.9±0.8; CSA: $1.3x10^6±1.3x10^5$, $p>0.05$ vs. 2K-1C). However, tempol decreased 2K-1C-induced increased nitrotyrosine levels ($5.9x10^5±1.3x10^4$ vs. $4.0x10^5±6.1x10^4$), dihydroethidium (16.9±1.7 vs. 8.9±1.1) and MMP-2 activity (57.5±6.4 vs. 39.4±2.6; $p<0.05$). All four groups presented S-glutathiolation of MMP-2, however, the levels of calponin-1 were only reduced in aortas from 2K-1C rats (17.2±1.5 vs. 28.5±1.2, $p<0.05$ vs. Sham groups) and tempol reverted such loss (23.6±1.6, $p<0.05$ vs. 2K-1C). As calponin-1 is a potential intracellular target of MMP-2 in VSMC, these results suggest that the intracellular activation of MMP-2 may be more pronounced in hypertension. Different concentrations of tempol (0.5, 1 or 1.5 mM) did not
directly inhibit MMP-2 activity in gelatin zymography (respectively: $4.6x10^6±7.4x10^4$, $4.9x10^6±1.2x10^5$ and $4.8x10^6±1.5x10^5$ vs. $4.6x10^6±2.2x10^5$; $p>0.05$). **Conclusion:** Increased oxidative stress may contribute to activate aortic MMP-2 by S-glutathiolation in early hypertension, thus resulting in calponin-1 loss and maladaptive remodeling.

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Angiotensin-(1-7) Attenuates Doxorubicin-Induced Aortic Dysfunction in Male and Female Rats by Distinct Mechanisms

PrimaryAuthor.AuthorBlock:**Omeed Rahimi**, Jay L, Kirby, Jasmina Varagic, E. Ann Tallant, Patricia E Gallagher, Wake Forest Sch of Med, Winston-Salem, NC

Doxorubicin (Dox), a commonly used and effective chemotherapeutic agent, often produces cumulative dose-dependent cardiovascular toxicity, resulting in long-term hypertrophy and fibrosis which can lead to heart failure. Adjunct therapies are thus needed to reduce Dox-induced cardiovascular toxicity and enhance long-term quality-of-life in cancer patients, especially in pediatric patients. Angiotensin-(1-7) [Ang-(1-7)] is an endogenous peptide hormone of the renin-angiotensin system that improves cardiac and vascular function by reducing hypertrophy and fibrosis in various animal models. In this study, juvenile Sprague-Dawley rats (male and female, n = 8-10) were administered Dox (cumulative dose of 22 mg/kg) for 6 week, in the presence and absence of Ang-(1-7) [24 µg/kg/h]. Aortic function was measured using a Vevo 2100 small animal ultrasound system. In both males and females, Dox administration increased pulse wave velocity (PWV), a measure of arterial stiffness, and co-treatment with Ang-(1-7) attenuated the Dox-induced increase (Males - 5.6 ± 0.5, Sham; 9.7 ± 1.4, Dox; 7.8 ± 0.6 m/s, Dox/Ang-(1-7), $p < 0.01$; Females - 5.1 ± 0.5, Sham; 14.3 ± 1.5, Dox; 7.7 ± 1.2 m/s, Dox/Ang-(1-7), $p < 0.001$); Ang-(1-7) alone had no effect. Dox increased aortic thickness and decreased aortic diameter at systole in males only, which was attenuated by Ang-(1-7) (aortic thickness - 0.28 ± 0.01, Sham; 0.33 ± 0.01, Dox; 0.28 ± 0.01

mm, Dox/Ang-(1-7), $p < 0.01$; aortic diameter - 2.8 ± 0.6, Sham; 2.3 ± 0.1, Dox; 2.5 ± 0.1 mm, Dox/Ang-(1-7); $p < 0.01$). No change in aortic thickness or diameter was observed following treatment with Ang-(1-7) alone. Conversely, Dox increased fibrosis in female aorta only, measured by immunohistochemistry with Picrosirius red, which was attenuated by Ang-(1-7) (5.4 ± 0.3, Sham; 7.2 ± 0.6, Ang-(1-7); 12.8 ± 2.0, Dox; 8.0 ± 1.0%, Dox/Ang-(1-7); $p < 0.001$). These results demonstrate that Dox causes aortic dysfunction in both males and females, albeit through different mechanisms—an increase in aortic hypertrophy in males and aortic fibrosis in females. Ang-(1-7) attenuated both the hypertrophy and fibrosis, suggesting that treatment with the heptapeptide hormone may serve as an effective adjuvant to improve Dox-induced aortic dysfunction.

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Modifiable Factors Affecting BP in Identical Twins

PrimaryAuthor.AuthorBlock:**Srividya Kidambi**, Xiaoqing Pan, Yingchuan Li, Pengyuan Liu, Michelle Liettl, Theodore Kotchen, Allen Cowley, Mawusi Kamassah, Yong Liu, Medical Coll of Wisconsin, Brookfield, WI; S A Burt, Kelly Klump, Supratik Rayamajhi, Michigan State Univ, East Lansing, MI; Cheryl Rockwell, Eric Gernaat, Michigan State Univ, East lansing, MI; Mary Donohue, Rakesh Reddy, Michigan State Univ, East Lansing, MI; Ralph Watson, Michigan State Univeristy, East lansing, MI; David Mattson, Mingyu Liang, Medical Coll of Wisconsin, Brookfield, WI

Background: Genetic factors determining blood pressure (BP) are largely unmodifiable. Understanding the effects of physiologic, environmental, and epigenetic (modifiable) factors on BP levels can improve strategies for hypertension (HTN) control. Monozygotic (MZ) twins with identical genetic background are a good model to study the effect of these factors on BP. Methods: Seventy pairs of MZ twins were from Milwaukee, WI and Michigan State University Twin Registry, East Lansing, MI (62% women; age of 44 ± 10 years). BPs (measured in triplicate and averaged), anthropometrics, physical activity (Rapid Assessment of Physical Activity), and sodium intake measurements (Block Sodium Screener) were obtained. DNA was obtained from T-lymphocytes for methylation (epigenetic) sequencing analysis. Differences in systolic (SBP) and diastolic (DBP) BPs between co-twins were used as continuous variables for these analyses. Results: Mean SBP was 124 ± 15 mm Hg (mean ± SD) and DBP was 78 ± 11 mm Hg. Average differences in SBP and DBP among co-twins were 8 ± 9 and 7 ± 6 mm Hg respectively. Twin pairs were considered concordant or discordant based on BP difference of 10 mm Hg between co-twins or one twin being hypertensive. Discordant twins as a group had higher BMI and waist circumference (WC) compared to concordant twins ($p<0.05$). Among discordant twins, the co-twin with higher BPs tended to have higher waist circumference (WC) (105 ± 15 vs. 98 ± 18 cm) and dietary salt intake (3900 ± 1437 vs. 3261 ± 1058 mg/d) even though they did not reach statistical significance. There were no differences in physical activity or education levels. Genome-wide methylation analyses revealed that 3 loci were associated with SBP difference and 2 were associated with DBP difference at an unadjusted significance level of $p < 10^{-4}$. Two sites reached an adjusted

significance level of 0.08 with SBP difference. Conclusions: In this study, we identified several differentially methylated sites of interest in relation to BP among MZ twins. In addition, higher central adiposity and sodium intake may contribute to BP levels among MZ twins. Understanding the relationship of modifiable risk factors such as WC and sodium intake with methylation changes may shed light into novel pathogenic pathways for HTN.

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Renal

PrimaryAuthor.AuthorBlock:Prasad Konkalmatt, Fei Han, Gaosi Xu, Xiaoxu Zheng, George Washington Univ, Washington, DC; John J Gildea, Robin A Felder, Univ of Virginia, Charlottesville, VA; Pedro A Jose, **Ines Armando**, George Washington Univ, Washington, DC

Some common single nucleotide polymorphisms (rs6276 and rs6277, SNPs) of the human *DRD2* gene are associated with decreased D2R expression and function and increased blood pressure or hypertension. Human renal proximal tubule cells (hRPTCs) from subjects carrying these SNPs (hRPTCs-SNPs) express elevated levels of proinflammatory and profibrotic proteins, indicating that the D2R has protective effects in these cells and that decreased D2R function may contribute to the susceptibility to renal disease associated with essential hypertension. Micro RNA 4301 (miR4301) is an intronic miRNA that resides in the second intron of the primary human *DRD2* transcript. A mouse homolog of miR4301 has not been identified to date. We hypothesized that miR4301 expression and function are decreased in hRPTCs-SNPs and that loss of miR4301 mediates, in part, the deleterious effects of decreased D2R function. We studied four cell lines carrying no SNPs (hRPTCs-WT) and four hRPTCs-SNPs lines. miR4301 expression was lower in hRPTCs-SNPs than in hRPTCs-WT (0.52±0.07- vs 1.03±0.14-fold; $P<0.05$). Silencing D2R via siRNA in hRPTCs-WT also decreased miR4301 expression (0.59±0.12- vs 1.01±0.11-fold; $P<0.05$). We measured the expression of several genes with pro-inflammatory and pro-fibrotic effects identified by Target 7.1 as miR4301 targets. The mRNA expressions of *SMAD1* (2.7-fold); *CASP2* (4.13-fold), *CASP7* (3.0-fold), *TGFβR1* (4.9-fold), and *CTGF*(7.3-fold) were increased in hRPTCs-SNPs, in comparison with hRPTCs-WT. Moreover, the mRNA expression of the miR4301 target *LEF1* in hRPTCs-WT transfected with miR4301 mimic was lower (0.70±0.02 vs 1.0±0.05-fold; $P<0.05$) than in cells transfected with control miR, while transfection with miR4301 inhibitor increased the expression of *LEF1* mRNA (1.30±0.03 vs 1.0±0.05-fold; $P<0.05$), compared with the control miR indicating that miR4301 represses *LEF1* expression. These results show that miR4301 mediates, in part, the protective effects of D2R expression and function on renal injury by repressing the expression of genes related to fibrosis and inflammation and suggest that the function of D2R-related proteins may be dependent, as well, on the regulation of specific miR4302.

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Pleiotropic Effects on Blood Pressure Traits Using Genome-wide Analysis of Gene-alcohol Interactions

PrimaryAuthor.AuthorBlock:**Aldi T Kraja**, Mary F Feitosa, Div of Statistical Genomics, Dept of Genetics, Ctr for Genome Sciences and Systems Biology, Washington Univ Sch of Med, Saint Louis, MO; Daniel Chasman, Deaprtment of Epidemiology, Brigham and Women's Hosp, MA, USA, Boston, MA; Yun J Sung, Div of Biostatistics, Washington Univ Sch of Med, Saint Louis, MO; Thomas W Winkler, Dept of Genetic Epidemiology, Inst of Epidemiology and Preventive Med, Univ Regensburg, Regensburg, Germany; Ioanna Ntalla, Dept of Health Sciences, Univ of Leicester, Leicestershire, United Kingdom; Hugues Aschard, Dept of Epidemiology, Harvard Medical Sch, Boston, MA; Kenneth Rice, Dept of Biostatics, Univ of Washington, Seattle, WA; Alisa K Manning, Harvard Univ and Massachusetts General Hosp, Boston, MA; Jeffrey O'Connell, Dept of Med, Univ of Maryland Sch of Med, Baltimore, MD; Sharon LR Kardia, Dept of Epidemiology, Univ of Michigan, Ann Arbor, MI; Patricia Munroe, William Harvey Res Inst, Barts and the London Sch of Med and Dentistry, London, United Kingdom; L. Adrienne Cupples, Dept of Biostatistics, Boston Univ Sch of Public Health, Boston, MA; Alana Morrison, Dept of Epidemiology, Human Genetics and Environmental Sciences, Sch of Public Health, The Univ of Texas Health Science Ctr at Houston, Houston, TX; W. James Gauderman, Dept of Preventive Med, Keck Sch of Med, Univ of Southern California, Los Angeles, CA; Daniel Levy, Framingham Heart Study, Framingham, MA and the Population Sciences Branch, Natl Heart, Lung, and Blood Inst, Bethesda, MD; D.C. Rao, Div of Biostatistics, Washington Univ Sch of Med, Saint Louis, MO; Xiaofeng Zhu, Dept of Epidemiology and Biostatistics, Case Western Reserve Univ, Cleveland, OH; Michael A Province, Div of Statistical Genomics, Dept of Genetics, Ctr for Genome Sciences and Systems Biology, Washington Univ Sch of Med, Saint Louis, MO; on behalf of the CHARGE Gene-Lifestyle Interactions, This work is supported in part by the NHLBI grant HL 118305, -, -, MO

We tested for pleiotropy in European ancestry subjects (N>90K) via GWAS of systolic and diastolic blood pressure (BP), mean arterial pressure, and pulse pressure, using gene (G)-alcohol consumption (E) interactions. The approach was a correlated meta-analysis (PMCID-PMC3773990) that combined simultaneously the 4 BP traits genome-wide GxE interactions summary meta-P values. This approach adjusts for correlations among single traits at the genomic level. A variant was considered pleiotropic when the overall correlated meta-analysis yielded $P \leq 5E\text{-}08$ and GxE meta-$P \leq E\text{-}04$ for at least two single traits. The novel pleiotropic variants localize in eight loci. *TTLL7* (1p31.1) is a tubulin modifier. *DYRK3* (1q32.1) is a transcription regulator. *MAPKAPK2* (1q32.1) is a stress-activated serine/threonine-protein kinase involved in cytokine production

especially for *TNF*, *IL6* and phosphorylates (among others) *LSP1*, identified in our GWAS GxE study for individual BP traits. *FSTL5* (4q32.2) is annotated as *calcium ion binding*. A locus at 11q13.1 includes *SNX32*, *EFEMP2,* and *FOSL1*. *FOSL1* variants may regulate expression of *SNX32*. *EFEMP2* is implicated in blood coagulation. *CATSPER2* (15q15.3) is a cation channel. *CCDC151* (19p13.2) is an outer dynein arm assembly. The functions of two other loci (17q22 and 18q22.3) are unknown. We also identified 4 pleiotropic loci (*SGK223*, *TNKS*, *GATA4*, *FTO*) that were found significant at our GxE meta-GWAS of single traits in 572K multi-ancestry individuals. In addition, we detected 24 pleiotropic BP-known loci. Some of these genes relate to alcohol consumption (e.g., *BLK*, *GATA4*, *FTO*). *TNKS*, *MAPKAPK2* and *FSTL5* interact with the *Wnt/β-catenin* signaling pathway, which contributes to hypertension. Several pleiotropic variants showed features of regulation by locating at promoter and enhancer histone marks, at DNAse, at proteins binding sites and being eQTL. The 36 novel and BP-known loci comprising 86 significant genes were enriched for *Hypertension*, *Cardiac arrhythmias*, *Myocardial infarction*, *Atrial fibrillation,* and *Left ventricular hypertrophy*. Our correlated meta-analysis of GxE interaction approach identified novel pleiotropic loci and validated known BP loci, thus providing insights into the mechanisms of hypertension.

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FOXO3, a Cardiovascular Protector, is at the Hub of a 46-gene Cell Resilience "Gene Factory" on Human Chromosome 6

PrimaryAuthor.AuthorBlock:**Brian J Morris**, Univ of Sydney, Sch of Medical Sciences, John A. Burns Sch of Med, Univ of Hawaii; Kuakini Medical Ctr, Dept of Res, Sydney, Australia; Timothy A. Donlon, Univ of Hawaii, John A. Burns Sch of Med and Kuakini Medical Ctr, Dept of Res, Honolulu, HI; Randi Chen, Kuakini Medical Ctr, Dept of Res, Honolulu, HI; Kamal H. Masaki, Univ of Hawaii, John A. Burns Sch of Med, Dept of Geriatric Med and Kuakini Medical Ctr, Dept of Res, Honolulu, HI; Richard C. Allsopp, Univ of Hawaii, John A. Burns Sch of Med,, Honolulu, HI; D. Craig Willcox, Okinawa Intl Univ; John A. Burns Sch of Med, Univ of Hawaii; Kuakini Medical Ctr, Dept of Res, Okinawa, Japan; Ayako Elliott, Kuakini Medical Ctr, Dept of Res, Honolulu, HI; Bradley J. Willcox, Univ of Hawaii, Dept of Geriatric Med, John A. Burns Sch of Med and Kuakini Medical Ctr, Dept of Res, Honolulu, HI

The transcription factor FoxO3 regulates multiple genes involved in cell resilience. We have previously implicated variation in non-coding DNA of the FoxO3 gene (*FOXO3*) with lower blood pressure, reduced inflammation, less hypertension, reduced coronary heart disease mortality, and longevity. The aim of the present study was to determine transcriptional, genetic and genomic mechanisms involving *FOXO3*. By DNA sequencing of chromosome 6q21 in lymphoblastoid cell lines of 95 men who had survived to ≥ 95 years of age we identified 110 *FOXO3* single nucleotide polymorphisms (SNPs). Thirteen SNPs were at binding sites for 18 transcription factors. Those SNPs appeared to be in physical contact, via RNA polymerase II

binding chromatin looping, with sites in the *FOXO3* promoter, and likely function together as a *cis*-regulatory unit. At the chromosome level, *FOXO3* was located at the center of a 7.3 Mb 46-gene chromatin domain flanked by gene deserts. We identified distant contact points between *FOXO3* and these 46 neighboring genes, through long-range physical contacts via CCCTC-binding factor zinc finger protein (CTCF) binding sites. The genes in this "archipelago" of neighbourhood genes mediate a similar repertoire of functions as FoxO3, including stress resistance, nutrient sensing, cell proliferation, autophagy, apoptosis and stem cell maintenance. The 7.3 Mb gene domain was highly conserved across species, indicating evolutionary importance. We believe that *FOXO3* serves as the hub for an "interactome" involved in healthy aging, including cardiovascular disease reduction, in those with favorable *FOXO3* genotypes. In support, we found that cellular stress (H_2O_2) could stimulate *FOXO3* expression in 20 lymphoblastoid cell lines, being 3-fold stronger for those with a favorable *FOXO3* genotype. In FISH experiments, stress-induced activation of *FOXO3* caused it to move towards its neighboring genes as suggested by our genomic data. In conclusion, we have shown, for the first time, that *FOXO3* is at the central hub of a gene network on chromosome 6 involved in cell protection and healthy aging. The concept of "gene factories" may apply more broadly to genome and genetic mechanisms involved in cardiovascular disease etiology.

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Hypertension and Gender Differences in Acute Ischemic Stroke Patients Treated With Rtpa

PrimaryAuthor.AuthorBlock:**Thomas I Nathaniel**, Jordan Gainey, Leah Wormack, Chibueze Ubah, Leanne Brechtel, Amber Frazier, Univ of South Carolina, Greenville, SC

Background.
Women and men have a similar incidence for ischemic cerebrovascular disease but women are more frequently hit by stroke later in life than men. It has been shown that women presented with severe stroke symptoms during admission, a poorer prognosis, are likely to have an overall poorer outcome after ischemic stroke when compared with men. However, some studies indicate similarities in outcome for men and women after stroke. Moreover, there is evidence that women treated with tPA benefit at least as much as men. Since a higher diastolic blood pressure increases the risk for a worse prospective functional status in men, but less significance in women, it is not clear whether hypertensive patients with acute ischemic stroke treated will reveal similarity or difference in functional outcome. We investigated this issue in the current study.
Method. We performed a retrospective analysis of 4500 acute ischemic stroke patients who presented to a health care system between January 2010 and June 2016 and received rt-PA. We develop a new tool to determine the possibility of erasing any gender difference, and identify the most important factor for the poorer outcomes in women or men and whether thrombolysis may counteract this effect.
Results. Our results reveal that poorer outcomes after stroke and the observed gender differences is due to age, at stroke onset, and that thrombolysis may neutralize this effect.
Conclusion. The important factor for the poorer

outcome and gender differences in hypertensive patients with acute ischemic stroke is mainly due to stroke severity and thrombolysis may play a major role in neutralizing this effect.

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P533

Distinct Molecular Compositions of Circulating Microparticles in Hypertension and Diabetes

PrimaryAuthor.AuthorBlock:Maddison Turner, Jean-Francois Thibodeau, Chet Holterman, Christopher Kennedy, **Dylan E Burger**, Univ Ottawa, Ottawa, ON, Canada

Background: Microparticles (MPs) are emerging as novel markers and mediators of vascular pathology. The purpose of this study was to examine the effect of hypertension and diabetes on the molecular composition of circulating MPs. **Methods:** We studied circulating MPs isolated from transgenic mice expressing active human renin in the liver (TtRhRen, a model of hypertension), OVE26 type 1 diabetic mice, and their wild-type (WT) littermates. At 20 weeks of age mice were sacrificed and blood samples were obtained by cardiac puncture. MPs were subsequently isolated from platelet-free plasma via differential centrifugation and protein content was assessed via mass spectrometry (MS). **Results:** TtRhRen mice exhibited increased blood pressure compared with OVE26 mice or their WT littermates. (144.2±7.6 vs 123.5±4.9 [OVE26] vs. 114.6±5.7 mmHg [WT], $p<0.05$). MS identified 300 independent proteins with at least 2 peptides per protein. Of these, 167 were found in all groups studied, 27 were exclusive to WT mice, 4 were exclusive to TtRhRen mice and 46 were exclusive to OVE26 mice. In addition, 16 proteins were found in MPs from WT and OVE26 mice but absent from TtRhRen mice while 40 were found in MPs from TtRhRen and OVE26 mice but absent in MPs from WT mice. Interestingly, proteins exclusive to MPs derived from hypertensive TtRhRen mice included two proteins previously implicated in the pathogenesis of hypertension: insulin-like growth factor-binding protein 5 and haptoglobin. **Conclusions:** Taken together our results suggest that circulating MPs display a distinct molecular composition, reflective of pathogenic state. Further examination of these changes may lead to the identification of novel biomarkers of vascular injury in hypertension and diabetes.

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Cardiac and Renal Involvement in Malignant-accelerated Hypertension: Implications on Long-term Survival

PrimaryAuthor.AuthorBlock:**Luiz Bortolotto**, Jose J De Lima, Dante Giorgi, Heart Inst, São Paulo, Brazil

Background: Malignant hypertension is a severe complication of arterial hypertension where end-organ damage is severe and occurs in a short period of time. Data about cardiac and renal involvement and implications on long-term survival of patients with malignant

hypertension are scarce. Methods: We performed a single-centre retrospective analysis of 176 patients with malignant hypertension, diagnosed in the period 1984-2007. Results: Incidence of malignant hypertension decreased along the different periods of the study, but cardiac and renal damage at presentation were common and severe. In 94.6% we observed left ventricle hypertrophy on echocardiogram, in 83.2% the glomerular filtration rate was below 60 ml/min/1.73m2 and 11% of patients required immediate dialysis. The survival rate was 95% at 6 months, 90% at 12 months, 81% at 36 months, and 67% at 5-year follow-up. In a Cox-regression analysis, the independent predictors of all-cause mortality were age, SBP at discharge, septum wall thickness values and GFR at admission. Conclusion: Malignant hypertension remains a severe complication of arterial hypertension with a high mortality rate at 5-year, that was independently associated to the severe cardiac and renal involvement present in most patients.

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Distinct Profile of Circulating Extracellular Vesicles in Normotensive Obese Humans

PrimaryAuthor.AuthorBlock:**Uta Erdbruegger**, Natalie Z Eichner, Nicole M Gilbertson, Christine Rudy, Luca Musante, Steven K Malin, Thu H Le, Univ of Virginia Health System, Charlottesville, VA

Hypertension (HTN) is a leading cause of cardiovascular disease. Early and non-invasive biomarkers of vascular dysfunction and end-organ damage in HTN are needed to optimize treatment for patients at risk for HTN. Extracellular vesicles (EVs) are potential candidate biomarkers that reflect occurrence of end-organ damage prior to symptom development. EVs are a heterogeneous population of submicron vesicles that shed from various cell types in the blood and carry markers from their parent cells. We hypothesized that obese adults with normotension have a distinct profile of circulating EVs. Twenty-two obese normotensive patients (Age: 62.5±8.9y, BMI 33.1±6.4 kg/m2) were enrolled in this cross-sectional study. After an overnight fast, systolic and diastolic blood pressure were 126±12.5mmHg and 68.7±8.2mmHg, respectively. Arterial stiffness was assessed by pulse wave velocity and augmentation index. Enumeration and phenotyping of platelet poor plasma EVs was performed using imaging flow cytometry. CD42 positive (platelet), CD31 (PECAM), CD105 (S-endoglin, endothelial), CD45 (leukocyte) positive and Annexin V (AnV) positive EVs were used as surface markers for circulating EVs. Annexin V (AnV) and S-Endoglin positive EVs (CD105+, AnV+) correlated significantly with the diastolic blood pressure ($p=0.03$, $r=0.46$). In addition, leukocyte derived EVs (CD45+, AnV+) correlated with pulse wave velocity ($p=0.04$, $r=0.44$). Other platelet derived EVs and AnV negative EVs did not correlate with blood pressure level or measures of vascular health. Subgroups of endothelial and leukocyte derived EVs correlate with diastolic blood pressure and arterial stiffness in obese normotensive adults. These findings suggest that the specific EV profile might reflect pathophysiologically significant pre-hypertensive blood pressure.

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Ambulatory Blood Pressure and Endothelial Dysfunction an Hematopoietic Cell Transplant Patients

PrimaryAuthor.AuthorBlock:Emily Pao, Nancy Gove, **Joseph Flynn**, Sangeeta Hingorani, Seattle Children's Hosp, Seattle, WA

Hematopoietic cell transplantation (HCT) is a common treatment for malignancies, metabolic, and genetic disorders. Albuminuria post-HCT, which may represent systemic endothelial injury or inflammation from graft vs host disease, increases the risk of kidney disease and non-relapse mortality at 1 year post-HCT. HCT patients are also known to have abnormal blood pressure (BP) and increased rates of cardiovascular complications. We sought to determine the relationships between albuminuria, endothelial dysfunction and ambulatory BP in HCT patients.
Patients ≥ 12 years of age who had their first allogeneic HCT between 2012-2015 and survived through day 80 were eligible. Peripheral endothelial function was assessed using the EndoPAT2000 device at day 80 post HCT along with a 24 hour ambulatory blood pressure monitor (ABPM) study. Clinical and lab data were collected and a urine sample for an albumin to creatinine ratio (ACR). Both logistic and linear regression analyses were used to identify associations between EndoPAT score and clinical variables including eGFR, 24 hour ABPM study, albuminuria, and serum lipids. Sixty patients completed the study. The median age was 48 years (range, 14-69). The median EndoPAT score (RHI normal ≥ 1.69) was 2.05 (range, 1.02- 4.45) and 28% (17/60) of the patients had abnormal endothelial function. Forty-two patients (72%) had hypertension based on the 24-hr ABPM results and 63% of the patients (38/60) had abnormal nocturnal dipping. Hypertension on ABPM ($p = 0.04$), abnormal nocturnal dipping ($p=0.04$), and elevated serum triglyceride levels ($p=0.03$) were associated with a lower EndoPAT score. Albuminuria was not associated with EndoPAT score or hypertension based on ABPM. Abnormal nocturnal dipping was associated with lower eGFR ($p=0.03$). There was a lack of agreement between office BP and use of medications and results on a 24 hour ABPM study ($p < 0.001$).
We did not find a correlation with lower EndoPAT scores and albuminuria; we did find associations between abnormal nocturnal dip, elevated triglyceride levels and hypertension diagnosed by 24 hour ABPM. Casual office BP readings do not accurately reflect the HCT patient's true BP suggesting that 24 hour ABPM studies are needed to diagnose and treat hypertension appropriately.

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Coenzyme Q10 Supplementation in Orthostatic Hypotension and Multiple-system Atrophy, Report on 5 Cases

PrimaryAuthor.AuthorBlock:**Christopher Rembold**, Univ VA Health Science Ctr, Charlottesville, VA

Multiple-system atrophy is a neurologic disorder characterized by orthostatic hypotension, Parkinsonian signs, and cerebellar signs. Mutations in COQ2, an enzyme involved

in coenzyme Q10 (CoQ10) synthesis were recently associated with familial and sporadic cases of multiple-system atrophy. I hypothesized that people with symptoms suggesting multiple-system atrophy might benefit from CoQ10 administration. Five patients with symptomatic orthostatic hypotension were treated in an unrandomized manner with 240±40 mg CoQ10 daily for 8±3 months - two had Parkinsonian and one had cerebellar signs. Prior to starting CoQ10, systolic BP fell 30±5 mmHg upon standing from a sitting position. After treatment with CoQ10, systolic BP fell 8±5 mmHg upon standing from a sitting position (p=0.013 for change in systolic BP fall by paired t test). These data suggest that orthostatic hypotension could possibly improve with CoQ10 administration and that a randomized clinical trial to test this hypothesis should be begun.

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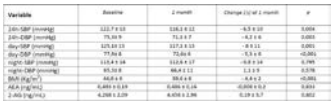
P543

Changes in Plasma Anandamide Concentrations are Associated With Reduction in Blood Pressure in Morbidly Obese Patients Undergoing Bariatric Surgery

PrimaryAuthor.AuthorBlock:**Anna Oliveras**, Hosp del Mar, Barcelona, Spain; Antoni Pastor, IMIM-Hosp del Mar Res Inst, Barcelona, Spain; Ana Marina Granados, Albert Goday, José M Ramon-Morós, Susana Vázquez, Maria Vera, Laia Sans, Hosp del Mar, Barcelona, Spain; Rafael de la Torre, IMIM-Hosp del Mar Res Inst, Barcelona, Spain; Julio Pascual, Hosp del Mar, Barcelona, Spain

The role of endocannabinoids in the vascular system is not well-known. Blood pressure (BP) levels decrease in obese patients after undergoing bariatric surgery, but the underlying mechanisms remain unclear.
Aim: to explore the associations between changes (Δ) in serum endocannabinoids [anandamide (AEA) and 2-arachidonoylglycerol (2-AG)] and Δ in ambulatory BP in morbidly obese patients 1 month after undergoing bariatric surgery (BxS).
Methods: Thirty-one patients (25 female, 16 hypertensives, mean age 45±8,6 yr, mean body mass index [BMI] 44±5,6 kg/m^2) with morbid obesity undergoing BxS were prospectively examined. Twenty-four hours-ambulatory BP parameters and serum AEA and 2-AG concentrations were evaluated at baseline and 1 month after BxS.
Results: 24h- systolic and diastolic BP decreased at 1 month (Table).



SBP=systolic blood pressure; DBP=diastolic blood pressure; BMI=body mass index; AEA=anandamide; 2-AG=2-arachidonoylglycerol
There were inverse correlations of Δ AEA with Δ 24h-SBP (P=-0,493; p=0,020) and with Δ 24h-DBP (P=-0,560; p=0.007). After adjusting for Δ BMI and baseline BP values, the correlation between Δ AEA and Δ 24h-DBP (β coef. =-0,38; 95%CI: -26,47 to -0,38) remained statistically significant (p=0,044; R^2=0,523).
Conclusion: in obese patients undergoing BxS, the decrease of 24h-DBP at 1 month is related to changes in AEA levels.

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Osteopontin Deficiency Decreases Autophagy and Exacerbates Tubular Injury in Acute Kidney Injury Induced by Folic Acid

PrimaryAuthor.AuthorBlock:**Tomoaki Nagao**, Takafumi Okura, Akiko Tanino, Ken-ichi Miyoshi, Masayoshi Kukida, Jitsuo Higaki, Ehime Univ Graduate Sch, Toon, Japan

Osteopontin (OPN), a secreted glycosylated phosphoprotein and pro-inflammatory cytokine, has been implicated in the pathology of several renal conditions, especially renal fibrosis in chronic kidney disease. OPN is slightly expressed in renal tubular cells in normal condition, but after acute tubular injury, OPN is highly induced in these cells. However, the role of induced OPN is still unclear.
The aim of this study was to clarify the roles of OPN in acute kidney injury (AKI).
AKI was induced in wild type (WT) and OPN knockout (KO) mice by using folic acid (FA) injection (0.35mg/kg). After 2days of injection, 34% of WT mice died, whereas 54% of KO died from renal failure. Kidneys from survived mice were removed and the renal histological changes, protein expression were examined. BUN and Creatinine levels were markedly elevated in WT-AKI and KO-AKI mice (BUN: WT-sham; 25.7±4.7mg/dl, WT-AKI; 315.0±173.2mg/dl, KO-AKI; 337.7±163.7mg/dl, Creatinine: WT-sham; 0.08±0.03 mg/dl, WT-AKI; 1.60±0.87 mg/dl, KO-AKI; 1.80±0.94 mg/dl). Renal OPN mRNA expression was increased in WT-AKI mice compared to WT-sham mice ($p<0.05$). High levels of OPN expression in renal tubular cells were induced in WT-AKI mice. TUNEL positive tubular cells were increased in KO-AKI mice compared to WT-AKI mice. In immunohistochemical analysis, Kidney injury molecules 1 (Kim-1) positive tubular cells were also highly
increased in KO-AKI mice compared to WT-AKI mice. In contrast, LC3B (autophagy related protein) positive tubular cells were decreased in KO-AKI mice compared to WT-AKI mice.
These results indicate that OPN deficiency exacerbates tubular injury via through the inhibiting autophagy in folic acid induced AKI mice.

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P545

Prehypertension is Associated With Metabolic Disturbance and Delayed Heart Rate Recovery: Potential Link Between Prehypertension and Metabolic-autonomic Dysfunction

PrimaryAuthor.AuthorBlock:**Mi-Hyang Jung**, Cardiovascular Ctr, Chuncheon Sacred Heart Hosp, Hallym Univ Coll of Med, Chuncheon, Korea, Republic of; Sang-Hyun Ihm, Dong-Hyeon Lee, Hae Ok Jung, Ho-Joong Youn, Div of Cardiology, Dept of Internal Med, The Catholic university of Korea, Seoul, Korea, Republic of

Background: Prehypertension is a highly prevalent condition, but its pathophysiology is still poorly understood. Heart rate recovery (HRR), noninvasive parameter acquired during treadmill test, is an index of autonomic dysfunction. We aimed to evaluate the association of prehypertension with autonomic dysfunction, and further, to identify the determinants of autonomic dysfunction.
Methods: We retrospectively identified 557 asymptomatic participants who did not have

hypertension and underwent comprehensive health check-ups including cardiopulmonary exercise test. They were categorized into two groups: optimal blood pressure (BP) (systolic BP<120mmHg and diastolic BP<80mmHg, n=277) and prehypertension (120≤systolic BP<140mmHg and/or 80 ≤diastolic BP<89mmHg, n=280). HRR at 1 minute (HRR_1) was also calculated as follows: peak heart rate (HR) minus HR after 1-minute recovery. **Results:** Prehypertension group exhibited more obese feature (waist circumference, 83.3±7.3 vs. 87.4±8.1cm; p<0.001) and metabolic disturbance (HOMA-IR, 0.93±0.85 vs. 1.26±1.06; uric acid 5.5±1.4 vs. 5.9±1.4; HDL-cholesterol 52.4±13.6 vs. 48.5±12.2 mg/dL; all p<0.005). They also had high pulse wave velocity (PWV, 1315.7±143.0 vs. 1453.1±187.1cm/sec, p<0.001). Although the resting HR and peak exercise HR did not differ between the groups, prehypertension group exhibited a significantly lower HRR_1 level, suggesting an impaired parasympathetic reactivation in the prehypertension (39.3±14.7 vs. 36.2±14.9 beats/min, p=0.016). In a multivariate linear regression analysis, resting HR (*ß*=-0.360, p<0.001), waist circumference (*ß*=-0.195, p=0.018), PWV (*ß*=-0.008, p=0.017), and HDL-cholesterol (*ß*=0.105, p=0.036) were independent determinants of the HRR_1. **Conclusions**: Prehypertension is associated with metabolic disturbance and autonomic dysfunction. HRR is mainly determined by waist circumference, arterial stiffness, and dyslipidemia even after adjustment for the resting HR. Our observation suggests the possible role of metabolic-autonomic abnormality in the pathogenesis of prehypertension.

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Sleep Quality in Medical Students A Cross-sectional Study

PrimaryAuthor.AuthorBlock:**Lilian S Costa**, Marina C Marins, Jocasta C Ansel, Carolina P Tavares, Fernanda T Queiroz, Jéssica B Rocha, Mariana M Banharo, Gabriela C Magalhães, Camilla S Moreira, Daniela C Schittini, Thaynã A Pavani, Beatriz V Aleix, Livia B Leig, Carlos Eduardo C Nascimento, Egberto B Santos Junior, Suellen S Oliveira, Luiza O Batista, Maria Clara A Palheiro, Renata M Jandre, Rodrigo I Salles, Bernardo P Freitas, Eduardo O Camara, Letícia N Martins, Isabelle M Freire, Karina B Lucca, Liga de Ciências Cardiovasculares da Faculdade de Medicina Souza Marques - LACCAV, Rio de Janeiro, Brazil

Introduction: Medical students, susceptible to sleep disorders, have irregular sleep-awake cycle, with repercussions on the quality of life and reduced academic performance, often with greater incidence of psychiatric disorders, estimated at 15% to 25% during your academic training. Objective: To evaluate the sleep habits in students of medical school in a private college of Rio de Janeiro, Brazil. Method: This subset study is a part of an observational study with cross-sectional delineation, with data collected through the application of an anonymous questionnaire, where they were asked about the number (and modification) of hours they sleep daily, in addition to reports of "stress and anxiety". These data collected formed the basis of an instrument for assessing the quality of life on the medical students of this College. Results: We analyzed data from 481 students: 82 (17%) at the first year, 118 (24.5%) at the second year, 99 (20.6%) at the third, 64 (13.3%) and 118 (24.5%) within the fifth and sixth years. The average age was 21.7

years (16-42) and 306 (63.6%) of female gender. As for the hours of sleep, 445 students (92.5%) report 5 to 8 hours of sleep, and 216 (44.9%) a minimum of 6 hours. In the sleep of the weekends, 394 (81.9%) reported change in the number of hours and, 313 (65.1%) referred to a reduction after the entrance into college. It was reported "some level of stress and anxiety," not related to the least number of hours sleepping (0.07). Comparing the data obtained among the 199 students of the first and second years (62.8% female-group A) to 117 at fifth and sixth years (70.9% female-group B), we observed similarity in relation to (1) number of 5 to 8 hours sleepping (A 92.9% x 90.5% B), (2) change the hours of sleepping on the weekends (A 81.9% x 79.5% B), and (3) reduction of sleepping hours after the entrance into College (A 81.9% x 78.6% B). With regard to the report of "stress and anxiety", we observed statistically significant difference between the groups (A 100% x 88% B, $p < 0.03$). Conclusion: The change in lifestyle imposed on joining a new school learning model, generates anxiety and loss of sleep hours among students. The development of resilience and adaptation to change, both individual and institutional, may have been responsible to varying degrees of stress.

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P547

Soluble Fiber Diet Alters Cardiovascular Responses To Stress In Normotensive Wky Rats

PrimaryAuthor.AuthorBlock:**Carla G Silva**, Tao Yang, Kacy Magee, Wendi Malphurs, Thomas Vickroy, Jasenka Zubcevic, Univ of Florida, Gainesville, FL

Gut microbiota is crucial for function of the gastrointestinal (GI) tract, and modulates the communication between the GI tract and the central nervous system. Microbial metabolites such as propionate have been shown to regulate blood pressure (BP), while butyrate, one of the major bacterial fermented byproducts, reportedly produces beneficial effects in multiple dysbiosis-related diseases. We previously showed that chronic supplementation with soluble fiber-rich, butyrolytic diet modified microbiota and increased BP in the spontaneously hypertensive rat (SHR). Here, we tested the impact of same diet on BP regulation in the Wistar Kyoto rats (WKY).
Methods: Male 4 weeks old WKY were placed on either the fructooligosaccharides/inulin-rich diet (fiber, N=6), or its calorie-matched control diet (control, N=6) (Research Diets, Inc.) for 14 weeks. Baseline BP was measured by tail cuff every week for the duration of the study, and by telemetry at the end of the study and during 20 minutes of restraint stress. Spectral analysis of BP waveform was performed during restraint stress to measure autonomic variables.
Results: We observed no significant difference in mean BP measured by tailcuff or by telemetry between the two groups. However, there was an increase in mean BP after 2 minutes of restraint (104.5 ± 7.45 mmHg vs. 116.3 ± 3.19 mmHg; p=0.0411, n=6), which was associated with a trend in increase in LF/HF

variable linked with vasovagal balance (1.99 ± 1.43 vs. 3.04 ± 1.46, p=0.17) in the fiber group when submitted to restraint stress. Conclusion: Unlike in the SHR, fiber-rich diet did not alter baseline BP in the normotensive WKY. However, chronic fiber diet produced autonomic imbalance and increased BP in response to restraint stress in the WKY.

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Spontaneous Report of Ilicit Drug Use among Medical Students. A Social Problem in Healthy Professionals

PrimaryAuthor.AuthorBlock:**Lilian S Costa**, Fabiana S D'Angelo, Tiago M Kobbaz, Graziella I Ribeiro, Vanessa M Morgado, Karen K Bachour, Laura B Alvarenga, Lucas V Concy, Daniela F Faria, Ingrid S Pavan, Carolina P Tavares, Fernanda T Queiroz, Jéssica B Rocha, Mariana M Banharo, Gabriela M Magalhães, Jocasta C Ansel, Camilla S Moreira, Liga de Ciências Cardiovasculares da Faculdade de Medicina Souza Marques - LACCAV, Rio de Janeiro, Brazil

Introduction: According to the World Health Organization, licit and illicit drugs when used have the ability to change the processes of consciousness, mood and thinking through your acting mechanism in the brain, which regulate mood, functions of thought and motivation. Research has shown high levels of drug use among students of medical school, which constitute alternatives to psychological problems caused by the stressful routine of a full-time course, which requires changes in lifestyle and in the acquisition of greater responsibility. Objective: To describe the prevalence of licit and illicit drug use in students of medical school to a private college in Rio de Janeiro, Brazil. Method: This subset study is part of an observational study with cross-sectional delineation performed on a sample from the first to the sixth year of medical students. The first stage of the study was designed to obtain data to prepare a socio demographic characteristics and evaluation of quality of life in these students. The data was collected from an anonymous questionnaire. Results: We evaluate 490 students: 84 (17.1%) of the first year, 119 (24.3%) of the second, 100 (20.4%) of the third, 66 (13.5%) of the fourth, 85 (17.3%) of the fifth and 36 (7.3%) of the sixth year. The average age was 21.7 years (16-42), 314 (64.1%) of female gender. In the total group, the percentage of alcohol consumption was 81.6%, there is no statistical significantly difference between the genders (81.3% male and 81.8% female) or between the basic cycles of first and second year course x internists from fifth and sixth years (79.3% x 80.8%, respectively). The consumption of illegal drugs found a percentage on the total group of 13.9%, with significant differences in both comparison groups ($p < 0.001$), 22.2% in male x 9.2% female gender and 12.8% basic cycle x 5.8% boarding school cycle. Conclusion: A sizable percentage of students reporting use of licit and illicit drugs, underscoring the increasing prevalence of alcohol use among women in the general population, especially the worrying percentage of use of illicit drugs among students. This underscores the need for strategies of University managers in implementing policies to reduce and control the consumption of drugs.

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P549

Profile of Physical Activity in Medical Students - Constructing a Quality of Life Model

PrimaryAuthor.AuthorBlock:**Lilian S Costa**, Gabriela C Magalhães, Carolina P Tavares, Fernanda T Queiroz, Jéssica B Rocha, Mariana M Banharo, Camilla S Moreira, Marina C Marins, Vanessa M Morgado, Liga de Ciências Cardiovasculares da Faculdade de Medicina Souza Marques - LACCAV, Rio de Janeiro, Brazil

Introduction: According to the World Health Organization , performing physical activity (PA) results in less chance of coronary heart disease development, hypertension, diabetes, obesity, heart attack, colon and breast cancer and depression, in addition to promote quality of life (QOL) improvement. Some studies show that students' perceptions about QOL during your college experience are essentially related to the levels of academic support. Thus, it is considered that the level of subject PA is crucial in promoting health and QOL in medical students and have important role to disseminate information in society, that could modify the community culture and habits where they are inserted. Objective: To identify the profile of PA on medical students of a private College in Rio de Janeiro, Brazil. Method: This subset study is part of an observational study with cross-sectional delineation, with data collected through the application of an anonymous questionnaire. The collection of these data was the basis of an instrument for assessing the QOL of the medical students of this College. For descriptive purposes, we analyze gender, age, BMI (body mass index), year, place and frequency of PA, type of exercise performed and time spent per week with PA. Results: Of the 490 students with an average age 21.7 years (16-42 years), 64.1% female, 75.1% practiced PA prior to entered the medical college, but 38.4% decreased the frequency after initiated the college, 22.2% perform regular PA three times a week, 44.5% practice anaerobic and aerobic PA and 28.6% are sedentary. Among the male participants, 83% x 70.7% female, practiced PA before starting the course of Medicine (p 0.003), both with reduced frequency after admission in college (38.1% x 38.5%, respectively, p NS). The combined aerobic and anaerobic activities predominates in both genders. Comparing the data obtained between the first and second years (Group A), with the fifth and sixth years students (Group B), statistically significant relative (1) sedentary (A 34.5% x 23.3% B) and reduction PA after admission in college (A 33% x 56.7% B). Conclusion: Studies confirm that the teaching model with full-time activities imposed in medicine schools, reduces the time required to adapt personal habits and maintain a good QOL.

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High Uric Acid Levels Correlate With Treatment- Resistant Hypertension

PrimaryAuthor.AuthorBlock:**Badhma Valaiyapathi 35205**, Mohammed Siddiqui, Suzanne Oparil, David A Calhoun, Tanja Dudenbostel, Univ of Alabama at Birmingham, Birmingham, AL

Background: Serum uric acid (sUA) levels have been found to be positively associated with increased risk of hypertension (HTN), independent of other cardiovascular risk factors. The role of sUA elevation in patients with resistant hypertension (RHTN) is unknown. We hypothesized that sUA levels are higher in RHTN patients compared to patients with controlled HTN.
Methods: This retrospective study included, 140 patients from the University of Alabama at Birmingham Hypertension Clinic. Patient characteristics including body mass index (BMI), office blood pressure (BP) and sUA levels were analyzed. RHTN was defined as office BP > 140/90 mmHg on ≥ 3 or more different antihypertensive agents including a diuretic. Patients with RHTN were compared with a control group with controlled hypertension. Patients with sUA levels <3 mg/dl, who were on treatment with allopurinol, and those with missing values were excluded from the study.
Results: Patient characteristics of 91 included patients were: 53.4% female, 40.7% African American, mean age 58.8 ± 12.4 years, mean BMI 33.1 ± 7.5 kg/m^2, mean sUA 6.6 ± 1.9 mg/dL. Mean sUA was higher among RHTN patients compared to the control group (p = 0.0031). Treatment resistance was found to be strongly correlated with sUA levels of ≥ 6 mg/dl (p = 0.0065).
Conclusion: In this retrospective study, sUA levels were found to be significantly higher among resistant HTN patients compared to controlled HTN patients, indicating that high sUA levels (≥ 6 mg/dl) may play a role in treatment resistance among hypertensive patients.

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Platelet Activation is Associated with Pulmonary Edema and Renal Injury in a Rat Model of Polymicrobial Sepsis

PrimaryAuthor.AuthorBlock:Jeanne Ishimwe, Maggie L McCalmon, Corbin A Shields, Ashley Gnam, Jan M Williams, **Denise C Cornelius**, UNIVERSITY OF MISS MED CENTER, Jackson, MS

Sepsis, life-threatening organ dysfunction due to a dysregulated host response to infection, is positively correlated with platelet activation. Furthermore, clinical studies have also shown that platelet activation is associated with sepsis severity, suggesting a role for platelets in sepsis pathophysiology. Despite this correlation, the underlying mechanisms by which activated platelets contribute to sepsis are under investigated. In preliminary studies, we set out to determine if platelet activation is associated with multi-organ dysfunction and injury in a rat model of chronic polymicrobial abdominal sepsis. Sepsis was induced via cecal ligation and puncture (CLP) followed by cecum removal 24 hours post-CLP. At 72 hours post-CLP, blood, urine, and tissues were collected for analysis. Platelet activation was measured via flow cytometry. Lung wet/dry ratio and plasma creatinine were measured to assess lung edema and renal injury, respectively. Platelet activation doubled in CLP rats versus Sham rats. Activated platelets increased from 3.8±1.7% of the gated population in Sham animals (n=5) to 9.2±1.9% of the gated population in CLP animals (n=5; p=0.07). Lung wet/dry ratio significantly

increased from 3.9±0.2 in Sham (n=8) to 6.7±1 in CLP rats (n=8; p<0.05). Furthermore, plasma creatinine increased by 33% from 0.55±0.3 mg/dL in Sham animals (n=6) to 0.73±0.06 mg/dL in CLP rats (n=8; p<0.05), indicating a decrease in renal function. These data demonstrate, for the first time, an increase in platelet activation in response to CLP, and identifies an association of activated platelets with pulmonary edema and reduced renal function in the cecal ligation and puncture rat model of abdominal polymicrobial sepsis. Future studies will investigate the underlying mechanisms by which activated platelets contribute to multi-organ dysfunction and injury in sepsis.

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Central Cytochrome P450 1B1 Generated 17Beta Estradiol Metabolite 2-Methoxyestradiol Minimizes Angiotensin II-induced Hypertension in Female Mice

PrimaryAuthor.AuthorBlock:**Purnima Singh**, Chiyoung Song, Scott A. Heldt, Kafait U. Malik, Univ of Tennessee, Memphis, TN

Recently we have shown that 17beta-estradiol (E2)-generated cytochrome P450 1B1 (CYP1B1) metabolite 2-methoxyestradiol (2-ME) protects against angiotensin II (Ang II)-induced hypertension in female mice. The demonstration that central E2 inhibits Ang II-induced hypertension together with the expression of CYP1B1 in various areas in the brain and the production of 2-ME from E2 by microglia cells led us to hypothesize that 2-ME contributes to the inhibitory effect of E2 on the brain on Ang II- induced hypertension in female mice. To test this hypothesis, we examined the effect of intracerebroventricular (ICV) administered E2 in ovariectomized (OVX) wild-type (*Cyp1b1*$^{+/+}$) and OVX *Cyp1b1*$^{-/-}$ mice on the action of systemic Ang II (700 ng/kg/min) for 14 days. E2 (1.5 µg/2µL/injection) or its vehicle (20% w/v 2-Hydroxypropyl-β-cyclodextrin dissolved in artificial CSF) was injected (400 nL/min every 2nd day) via a cannula implanted in the brain. Mean arterial blood pressure (MAP) was measured by radiotelemetry (n=3-4). E2 but not its vehicle attenuated Ang II-induced increase in MAP on Day 12 in OVX *Cyp1b1*$^{+/+}$ (111±2 vs. 154±5, mmHg, P<0.05). ICV injections of 2-ME but not E2 (1.5 µg/2µL/injection every 2nd day) attenuated the increase in MAP by Ang II in OVX *Cyp1b1-/-* mice (111±1 vs. 141±6, mmHg, P<0.05). Administration of ganglionic blocker hexamethonium (30 mg/Kg, IP) on day 14 of Ang II infusion resulted in greater reduction in MAP (P<0.05, n=4) in centrally injected E2 in OVX *Cyp1b1*$^{-/-}$, and vehicle-injected OVX *Cyp1b1*$^{+/+}$ mice (Δ90±1 and Δ91±14, mmHg) than in E2 injected OVX *Cyp1b1*$^{+/+}$ (Δ68±5 mmHg), and 2-ME injected OVX *Cyp1b1*$^{-/-}$ mice (Δ60±4 mmHg). Furthermore, in intact *Cyp1b1*$^{-/-}$, but not OVX *Cyp1b1*$^{-/-}$ mice reconstitution of CYP1B1 in the brain by transduction with adenovirus (AdV)-CYP1B1-cDNA (ICV 2µL of 1.0 X 10^{12}particle/mL) reduced systolic blood pressure (SBP) measured by tail-cuff on Day12 (136±2 vs. 166±6 mmHg, P<0.05, n=4-5). ICV injection of AdV-GFP-cDNA in these mice did not alter Ang II-induced increase in SBP (168±8 and 171±2 mmHg, n=4). These data suggest that central effect of E2 to attenuate Ang II-induced hypertension is dependent on brain CYP1B1 and is most likely mediated via generation of 2-ME, which decreases sympathetic outflow in female mice.

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